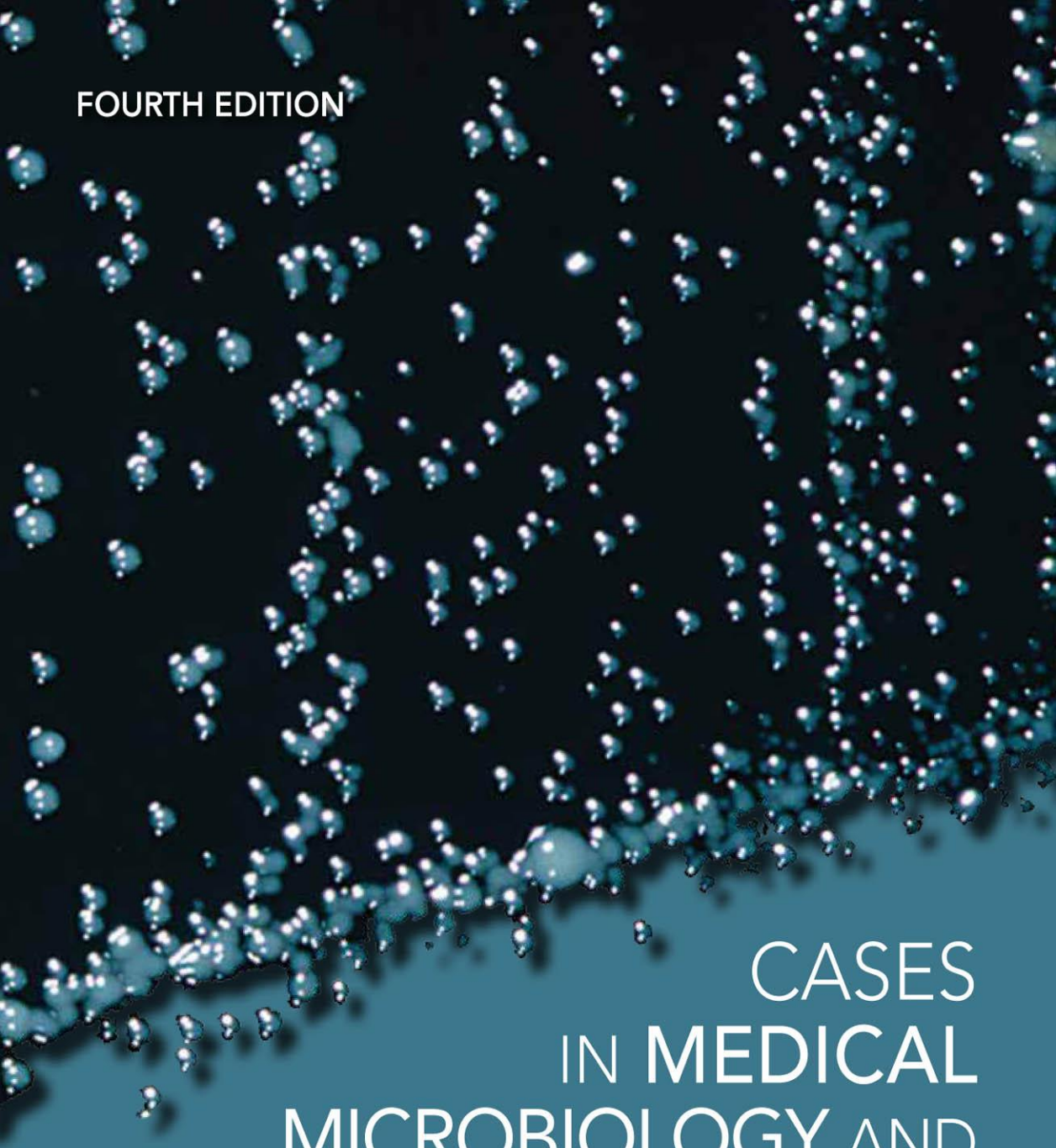


FOURTH EDITION

CASES IN MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Peter H. Gilligan, Ph.D.
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Melissa B. Miller, Ph.D.

Table of Normal Values[a]

WBC	4,000–12,000/μl	[4–12 × 10^9/liter]
Neutrophils	2,000–7,500/μl	[2–7.5 × 10^9/liter]
Eosinophils	40–400/μl	[0.04–0.40 × 10^9/liter]
Platelets	150,000–400,000/μl	[150–400 × 10^9/liter]
pO_2	85–100 mmHg	[11.3–13.3 kPa]
CD4 count	430–1,185/μl (adults)	[Same]

	Male	Female
Hemoglobin	13.4–17.4 g/dl	12.3–15.7 g/dl
[Haemoglobin]	[Same]	[Same]
Hematocrit	40–54%	38–47%
[Haematocrit]	[0.4–0.54 liter/liter]	[0.38-0.47 liter/liter]
Erythrocyte sedimentation rate	0–20 mm/h	0-30 mm/h

[ESR is usually calculated by age: male (ESR = 0.5 × age); female (ESR = 0.5 × {age + 10}); alternatively, the American values given here usually apply.]

	Male	Female	Newborn	Age 1
ALT	10–53 U/liter	7–30 U/liter		
AST	11–40 U/liter	9–26 U/liter	35–140 U/liter	20–60 U/liter
Creatinine	0.8–1.5 mg/dl	0.6–1.2 mg/dl	Lower for children	

[Creatinine (male and female) = 70–150 μmol/liter]

Creatinine kinase	61–200 U/liter	30–125 U/liter

Albumin	3.5–5.0 g/dl	[35–50 g/liter]
Serum glucose (fasting)	65–110 mg/dl	[<3.6–6.1 mmol/liter]
Alkaline phosphatase	39–117 U/liter	[Same]
Total bilirubin	0–1.2 mg/dl	[0–20 μmol/liter]
Lactate dehydrogenase	108–215 U/liter	[Same]
CSF glucose	50–75 mg/dl	[2.8–4.2 mmol/liter, or 2/3 blood glucose]
CSF protein	15–45 mg/dl	[0.15–0.45 g/liter]
CSF total nucleated cells	0–3/μl	[Same]
Body temperature	37°C	
Heart rate	60–100/min; higher for infants and children	
Respiratory rate	9–18/min; higher for infants and children	
Blood pressure	90–150/50–90; lower for infants and children	

[a]Values in brackets indicate European equivalents. If no value is given, the American value is used.

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by

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For Lynn, whose idea this book was.
Peter

To those who have taught me in the areas of infectious diseases and clinical microbiology.
Dan

For my family, who endured many hours of my writing at home.
Melissa

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INTRODUCTION

TO THE FOURTH EDITION

It has been almost a decade since the 3rd edition of this text was published. Much has happened in the world of infectious diseases during this time. First, there has been recognition that the problems of infectious diseases are truly global and that infectious diseases in one part of the world can be quickly transmitted to another. Prime examples of this were the severe acute respiratory syndrome (SARS), the 2009 H1N1 influenza A virus outbreak, and multidrug-resistant Gram-negative bacilli (MDR-GNB). Genes for multidrug resistance can be carried on extrachromosomal genetic elements, facilitating the spread of these drug resistance determinants to highly virulent organisms such as was seen in the Shiga toxin-producing *Escherichia coli* (STEC) outbreak due to the O104 serotype in Germany in 2011. These emerging pathogens are literally a plane ride away, no matter where they are found globally, and can be disseminated worldwide in a matter of days to weeks.

MDR-GNB, environmental mycobacteria, and molds are emerging as important pathogens in the ever-expanding population of immunocompromised hosts. These organisms, although of comparatively low virulence when compared to highly adapted human pathogens such as *Streptococcus pneumoniae* or group A streptococci, have distinct characteristics that make them very worrisome. First, they have evolved over millions of years, adapting to harsh environments which contain antimicrobial molecules. As a result, organisms such as *Acinetobacter baumannii*, *Mycobacterium abscessus* group, and *Fusarium* spp. have high levels of intrinsic drug resistance. Additionally, they have comparatively large amounts of DNA, giving them a broad genetic repertoire which allows them to survive in hostile environments such as hospital surfaces and equipment. Finally, many MDR-GNBs are genetically promiscuous, taking up DNA which may contain resistance genes from other species or genera of bacteria. This promiscuity has led to a new concept in antimicrobial resistance, the "antimicrobial resistome," which describes all the antimicrobial-resistant genes in a particular environment.

Rapid expansion in our understanding of molecular biology has greatly enhanced our knowledge of the etiology and epidemiology of infectious diseases. The evolution of molecular diagnostics makes it possible to design a nucleic acid amplification test (NAAT) in a matter of days to detect newly emerging pathogens, such as was done with the 2009 H1N1 influenza A virus. Other applications of NAAT testing allow us to rapidly detect viruses which are not cultivable or were unknown when the 3rd edition of this book was published. DNA sequencing has led to a clearer understanding of how organisms such as members of the *Burkholderia cepacia* and *Mycobacterium chelonae/abscessus* complexes are involved in numerous disease processes. Using the tools of direct 16S rRNA gene sequencing, we have greatly improved the etiologic diagnosis of bacterial

endocarditis and septic arthritis, leading to an improvement in our understanding of these disease entities.

One of the most significant advances in the study of infectious disease in the past decade has been the Human Microbiome Project. Microbiome studies have shown that many of the microorganisms that are present in our bodies are not cultivable. This observation challenges our basic assumptions of defining a human pathogen based on its ability to grow *in vitro* or in animal models. The Human Microbiome Project is increasing our understanding of the role of microbial communities in chronic infection, such as those seen in chronic lung disease in cystic fibrosis patients and in chronic wounds of the extremities in diabetics. It is also likely that probing the microbiome will give us greater understanding of such disparate conditions as obesity, inflammatory bowel disease, and perhaps a variety of rheumatologic disorders.

The past decade offered examples of the impact that public health measures can have on the dissemination of infectious diseases following natural disasters. One of the most destructive hurricanes in U.S. history, Katrina, caused massive damage in New Orleans in August 2005 but was responsible for few deaths due to infection and no significant disease outbreaks, despite a complete collapse of that city's infrastructure and significant damage to medical facilities. This is a testament to the public health interventions that were put in place soon after this catastrophe. This success is in stark contrast to the cholera outbreak that occurred following the magnitude 7 earthquake in Haiti in January 2010. Ironically, Haiti was essentially cholera free until the earthquake. The organism was shown to have been brought to Haiti by UN soldiers from Nepal who were there for humanitarian purposes. This outbreak began several months after the earthquake and the epidemic is still ongoing; as of this writing, more than 8,500 people have died. The reason for this difference is clearly one of resources. Haiti continues to struggle with repairing and upgrading its infrastructure to provide basic sanitation and clean water for its population, while New Orleans and its environs are essentially back to "normal."

As discouraging as the emergence of MDR-GNBs and the failure to control disease epidemics due to scarce resources might be, much has been accomplished in the past decade in improving the lives of those afflicted with or at risk for infectious disease. Two advances clearly stand out. First, the demonstration that the spread of HIV could be greatly reduced by pre-exposure prophylaxis gives hope that this epidemic that has caused so much suffering can be blunted. Second, new biologics including vaccines and monoclonal antibody preparations are playing an important role in not only infectious diseases but other diseases where there is a malfunctioning of the immune system.

Two vaccines of particular note have been the conjugate 7-valent, now 13-valent, *Streptococcus pneumoniae* vaccine and a malaria vaccine. The conjugate pneumococcal vaccine has been shown to reduce disseminated disease not only in its target group, young children, but also in the entire population—a clear example of herd immunity. A prototype malaria vaccine has shown success in phase 3 clinical trials and has great promise for

reducing malaria disease burden especially among young children, the vaccine's targeted population.

New monoclonal antibodies show tremendous promise for the treatment of a variety of diseases due to immune dysregulation while at the same time placing individuals at peril for unintended consequences of this therapy. As a result, care providers are faced with "black box" warnings which advise of potentially fatal infectious disease complications of these promising therapies.

The 4th edition of this text provides cases that will illustrate many of these issues. The goal of this edition continues to be to challenge students to develop a working knowledge of the variety of microorganisms that cause infections in humans. This working knowledge is rapidly expanding due to the rapid and increased deployment of NAAT and sequence analysis for detection and identification of microorganisms. As a result, many of the cases have a significant molecular diagnostic component. The "Primer on the Laboratory Diagnosis of Infectious Diseases" has been updated and expanded to reflect the increasing importance of molecular-based assays.

The basic format of this edition is consistent with that of the previous three editions. The cases are presented as "unknowns" and represent actual case presentations of patients we have encountered during our professional duties at two university teaching hospitals. Each case is accompanied by several questions to test knowledge in four broad areas:

- The organism's characteristics and laboratory diagnosis
- Pathogenesis and clinical characteristics of the infection
- Epidemiology
- Prevention, and in some cases, drug resistance and treatment

This edition features a new section titled "Advanced Cases," which replaces the section titled "Emerging and Re-Emerging Infectious Diseases." The types of cases that are seen by our infectious disease consult services and discussed in our weekly infectious disease case management conferences will be found here. These include newly recognized disease agents as well as highly complex cases where the interaction of the immune system and human pathogens can be more closely examined. The Advanced Cases section has all new cases.

This edition contains 74 cases, of which 42 are new. The new cases explore many of the issues described above in this introduction. The 32 cases that have been retained have been updated to reflect the current state of the art as it relates to the organism causing the infection.

The most significant change in the 4th edition is that we bid adieu to one of the authors of the first three editions, Dr. Lynn Smiley, and welcome a new author, Dr. Melissa Miller. This work was Dr. Smiley's idea, an idea that she helped bring to fruition through

three editions. She now passes the mantle to Dr. Miller. Dr. Miller, Director of the Molecular Microbiology Laboratory at UNC Health Care, brings a wealth of knowledge on the molecular aspects of infectious diseases, especially in the fields of virology and antimicrobial resistance. This expertise is essential to produce a contemporary text in medical microbiology and infectious disease. We welcome her!

TO THE STUDENT

This text was written for you. It is an attempt to help you better understand the clinical importance of the basic science concepts you learn either in your medical microbiology or infectious disease course or through your independent study. You may also find that this text is useful in reviewing for Part I of the National Board of Medical Examiners exam. It should be a good reference during your Infectious Disease rotations.

Below is a sample case, followed by a discussion of how you should approach a case to determine its likely etiology.

SAMPLE CASE

A 6-year-old child presented with a 24-hour history of fever, vomiting, and complaining of a sore throat. On physical examination, she had a temperature of 38.5°C, her tonsillar region appeared inflamed and was covered by an exudate, and she had several enlarged cervical lymph nodes. A throat culture plated on sheep blood agar grew many beta-hemolytic colonies. These colonies were small with a comparatively wide zone of hemolysis.

What is the likely etiologic agent of her infection?

The first thing that should be done is to determine what type of infection this child has. She tells you that she has a sore throat, "my throat hurts." On physical examination, she has sign of an inflamed pharynx with exudate, which is consistent with her symptoms. (Do you know what an exudate is? If not, it's time to consult the glossary in the back of this text.) She also has enlarged regional lymph nodes, which support your diagnosis of pharyngitis (sore throat).

What is the etiology of her infection? The obvious response is that she has a "strep throat," but in reality there are many agents which can cause a clinical syndrome indistinguishable from that produced by group A streptococci, the etiologic agent of "strep throat." For example, sore throats are much more frequently caused by viruses than streptococci. Other bacteria can cause pharyngitis as well, including *Mycoplasma* spp., various *Corynebacterium* spp., *Arcanobacterium* sp., and *Neisseria* gonorrhoeae. All of these organisms would be in the differential diagnosis, along with other perhaps more obscure causes of pharyngitis.

However, further laboratory information narrows the differential diagnosis considerably; small colonies that are surrounded by large zones of hemolysis are consistent with beta-hemolytic streptococci, specifically group A streptococci. On the basis of presenting signs and symptoms and the laboratory data, this child most likely has group A streptococcal pharyngitis.

Specific aids have been added to the book to assist you in solving the cases.

1. The book begins with "A Primer on the Laboratory Diagnosis of Infectious Diseases." The purpose of this section is to explain the application and effectiveness of different diagnostic approaches used in the clinical microbiology laboratory. We recommend that you read this primer before beginning your study of the cases.
2. At the beginning of each book section is a brief introduction and a list of organisms. Only organisms on this list should be considered when solving the cases in that section. These lists have been organized on the basis of important characteristics of the organisms.
3. A table of normal values is available inside the front cover of this book. If you are unsure whether a specific laboratory or vital sign finding is abnormal, consult this table.
4. A glossary of medical terms which are frequently used in the cases is available at the end of the text. If you do not understand a specific medical term, consult the glossary. If the term is not there, you will have to consult a medical dictionary or other medical texts.
5. Figures demonstrating microscopic organism morphology are presented in many of the cases, as are key radiographic, laboratory, clinical, or pathologic findings. They provide important clues in helping you determine the etiology of the patient's infection. Because many medical schools have abandoned "wet" labs where medical students get to do "hands-on" microbiology, we felt it was important to have a richly illustrated text.

A FINAL THOUGHT

The temptation for many will be to read the case and its accompanying questions and then go directly to reading the answers. You will derive more benefit from this text by working through the questions and subsequently reading the case discussion.

Have fun and good luck!

A PRIMER

ON THE LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

The accurate diagnosis of infectious diseases often but not always requires the use of diagnostic tests to establish their cause. The utilization of diagnostic tests in the managed care environment is carefully monitored and is frequently driven by standardized approaches to care called "clinical pathways" or "clinical care algorithms." These pathways include using a predefined set of diagnostic tests for patients who present with signs and symptoms characteristic of certain clinical conditions, such as community-acquired pneumonia. Currently, the Infectious Diseases Society of America has published more than 30 different "practice guidelines" dealing with various infectious diseases, including HIV, tuberculosis, group A streptococcal pharyngitis, diarrheal disease, and pneumonia, from which clinical pathways can be derived. Clinical pathways and practice guidelines fall under the concept of "evidence-based medicine." Evidence-based medicine relies on review and interpretation of data in the medical literature as a basis for clinical decision making.

In some patients, such as an otherwise healthy child with a rash typical of varicella (chicken pox), the etiology of the infection can be established with a high degree of certainty by physical examination alone. The use of diagnostic testing in this setting would be viewed as wasteful of the health care dollar. On the other hand, a 4-year-old who presents with enlarged cervical lymph nodes and a sore throat should have a diagnostic test to determine whether he or she has pharyngitis due to group A streptococci. The reason why such testing is necessary is that certain viral syndromes are indistinguishable clinically from group A streptococcal pharyngitis. Because group A streptococcal pharyngitis should be treated with an antibiotic to prevent poststreptococcal sequelae, and viral infections do not respond to antibiotics, determining the cause of the infection in this particular case is central to appropriate patient management. Far too often, antibiotics are given without diagnostic testing in a child with a sore throat. As a result, many children with viral pharyngitis are given antibiotics. This inappropriate use of antibiotics increases antibiotic selective pressure. This can result in greater antimicrobial resistance among organisms in the resident microbiota of the throat, such as *Streptococcus pneumoniae*. In addition, patients may develop antibiotic-associated complications, such as mild to severe allergic reactions or gastrointestinal distress including diarrhea. One of the goals of the fourth edition of this text is to help you think in a cost-effective way about how best to use laboratory resources. As an introduction to this edition, we will present a general overview of the various laboratory approaches that are used in the diagnosis and management of infectious diseases.

ACCURACY IN LABORATORY TESTING

The clinical microbiology laboratory must balance the requirements of timeliness with those of accuracy.

As an example, consider the identification of a Gram-negative bacillus from a clinical specimen. If the organism is identified with the use of a commercially available identification system, an identification and an assessment of the probability of that identification will be made on the basis of biochemical test results and a comparison of these results with a database. So, if the result states that the organism is *Enterobacter cloacae* with 92% probability, the laboratory may very well report this identification. Assuming that the 92% probability figure generated by the commercial system is on target (many commercial systems do a worse job with anaerobic bacteria), this means that there is a probability of 8%, or about 1 time in 12, that this identification will be incorrect.

Certainly, it would be possible for the laboratory to perform additional testing to be more certain of the identification. The problem is that by doing so there would be a delay, perhaps a clinically significant one, in the reporting of the results of the culture. In some cases such a delay is unavoidable (e.g., when the result of the identification in the commercial system is below an arbitrary acceptable probability and manual methods must be used) or clinically essential (e.g., when a specific identification is required and the isolate must be sent to a reference laboratory for identification; an example is *Brucella* spp., which require prolonged therapy and are potential agents of bioterrorism).

Similarly, the methods most commonly used in the clinical laboratory for susceptibility testing are imperfect. The worst errors, from the clinical point of view, are those in which the laboratory reports an organism as susceptible to a particular antibiotic to which, in fact, it is resistant. In some cases, additional tests are employed to minimize the risk of this occurring. For example, in addition to standard testing using either an automated or a manual method, recommended susceptibility testing of *Enterococcus* includes the use of Mueller-Hinton agar in which the antibiotic vancomycin is present at a known concentration. Even if the results of the standard susceptibility testing indicate susceptibility to vancomycin, if there is growth of the *Enterococcus* isolate on the vancomycin-containing Mueller-Hinton plate, the organism is reported as resistant to vancomycin.

Unfortunately, very few such checks exist to correct erroneous bacterial susceptibility assays. In general, there is a delay in the ability of automated susceptibility methods to reliably identify newly described mechanisms of antibiotic resistance. As a result, manual methods are often required. The performance of automated susceptibility testing methods varies, and certain combinations of organism and antibiotic have an unacceptably high error rate. In such cases, backup methods, such as disk diffusion or MIC testing, should be employed. Laboratories with a significant number of susceptibility tests to perform commonly use automated susceptibility methods because of the labor-intensive nature of manual testing and the speed with which automated systems are able to report results—often in a few hours as compared with overnight incubation, as is the case with manual methods.

Diagnostic tests vary in their **sensitivity** and **specificity**. As an example, consider a hypothetical STI (sexually transmitted infection) clinic in which the rapid plasma reagin (RPR) test, a screening test for syphilis, is being evaluated in 1,000 patients with genital ulcer disease who are suspected of having primary syphilis:

		PRIMARY SYPHILIS: PRESENT	PRIMARY SYPHILIS: ABSENT	
RPR TEST RESULT	**POSITIVE**	**420**	**60**	Positive predictive value = 420/(420 + 60) = 0.88 Positive predictive value = 88%
	NEGATIVE	**220**	**300**	Negative predictive value = 300/(300 + 220) = 0.58 Negative predictive value = 58%
		Sensitivity = 420/(420 + 240) = 0.66 Sensitivity = 66%	Specificity = 300/(300 + 60) = 0.83 Specificity = 83%	

On the basis of these data, the sensitivity of this test (the true-positive rate, calculated as true-positive results divided by the number of patients with disease) in primary syphilis is 66%. The specificity (1 minus the false-positive rate) is 83%. Note that in this high-prevalence population (the prevalence here is the total number of cases in which primary syphilis is present—640 divided by the total number of individuals, 1,000—and is thus 0.64 or 64%), the predictive value of a positive test is fairly good, at 88%. **The positive predictive value of an assay varies with the prevalence of the disease in the population.** This is a key point. An example of this in our syphilis serology example in a low-prevalence population will serve to illustrate the point.

The same RPR serologic assay is being used in a hypothetical population of octogenarian nuns, none of whom are or have been sexually active in at least 6 decades.

		SYPHILIS: PRESENT	SYPHILIS: ABSENT	
RPR TEST RESULT	**POSITIVE**	**1**	**169**	Positive predictive value = 1/170 = 0.006 Positive predictive value = 0.6%
	NEGATIVE	**0**	**830**	Negative predictive value = 830/830 = 1.00 Negative predictive value = 100%
			Specificity = 830/999 = 0.83 Specificity = 83%	

In this patient population, there is only one true case of syphilis, presumably acquired many years previously. The specificity of the test in this patient population is the same as it is in the individuals attending the STI clinic (in reality, it is likely to be different in different populations and also in different stages of syphilis). Because there is one case of syphilis, and 169 of the positive RPR results are false-positive test results, the positive predictive value in this patient population is only 0.6%. Clearly, this is a patient population in which the decision to test for syphilis using the RPR assay is not cost-effective.

In making a decision to order a specific test, the physician should know what he or she will do with the test results—essentially, how the results will alter the care of the patient. In a patient who the physician is certain does not have a specific disease, if the test for that disease has an appreciable rate of false-positive results, a positive test result is likely to be false positive and should not alter clinical care. Conversely, if the physician is certain that a patient has a disease, there is no good reason to order a test with a low sensitivity, as a negative result will likely be false negative. Tests are best used when there is uncertainty and when the results will alter the posttest probability and, therefore, the management of the patient.

SPECIMEN SELECTION, COLLECTION, AND TRANSPORT

Each laboratory test has three stages.

1. **The preanalytical stage:** The caregiver selects the test to be done, determines the type of specimen to be collected for analysis, ensures that it is properly labeled with the patient's name, and facilitates rapid and proper transport of this specimen to the laboratory.
2. **The analytical stage:** The specimen is analyzed by the laboratory for the presence of specific microbial pathogens. The remaining sections of this chapter describe various analytic approaches to the detection of pathogens.
3. **The postanalytical stage:** The caregiver uses the laboratory results to determine what therapies, if any, to use in the care of the patient.

The preanalytical stage is the most important stage in laboratory testing! If the wrong test is ordered, if the wrong specimen is collected, if the specimen is labeled with the wrong patient's name, or if the correct specimen is collected but is improperly transported, the microbe causing the patient's illness may not be detected in the analytical stage. As a result, at the postanalytical stage, the caregiver may not have the appropriate information to make the correct therapeutic decision. The maxim frequently used in laboratory medicine is "garbage in, garbage out."

Specimen selection is important. A patient with a fever, chills, and malaise may have an infection in any one of several organ systems. If a patient has a urinary tract infection and if urine is not selected for culture, the etiology and source of the infection will be

missed. Careful history taking and physical examination play an important role in selecting the correct specimen.

Continuing with the example of a patient with a fever due to a urinary tract infection, the next phase in the diagnosis of infection is the collection of a urine specimen. Because the urethra has resident microbiota, urine specimens typically are not sterile. A properly collected urine specimen is one in which the external genitalia are cleansed and midstream urine is collected. Collection of midstream urine is important because the initial portion of the stream washes out much of the urethral microbiota. Even with careful attention to detail, clean-catch urine can be contaminated with urethral microbiota, rendering the specimen uninterpretable at the postanalytical stage.

An important concept when considering the transport of clinical specimens for culture is to recognize that they contain living organisms whose viability is influenced by transport conditions. These organisms may be killed by changes in temperature, drying of the specimen, exposure to oxygen, lack of vital nutrients, or changes in specimen pH. Transport conditions that support the viability of any clinically significant organisms present in the specimen should be established. It should also be noted that the longer the transport takes, the less likely it is that viability will be maintained. Rapid transport of specimens is important for maximal accuracy at the analytical stage.

If the correct test is selected, the proper specimen is collected and transported, but the specimen is labeled with the wrong name, the test findings might be harmful to two different patients. The patient from whom the specimen came might not receive the proper therapy, while a second patient whose name was mistakenly used to label the specimen might receive a potentially harmful therapy.

DIRECT EXAMINATION

Macroscopic

Once a specimen is received in the clinical laboratory, the first step in the determination of the cause of an infection is to examine it. Frequently, infected urine, joint, or cerebrospinal fluid specimens will be "cloudy" because of the presence of microorganisms and white blood cells, suggesting that an infectious process is occurring. Occasionally, the organism can be seen by simply looking for it in a clinical specimen or by looking for it on the patient. Certain worms or parts of worms can be seen in the feces of patients with ascariasis or tapeworm infections. Careful examination of an individual's scalp or pubic area may reveal lice, while examination of the anal region may result in the detection of pinworms. Ticks can act as vectors for several infectious agents, including Rocky Mountain spotted fever, Lyme disease, and ehrlichiosis. When they are found engorged on the skin, physicians may remove and submit these ticks to the laboratory to determine their identity. This is done because certain ticks (deer ticks) act as a vector for certain infectious agents (*Borrelia burgdorferi*, the organism that causes Lyme disease). Knowing the vector may help the physician determine the patient's diagnosis.

Microscopic

Because most infectious agents are visible only when viewed with the aid of a light microscope, microscopic examination is central to the laboratory diagnosis of infectious diseases. Microscopic examination does not have the overall sensitivity and specificity of culture or the newer molecular diagnostic techniques. However, microscopic examination is very rapid, is usually relatively inexpensive (especially when compared with molecular techniques), is available around the clock in at least some formats in most institutions, and in many clinical settings, but by no means all, is highly accurate when done by highly skilled laboratorians. The organisms can be detected either unstained or by using a wide variety of stains, some of which are described below. Microbes have characteristic shapes that are important in their identification. Morphology can be very simple, with most clinically important bacteria generally appearing as either bacilli (Fig. 1a) or cocci (Fig. 1b). The bacilli can be very long or so short that they can be confused with cocci (coccobacilli); they can be fat or thin, have pointed ends, or be curved. The arrangement of cocci can be very helpful in determining their identity. These organisms can be arranged in clusters (staphylococci), pairs or diplococci (*S. pneumoniae*), or chains (various streptococcal and enterococcal species).

Fungi are typically divided into two groups based on morphology. One is a yeast (Fig. 2), which is a unicellular organism, and the other is a mold, which is a multicellular organism with complex ribbon-like structures called hyphae (Fig. 3). Organisms that are referred to as parasites may be unicellular—the protozoans (Fig. 4)—or highly complex—the nematodes and cestodes (Fig. 5). Parasites are typically identified on the basis of morphology alone.

Because of their small size, viruses cannot be visualized by light microscopy. Alternative approaches described below are needed to detect these microbes in clinical specimens.

Wet mounts

The wet mount technique is extremely simple to perform. As the name implies, the clinical specimen is usually mixed with a small volume of saline, covered with a glass coverslip, and examined microscopically. It is most commonly utilized to examine discharges from the female genital tract for the presence of yeasts or the parasite *Trichomonas vaginalis*. Wet mounts are also used to make the diagnosis of oral thrush, which is caused by the yeast *Candida albicans*. Using a special microscopic technique—dark-field microscopy—scrapings from genital ulcers and certain skin lesions can be examined for the spirochete *Treponema pallidum*, the organism that causes syphilis. This technique is not particularly sensitive but is highly specific in the hands of an experienced microscopist. It is typically done in STI clinics where large numbers of specimens are available, enabling the microscopist to maintain his or her skill in detecting this organism.

The wet mount can be modified by replacing a drop of saline with a drop of a 10% KOH solution to a clinical specimen. This technique is used to detect fungi primarily in

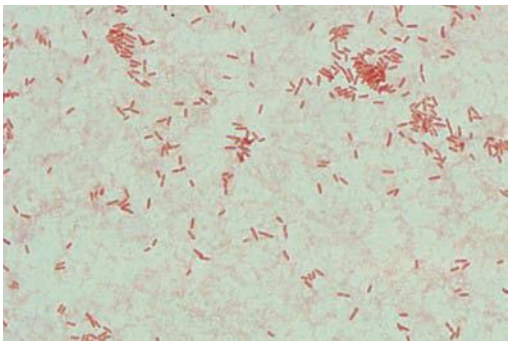


Figure 1a

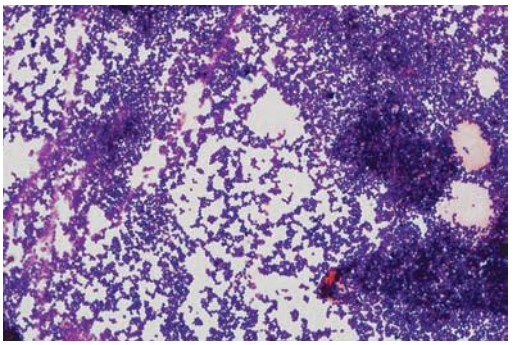


Figure 1b

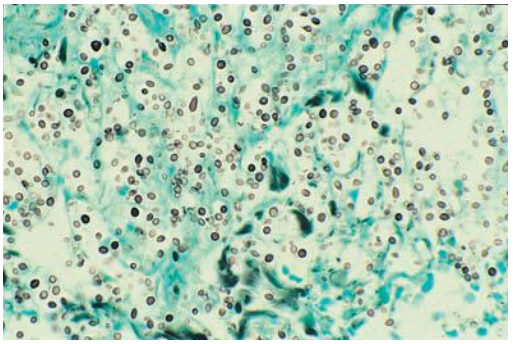


Figure 2

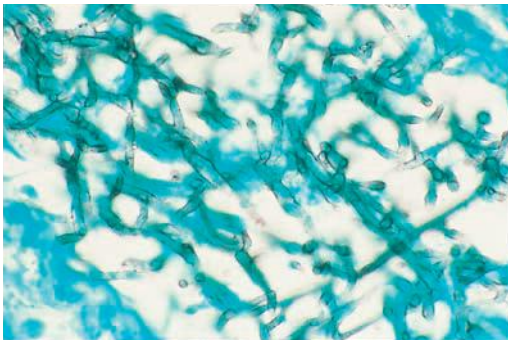


Figure 3

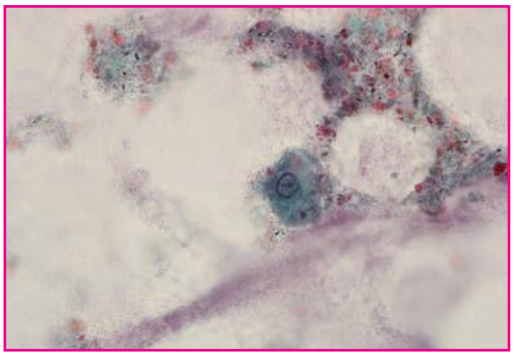


Figure 4



Figure 5

sputum or related respiratory tract specimens, skin scrapings, and tissues. The purpose of the KOH solution is to "clear" the background by "dissolving" tissue and bacteria, making it easier to visualize the fungi.

Another modification of the wet mount is to mix a drop of 5% Lugol's iodine solution with feces. This stains any protozoans or eggs of various worms that may be present in the stool, making them easier to see and identify.

Gram stain

The most frequently utilized stain in the microbiology laboratory is the Gram stain. This stain differentiates bacteria into two groups. One is referred to as Gram positive because of its ability to retain crystal violet stain, while the other is referred to as Gram negative because it is unable to retain this stain (see Fig. 1). These organisms can be further subdivided based on their morphological characteristics.

The structure of the bacterial cell envelope determines an organism's Gram stain characteristics. Gram-positive organisms have an inner phospholipid bilayer membrane surrounded by a cell wall composed of a relatively thick layer of the polymer peptidoglycan. Gram-negative organisms also have an inner phospholipid bilayer membrane surrounded by a peptidoglycan-containing cell wall. However, in the Gram-negative organisms, the peptidoglycan layer is much thinner. The cell wall in Gram-negative organisms is surrounded by an outer membrane composed of a phospholipid bilayer. Embedded within this bilayer are proteins and the lipid A portion of a complex molecule called lipopolysaccharide. Lipopolysaccharide is also referred to as **endotoxin** because it can cause a variety of toxic effects in humans.

Because of their size or cell envelope composition, certain clinically important bacteria cannot be seen on Gram stain. These include all species of the genera *Mycobacterium*, *Mycoplasma*, *Rickettsia*, *Coxiella*, *Ehrlichia*, *Chlamydia*, and *Treponema*. Yeasts typically stain as Gram-positive organisms, while the hyphae of molds may inconsistently take up stain but generally will be Gram positive.

Gram stains can be performed quickly, but attention to detail is important to get an accurate Gram reaction. One clue to proper staining is to examine the background of the stain. The presence of significant amounts of purple (Gram positive) in the epithelial cells, red or white blood cells, or proteinaceous material, all of which should stain Gram negative, suggests that the stain is under-decolorized and that the Gram reaction of the bacteria may not be accurate. This type of staining characteristic is frequently seen in "thick" smears. The detection of over-decolorization is much more difficult and is dependent on the observation skills of the individual examining the slide.

Staining of acid-fast organisms

Mycobacterium spp., unlike other bacteria, are surrounded by a thick mycolic acid coat. This complex lipid coat makes the cell wall of these bacteria refractory to staining by the dyes used in the Gram stain. As a result, bacteria within this genus usually cannot be visualized or, infrequently, may have a beaded appearance on Gram stain. Certain stains, such as carbol fuchsin or auramine-rhodamine, can form a complex with the mycolic acid. This stain is not washed out of the cell wall by acid-alcohol or weak acid solution, hence the term "acid-fast" bacterium.

Auramine and rhodamine are nonspecific fluorochromes. Fluorochromes are stains that "fluoresce" when excited by light of a specific wavelength. Bacteria that retain these

dyes during the acid-fast staining procedure can be visualized with a fluorescent microscope (Fig. 6). In clinical laboratories with access to a fluorescent microscope, the auramine-rhodamine stain is the method of choice because the organisms can be visualized at a lower magnification. By screening at lower magnification, larger areas of the microscope slide can be examined more quickly, making this method more sensitive and easier to perform than acid-fast stains using carbol fuchsin and light microscopy.

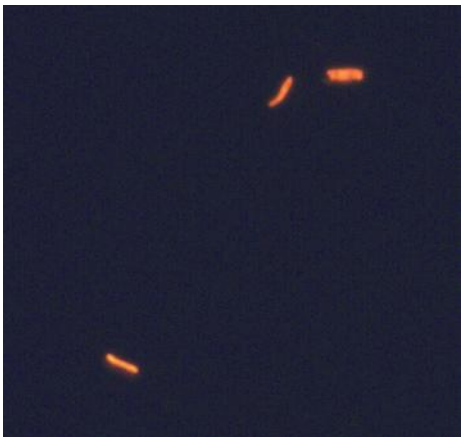


Figure 6

Several other organisms are acid-fast, although they typically are not alcohol-fast. As a result, they are stained using a modified acid-fast decolorizing step whereby a weak acid solution is substituted for an alcohol-acid one. This technique is frequently used to distinguish two genera of Gram-positive, branching rods from each other. *Nocardia* species are acid-fast when the modified acid-fast staining procedure is used, while *Actinomyces* species are not. *Rhodococcus equi* is a coccobacillus that may also be positive by modified acid-fast stain when first isolated. The modified acid-fast stain has also been effective in the detection of two gastrointestinal protozoan parasites, *Cryptosporidium* and *Cyclospora*. It should be noted that *Cyclospora* stains inconsistently, with some organisms giving a beaded appearance while others do not retain the stain at all.

Trichrome stain

The trichrome stain is used to visualize protozoans in fecal specimens. This stain is particularly effective at staining internal structures, the examination of which is important in determining the identity of certain protozoans, such as *Entamoeba histolytica*. Modification of the trichrome stain is used in the detection and identification of microsporidia.

Direct fluorescent-antibody stains

The development of monoclonal antibodies has enhanced both the sensitivity and the specificity of staining techniques that use antibodies to detect microbes in clinical specimens. The most widely used staining technique that incorporates the use of antibodies is the direct fluorescent-antibody (DFA) stain. In this technique, a highly specific antibody is coupled to a fluorochrome, typically fluorescein, which emits an "apple-green" fluorescence. The antibody binds specifically either to antigens on the surface of the microbes or to viral antigens expressed by virally infected cells, which can be visualized under the fluorescent microscope (Fig. 7). This technique is rapid, usually requiring 1 to 2 hours. In the hands of a skilled operator, the test is highly specific, although it frequently has a sensitivity of only 60 to 70% compared with bacterial culture. Because of its rapidity, the

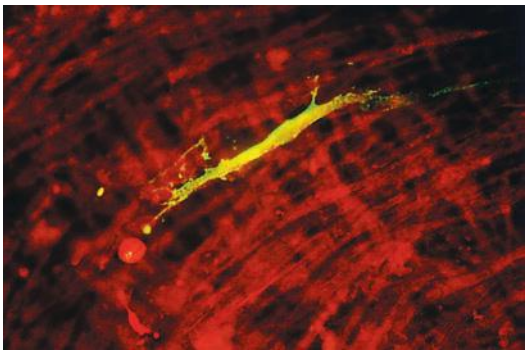


Figure 7

test has been used to detect some relatively slow-growing or difficult-to-grow bacteria, such as *Bordetella pertussis* and *Legionella pneumophila*. For respiratory viruses and herpesviruses, the sensitivity of this technique approaches 90% of the sensitivity of culture. However, the development of molecular amplification techniques for the detection of viral agents has demonstrated that DFA sensitivities can be as low as 50%, but may range up to 80% for some viruses. As result, many laboratories have replaced DFA with molecular amplification for organisms such as *B. pertussis*, herpesviruses, and respiratory viruses.

DFA staining is frequently used for the detection of microbes that cannot be cultured. DFA is the method of choice for detection of the nonculturable fungus *Pneumocystis jirovecii*, a common cause of pneumonia in people with AIDS. DFA is much more sensitive than other commonly used staining techniques, such as silver, Giemsa, or toluidine blue O staining. Likewise, for the gastrointestinal protozoans *Giardia lamblia* and *Cryptosporidium parvum*, DFA staining has been found to be much more sensitive than examination of wet mounts or the use of trichrome (for *Giardia*) or modified acid-fast stain (for *Cryptosporidium*). Molecular amplification techniques are also beginning to be deployed to detect these organisms as well and may soon replace DFA testing.

Infectious disease diagnosis from peripheral blood smears and tissue sections

Not all staining used in the diagnosis of infectious disease is done in the microbiology laboratory. The hematologist and the anatomical pathologist can play important roles in the diagnosis of certain infectious diseases.

The peripheral blood smear is the method of choice for detection of one of the most important infectious diseases in the world, malaria, which is caused by protozoans within the genus *Plasmodium*. The various developmental stages of these parasites are detected in red blood cells. Other, less frequently encountered parasites seen in a peripheral blood smear include *Babesia* species, trypanosomes, and the microfilariae.

Bacterial and fungal pathogens may be seen in peripheral smears on occasion. The most likely of these is *Histoplasma capsulatum*, which is seen as small, intracellular yeasts in peripheral white blood cells. *Ehrlichia* and *Anaplasma* can produce characteristic inclusions (morulae), which can be seen in peripheral mononuclear cells and granulocytic cells, respectively.

Examination of tissue by the anatomical pathologist is an important technique for detecting certain infectious agents. Tissue cysts due to toxoplasmosis can be detected in

brain biopsy material from patients with encephalitis. The diagnosis of Creutzfeldt-Jakob disease is based on the finding of typical lesions on brain biopsy. The finding of hyphal elements in lung tissue is an important tool in the diagnosis of invasive aspergillosis and pulmonary zygomycosis. The observation of ribbon-like elements in a sinus biopsy is pathognomonic for the diagnosis of rhinocerebral zygomycosis, a potentially fatal disease most frequently seen in diabetic patients.

Antigen detection

Visual examination of a clinical specimen is not the only means by which an infectious agent can be directly detected. A variety of tests have been developed that, like DFA, are dependent on the availability of highly specific antibodies to detect antigens of specific bacteria, fungi, viruses, and parasites. The most widely used antigen detection tests are various formats of the enzyme immunoassay or the latex agglutination assay. These tests take anywhere from 10 minutes to 2 hours. The test most widely used is a 10- to 15-minute enzyme immunoassay for the detection of group A streptococci. The sensitivity of these various formats has been reported to be 80 to 90%, with specificity usually greater than 95%. In the United States, there are more than 50 different test formats marketed for the detection of this organism. The test is done in a wide variety of laboratories, clinics, and physicians' offices. Antigen detection tests are widely used in the United States to detect a variety of infectious agents, including *Cryptococcus neoformans*, *Clostridium difficile* toxin, respiratory syncytial virus, rotavirus, influenza virus, and *Giardia* and *Cryptosporidium* spp. It should be noted, however, that as more molecular tests become commercially available and are used as reference methods, the sensitivities of many of the rapid antigen tests deteriorate. For example, published sensitivities for rapid antigen tests for influenza are as low as 10% and those for respiratory syncytial virus are as low as 59%.

MOLECULAR DIAGNOSTICS

In addition to standard methods of culturing and identifying pathogenic microorganisms, there are now a number of molecular methods available that are able to detect the presence of the specific nucleic acid of these organisms. These methods are used in demonstrating the presence of the organism in patient specimens as well as in determining the identification of an isolated organism. In some cases, these methods are able to determine the quantity of the nucleic acid.

As an example, bacteria of a particular species will have a chromosomal nucleic acid sequence significantly different from that of another bacterial species. On the other hand, the nucleic acid sequence within a given species has regions that are highly conserved. For example, the base sequence of the *Mycobacterium tuberculosis* rRNA differs significantly from the base sequence in the *Mycobacterium avium* complex rRNA, yet the sequence of bases in this region among members of the *M. tuberculosis* complex is highly conserved.

These properties form the basis for the use of genetic probes to identify bacteria to the species level. There are a number of commercially available genetic probes that can detect specific sequences in bacteria, mycobacteria, and fungi.

Nucleic acid hybridization is a method by which there is the *in vitro* association of two complementary nucleic acid strands to form a hybrid strand. The hybrid can be a DNA-RNA hybrid, a DNA-DNA hybrid, or, less commonly, an RNA-RNA hybrid. To do this, one denatures the two strands of a DNA molecule by heating to a temperature above which the complementary base pairs that hold the two DNA strands together are disrupted and the helix rapidly dissociates into two single strands. A second nucleic acid sequence is introduced that will bind to regions that are complementary to its sequence. The stringency, or specificity, of the reaction can be varied by reaction conditions such as the temperature.

In addition to the direct demonstration of a nucleic acid sequence by hybridization, amplification assays (the process of making additional copies of the specific sequence of interest) are of increasing importance in clinical microbiology. The most commonly used amplification assay is PCR (Fig. 8). PCR uses a DNA polymerase that is stable at high temperatures that would denature and inactivate most enzymes. This thermostable DNA polymerase most often is isolated from the bacterium *Thermus aquaticus*. Its stability at high temperature enables the enzyme to be used without the need for replacement after

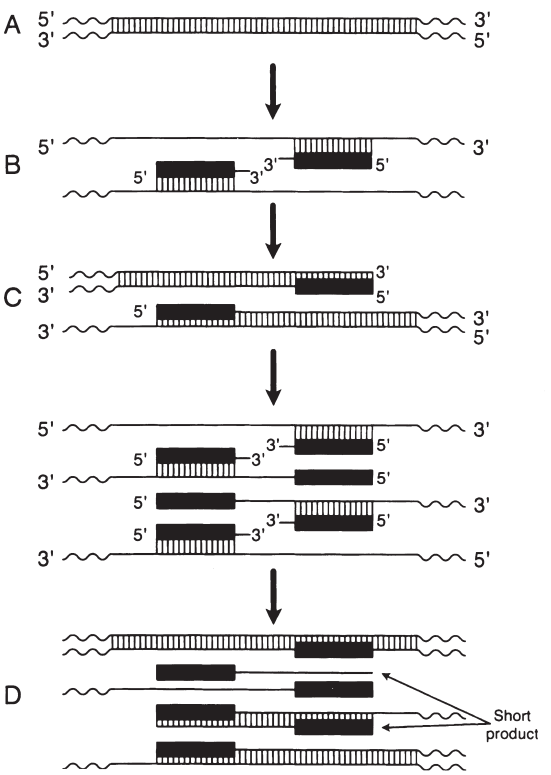


Figure 8 PCR. (A) In the first cycle, a double-stranded DNA target sequence is used as a template. (B) These two strands are separated by heat denaturation, and the synthetic oligonucleotide primers (solid bars) anneal to their respective recognition sequences in the 5′ → 3′ orientation. Note that the 3′ ends of the primers are facing each other. (C) A thermostable DNA polymerase initiates synthesis at the 3′ ends of the primers. Extension of the primer via DNA synthesis results in new primer-binding sites. The net result after one round of synthesis is two "ragged" copies of the original target DNA molecule. (D) In the second cycle, each of the four DNA strands in panel C anneals to primers (present in excess) to initiate a new round of DNA synthesis. Of the eight single-stranded products, two are of a length defined by the distance between and including the primer-annealing sites; these "short products" accumulate exponentially in subsequent cycles. (Reprinted from *Manual of Clinical Microbiology*, 7th ed, ©1999 ASM Press, with permission.)

the high-temperature conditions of the DNA denaturation step that occurs during each cycle of PCR:

1. The target DNA sequence is heated to a high temperature that causes the double-stranded DNA to denature into single strands.
2. An annealing step follows, at a lower temperature than the denaturation step above, during which sets of primers, with sequences designed specifically for the PCR target sequences, bind to these target sequences.
3. Last is an extension step, during which the DNA polymerase completes the target sequence between the two primers.

Assuming 100% efficiency, the above three steps generate two copies of the target sequence. Multiple cycles (such as 30) in a thermal cycler result in a tremendous amplification of the number of sequences, so that the sequence is readily detectable using any of a variety of methods—gel electrophoretic, colorimetric, chemiluminescent, or fluorescent.

When the specific target nucleic acid is RNA rather than DNA, a cDNA sequence is made with the enzyme reverse transcriptase (RT) before PCR amplification in a procedure known as RT-PCR. Examples of pathogens for which RT-PCR is used include the RNA-containing viruses HIV-1 and hepatitis C virus (HCV).

An additional feature of PCR is that the amplified nucleic acid products can be directly sequenced. These sequences can be compared with sequences found in publicly accessible databases. This allows, for example, the identification of a bacterial organism to the level of species on the basis of a sequence of hundreds of bases in the rRNA or, if the sequence is less closely related to sequences within the database, to the level of genus. In some cases, the organism may be an entirely new one. This method of PCR and sequencing of the product for the purposes of bacterial identification is being used in clinical microbiology for the identification of slow-growing or difficult-to-identify organisms such as *Mycobacterium* spp., *Nocardia* spp., and anaerobic organisms. However, mass spectrometry has recently entered clinical microbiology and will likely replace ribosomal gene sequencing as the method of choice for these organisms, as well as all other bacteria and fungi. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) allows the identification of organisms by their protein spectra. Although initial instrumentation is expensive, identifications can be performed for less than $1 and in at little as 20 minutes. Many clinical laboratories are already using MALDI-TOF as the primary method for identifying bacteria.

After the invention of PCR, a number of other amplification assays were developed, some of which have entered the clinical microbiology laboratory. Transcription-mediated amplification (TMA), which does not require a thermal cycler, relies on the formation of cDNA from a target single-stranded RNA sequence, the destruction of the RNA in the RNA-DNA hybrid by RNase H, and the formation of double-stranded cDNA (which can serve as transcription templates for T7 RNA polymerase). A similar procedure occurs

during the nucleic acid sequence-based amplification (NASBA) assay. Strand-displacement amplification (SDA) does not require a thermal cycler and has two phases in its cycle: a target generation phase during which a double-stranded DNA sequence is heat denatured, resulting in two single-stranded DNA copies; and an exponential amplification phase in which a specific primer binds to each strand at the cDNA sequence. DNA polymerase extends the primer, forming a double-stranded DNA segment that contains a specific restriction endonuclease recognition site, to which a restriction enzyme binds, cleaving one strand of the double-stranded sequence and forming a nick, followed by extension and displacement of new DNA strands by DNA polymerase.

All of these assays—PCR, TMA/NASBA, and SDA—have one thing in common: they amplify the target nucleic acid sequence, making many, many copies of the sequence. As you might imagine, there is the possibility that small quantities of the billions of amplified target nucleic acid sequences can contaminate a sample that will then undergo amplification testing, resulting in false-positive results. Steps are taken to minimize contamination, including physical separation of specimen preparation and amplification areas, positive displacement pipettes, and both enzymatic (in PCR) and nonenzymatic methods to destroy the amplified products.

An alternative method of demonstrating the presence of a specific nucleic acid sequence that does not require the amplification of the target is by amplification of the signal. One example is branched DNA (bDNA) technology (Fig. 9), which is used particularly in quantitative assays, such as HIV and HCV viral load determinations. In this assay,

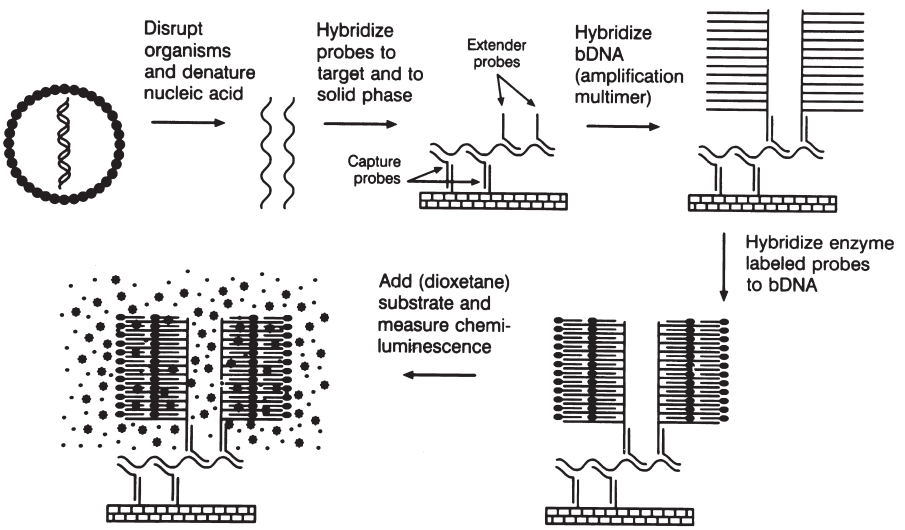


Figure 9 bDNA-based signal amplification. Target nucleic acid is released by disruption and is captured onto a solid surface via multiple contiguous capture probes. Contiguous extended probes hybridize with adjacent target sequences and contain additional sequences homologous to the branched amplification multimer. Enzyme-labeled oligonucleotides bind to the bDNA by homologous base pairing, and the enzyme-probe complex is measured by detection of chemiluminescence. All hybridization reactions occur simultaneously. (Reprinted from *Manual of Clinical Microbiology*, 7th ed, ©1999 ASM Press, with permission.)

specific oligonucleotides hybridize to the sequence of interest and capture it onto a solid surface. In addition, a set of synthetic enzyme-conjugated branched oligonucleotides hybridize to the target sequence. When an appropriate substrate is added, light emission is measured and compared with a standard curve. This permits quantitation of the target sequence. As there is no amplified sequence to be concerned about, the risk of contamination is dramatically reduced. Another example that is widely used is a hybrid capture test for human papillomavirus (HPV) detection. In this test, HPV DNA is denatured and bound to complementary RNA probes. This hybrid is then "captured" by immobilized anti-hybrid antibodies. A chemiluminescent reaction allows for the detection of DNA-RNA hybrids and therefore HPV DNA in the sample.

There are several commercially available molecular diagnostic assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Although first-generation molecular tests included direct hybridization assays, nucleic acid amplification tests are now the laboratory standard due to their increased sensitivity. Depending on the manufacturer of the tests, specimens of cervical, vaginal, and urethral swabs and urine are acceptable. Because *N. gonorrhoeae* is a fastidious organism that may not survive specimen transport, nucleic acid amplification tests are of particular benefit in settings in which there may be a delay in the transport of the specimen to the laboratory; i.e., the viability of the organisms is not required to detect the presence of its nucleic acid. Similarly, the previous gold standard for the detection of *C. trachomatis*—tissue culture—was labor-intensive, required the use of living cell lines, and required rapid specimen transport on wet ice to ensure the viability of the organisms in the specimen. In almost all clinical laboratories, *C. trachomatis* tissue culture has been replaced by amplification technologies, which have been shown to be significantly more sensitive. As you might imagine, since these assays do not require the presence of living organisms, patients who have been treated with appropriate antibiotics may continue to have a positive assay for some time because of the presence of dead, and therefore noninfectious, organisms that contain the target nucleic acid.

Quantitative assays are now available for several different pathogens. These include tests that determine the level of HIV RNA in patients with HIV infection and are now recognized as one component of the standard clinical management of these patients. With the availability of highly active antiretroviral therapy but the potential for antiviral drug resistance, it is important to be able to closely monitor the plasma level of HIV RNA, also known as the viral load. A clinical response to antiretroviral therapy can be demonstrated by a decrease in the viral load. Similarly, an increase in the viral load may indicate either the development of viral resistance to one or more of the antiviral agents being used to treat the patient or merely patient noncompliance with therapy. Modification of therapy may be made on the basis of a rising HIV viral load and the results of HIV genotyping studies.

HIV genotyping is a test that determines the specific nucleic acid sequence present in the virus infecting a patient. There are a number of ways that this test can be performed, and direct sequencing of amplified cDNA (using RT-PCR) is one example. These results are routinely compared with a database that contains nucleic acid sequences from viral

strains that are known to be both sensitive and resistant to specific antiretroviral medications. This comparison permits the clinician to note what, if any, mutations are present in the virus infecting the patient and to predict with some reasonable degree of probability whether the viral isolate is resistant to antiretroviral medications, including those being taken by the patient. These data can help the physician make a rational choice of an antiretroviral regimen in a patient whose therapy is failing. One difficulty with this test is that patients are often infected with a mixture of different HIV viral populations, both because of the high frequency of mutation that occurs with HIV and because of the selection of resistant subpopulations while the patient receives antiretroviral therapy. As a result, there may be resistant subpopulations that are below the level of detection of the standard HIV genotyping assay and that could become clinically relevant under the selective pressure of continued antiretroviral therapy.

Detection of HCV RNA using RT-PCR can be used both diagnostically and for following the effectiveness of therapy. The PCR product generated during the HCV RNA assay can be used for genotyping using a variety of hybridization assays in which specific nucleic acid sequences associated with specific genotypes are detected. Genotype 1 is more refractory to therapy than genotypes 2 and 3. Therefore, therapy is much more prolonged (48 versus 24 weeks) for genotype 1 than for 2 and 3. Further, treatment with the newer HCV protease inhibitors is currently only available for patients with genotype 1.

CULTURE

Detection of bacterial and fungal pathogens by culture

Culture on manufactured medium is the most commonly used technique for detecting bacteria and fungi in clinical specimens. Although not as rapid as direct examination, it is more sensitive and much more specific. For the majority of human pathogens, culture requires only 1 to 2 days of incubation. For particularly slow-growing organisms, such as *M. tuberculosis* and some fungi, the incubation period may last for weeks. By growing the organism, it is available for further phenotypic and genotypic analysis, such as antimicrobial susceptibility testing, serotyping, virulence factor detection, and molecular epidemiology studies.

Environmental and nutritional aspect of bacterial and fungal culture

Certain basic strategies are used to recover bacterial and fungal pathogens. These strategies are dependent on the phenotypic characteristics of the organisms to be isolated and the presence of competing microbiota in a patient's clinical specimen. Most human pathogens grow best at 37°C, human body temperature. Most bacterial and fungal cultures are performed, at least initially, at this temperature. Certain skin pathogens, such as dermato-

phytes and some *Mycobacterium* spp., grow better at 30°C. When seeking these organisms, cultures may be done at this lower temperature. A few clinically significant microorganisms will grow at low temperatures (4°C), while others prefer higher temperatures (42°C). These incubation temperatures may be used when attempting to recover a specific organism from specimens with a resident microbiota, such as feces, as few organisms other than the target organism can grow at these temperature extremes.

Another important characteristic of human bacterial and fungal pathogens is the impact of the presence of oxygen on the growth of these organisms. Microbes can be divided into three major groups based on their ability to grow in the presence of oxygen. Organisms that can only grow in the presence of oxygen are called **aerobes**. Fungi and many bacteria are **aerobic** organisms. Organisms that can only grow in the absence of oxygen are called **anaerobes**. The majority of bacteria that make up the resident microbiota of the gastrointestinal and female genital tracts are anaerobic organisms. Some bacteria can grow either in the presence or in the absence of oxygen. These organisms are called **facultative** organisms. A subgroup of facultative organisms is called **microaerophiles**. These organisms grow best in an atmosphere with reduced levels of oxygen. *Campylobacter* spp. and *Helicobacter* spp. are examples of microaerophiles.

Besides temperature and oxygen, nutrients are an important third factor in the growth of microbes. Many bacteria have very simple growth requirements. They require an energy and carbon source, such as glucose; a nitrogen source, which may be ammonium salts or amino acids; and trace amounts of salts and minerals, especially iron. Some human pathogens have much more complex growth requirements, needing certain vitamins or less well-defined nutrients such as animal serum. Organisms with highly complex growth requirements are often referred to as being **fastidious**. A fastidious bacterium that is frequently encountered clinically is *Haemophilus influenzae*. This bacterium requires both hemin, an iron-containing molecule, and NAD for growth.

Media

The selection of media to be used in isolation of pathogens from clinical specimens is dependent on several factors. First, the nutritional requirements of the specific pathogen must be met. For example, fastidious organisms require a medium that is enriched with specific nutrients, such as animal blood, serum, or other growth factors. If the clinical specimen is obtained from a site that has a resident microbiota, certain strategies will be necessary to isolate a specific pathogen from the accompanying resident microbiota. Often in this setting, a special type of medium called selective medium is used to recover these pathogens. This medium selects for the growth of a specific group of organisms. This is done by adding substances, such as dyes, antibiotics, or bile salts, that inhibit the growth of one group of organisms while permitting the growth of another. For example, MacConkey agar is a selective medium that contains bile salts and the dye crystal violet. These two substances are inhibitory for Gram-positive organisms as well as some Gram-negative ones. A wide variety of Gram-negative rods grow on this medium. Some selective media are also differential. MacConkey agar is an

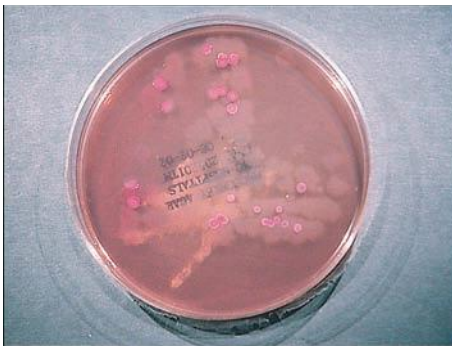


Figure 10

example of a selective and differential medium. The Gram-negative bacilli that grow on this agar can be differentiated from one another on the basis of the organism's ability to ferment the carbohydrate lactose. Organisms that ferment lactose are called lactose positive, and organisms that are unable to ferment lactose are called lactose negative (Fig. 10). When selecting media for culturing clinical specimens from sites with a resident microbiota, typically both enriched and selective media are used. If Gram-negative bacilli are a component of this microbiota, than a selective-differential medium might be used as well.

Certain organisms will not grow on media commonly used to culture clinical specimens, because the media may not be enriched enough or may contain inhibitory substances. When these organisms are sought, the laboratory must be notified so that special isolation medium can be used. Two important respiratory tract pathogens, *B. pertussis* and *L. pneumophila*, are examples of organisms that do not grow on standard laboratory media and require special media for their isolation.

Organism identification and susceptibility testing

Once organisms are isolated, they may be identified, and in some cases susceptibility testing needs to be performed. Bacteria and fungi grow as colonies on agar plates. The appearance of these colonies is often useful in determining the identity of the organism. Colonies may appear flat or raised, smooth or rough; may pit the agar; or may hemolyze red blood cells in blood-containing agar. Molds, for example, have very characteristic "fuzzy" growth on agar. Colonies of organisms such as *S. aureus* may be pigmented or may secrete a diffusible pigment, as seen with *Pseudomonas aeruginosa*. Skilled microbiologists often have a very good idea of the identification of a microorganism based solely on its colonial appearance.

In specimens that come from an area of the body with a resident microbiota, it is important to separate the colonies of organisms that may represent the resident microbiota from the colonies of organisms that may be pathogens. Much of the time, this can be done on the basis of colonial appearance. However, some potential pathogens, such as *S. pneumoniae*, a common cause of bacterial pneumonia, cannot be readily differentiated from viridans group streptococci, a member of the resident oropharyngeal microbiota. In patients with suspected bacterial pneumonia, a sputum specimen may be obtained. Sputum consists of secretions coughed up from the lower airways that are expectorated through the oropharynx and submitted for culture. Because they pass through the oropharynx, sputum specimens almost always contain viridans group streptococci. The appearance of colonies produced by viridans group streptococci is very similar to that produced by

S. pneumoniae. To determine whether or not these colonies are *S. pneumoniae*, one must do tests based on the phenotypic characteristics of the organism; these are referred to as **biochemical** tests. The biochemical test that is done most often to distinguish between these two organisms is the disk diffusion test, in which the organism's susceptibility to the compound optochin is examined. *S. pneumoniae* (Fig. 11) is susceptible to optochin, while the viridans group streptococci are not. On the basis of this easily performed test, the identity of *S. pneumoniae* can be determined from a sputum specimen.



Figure 11 Left disk, optochin; right disk, oxacillin.

Bacteria are typically identified on the basis of colonial morphology, Gram stain reaction, the primary isolation media on which the organism is growing, and biochemical and serologic tests of various degrees of complexity. Figures 12 and 13 are flow charts that give fairly simple means of distinguishing commonly encountered human pathogens. Yeasts are identified in much the same way that bacteria are, while molds are generally identified on the basis of the arrangement of microscopic reproductive structures called conidia. It is important to accurately identify bacteria and fungi because certain organisms (e.g., *B. pertussis*) are the cause of certain clinical syndromes (in this case, whooping cough). Other bacteria (e.g., *Staphylococcus epidermidis*) may represent contamination in a clinical specimen (e.g., a wound culture). The accurate identification of a bacterium or fungus may help determine what role a particular microbe may be having in the patient's disease process.

Antimicrobial susceptibility typically is performed on rapidly growing bacteria if the organism is believed to play a role in the patient's illness and if the profile of antimicrobial agents to which the organism is susceptible is not predictable. Let's take three clinical scenarios to explain this concept.

First, a patient with a "strep throat" has group A streptococci recovered from his throat. Although the organism is clearly playing a role in the illness of this patient, antimicrobial susceptibility testing is not warranted. This organism is uniformly susceptible to first-line therapy—penicillin—and is susceptible more than 98% of the time to second-line therapy—the macrolide antibiotics such as erythromycin—although recent reports suggest that erythromycin resistance is becoming more frequent in this organism.

Second, a patient presents with a leg abscess from which *S. aureus* is recovered. Susceptibility testing is indicated because some strains are resistant to the first-line drugs used to treat this infection—semisynthetic penicillins, including oxacillin and dicloxacillin—and the second-line drug, clindamycin. In this situation, the patient may be started on **empiric** antimicrobial therapy until the susceptibility of the organism is known. If the organism is resistant to the agent used for empiric therapy, then the patient should be treated with an alternative antimicrobial agent to which the organism is susceptible.

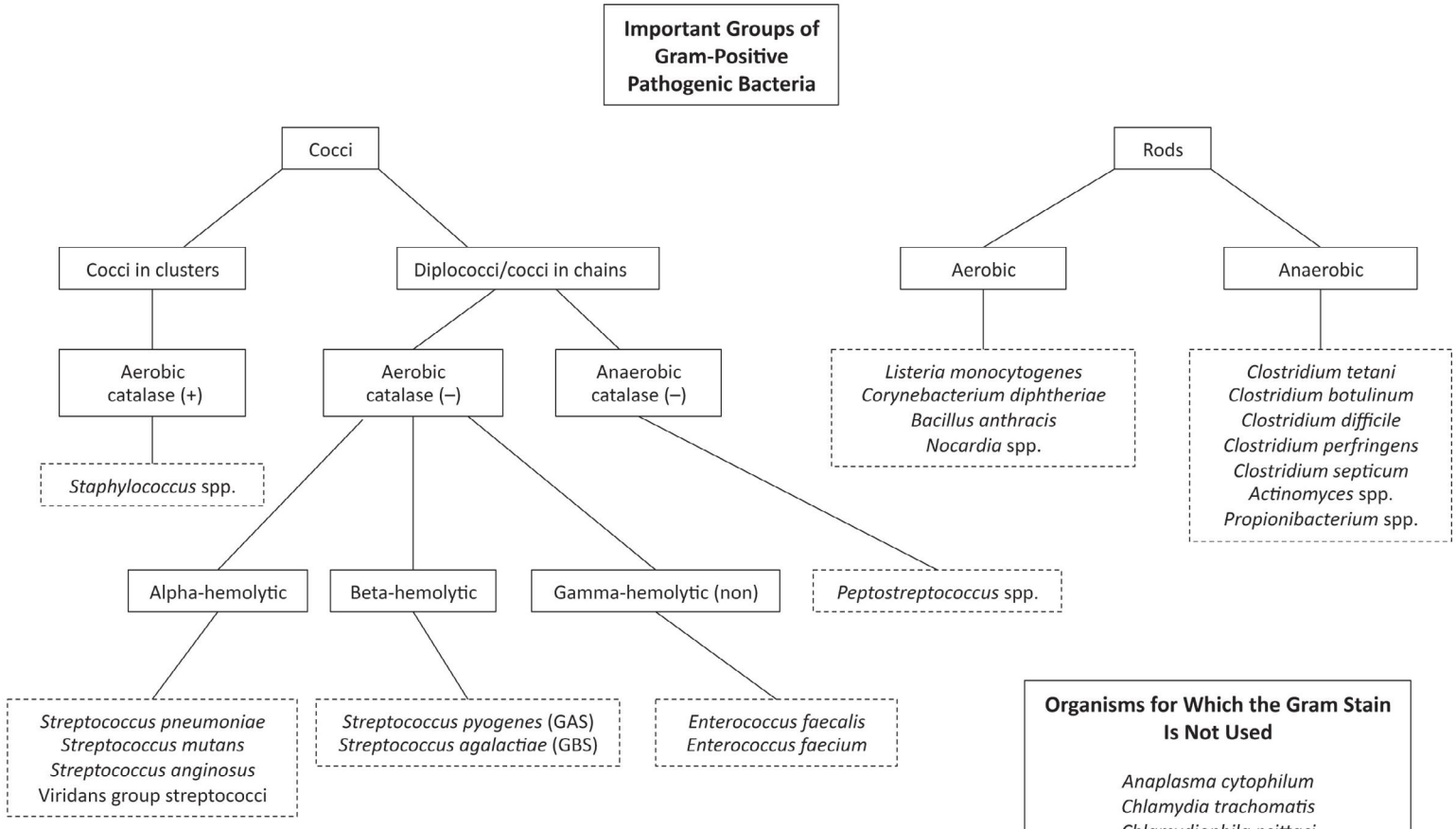

Important Groups of Gram-Positive Pathogenic Bacteria
Cocci
Rods
Cocci in clusters
Diplococci/cocci in chains
Aerobic
Anaerobic
Aerobic catalase (+)
Aerobic catalase (–)
Anaerobic catalase (–)
Listeria monocytogenes
Corynebacterium diphtheriae
Bacillus anthracis
Nocardia spp.
Clostridium tetani
Clostridium botulinum
Clostridium difficile
Clostridium perfringens
Clostridium septicum
Actinomyces spp.
Propionibacterium spp.
Staphylococcus spp.
Alpha-hemolytic
Beta-hemolytic
Gamma-hemolytic (non)
Peptostreptococcus spp.
Streptococcus pneumoniae
Streptococcus mutans
Streptococcus anginosus
Viridans group streptococci
Streptococcus pyogenes (GAS)
Streptococcus agalactiae (GBS)
Enterococcus faecalis
Enterococcus faecium
Organisms for Which the Gram Stain Is Not Used
Anaplasma cytophilum
Chlamydia trachomatis
Chlamydiophila psittaci
Chlamydiophila pneumoniae
Treponema pallidum
Borrelia burgdorferi
Rickettsia rickettsii
Ehrlichia chaffeensis
Mycobacterium tuberculosis
Mycobacterium sp.
Mycoplasma spp.

Figure 12

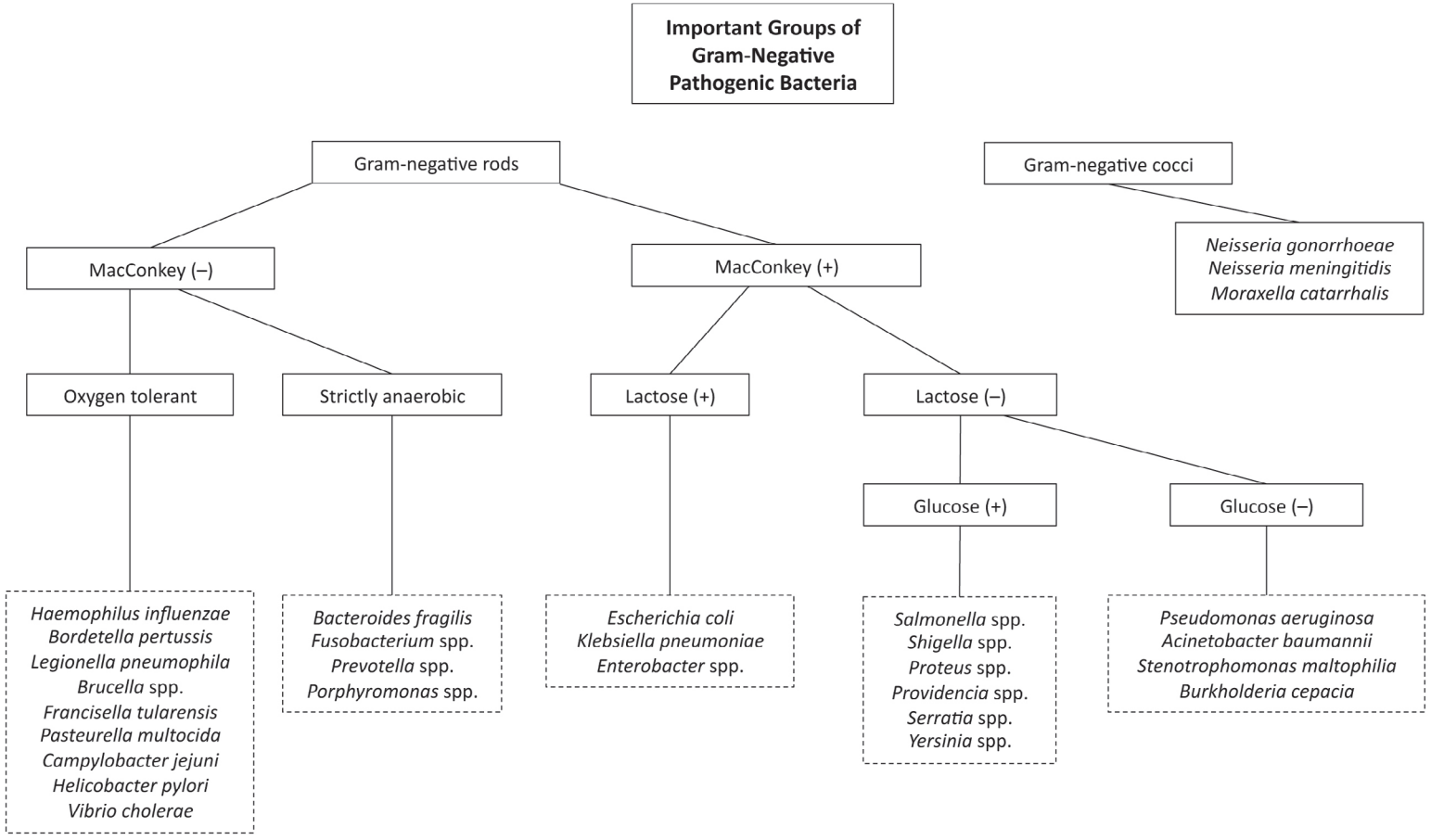

Important Groups of Gram-Negative Pathogenic Bacteria
Gram-negative rods
Gram-negative cocci
MacConkey (–)
MacConkey (+)
Neisseria gonorrhoeae
Neisseria meningitidis
Moraxella catarrhalis
Oxygen tolerant
Strictly anaerobic
Lactose (+)
Lactose (–)
Glucose (+)
Glucose (–)
Haemophilus influenzae
Bordetella pertussis
Legionella pneumophila
Brucella spp.
Francisella tularensis
Pasteurella multocida
Campylobacter jejuni
Helicobacter pylori
Vibrio cholerae
Bacteroides fragilis
Fusobacterium spp.
Prevotella spp.
Porphyromonas spp.
Escherichia coli
Klebsiella pneumoniae
Enterobacter spp.
Salmonella spp.
Shigella spp.
Proteus spp.
Providencia spp.
Serratia spp.
Yersinia spp.
Pseudomonas aeruginosa
Acinetobacter baumannii
Stenotrophomonas maltophilia
Burkholderia cepacia

Figure 13

The third scenario is more subtle. A patient comes to the hospital with a high fever. He has two sets of blood cultures drawn in the emergency department. Two days later, *S. epidermidis* is recovered from one of these blood culture sets. As with *S. aureus*, this organism may show resistance to a variety of antimicrobial agents that are used to treat infected patients. However, no susceptibility testing is done by the laboratory, and this practice is acceptable to the clinician caring for the patient. Why? *S. epidermidis* is a component of skin microbiota and may have contaminated the culture. If the laboratory had performed the susceptibility testing without considering that this isolate was a potential contaminant, they would be validating that the isolate was clinically significant. In this setting, the laboratory should only do susceptibility testing if instructed to by the caregiver, who is in a better position to know if this organism is clinically important.

There are several approaches to antibacterial susceptibility testing. All the approaches are highly standardized to ensure that the susceptibility results will be consistent from laboratory to laboratory. Screening of selected organisms for resistance to specific antimicrobial agents is one strategy that is frequently used, especially with the emergence of resistance in three organisms: *S. aureus* to cefoxitin to predict oxacillin resistance, *S. pneumoniae* to penicillin, and *Enterococcus faecium* and *Enterococcus faecalis* to vancomycin. Other strategies are to determine susceptibility to a preselected battery of antimicrobial agents using automated or manual systems that determine the MIC of antibiotics to the organism being tested or by using the disk diffusion susceptibility testing technique.

A novel approach to susceptibility testing is to perform MIC determinations using the E-test. The E-test is a plastic strip that contains a gradient of a specific antimicrobial agent. This strip is applied to a lawn of bacteria on an agar plate. Where the zone of inhibition intersects with the strip is the MIC value of that antibiotic for the organism tested. This test has many applications but is used most frequently for determining penicillin MIC values for *S. pneumoniae* isolates that show resistance to penicillin in the screening test previously described (Fig. 14).

Susceptibility testing is performed with increasing frequency on *Candida* spp. other than *C. albicans* but is rarely done on other yeasts and almost never on molds. Because of their slow growth, special susceptibility testing techniques are used for the mycobacteria.

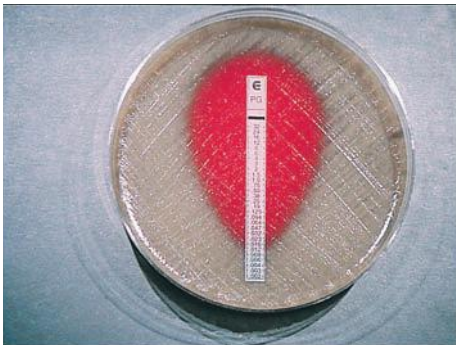


Figure 14

Tissue culture for Chlamydia *and viruses*

Both *Chlamydia*, a bacterium, and the viruses are obligate intracellular parasites. As such, they do not grow on artificial media, as fungi and other bacteria do. Rather, they can only grow by parasitizing living animal cells (including human cells) that are maintained by continuous tissue culture. Animals such as mice, or chicken eggs, can be inoculated in an attempt to isolate certain viruses, but this approach is rarely done. Tissue culture for *Chlamydia* may still be attempted, especially in situations where the detection of *C. trachomatis* is at issue in a legal proceeding, such as a case of sexual abuse of a child. However, molecular detection has become the standard method for diagnosis of *C. trachomatis* infection.

Tissue culture is still an important technique for the detection of viruses in many laboratories, though laboratories are converting to molecular methods for viral detection at an increasing rate. Herpes simplex virus can be isolated from skin and genital tract lesions, often within the first 24 hours of incubation. Another herpesvirus, varicella-zoster virus, the etiologic agent of chicken pox and herpes zoster, can also be isolated from skin lesions, but it typically requires 3 to 7 days to grow. The enteroviruses are the major etiologic agents of aseptic meningitis and can be isolated from cerebrospinal fluid, but at a significantly reduced rate compared with molecular detection.

A modification of the tissue culture technique is done to detect cytomegalovirus and several respiratory viruses in clinical specimens called rapid centrifugation cultures or shell vial cultures. In this method, the specimen is centrifuged onto tissue culture cells that are growing on a round glass coverslip inside a vial referred to as a shell vial. The cells are incubated for a brief period of time (24 to 72 hours) and then stained with fluorescent antibodies to detect the virus. This technique is much more rapid and sensitive than conventional tissue culture but is still less sensitive than molecular detection.

SEROLOGY

It is not always possible to isolate a microorganism by culture, visualize it microscopically, or detect it by antigenic or molecular detection techniques. In those situations, an alternative approach is to determine if the patient has mounted an immune response against a specific agent as evidence that he or she has been infected with that agent. The immune response is generally measured by detecting antibodies in the serum of patients—thus the name serology.

Serology has both advantages and disadvantages. As advantages, (i) specimens for testing are readily available; (ii) antibodies are relatively stable molecules, so transport is not a major concern as it is with culture; and (iii) tests have been designed that can detect most known agents, such as HIV and HCV, which are difficult to detect by other means. Depending on the target antigen against which the immune response is measured, the test can show both high sensitivity and high specificity. Compared with other techniques, these tests are relatively inexpensive and easy to perform, in part because they have been automated. As a result, they can be used to screen large numbers of specimens for selected infectious agents. For example, this approach is used to screen blood products used for

transfusions to ensure that the transfused patient does not receive blood contaminated with hepatitis B and C viruses, HIV, or *T. pallidum*, the agent of syphilis.

Serologic tests also have several disadvantages and should be interpreted with some caution. To have a positive test, the patient must have mounted an immune response. Serum obtained from an acutely ill patient may have been taken during the window period in an infection before the patient had time to mount an immune response. Therefore, to get the most accurate result, **acute** and **convalescent** specimens should be obtained. The convalescent specimen should show a significant increase (or, in some cases, decrease) from the antibody level of an acute specimen. This is usually a 4-fold change in the titer. Because the convalescent specimen should be obtained a minimum of 2 weeks after the acute specimen, serologic diagnosis is often retrospective. Because obtaining a convalescent specimen is often difficult logistically, the only value that may be available is that from the acute specimen. Patients may have relatively high antibody levels because of previous infection with the test organism and, as a result, may have a false-positive result. Antigenic cross-reactions between the test organism and other antigens may also lead to false-positive results. Some immunocompromised patients are unable to mount a response and may never have a positive serologic test.

Serologic tests can be done in combination using a **screening** test followed by a **confirmatory** test. This approach is used most commonly in the diagnosis of syphilis, HIV infection, and Lyme disease. The screening test should be highly sensitive so that all true-positive results will be detected. This test may not be highly specific, meaning that some results may be false positives. It should also be easily performed, so that large numbers of specimens can be tested fairly inexpensively. The confirmatory test needs to be highly specific so that the correct diagnosis can be applied to the patient who screens positive for the infectious agent. It tends to be much more expensive and technically complex than the screening test. Western blotting or an equivalent technique is used in the confirmatory tests for Lyme disease, HIV infection, and HCV infection. In this technique, a patient is considered to be positive for the agent only if the patient has antibodies to multiple specific antigens.

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SECTION ONE

UROGENITAL TRACT INFECTIONS

INTRODUCTION TO SECTION I

We begin this text with a discussion of infections of the genitourinary tract for two reasons. First, the number of microorganisms that frequently cause infection in these organs is somewhat limited. Second, **urinary tract infections (UTIs)** and **sexually transmitted infections (STIs)** are two of the most common reasons why young adults, particularly women, consult a physician. UTIs are examples of **endogenous** infections, i.e., infections that arise from the patient's own microbiota. In the case of UTIs, the microbes generally originate in the gastrointestinal tract and colonize the periurethral region before ascending the urethra to the bladder. STIs are **exogenous** infections; i.e., the infectious agent is acquired from a source outside the body. In the case of STIs, these agents are acquired by sexual contact.

UTIs are much more common in women than in men for a number of reasons. The urethra is shorter in women than in men, and straight rather than curved as in men, making it easier for microbes to ascend to the bladder. Prostatic secretions have antibacterial properties, which further protects the male. The periurethral epithelium in women, especially women with recurrent UTIs, is more frequently colonized with microorganisms that cause UTIs. It should also be noted that the incidence of UTIs is higher in sexually active women, as coitus can introduce organisms colonizing the periurethral region into the urethra. The incidence of nosocomial UTIs, however, is similar in women and men. In these infections, catheterization is the major predisposing factor.

The incidence of STIs is similar in both heterosexual men and women; however, the morbidity associated with these infections tends to be much greater in women. In particular, irreversible damage to reproductive organs, caused by both *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, is all too common. Infections with these two organisms are almost always symptomatic in males, though the few men who do not have symptoms can be responsible for infecting many partners. By contrast, a significant number of women may be infected asymptomatically at first. They may manifest signs and symptoms of infection only when they develop **pelvic inflammatory disease**, which can result in sterility. Fetal loss or severe perinatal infection may be caused by two other STI agents, herpes simplex virus and *Treponema pallidum*, the etiologic agent of syphilis.

Important agents of genitourinary tract infections are listed in Table 1. Only organisms in this table should be considered in your differential diagnosis for the cases in this section. You should note that not all organisms that can be spread sexually, such as hepatitis B virus and *Entamoeba histolytica*, are listed. This is because these infections do not have genitourinary tract manifestations.

TABLE I SELECTED GENITOURINARY TRACT PATHOGENS

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Bacteria			
Actinomyces spp.	Anaerobic, Gram-positive bacilli	Endogenous	PID[a] associated with intrauterine device usage
Aerococcus spp.	Catalase-negative, Gram-positive cocci	Endogenous	Community- or health care-associated UTI[b]
Bacteroides fragilis	Anaerobic, Gram-negative bacillus	Endogenous	Pelvic abscess
Chlamydia trachomatis	Obligate intracellular pathogen (does not Gram stain)	Direct sexual contact	Urethritis, cervicitis, PID
Enterobacter spp.	Lactose-fermenting, Gram-negative bacilli	Endogenous	Community- or health care-associated UTI
Enterococcus spp.	Catalase-negative, Gram-positive cocci	Endogenous	Health care-associated UTI
Escherichia coli	Lactose-fermenting, Gram-negative bacillus	Endogenous	Community- or health care-associated UTI
Haemophilus ducreyi	Fastidious, pleiomorphic, Gram-negative bacillus	Direct sexual contact	Chancroid (painful genital ulcer)
Klebsiella pneumoniae	Lactose-fermenting, Gram-negative bacillus	Endogenous	Community- or health care-associated UTI
Morganella morganii	Lactose-nonfermenting, Gram-negative bacillus	Endogenous	Community- or health care-associated UTI
Mycoplasma hominis	Lacks a cell wall (does not Gram stain)	Endogenous; direct sexual contact	Pyelonephritis, PID
Neisseria gonorrhoeae	Gram-negative, intracellular diplococcus	Direct sexual contact	Urethritis, cervicitis, PID
Proteus mirabilis	Lactose-nonfermenting, swarming, Gram-negative bacillus	Endogenous	Community- or health care-associated UTI
Pseudomonas aeruginosa	Lactose-nonfermenting, Gram-negative bacillus	Catheterization	Health care-associated UTI
Staphylococcus saprophyticus	Catalase-positive, Gram-positive coccus	Endogenous	Community-associated UTI
Treponema pallidum	Spirochete (does not Gram stain)	Direct sexual contact; vertical, mother to child	Chancre (painless genital ulcer); primary, secondary, tertiary syphilis; neonatal syphilis
Ureaplasma urealyticum	Lacks a cell wall (does not Gram stain)	Endogenous; direct sexual contact	Urethritis, urethroprostatitis, epididymitis, chorioamnionitis

(continued next page)

TABLE 1 SELECTED GENITOURINARY TRACT PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Fungi			
Candida spp.	Yeasts with pseudohyphae	Endogenous	Vaginitis, health care-associated UTI, balanitis
Parasites			
Phthirus pubis	Crab lice	Direct sexual contact	Pubic hair infestation
Trichomonas vaginalis	Protozoan	Direct sexual contact	Vaginitis
Viruses			
Adenoviruses	Nonenveloped DNA viruses	Exogenous exposure	Hemorrhagic cystitis
Herpes simplex viruses (HSV-1 and -2)	Enveloped DNA viruses	Direct sexual contact; vertical, mother to child	Recurrent genital ulcers, fetal/neonatal infections, encephalitis
Human immunodeficiency viruses (HIV-1 and -2)	Retroviruses	Direct sexual contact; blood and body fluids; vertical, mother to child	AIDS, neonatal infection, dementia
Human papillomavirus	Nonenveloped DNA virus	Direct sexual contact	Genital warts, cervical and anal carcinoma

[a] PID, pelvic inflammatory disease.

[b] UTI, urinary tract infection.



The patient was a 19-year-old female with a history of a urinary tract infection (UTI) 4 months prior to admission for which she was treated with oral ampicillin without complications. Five days prior to this admission she began to note nausea without vomiting. One day later she developed left flank pain, fevers, and chills and noted increased urinary frequency. She noted foul-smelling urine on the day prior to admission. She presented with a temperature of 38.8°C, and physical examination showed left costovertebral angle tenderness. Urinalysis of a clean-catch urine sample was notable for >50 white blood cells per high-power field, 3 to 10 red blood cells per high-power field, and 3+ bacteria. Urine culture was subsequently positive for $>10^5$ CFU of an organism per ml (seen growing on culture in Fig. 1.1 [sheep blood agar] and Fig. 1.2 [MacConkey agar]). Note that the organism is beta-hemolytic.

1. What do the urinalysis findings indicate? Explain your answer.
2. Why were the numbers of organisms in her urine quantitated on culture? How would you interpret the culture results in this case?
3. Which Gram-negative rods are lactose fermenters? Which one is also often beta-hemolytic?
4. This bacterium was resistant to ampicillin. What in this patient's history might explain this observation? Multidrug-resistant strains of this organism are beginning to be seen as an important cause of UTI. Describe the mechanism of resistance that these organisms most likely will have.
5. UTIs are more frequent in women than men. Why?
6. Did this woman have cystitis or pyelonephritis? Why is it important to differentiate between the two?
7. Briefly explain the evolution of the organism causing this infection in terms of its ability to infect the urinary tract. What virulence factors have been shown to play a pathogenic role in this infection?

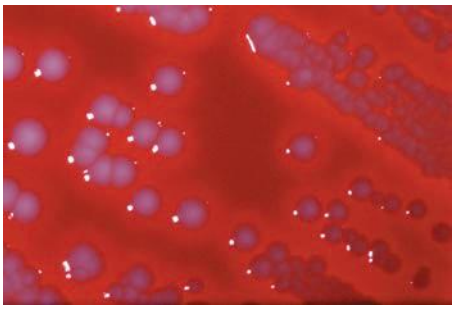


Figure 1.1

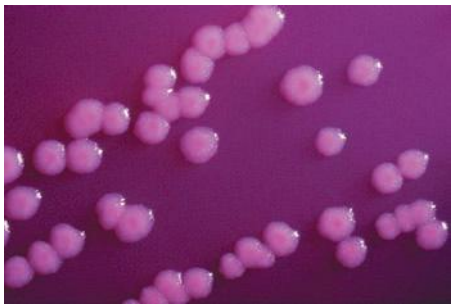


Figure 1.2



CASE DISCUSSION

1. Urine from normal individuals usually has <10 white blood cells per high-power field. Pyuria (the presence of >10 white blood cells per high-power field in urine) and hematuria (the presence of red blood cells in urine), as seen in this patient, are reasonably sensitive but not always specific indicators of UTI. The presence of bacteriuria (bacteria in urine) in this patient further supports this diagnosis. However, the presence of bacteriuria on urinalysis should always be interpreted with caution. Clean-catch urine, which is obtained by having the patient cleanse her external genitalia, begin a flow of urine, and then "catch" the flow of urine in "midstream," is rarely sterile because the distal urethra is colonized with bacteria. Urine is an excellent growth medium. Therefore, if urine is not analyzed fairly quickly (within 1 hour), the organisms colonizing the urethra can divide (two to three generations per hour) if the urine specimen is left at room temperature rather than refrigerated or immediately planted on culture media. Organisms colonizing the urethra may be present in sufficient numbers to be visualized during urinalysis even when the patient is not infected.

2. In a normal individual, urine within the bladder is sterile. As it passes through the urethra, which has a resident microflora, it almost always becomes contaminated with a small number ($<10^3$ CFU/ml) of organisms. As a result of urethral contamination, essentially all clean-catch urine samples will contain a small number of organisms, so culturing urine nonquantitatively will not allow differentiation between colonization of the urethra and infection of the bladder. It should be noted that only a small number of clinical specimens other than urine are cultured quantitatively.

Patients in whom the bladder is infected tend to have very large numbers of bacteria in their urine. These organisms usually, but not always, are of a single species. Studies have shown that most individuals with true UTIs have $>10^5$ CFU/ml in clean-catch urine specimens. There are exceptions to this generalization. In a woman with symptoms consistent with UTIs, bacterial counts as low as 10^2 CFU/ml of a uropathogen—e.g., *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus* spp., or *Staphylococcus saprophyticus*—may indicate that she has a UTI. Colony counts of 10^2 CFU/ml of a uropathogen are highly sensitive for diagnosing UTIs but are of low specificity; colony counts of $>10^5$ CFU/ml are highly specific, but the sensitivity in the setting of acute, uncomplicated cystitis in women is only ~50%.

3. The lactose-fermenting, Gram-negative bacilli that are most commonly isolated from urine are the "KEE" organisms (*Klebsiella* spp., *E. coli*, and *Enterobacter* spp.). *E. coli* is recovered from ~80 to 85% of outpatients and ~40 to 50% of inpatients with UTI. The observation that the organism is beta-hemolytic indicates that, in all likelihood, the organism is *E. coli*. Approximately 55% of *E. coli* isolates recovered from urine of patients with pyelonephritis are beta-hemolytic, whereas *K. pneumoniae* and *Enterobacter*

spp. are rarely, if ever, beta-hemolytic. Another common Gram-negative rod that is frequently beta-hemolytic is *Pseudomonas aeruginosa*, which is very unlikely to be the cause of community-acquired cystitis or pyelonephritis in an otherwise healthy woman. This organism is incapable of fermenting carbohydrates and should not be confused with lactose-fermenting isolates of *E. coli*. A spot indole test was done on the patient's isolate and was positive, confirming the identity of this organism as *E. coli*.

4. The patient had a previous UTI, at which time she received oral ampicillin. One of the deleterious effects associated with the use of antimicrobial agents is the selection of antibiotic-resistant bacteria. This occurs with some degree of frequency in gut flora, where plasmids coding for resistance may be mobilized in response to antimicrobial pressure, leading to the transfer of resistance to previously susceptible organisms, such as in this *E. coli* isolate. Not only may resistance to the agent supplying the selective pressure result, but also the plasmid may contain genes that code for resistance to other antimicrobial agents, the end result being a multidrug-resistant organism.

During the past 10 years, the emergence of multidrug-resistant *E. coli* causing both community-acquired as well as health care-associated UTIs has made the selection of empiric antimicrobial therapy much more difficult. Globally, ~20% of *E. coli* strains causing UTIs produce extended-spectrum β-lactamases (ESBLs). Mutations in the active site of the β-lactam "extend" the activity of the β-lactamases so that they are active against all penicillins and cephalosporins. ESBLs are carried on plasmids that frequently also encode resistance to trimethoprim-sulfamethoxazole, fluoroquinolones, and aminoglycosides. Both fluoroquinolones and trimethoprim-sulfamethoxazole are widely used as empiric therapy for cystitis in women. The increasing resistance being seen in *E. coli*, due in part to ESBL-producing strains, greatly limits the choice of oral agents to treat uncomplicated cases of UTI. For now, ESBL-producing *E. coli* isolates remain susceptible to the oral agents fosfomycin and to a lesser degree nitrofurantoin, but how long this will continue to be true is difficult to predict. ESBL-producing organisms remain susceptible to carbapenems such as ertapenem and imipenem. These parenterally administered antimicrobials are widely used to treat systemic infections such as pylonephritis due to ESBL-producing organisms. However, carbapenemases have also emerged and can be encoded on plasmids that carry resistance genes similar to those found on ESBL-encoding plasmids. These carbapenemase-encoding plasmids have been found in *E. coli*. Nitrofurantoin is not active against carbapenemase-producing strains, while fosfomycin has some degree of activity and may be useful in treating cystitis. However, fosfomycin is poorly absorbed systemically and should not be used to treat patients with pyelonephritis, such as the patient in this case, or urosepsis.

5. In adults, 90% of uncomplicated UTIs occur in women. It is one of the most common reasons why adolescent and adult women seek health care, resulting in ~10 million physician visits annually in the United States. The simplistic view of why women have

more UTIs than do men is that the shorter urethra in women results in a greater likelihood that organisms will ascend the urethra and enter the bladder. Sexual activity is thought to play a significant role in the introduction of uropathogens into the urethra. In addition, the use of spermicides, with both diaphragms and coated condoms, has been shown to predispose women to UTIs. However, other factors that may play a role in this gender difference have been identified. It has been observed that prostatic fluid inhibits the growth of common urinary tract pathogens in urine, providing a unique defense mechanism for men. It has also been observed that specific uropathogens bind to vaginal and periurethral epithelial cells. Binding in the periurethral region by these organisms is often seen in women prior to the development of UTI, as well as in women who have recurrent UTIs. Binding of uropathogens to the periurethral epithelium is highest when estrogen levels reach their peak during the menstrual cycle. These observations may further explain why a preponderance of UTIs are seen in women.

6. The clinical presentation in this patient is consistent with acute pyelonephritis. Pyelonephritis is an infection of the kidney, whereas cystitis is an infection of the bladder. The findings of fever, chills, and left flank pain, with corresponding costovertebral angle tenderness, are all consistent with pyelonephritis. If white blood cell casts were seen in the patient's urinalysis, that finding would further support the diagnosis of pyelonephritis. Culture results would not be useful in differentiating between the two types of infections. Radiographic or cystoscopic studies would be necessary to make a definitive diagnosis of pyelonephritis, but clinical judgment is usually sufficient. The reason it is important to distinguish between pyelonephritis and cystitis is that antimicrobial treatment strategies differ. Cystitis therapy is usually brief, typically a 3-day course of trimethoprim-sulfamethoxazole unless there is a high rate of resistance to this agent in the community, while pyelonephritis therapy may be more prolonged, typically lasting 7 days to 2 weeks. The outcome of antimicrobial therapy is dependent in great part on the susceptibility of the *E. coli* strain. If patients are treated empirically with an antimicrobial agent to which their isolate is resistant, their outcome will be less likely to be favorable than in those patients who receive an antimicrobial agent to which their isolate is susceptible.

7. "Pathogenicity islands" are an exciting recent concept for understanding the evolution of human microbial pathogens. They are relatively large segments of DNA that encode virulence factors that have been inserted by recombination into chromosomal regions that appear to more readily allow "foreign" DNA. What that means practically is that organisms such as *E. coli* can quickly evolve from harmless gastrointestinal tract commensals to agents capable of causing UTI by incorporating DNA that encodes virulence factors. Acquisition of virulence factors by gene transfer is a common theme in *E. coli* pathogenicity, not only in strains causing UTI but also in strains that cause diarrheal disease. Two virulence factors known to be important in the pathogenesis of *E. coli* pyelonephritis, P fimbriae and hemolysin, have been found on pathogenicity islands. Pathogenicity

islands are found much more frequently in *E. coli* strains that cause cystitis and pyelonephritis than in fecal isolates.

The fimbriae are the major means of adhesion of uropathogenic *E. coli*, allowing them to bind to the various types of epithelial cells that line the urinary tract. Two different fimbriae found on the surface of uropathogenic *E. coli*, types P and 1, have been well studied. The P fimbriae are so designated because they agglutinate red blood cells possessing the P blood group antigen. They bind to uroepithelial cells and are resistant to phagocytosis. More than 80% of *E. coli* isolates causing pyelonephritis have pathogenicity islands that encode these fimbriae. Type 1 fimbriae are distinct from the P fimbriae. Both agglutination of red blood cells and binding to uroepithelial cells by *E. coli* possessing type 1 fimbriae can be blocked by preincubating the organism with mannose, while binding of type P-fimbriated *E. coli* is not blocked by mannose. Type 1-fimbriated *E. coli* strains are thus said to be mannose sensitive, while type P strains are said to be mannose insensitive. Type 1 fimbriae are found more frequently in patients with cystitis and less frequently in patients with pyelonephritis. Our patient likely had a P-fimbriated *E. coli* strain because she had pyelonephritis.

Another important virulence factor of uropathogenic *E. coli* is hemolysin. Hemolysin production is detected in ~55% of *E. coli* recovered from patients with pyelonephritis. Studies with renal tubular cells in primary culture have shown them to be quite sensitive to the cytotoxic activity of this virulence factor.

Aerobactin is a third virulence factor, found in ~75% of *E. coli* strains causing pyelonephritis. Aerobactin is a siderophore. Siderophores are molecules produced by bacteria and scavenge iron, an essential nutrient for bacteria, from the host. Strains of *E. coli* that produce aerobactins have been shown to grow faster in urine than nonproducing strains, although how important this is in the pathogenesis of UTI is unclear.

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The patient was a 15-year-old male who was brought to the emergency room by his sister. He gave a 24-hour history of dysuria and noted some "pus-like" drainage in his underwear and on the tip of his penis. Urine appeared clear, and urine culture was negative although urinalysis was positive for leukocyte esterase and multiple white cells were seen on microscopic examination of urine. He gave a history of being sexually active with five or six partners in the past 6 months. He claimed that he and his partners had not had any sexually transmitted infections. His physical exam was significant for a yellow urethral discharge and tenderness at the tip of the penis. (A Gram stain done in the emergency room is shown in Fig. 2.1.) He was given antimicrobial agents and scheduled for a follow-up visit 1 week later. He did not return.

1. Based on the Gram stain results, with what organism is this patient infected? What is the reliability of the Gram stain for establishing the diagnosis in this patient? How reliable is the Gram stain for detection of this organism in vaginal specimens from infected women? What other direct detection technique is available for laboratory diagnosis of the organism causing this patient's infection?
2. Are his urinalysis and urine culture findings consistent with his illness? Explain.
3. Why did his partners have a negative history for sexually transmitted infections? For what complications are his sexual partners (whom he may have infected and/or who infected him) at increased risk?
4. What virulence factor(s) made by this organism is responsible for his symptoms?
5. Given his history, for what organisms is he at increased risk? Why do you think this patient was asked to return for a follow-up visit?
6. What antimicrobial agent(s) was he given in the emergency room? How has antimicrobial therapy for this infection evolved over the past 25 years and why was that evolution necessary?
7. Why is there no reliable vaccine against the organism causing this individual's infection?

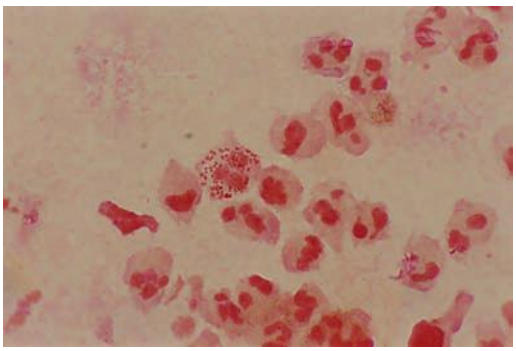


Figure 2.1



CASE DISCUSSION

1. The organism seen on Gram stain is a Gram-negative, intracellular diplococcus consistent with *Neisseria gonorrhoeae*. In males with symptomatic urethritis, a Gram stain of a urethral discharge is a highly reliable test for diagnosis of *N. gonorrhoeae* urethral infection. The Gram stain will be positive for Gram-negative, intracellular diplococci in approximately 95 to 100% of infected male patients. Gram stains of vaginal specimens are positive in only 50 to 60% of females and there are specificity concerns because of the presence of saprophytic *Neisseria* spp. in the vaginal microbiota, making direct Gram stain an unreliable test for women suspected of having a gonococcal infection. A number of FDA-approved nucleic acid amplification tests (NAATs), including ones that use PCR and transcription-mediated amplification, are commercially available. In males, these assays can be performed on either urine or urethral swabs. In females, the assays can be performed on endocervical swabs, vaginal swabs, or urine. Less is known about the performance of these methods in throat or rectal specimens. These methods are more sensitive than culture in part due to the fastidious nature of the organism. Historically, false-positive results have been reported in some NAATs for closely related but saprophytic *Neisseria* spp. The NAATs that are now in use have a greater specificity than did the earlier NAATs. As clinical laboratories become more centralized in the era of managed care, the NAATs are replacing *N. gonorrhoeae* culture. The reason for this changing diagnostic approach is that maintaining the viability of this fastidious organism for culture is difficult when specimens have to travel significant distances to a central laboratory. Bacterial nucleic acid, on the other hand, is comparatively stable, making transport of these specimens for molecular amplification much easier and the detection of gonococci theoretically more sensitive. Given the potential implications of a false-positive result, due to either the presence of saprophytic *Neisseria* spp. or laboratory contamination, it is important for health care providers to understand the issues surrounding the specificity of the particular amplification assay that is being used in the diagnostic laboratory.

There is an important distinction between the use of a NAAT in a patient with signs and symptoms that are strongly suggestive of gonorrhea, as is the case here, and the use of this testing to screen a population of patients. In 2002, the Centers for Disease Control and Prevention (CDC) recommended additional testing to improve the positive predictive value of NAAT screening tests for sexually transmitted infections, particularly in low-prevalence settings. Based on data that demonstrated >90% agreement between initial and confirmatory testing, the CDC no longer recommends routine repeat testing for *Chlamydia trachomatis*, and additional testing for *N. gonorrhoeae* should only be performed when a NAAT is used that cross-reacts with other *Neisseria* spp. However, if a positive test would lead to substantial adverse medical, social, psychological, or legal impact for a patient, additional testing may be warranted.

2. In patients with gonococcal urethritis, white blood cells wash from the urethra during urination. The white blood cells can be detected in urine by dipstick testing for leukocyte

esterase (an enzyme produced by leukocytes) or by microscopic examination. *N. gonorrhoeae* is generally not recovered on urine culture because of the media and incubation conditions used (usually sheep blood agar and media selective for enteric Gram-negative rods, with incubation times usually <48 hours and incubation under ambient air). *N. gonorrhoeae* requires an enriched medium such as chocolate agar and incubation times of at least 36 to 48 hours in 5% CO_2 for growth to be detected visibly. Therefore, a negative urine culture is consistent with the patient's disease. A patient with positive urinalysis for leukocytes who does not have an organism recovered on urine culture is said to have "sterile pyuria." *N. gonorrhoeae* is a common cause of sterile pyuria, as is *C. trachomatis*.

3. Obtaining an accurate sexual history, especially from adolescents, may be difficult. The individual may not recognize signs and symptoms of sexually transmitted infections or may be too embarrassed or ashamed to seek medical care for them. However, given an incubation time of approximately 2 to 5 days for *N. gonorrhoeae* and an acute symptomatic history of 24 hours, it is most likely that this patient was recently infected. If the patient was "serially monogamous" (that is, sexually active exclusively with only one partner for varying lengths of time), it is likely that he was infected by one of his recent partners and that his previous partners had not been infected. A significant percentage of infected women may be infected asymptomatically, and it is possible that the sexual partner who infected him was asymptomatic.

Complications of *N. gonorrhoeae* infection are more common in women because of increased rates of asymptomatic infections. These complications tend to be severe. The major complication seen in women infected with *N. gonorrhoeae* is pelvic inflammatory disease (PID). PID can cause fallopian tube scarring and obstruction, which may result in infertility. Ectopic pregnancy is also more common in women with a history of PID. Though it is uncommon, both men and women can have disseminated gonococcal infection, which can present with a rash and septic arthritis.

4. *N. gonorrhoeae* induces an intense inflammatory response, which is manifested clinically in males as exudate from the urethra. Two virulence factors are important in this process: pili and lipooligosaccharide. Pili mediate attachment and stimulate nonspecific phagocytosis by epithelial cells in the urethra. Lipooligosaccharide (endotoxin) can stimulate an inflammatory reaction to these phagocytized organisms.

5. This individual is at increased risk for a number of sexually transmitted infections. Coinfections with *C. trachomatis* are common. Less frequent but still problematic would be syphilis (*Treponema pallidum*), herpes simplex virus, human papillomavirus, and HIV. Because of his history of multiple sexual partners and the diagnosis of a sexually transmitted infection, this individual is at increased risk for becoming infected with HIV. Sexually active teenagers are one of the populations in which HIV is most rapidly spreading in the United States. Emergency rooms are often hectic, with physicians needing to see many patients as rapidly as possible. This physician did not feel he could adequately counsel and

get consent for HIV serologic testing in such an environment. The physician asked the patient to return to the clinic so appropriate counseling and HIV testing could be done. This patient did not return.

6. The current CDC guidelines for treating uncomplicated gonococcal urethritis are to administer a single dose of an oral cephalosporin (cefixime) or an intramuscular injection of ceftriaxone, plus doxycycline or azithromycin to treat a presumed coinfection with *C. trachomatis*. Many centers, especially in areas of high HIV incidence, have abandoned intramuscular administration of antimicrobial agents for treatment of gonococcal disease in favor of oral therapy. The reason is concern among health professionals over needlestick injuries after injection of patients who are at high risk for HIV infection. Why not treat both the gonococcal and *C. trachomatis* infections with doxycycline? There are two reasons. First, CDC surveillance data in 1997 showed that 26% of gonococcal isolates were resistant to doxycycline. Second, compliance when antimicrobial agents must be taken twice daily for 7 days is often poor.

In addition to resistance to the tetracyclines, gonococcal resistance to penicillin therapy has become so widespread in the past 25 years that penicillin is no longer a reasonable therapeutic option for treating infections with this organism. Initially, penicillin resistance was due to a plasmid-encoded β-lactamase; β-lactamase is an enzyme that degrades the β-lactam ring in penicillin, inactivating the drug. Subsequently, isolates were recovered that had chromosomal mutations that encoded modification in penicillin-binding proteins, making the binding of penicillin to the gonococci much less efficient. This decreased binding resulted in resistance to penicillin. Additionally, by 2008, gonococcal antimicrobial susceptibility surveillance studies showed widespread resistance to the fluoroquinolones, a first-line drug class in the 2002 CDC guidelines. As a result, it is no longer recommended for treatment of gonococcal infection. This is not surprising, since single mutations resulting in fluoroquinolone resistance have been reported in other organisms. However, this is a significant setback for public health efforts to control gonococcal infections since fluoroquinolones such as ciprofloxacin are inexpensive and easy to administer as a single oral dose.

With resistance to different classes of antimicrobials becoming increasingly widespread, how do we monitor drug resistance development in the few antimicrobials to which the gonococci remain susceptible? Molecular methods that are increasingly used for diagnosis of gonococcal infections do not determine the antimicrobial resistance pattern of these organisms. Therefore, the CDC surveillance studies of gonococcal resistance are critical for the recognition of when increased resistance to cefixime and ceftriaxone emerges. Examples of either *in vitro* resistance or treatment failures with these antimicrobials have already been recognized. Public health experts are concerned that we are reaching a time when only more complex and expensive treatment regimens will be effective against this organism. Right now both spectinomycin, which is expensive, and azithromy-

cin are available for treatment of gonococcal infection in patients who have cephalosporin treatment failure, infection with a cephalosporin-resistant organism, or cephalosporin allergies. However, low-level azithromycin resistance is widespread in Europe and high-level azithromycin isolates have been found in the United States.

7. The most successful bacterial vaccines elicit an immune response against either toxins produced by the organism (tetanus and diphtheria) or surface components of the bacteria (*Haemophilus influenzae* type b capsular polysaccharide or filamentous hemagglutinin in the acellular pertussis vaccine). Since the gonococcus does not produce a conventional exotoxin, the obvious target would be a surface component. Unfortunately, surface components of gonococci such as pili can undergo rapid antigenic variation because of frequent rearrangement of the pilin genes, making it impossible to produce a reliably protective vaccine antigen. Conserved and phenotypically stable determinants on the surface of the gonococcus have not yet been used in vaccine development. Whether they will be efficacious in providing mucosal immunity is beyond the scope of this discussion.

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This 16-year-old female presented to the emergency room of an urban medical center with complaints of crampy abdominal pain for days and vaginal bleeding. She denied symptoms of urinary tract infection or abnormal vaginal discharge and had not noted any chills or fever. She had no nausea or vomiting. The pain increased in the 24 hours prior to presentation, and at the time of examination she also noted pain in the right upper quadrant. She was sexually active with one male partner in the preceding 3 months and claimed to use condoms as a method of birth control. She is the mother of one child.

On examination, her temperature was 38.3°C, and there was exquisite tenderness in the right upper quadrant as well as the left lower quadrant. No rebound tenderness or guarding was noted. On pelvic exam, cervical motion tenderness was present, as well as right and left adnexal tenderness. No masses were palpated.

1. Clinically, this patient was believed to have pelvic inflammatory disease (PID) and was admitted to the hospital for antibiotic treatment. What bacteria have been associated with PID?
2. An endocervical swab was collected from this patient, and a nucleic acid amplification test (NAAT) was performed for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Why have NAATs become the method of choice for diagnosis of these two organisms? What are strengths and weaknesses of this method?
3. The NAAT was positive for the more common cause of PID of the two. What was the organism? Why does PID occur in a limited proportion of women who have genital infections with this organism? What serious consequence can PID have for the infected individual?
4. Briefly describe the life cycle of the organism infecting this patient. Why was this organism once classified as a virus?
5. In addition to PID, in which this organism may well have a significant role, in what other clinical situations might this organism be expected to be recovered?
6. How effective are β-lactam antibiotics in treating infections caused by this organism? What is the rationale for using a β-lactam in addition to doxycycline in this patient's therapy? What else should be done epidemiologically in cases of PID?
7. What type of screening strategy has been used successfully to prevent PID? What populations have a high prevalence of chlamydial infection? What are the potential consequences of PID?



CASE DISCUSSION

1. PID, which includes any combination of endometritis, salpingitis, tubo-ovarian abscess, and pelvic peritonitis, has been associated with the sexually transmitted bacterial agents *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. In addition, normal vaginal flora, including anaerobes and facultative aerobes, may be isolated from patients with PID who either have no documented gonococcal or chlamydial infection or have an infection documented with one of these pathogens. Knowledge about the role of *Mycoplasma* and *Ureaplasma* species in the pathogenesis of PID is evolving, but both genera have been found in patients with PID in the absence of *N. gonorrhoeae* and *C. trachomatis*.

2. NAATs are the preferred method for diagnosing sexually transmitted infections due to *N. gonorrhoeae* and *C. trachomatis* owing to their increase in sensitivity, decreased time to result (compared with culture), and ease of specimen transport. For the routine diagnosis of sexually transmitted infections in adults by NAAT, a vaginal or endocervical swab should be used for women and a urine or urethral swab for men. In addition, the Centers for Disease Control and Prevention (CDC) recommends routine screening of rectal and oral swabs from men who have sex with men. However, it should be noted that there are no NAATs currently FDA approved for rectal or oral swabs, and not all NAATs are approved for vaginal swabs. A major disadvantage for some NAATs is decreased specificity compared with culture, particularly for *N. gonorrhoeae*. Depending on the target amplified by the NAAT, there is cross-reactivity with nonpathogenic *Neisseria* species. As a result, when screening for gonorrhea in a low-prevalence population, it would be expected that a large fraction of the unconfirmed positive results are false positives, which may be associated with adverse medical, social, or psychological consequences for these patients. It is recommended not to use off-label specimens (i.e., rectal/oral swabs or specimens from children) when using NAATs with decreased specificity. Other disadvantages of these assays include their higher cost, the potential for contamination resulting in a positive result in a patient without an infection, and in some assays the possible nonspecific inhibition of the assays by blood or other components of cervical secretions and by compounds present in urine. Additionally, the use of NAATs has limited the availability of isolates for antimicrobial resistance surveillance. This is a particular concern with *N. gonorrhoeae*. Nonetheless, the increased sensitivity and ease of screening large numbers of patients simultaneously for both chlamydia and gonorrhea by NAAT outweigh the potential limitations.

3. *C. trachomatis* was the organism identified in this patient's infection. *C. trachomatis* is the most common bacterial cause of sexually transmitted infections (*N. gonorrhoeae* is the second most common). It is one of the causes of PID, which is often a polymicrobial infection (see answer 1, above). Only a subset of women infected with *C. trachomatis* subsequently develop PID. The pathogenesis of the development of PID in cases of *C. trachomatis* infec-

tions is an active area of research. Following the infection of epithelial cells by *C. trachomatis*, proinflammatory cytokines are secreted. It may be that the pathogenesis of PID is the result of an inappropriately increased host inflammatory response, causing tissue injury. A small number of published investigations have looked at the possibility that the variability in the response to *C. trachomatis* genital infection is the result of variations in human innate immune receptor genes such as members of the Toll-like receptor family.

Complications of PID include infertility, chronic pelvic pain, and ectopic pregnancy.

4. *Chlamydia* was once incorrectly classified as a virus because it is an obligate intracellular pathogen and as such cannot be cultured on enriched agar media like most bacteria. McCoy cells are used to culture *C. trachomatis*. After the infectious elementary body infects the McCoy cells, the organism is taken into the cell by a process called receptor-mediated endocytosis. The bacterium develops into a reticulate body within a membrane-bound structure called an inclusion. Reticulate bodies, the reproductive form of the organism, multiply by binary fission. The reticulate bodies then condense to form elementary bodies. Elementary bodies are released from the cell by lysis, release of intact inclusions, or exocytosis. The presence of chlamydial inclusions is demonstrated by staining these cells with a fluorescein-tagged monoclonal antibody that binds specifically to the chlamydial antigens present within the infected McCoy cells. These can then be viewed with a fluorescent microscope, where they will give a characteristic apple-green fluorescence, and the etiologic diagnosis can be established. *Chlamydia* culture is now only rarely used in clinical laboratories as a result of the availability of the less labor-intensive and more sensitive molecular methods (see answer 2, above).

5. *C. trachomatis*, the most common sexually transmitted bacterial pathogen in the United States, is also an etiologic agent of both nongonococcal urethritis and epididymitis in males and cervicitis, endometritis, and salpingitis in women, and it can cause pneumonia and conjunctival disease in neonates if they have passed through an infected birth canal. It is worth noting that many patients are minimally symptomatic or asymptomatic with genital infection due to *C. trachomatis* and may not seek medical attention. Other serotypes of *C. trachomatis*, found rarely in the United States, cause lymphogranuloma venereum. Lymphogranuloma venereum is a genital tract infection characterized by enlarged, tender, and erythematous inguinal lymph nodes and is frequently accompanied by systemic symptoms of fever, headache, and malaise. Still other serotypes of *C. trachomatis* cause trachoma, a leading cause of blindness in the developing world.

6. The CDC notes that "all regimens used to treat PID should also be effective against *N. gonorrhoeae* and *C. trachomatis* because negative endocervical screening for these organisms does not rule out upper-reproductive-tract infection."

Empiric therapy for sexually active women in whom PID is clinically suspected includes, most commonly, a β-lactam antimicrobial agent to treat *N. gonorrhoeae* and

anaerobes, plus doxycycline to treat *C. trachomatis*. The combination is necessary because of the poor activity of β-lactams against *C. trachomatis*. β-Lactams characteristically have poor intracellular penetration. The intracellular location of the replicative phase of *C. trachomatis* (the reticulate bodies) protects it from the activity of β-lactam antibiotics. Other combinations of antibiotics have been used with success in the treatment of PID, including intravenous clindamycin and gentamicin. Oral treatment of PID can be used in those patients who are able to be managed as outpatients. However, fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin) are no longer recommended in the oral treatment of PID as a result of an increase in the resistance of *N. gonorrhoeae* to these antibiotics. Resistance to antibiotics in *N. gonorrhoeae* has become more of a problem in the past decade. The first isolate with high-level resistance to azithromycin was identified in 2011 in Hawaii, and resistance to the oral cephalosporin cefixime has increased in the United States to the point that it is no longer a recommended treatment for gonococcal infections. The 2010 CDC recommendations note that when considering alternative regimens, the addition of metronidazole should be considered because anaerobic organisms are suspected in the etiology of PID. As a result, it has become more difficult in recent years to determine an efficacious oral antibiotic regimen for PID.

In addition, it is important for sex partners of women who have PID to be evaluated because of the high risk of infection with *C. trachomatis* and *N. gonorrhoeae* even if these pathogens have not been isolated from the affected woman. The 2010 CDC guidelines state:

> Male sex partners of women with PID should be examined and treated if they had sexual contact with the patient during the 60 days preceding the patient's onset of symptoms . . . Patients should be instructed to abstain from sexual intercourse until therapy is completed and until they and their sex partners no longer have symptoms. Evaluation and treatment are imperative because of the risk for reinfection of the patient and the strong likelihood of urethral gonococcal or chlamydial infection in the sex partner. Male partners of women who have PID caused by *C. trachomatis* and/or *N. gonorrhoeae* frequently are asymptomatic. Sex partners should be treated empirically with regimens effective against both of these infections, regardless of the etiology of PID or pathogens isolated from the infected woman.

In non-PID cases of genital infection by *C. trachomatis*, the two oral antibiotics that are options in the current recommendations are doxycycline (a tetracycline) and azithromycin. Of note, in patients who are likely to have poor treatment compliance or are unlikely to return for follow-up, azithromycin, which is given as a single dose, is preferred to doxycycline, which is taken twice daily for 7 days. In addition, tetracyclines should be avoided in pregnancy.

7. The use of criteria to identify women among a low-prevalence population who are at increased risk for chlamydial infections, to test these women for cervical chlamydial infections, and to treat those who are found to be infected has significantly reduced the incidence of PID in a low-prevalence population. Adolescent inner-city females are a very high-prevalence population for *C. trachomatis*, and on the basis of a prospective longitudinal study, the screening of all sexually active adolescent females every 6 months has been advocated. Similarly, the high prevalence of both chlamydial and gonococcal infection in women entering jails and adolescents entering juvenile detention centers suggests that screening of these women may be worthwhile.

Untreated lower genital tract infections in women may lead not only to PID but to complications of PID, including infertility, ectopic pregnancy, and chronic pelvic pain, as noted above.

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The patient was a 20-year-old female who presented to the emergency room with a 4-day history of fever, chills, and myalgia. Two days prior to this she had noted painful genital lesions. On the day of admission she developed headache, photophobia, and a stiff neck. Previously she had been in good health. She admitted to being sexually active but had no history of sexually transmitted infections.

On physical examination, she was alert and oriented. Her vital signs were normal except for a temperature of 38.5°C (101.3°F); pulse rate was 80 beats/min, and blood pressure was 130/80 mm Hg. A general examination was unremarkable except for slight nuchal rigidity. Her throat was clear, and there was no lymphadenopathy. A pelvic examination revealed extensive vesicular and ulcerative lesions on the left labia minora and majora with marked edema. The cervix had exophytic (outward-growing) necrotic ulcerations.

General laboratory tests were unremarkable. A vaginal swab was collected for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* nucleic acid amplification test (NAAT), a swab of the lesions was sent for herpes simplex virus (HSV) NAAT, and an RPR (rapid plasma reagin) was performed. A lumbar puncture was also done, which had a normal opening pressure. The cerebrospinal fluid (CSF) showed a mild pleocytosis with a leukocyte count of 41/μl with 21% polymorphonuclear leukocytes and 79% mononuclear cells, a glucose level of 46 mg/dl, and a protein level of 68 mg/dl (slightly elevated). The RPR and a CSF VDRL test were negative. A NAAT was positive from the lesion as well as from her CSF. The patient's condition improved after 2 days of intravenous therapy. She was discharged home on oral medication.

1. What is the differential diagnosis of ulcerative genital lesions? Which rapid test was used so that specific therapy could be started?
2. Which complication of her underlying illness did she develop?
3. If she had been pregnant at the time of her infection, for what would her fetus be at risk?
4. Briefly describe the natural history of this infection.
5. Briefly describe the epidemiology of the agent causing her infection.
6. There are two different serotypes of the agent causing her infection. What similarities do they share and what are the differences between these agents?



CASE DISCUSSION

1. In the United States, the most likely diagnosis is either genital herpes or syphilis. In studies of patients with genital lesions in the industrialized world, HSV is the most frequently recovered agent. Other agents that are common causes of genital lesions include *Haemophilus ducreyi* (the etiologic agent of chancroid), human papillomavirus (genital warts), and the lymphogranuloma venereum-causing serotypes of *C. trachomatis*. Genital herpes lesions are painful, whereas lesions due to *Treponema pallidum* are usually painless. Genital infections such as chancroid or lymphogranuloma venereum can result in painful or painless ulcers, respectively, but they often result in suppurative lymphadenopathy. The diagnosis of HSV infection can be confirmed by swabbing the base of the lesion and performing either viral culture or NAAT. Using a shell vial culture technique, the virus can usually be detected within 24 hours. However, detection of HSV antigen by immunofluorescence or DNA from the lesion by NAAT is more rapid than culture. In addition, NAAT testing of lesions may be more sensitive than culture, though it is critical to monitor for laboratory contamination since these specimens contain high viral titers. To date, there is only one FDA-cleared NAAT for HSV, which is only approved for vaginal lesion swabs. Tzanck preparations, in which smears taken from the edge of the lesion are examined for the presence of cells showing pathologic changes consistent with HSV infection, can also be used in the diagnosis of genital lesions. This technique, although inexpensive, lacks both the sensitivity and specificity of culture, immunofluorescence, or NAAT. HSV was detected in this patient by an HSV NAAT performed on a swab of her genital lesion, which was positive for HSV-2.

2. Among women with primary genital herpes due to HSV-2, approximately one in three will have self-limited, aseptic meningitis. These patients typically have a pleocytosis with a lymphocytic predominance and an elevated protein level, as was seen in this case. In this clinical setting, CSF would not always be obtained. A NAAT was positive from the lesion as well as from her CSF. While HSV NAAT testing on lesions performs similarly to culture, NAAT testing on CSF is much more sensitive than culture. When CSF cultures were standard laboratory practice, the rate of isolation of HSV-2 was 0.5 to 3.0% in patients with aseptic meningitis. Now that NAAT testing of CSF is the reference method, the rate of detection of HSV-2 has increased to 5 to 17%.

3. Her fetus would be at risk for neonatal herpes. Neonatal herpes is a relatively infrequent infection, occurring in between 1 in 1,700 and 1 in 12,500 births. However, it is estimated that 25 to 50% of women who have acquired HSV during pregnancy and have vaginal deliveries will transmit the disease to their child, whereas ≤1% with recurrent infection will do so. Notably, most cases of genital HSV in women are asymptomatic, though cervical viral shedding still occurs. Other factors that increase the likelihood of infection are prolonged rupture of membranes, a mother who is seronegative for HSV-2

(suggesting acute infection), and the use of fetal scalp monitors. Of neonates with herpes infections, ~80% are infected during passage through an infected birth canal, while ~6 to 14% are infected in utero and the remaining are infected postpartum.

Most neonatal HSV infections occur in the second to third week of life. There are three forms of neonatal HSV infection: (i) skin, eyes, and mouth disease; (ii) central nervous system (CNS) disease; and (iii) disseminated disease. The most benign form, which is seen in 45% of cases, causes infection localized to the skin, eyes, and mouth. If recognized, it can be effectively treated with antiviral agents such as acyclovir. CNS-associated infections account for 30% of cases. These children have nonspecific CNS symptoms not unlike those of neonatal bacterial meningitis, including seizures, lethargy, high fevers, poor feeding, and irritation. Lesions may or may not be present. Mortality approaches 50% in untreated children, and long-term neurologic sequelae such as developmental delay, epilepsy, blindness, and cognitive disabilities are seen in half of the survivors. The most severe manifestation of disease is disseminated infection, which occurs in ~25% of cases. In this infection, multiple organs, including the brain, may be infected. These individuals typically have a viral exanthem in the setting of CNS infection and/or multiorgan failure. Up to 50% of cases do not have a rash. If the infection is untreated, mortality is very high, reaching 80%. Even with appropriate therapy, mortality for disseminated disease is 30%, and those who survive often have profound neurologic sequelae as mentioned above.

4. HSV, like all herpesviruses, causes a lifelong, latent infection. In genital tract infections, the virus enters a latent state in the sacral nerve ganglia. Recurrences occur when the virus replicates in the neuron and is carried along the peripheral nerves to the epithelium. Of adults with HSV-2, only 10 to 25% have a clinical history of genital herpes lesions. HSV-infected individuals can intermittently shed HSV in the absence of symptoms and therefore contribute to the transmission of HSV. Both condom use and antiviral suppression decrease transmission. Symptomatic recurrences may occur as frequently as 8 to 10 times per year, although the majority of individuals have significantly fewer episodes. Recurrences are generally milder than the primary episode of disease.

5. HSV-2 infects ~16% of individuals in the United States. Infections are more common in females (21%) than in males (12%) and are more common in black individuals (39%, versus 12% for whites). Other risk factors for HSV-2 infection include early age of first sexual encounter, a high number of sexual partners, history of other sexually transmitted infections, and lower socioeconomic status. Infection rates among commercial sex workers may approach 100%. Although HSV-2 infection rates increased significantly from 1976 to 1994, with the highest rate of increase in individuals <30 years old, this trend has reversed in recent years.

6. There are two distinct serotypes of HSV—HSV-1 and HSV-2. HSV-1 is an infection primarily of the oropharyngeal mucosa, with latent infection occurring in the trigeminal

ganglion, while HSV-2 primarily infects the genital mucosa, though either serotype can be seen in these anatomic sites. HSV-1 infections are typically acquired in early childhood, while HSV-2 infections occur after the individual becomes sexually active. There has been a noticeable increase in HSV-1 genital infections over the last 2 decades, with some studies quoting an incidence of up to 50%. Possible explanations for this increase include increased oral-genital contact; increased HSV-1 acquisition in childhood, providing more viral exposure in adolescence; and/or a decrease in HSV-1 infection in childhood, making children more susceptible when they become sexually active. Although both serotypes are neurotropic, HSV-1 appears to cause more severe CNS infection affecting the temporal and frontal lobes. In contrast to aseptic meningitis associated with primary genital HSV-2 infection and neonatal CNS infection, herpes encephalitis in adults and older children is most often due to HSV-1 infection. Herpes encephalitis is a rare, sporadic CNS viral infection and is the most common cause of nonepidemic viral encephalitis in adults in the United States. Patients present with fever, headache, and encephalopathic findings such as altered consciousness, behavioral and speech disturbances, and focal or diffuse neurologic signs. The diagnosis can be confirmed by detecting HSV directly using fluorescent antibody staining of tissue obtained by brain biopsy. Because brain biopsy is dangerous, alternative means of making this diagnosis have been sought. Two studies have carefully evaluated the sensitivity of HSV PCR compared with histopathologic evaluation of brain tissue, reporting sensitivities of 97 to 98% for PCR. Therefore, HSV PCR of CSF has become the standard method for diagnosing HSV CNS infection. It is not clear why certain patterns of CNS infection with either HSV-1 or HSV-2 result in different CNS manifestations. The age of the patient, the route of viral dissemination (e.g., neural versus hematogenous), preexisting immunity, and/or specific viral properties may be factors.

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This 26-year-old woman was referred to a public health clinic as a result of contact tracing in a case of gonorrhea. The woman, who had recently had unprotected sexual intercourse, had no symptoms. Physical examination was normal. Pelvic examination demonstrated a white vaginal discharge but was otherwise unremarkable. A cervical swab was obtained and submitted for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing by a nucleic acid amplification test (NAAT). Examination of a wet mount of the vaginal discharge revealed the presence of a protozoan with a characteristic jerky motility. Figure 5.1 shows a Giemsa stain of the organism.

1. What organism did the wet preparation demonstrate? What other organism can cause vaginitis and can be detected by wet mount?
2. What other methodologies are available for detection of this organism?
3. How is infection with this organism most commonly acquired? What clinical presentations occur in women infected with this organism? In men infected with this organism?
4. This patient was asymptomatic when examined. She had had sexual contact with a partner who had a positive culture for *N. gonorrhoeae*. What would be appropriate antimicrobial therapy for this patient?
5. Why is infection with this organism of special concern in pregnant women? Would therapy be any different if this woman were pregnant?
6. What else should be done to prevent this patient from becoming reinfected with the organism identified on the wet preparation?

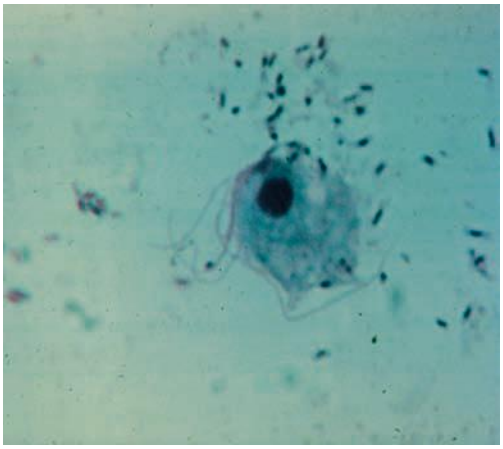


Figure 5.1



CASE DISCUSSION

1. The wet preparation demonstrated the trophozoites of the protozoan *Trichomonas vaginalis*. Examination of freshly prepared wet mounts of vaginal fluid, prostatic secretions, or urine from infected patients will reveal the organism in 40 to 80% of infected individuals. The organism is 7 to 23 μm in size, with a typical jerky motility. Microscopic examination for *T. vaginalis* is highly specific because its unique morphology makes it unlikely to be confused with any other organism that might typically be seen in genital tract secretions. Wet-mount examination is widely used by laboratories because it is inexpensive, rapid, easily performed, and requires relatively simple equipment (light microscope). However, because detection is based on motile live organisms, the test is best done in the clinic, unless a rapid transit time to the laboratory is possible. Trichomonads die quickly and test sensitivity declines sharply, making a specimen >15 minutes old of limited clinical value with this technique. Wet mounts can also be used to diagnose *Candida* vaginitis. In this form of vaginitis, yeast and pseudohyphae will be seen on wet mount. *Candida* vaginitis is frequently seen during or following antimicrobial therapy that alters the vaginal microbiota.

2. Rapid enzyme immunoassay (EIA), DNA hybridization, culture, and NAAT techniques have been developed to detect this organism. The most widely used rapid EIA test is an immunochromatographic "dipstick" test similar to a home pregnancy test. The test is performed on a vaginal swab. EIA is more sensitive than wet-mount examination and is more specific because of an objective colorimetric endpoint. Although more expensive than wet mount, EIA is relatively inexpensive compared to NAAT although not as sensitive.

A commercial DNA hybridization test is available that detects not only *T. vaginalis* but also other organisms associated with vaginitis (*Gardnerella* and *Candida*). Compared with wet mount and culture for *Trichomonas*, the hybridization test is 90% sensitive and 99% specific, but compared with NAAT it is only 63% sensitive.

Culture is done by growing the organism in enriched broth. A commercial test is available that uses a specially designed pouch that allows the direct examination of the broth microscopically for trophozoites. Culture is more sensitive than direct examination, but because of its complexity, expense, and length of time to result, it is primarily a research tool and is not commonly used clinically.

NAAT for *T. vaginalis* has been found to be more sensitive than direct examination, EIA, and DNA hybridization. It is both more rapid and more sensitive than culture. False-positive reactions with NAAT are of concern. A commercial NAAT was recently FDA-cleared which will likely promote more frequent clinical testing for this organism.

3. *T. vaginalis* is typically transmitted via sexual contact. Since *Trichomonas* infection is not a reportable disease, the number of cases that occur annually is unknown. However, it

is estimated that 3 million women are infected annually in the United States, making this parasite an important health issue. Women can be asymptomatically infected, but most infections result in a vaginal discharge. Symptoms of itching or burning are frequently associated with this discharge. The infection can also involve the urethra, resulting in symptoms of dysuria. In men, most cases are asymptomatic, though some men have symptoms of urethral involvement, including a urethral discharge. Involvement of the prostate or seminal vesicles may occur as well. Confirming the diagnosis of *T. vaginalis* infection in men is difficult. Direct microscopic examination is insensitive and the EIA tests are not approved for use in specimens collected from males. The sensitivity of culture for men is low compared with the sensitivity of culture for infected women. Even NAAT may be falsely negative unless multiple specimens are tested. Further, an FDA-cleared NAAT is not currently available for use with male specimens.

4. Clearly, this woman must be treated for the *T. vaginalis* infection, the diagnosis having been established on the basis of a microscopic examination of her discharge. The drug of choice for this infection is metronidazole (Flagyl). It should be noted that there are an increasing number of reports of treatment failures due to metronidazole-resistant strains of *T. vaginalis*, though there are few studies on the surveillance of resistance. One study, published in 2006 from Birmingham, AL, tested clinical isolates of *T. vaginalis* and found that 17 of 178 (9.6%) were resistant *in vitro*. However, the laboratory results did not correlate well with the clinical response to treatment with metronidazole. Tinidazole has been approved for use to treat *T. vaginalis*. Clinical studies suggest that it is superior for the treatment of *T. vaginalis* in women, but there is a paucity of data on the effectiveness of this agent in *T. vaginalis*-infected men. *Trichomonas*-infected women who fail metronidazole therapy should be treated with tinidazole.

Even though this patient was asymptomatic, she was at a very high risk for a coinfection with *N. gonorrhoeae* because that organism had been detected in her male sexual partner. This finding prompted her visit to the clinic. Her presumptive gonococcal infection was treated with an intramuscular injection of ceftriaxone. In addition, since gonococcal infections are often associated with infection by *C. trachomatis*, she was given oral doxycycline. Her cervical swab NAAT was subsequently positive for both *N. gonorrhoeae* and *C. trachomatis*. Remember that patients can be simultaneously infected with multiple sexually transmitted infectious agents and that both *C. trachomatis* and *N. gonorrhoeae* more frequently cause asymptomatic infections in women than in men.

The patient was also offered testing for HIV infection. Recent studies have shown that *T. vaginalis* infection, as well as other sexually transmitted infections, increases the likelihood of HIV transmission.

5. *T. vaginalis* has been associated with preterm labor, premature rupture of membranes, and low-birth-weight babies. The use of metronidazole during pregnancy has been con-

troversial because this drug has been shown to be mutagenic in bacteria and carcinogenic in laboratory animals. Retrospective studies have shown that women treated with metronidazole during pregnancy do not have a higher rate of delivery of children with birth defects than those women who did not receive this drug during pregnancy. Nevertheless, some experts would caution against using metronidazole during the first trimester.

6. The patient's partner, who had been treated for gonorrhea and chlamydia, had not been treated for infection with *T. vaginalis*. As with other sexually transmitted infections, treatment of both people within a sexual relationship is necessary to prevent reinfection by the untreated person. Treatment of only the person presenting and not the partner can result in a "ping-pong ball" phenomenon, where the infection "bounces" back and forth between the two partners. In addition, the patient was advised on the risks of unprotected sex and informed that condom use may help to prevent disease transmission.

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A 40-year-old woman presented to her primary care physician for a routine annual health exam. She had no concerns beyond stress related to marital problems. She reported regular menstrual cycles every 4 weeks and a normal Pap history with no record of previous sexually transmitted infections. Her review of systems and pelvic examination were normal. Cervical sampling for a Papanicolaou-stained (Pap) smear was performed. Cytologic examination showed atypical squamous cells of uncertain significance (ASC-US), so the pathologist ordered a human papillomavirus (HPV) molecular detection test, which returned positive. Molecular testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was negative.

Due to her HPV-positive ASC-US result, the patient was referred to a gynecologist for evaluation. During this visit she stated she and her husband of 20 years had separated during the last year, and she had a new sexual partner. A colposcopy was performed in which cervical lesions were identified and biopsied. Pathologic examination of the biopsy showed benign endocervical epithelium with acute inflammatory cells, but no squamous epithelium was present for evaluation. Since the biopsy showed no evidence of dysplasia or HPV cytopathic effect, the patient was asked to follow up in 6 to 12 months.

At 6 months, her physical exam was normal with the exception of presumed bacterial vaginosis. A Pap smear was repeated and showed ASC-US, which was HPV positive. A colposcopy was performed, and lesions were biopsied. Pathologic examination of two biopsies showed squamous and endocervical mucosa present with reactive epithelium changes, but no dysplasia or HPV cytopathic effect was identified. Six months later a repeat Pap was normal and HPV testing was negative, so she was told to return in 1 year.

At her next annual exam, a Pap examination showed atypical squamous cells including both low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively). She was again HPV positive. Three biopsies were obtained which showed high-grade dysplasia that could not be further characterized due to scant sampling, but was likely CIN 2 or CIN 3 (cervical intraepithelial neoplasia, a cervical cancer precursor). Subsequently, she underwent a loop electrosurgical excision procedure (LEEP) which did not show any remaining dysplasia at the margins.

1. What is the most common outcome of HPV infection? In what patient population is HPV most prevalent?
2. Describe the range of infectious complications associated with HPV.
3. What are the pathologic changes associated with persistent HPV infection of the female genital tract?

4. Several molecular tests are available for the detection of HPV DNA. What are the challenges associated with these tests? What are the advantages of molecular tests for HPV?
5. What guidelines exist for the monitoring of HPV infection and atypical Pap results? At what intervals should testing take place?
6. How can HPV infections be prevented?

CASE DISCUSSION



1. HPV is the most common sexually transmitted infection, resulting in ~14 million new infections annually in the U.S. Although there are an estimated 79 million HPV infections currently in the U.S., about 90% are asymptomatic and resolve within 2 to 3 years with no associated morbidity. The peak prevalence for HPV infections is seen in sexually active individuals 15 to 24 years old; this group represents 50% of all new HPV and other sexually transmitted infections. For this reason, it is not recommended that women under 30 years of age be routinely tested for HPV. In this patient population, HPV infection is most commonly transient and poses no risk for the development of cancer.

2. There are over 150 types of HPV, 40 of which can be sexually transmitted. HPV can cause either a cutaneous or mucosal infection depending on the tropism of the specific virus. Cutaneous infections present as non-genital warts, which include common warts, plantar warts, and flat warts. HPV types 1, 2, 3, 7, and 10 are most commonly associated with cutaneous warts. Although relatively common in all age groups, warts occur with a peak incidence in children aged 12 to 16. Mucosal infections include genital warts; cancers of the cervix, anus, external genitalia, and oropharnyx; and recurrent respiratory papillomatosis. Among sexually active individuals, genital warts range in prevalence from 1 to 10% with a peak incidence in 20- to 24-year-old persons. Risk factors associated with genital warts include infection with HPV types 6 and 11, introduction of new sexual partners, and an increased number of sexual partners. Cervical cancer is most commonly caused by persistent infection with types 16 and 18, which, combined, cause ~70% of cervical cancers. The remainder is caused by other high-risk HPV types. (See question 3 for further discussion of HPV and cervical cancer.) The incidence of HPV-associated anal cancer has been on the rise during the past 30 years and is primarily due to type 16. Risk factors for this uncommon cancer include female gender, HPV infection, increased number of partners, genital warts, cigarette smoking, receptive anal intercourse, and HIV infection. Some cancers of the external genitalia (penile, vulvar, and vaginal cancers) are associated with HPV infections and tend to occur in younger patients than HPV-negative cancers. Squamous cell carcinomas of the head and neck may also be due to HPV, but like cancers of the external genitalia, not all are associated with HPV. HPV-associated head and neck cancers are primarily found in the oropharynx and the base of the tongue and tonsil. Oral cancers due to HPV infection occur in younger individuals with increased sexual risk factors and are more common in men. Lastly, recurrent respiratory papillomatosis (RRP) is thought to be due to HPV acquisition during birth and presents as laryngeal warts in childhood, although adult cases have also been reported. RRP is associated with HPV types 6 and 11 and is generally benign. However, if not removed, laryngeal warts can lead to obstruction and can occasionally be aggressive and malignant.

3. The development of cervical cancer usually takes several years of persistent HPV infection. Thus, the patient's recent change in sexual partners is likely not the initial source of the HPV infection causing her cervical changes. Disease progression is linked to high-risk oncogenic HPV types (e.g., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69, 82), whereas low-risk types are only rarely associated with the development of cervical cancer and, therefore, are not routinely detected by HPV tests (e.g., 6, 11, 40, 42, 43, 44, 54, 61, 72, 81). Two main classification systems exist to describe HPV-associated changes in the cervical epithelium. The Bethesda system is primarily used to described changes seen by cytology (i.e., liquid-based Pap testing), whereas the CIN system is primarily used to describe the neoplasia seen by histology (i.e., biopsies obtained during colposcopy). Table 6.1 summarizes dysplasia classification and the associated interpretations. It should be noted that persistent HPV infection with a high-risk type most often does not progress through all of these stages. All precancerous stages have a significant likelihood of regression, with a greater percentage of the low-grade abnormalities regressing compared to high-grade dysplasia. It has been reported that up to 43% of CIN 2 and 32% of CIN 3 may regress without intervention. Invasive cancer is more commonly diagnosed in women over 40 years old, typically 8 to 13 years after identification of a high-grade lesion.

4. There are currently four FDA-approved tests for the detection of HPV DNA from liquid cytology specimens. The detection chemistries range from hybrid capture and Invader chemistry (signal amplification) to PCR and transcription-mediated amplification (target amplification of DNA and RNA, respectively). The initial clinical trials were performed with the hybrid capture system. Using CIN 2 or greater as an endpoint, hybrid capture had a 96% sensitivity (compared to 55% sensitivity of Pap smear). The development of amplification-based tests has led to an increase in analytic sensitivity, but no apparent increase in clinical sensitivity. The detection of HPV DNA by molecular screening has reduced cervical cancer rates by providing detection often prior to traditional cytology. Further, a negative HPV test in the setting of ASC-US prevents many unnecessary colposcopies. An additional advantage is the ability to detect only high-risk HPV types, which increases the clinical specificity of HPV detection. The more recently approved tests also have the ability to provide type-level results for types 16 and 18 such that positive women (even with normal Pap smear) will be followed by colposcopy due to the increased oncogenic potential of these types. One concern with the molecular methods is sample contamination, particularly if liquid cytology specimens are processed via automation. Some versions of automated processors have been shown to cross-contaminate specimens, but more recent automation appears not to have that problem. Nonetheless, to minimize the possibility of laboratory contamination, it is prudent to aliquot from the liquid cytology vial for HPV testing prior to placing the vial on an automated processor. Another concern is that a negative HPV test in a low-risk patient increases the time until the next Pap/HPV test to 5 years. Many physicians are concerned that patients will cease

to present for annual health maintenance, which includes screens for many other important women's health issues, such as breast cancer.

5. Three guidelines exist for cervical cancer screening. Guidelines updated in 2012 are available from the American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP); from the American College of Obstetricians and Gynecologists (ACOG); and from the U.S. Preventative Services Task Force (USPSTF). All three guidelines agree that women younger than 21 years of age should not be screened by any method and that women 21 to 29 years of age should be screened by cytology alone every 3 years. For women 30 to 65 years of age, it is recommended that co-testing by cytology and HPV molecular

TABLE 6.1 THE BETHESDA CLASSIFICATION SYSTEM FOR CERVICAL SQUAMOUS CELL DYSPLASIA[a]

BETHESDA SYSTEM 1999	BETHESDA SYSTEM 1991	CIN SYSTEM	INTERPRETATION
Negative for intraepithelial lesions or malignancy	Within normal limits	Normal	No abnormal cells
ASC-US (atypical squamous cells of undetermined significance)	ASCUS (atypical squamous cells of undetermined significance)		Squamous cells with abnormalities greater than those attributed to reactive changes but that do not meet the criteria for a squamous intraepithelial lesion
ASC-H (atypical squamous cells, cannot exclude HSIL)			
LSIL (low-grade squamous intraepithelial lesions)	LSIL (low-grade squamous intraepithelial lesions)	CIN 1	Mildly abnormal cells; changes are almost always due to HPV
HSIL (high-grade squamous intraepithelial lesions) with features suspicious for invasion (if invasion is suspected)	HSIL (high-grade squamous intraepithelial lesions)	CIN 2/3	Moderately to severely abnormal squamous cells
Carcinoma	Carcinoma	Invasive squamous cell carcinoma; invasive glandular cell carcinoma (adenocarcinoma)	The possibility of cancer is high enough to warrant immediate evaluation but does not mean that the patient definitely has cancer

[a]From reference 1.

detection occur every 5 years. In addition, the ACS/ASCCP/ASCP guidelines state that primary HPV testing in the absence of cytology for women 30 to 65 years old is not recommended. Screening can be discontinued in posthysterectomy patients and after 65 years of age if the woman has a history of adequate screening. Screening should take place as above, independent of the woman's vaccination status. These recommendations do not apply to women who have been diagnosed with a high-grade dysplasia or cervical cancer, are immunocompromised, or were exposed to diethylstilbestrol in utero, who need more frequent screening. Diethylstilbestrol is a synthetic nonsteroidal estrogen that was used in the U.S. from 1938 to 1971 to prevent miscarriage and other pregnancy complications and has been shown to be associated with increased reproductive cancers.

Additional guidelines exist for managing patients with abnormal cytology results and/or a positive HPV test. In a woman with a normal Pap smear but positive high-risk HPV test, HPV genotyping should be considered. If HPV genotyping is not performed or it is not HPV 16/18, then the woman should return in a year to determine if the HPV infection is persistent. However, if the genotype is HPV 16/18, colposcopy should be considered. ASC-US with a negative HPV testing indicates only repeat testing in a year. A woman with ASC-US and a positive HPV test, LSIL, or HSIL should proceed to colposcopy. If the biopsy obtained during colposcopy is abnormal, further treatment is needed, which includes LEEP, cryotherapy, laser therapy, or cone biopsy.

6. HPV infection requires genital contact. Thus, abstinence or a monogamous relationship with an uninfected partner will prevent HPV infection. Condom use has been shown to reduce transmission, but it does not completely prevent infection. Two vaccines are available for the prevention of HPV infection. Both vaccines protect against HPV 16 and 18 which together cause ~70% of cervical and anal cancers. One of the vaccines also prevents infection with HPV types 6 and 11, which cause ~90% of genital warts. The quadrivalent vaccine requires three injections over 6 months and is approved for females and males aged 9 to 26. Likewise, the bivalent vaccine requires three injections over 6 months, but is approved only for females aged 9 to 25. Neither vaccine has been shown to provide protection against other high-risk HPV types, which is why vaccinated women should continue to get routine cervical cancer screening by Pap smear and HPV molecular detection.

The HPV vaccines are composed of HPV surface components that aggregate to form virus-like particles (VLPs). These VLPs contain no DNA, so there is no risk of developing HPV infection from vaccination. However, the VLPs stimulate antibody production, which protects the host against future HPV infections with the specific HPV types in the vaccine. Longitudinal outcome studies are still being performed on these relatively new vaccines, but the data to date indicate nearly 100% protection from persistent HPV 16/18 infections and the associated precancerous changes up to 8 years post-vaccination. HPV vaccination is recommended for 11- to 12-year-old girls and boys. In addition, females aged 13 to 26 and males aged 13 to 21 should receive the vaccine series if not previously vaccinated. Men who have sex with men should receive the vaccine through 26 years of age.

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SECTION TWO

RESPIRATORY TRACT INFECTIONS

INTRODUCTION TO SECTION II

Respiratory tract infections are a major reason why children and the elderly seek medical care. These infections are more common in cold-weather months in locales with temperate climates. Respiratory tract infections are primarily spread by inhalation of aerosolized respiratory secretions from infected hosts. Some respiratory tract pathogens, such as rhinoviruses and respiratory syncytial virus (RSV), can also be spread by direct contact with mucous membranes, but this mode of transmission is much less common than inhalation. Organisms that are part of the endogenous microbiota of the oropharynx may, under certain conditions (such as aspiration of oropharyngeal secretions), be able to cause clinical disease. Animal exposure may result in some of the less common but more severe bacterial causes of respiratory infection, including inhalation anthrax, pneumonic plague, tularemia pneumonia, and hantavirus pulmonary syndrome. These zoonotic agents are also potential agents of bioterrorism. For the purposes of our discussions, we will divide these types of infections into two groups, upper tract and lower tract infection.

The most common form of upper respiratory tract infection is pharyngitis. Pharyngitis is seen most frequently in children from 2 years of age through adolescence. The most common etiologic agents of pharyngitis are viruses, particularly adenoviruses, coronaviruses, enteroviruses, and rhinoviruses, and group A streptococci. Pharyngitis due to group A streptococci predisposes individuals to the development of the poststreptococcal sequelae rheumatic fever and glomerulonephritis. Because rheumatic fever can be prevented by penicillin treatment of group A streptococcal pharyngitis, aggressive diagnosis and treatment of pharyngitis due to this organism is needed.

Otitis media is a common infectious problem in infants and young children. The most frequently encountered agents of this infection are the bacteria *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. These organisms, along with selected viruses and anaerobic bacteria from the oral cavity, are the most important pathogens in sinusitis.

S. pneumoniae, *H. influenzae*, *M. catarrhalis*, and adenoviruses are the common etiologic agents of conjunctivitis. Less commonly, *Chlamydia trachomatis* can cause conjunctivitis in neonates. External otitis, a common problem in swimmers, is more common in warm-weather months. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most common agents of this relatively benign condition. Malignant external otitis is a serious medical condition seen primarily in diabetics, the elderly, and the immunocompromised. The infection can spread from the ear to the temporal bone. The most common etiology of malignant otitis externa is *P. aeruginosa*. Two other life-threatening infections of the upper respiratory tract are rhinocerebral mucormycosis (zygomycosis) and bacterial epiglottitis. Rhinocerebral mucormycosis is most common in diabetics, especially those with ketoacidosis. In this infection of the sinuses, fungi within the zygomycetes, such as *Mucor* and *Rhizopus* spp., invade blood vessels, resulting in necrosis of bone and thrombosis of the cavernous sinus and internal carotid artery. Treatment of this infection requires aggressive surgical debridement of the infected tissue in addition to antifungal therapy. Epiglottitis

is most commonly caused by *H. influenzae* type b but can also be associated with *S. pneumoniae*, other streptococci, and staphylococci. In this disease, the airway may become compromised because of swelling of the epiglottis, with death due to respiratory arrest. With the widespread use of *H. influenzae* type b vaccine, the incidence of this disease has greatly decreased, but it is still occasionally seen in both children and adults.

Three childhood infections with respiratory manifestations or complications that were common in the early part of the 20th century—diphtheria, whooping cough, and measles—are now rare diseases in the developed world. This is due to the development and use of vaccines in children that are effective against the etiologic agents of these diseases, *Corynebacterium diphtheriae*, *Bordetella pertussis*, and measles virus, respectively.

Viruses play an important role in upper respiratory tract infections. The common syndrome of cough and "runny" nose is usually due to rhinoviruses, but enteroviruses and coronaviruses are frequent causes. More severe upper respiratory infections such as the "croup" are due to RSV, influenza viruses, parainfluenza viruses, and metapneumovirus. These viruses can also cause lower tract infection and are important causes of morbidity and mortality in the very young and very old.

When discussing lower respiratory tract infections, it is important to look at four different groups of patients: patients with community-acquired infections; patients with health care-associated infections; patients with underlying lung disease; and immunocompromised individuals, especially those with AIDS.

Common agents of community-acquired lower respiratory tract infections include *S. pneumoniae*; *Klebsiella pneumoniae*, especially in alcoholics; *Mycoplasma pneumoniae*, especially in school-age students through young adulthood; *Mycobacterium tuberculosis*, especially in individuals born in countries with a high prevalence of tuberculosis; RSV in infants and young children; and influenza A virus. The dimorphic fungi *Histoplasma capsulatum* and *Coccidioides posadasii/immitis* usually cause mild, self-limited diseases in patients residing in specific geographic locales. *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *M. catarrhalis* may cause bronchitis and/or pneumonia in adults following viral pneumonia. Aspiration due to seizure disorders, semiconscious states from excessive consumption of alcohol or other drugs, or impairment of the gag reflex, as may occur following a stroke, may result in aspiration pneumonia or lung abscess caused by the organisms residing in the oral cavity. The anatomic location of the lung process depends on the patient's position at the time of aspiration.

Health care-associated infections due to the organisms listed above certainly occur. Particular emphasis is placed on preventing the spread of *M. tuberculosis* in all patient populations and on preventing health care-associated spread of RSV in pediatric patients. Health care-associated pneumonia due to methicillin-resistant *S. aureus* and multidrug-resistant Gram-negative bacilli, such as *P. aeruginosa* and *Acinetobacter baumannii*, is a concern for intubated patients. Because of their ability to survive within hospital water and air conditioning systems, the potential for outbreaks of pneumonia due to *Legionella* spp. is a constant threat.

Patients with chronic obstructive pulmonary disease brought on by frequent smoking develop bronchitis. *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, and *P. aeruginosa* are common causes of this type of infection. Patients with cystic fibrosis have chronic airway infections that are primarily responsible for their premature death. *S. aureus* and mucoid strains of *P. aeruginosa* are the most important agents of such chronic airway disease. Both of these patient populations have an increased risk of developing allergic bronchopulmonary aspergillosis. Patients with cavitary lung disease, frequently due to prior *M. tuberculosis* infection, are at increased risk for another type of infection, an aspergilloma or fungus ball caused by *Aspergillus* spp. This fungus grows in the form of a ball in the preformed cavity. A distinction between actual tissue invasion with this fungus and noninvasive disease is clinically difficult but is important.

The diagnosis of the etiology of lung infection in immunocompromised patients is one of the most daunting in clinical microbiology and infectious disease. It has been greatly facilitated by the use of the flexible bronchoscope, which provides a relatively noninvasive means to sample the airways and alveoli. Immunocompromised patients are typically at risk for essentially all recognized respiratory tract pathogens. However, a distinction must be made between different types of immunosuppression—defects in cell-mediated immunity, humoral immunity, and neutrophil number or function—because different types of immunosuppression predispose patients to infection with different pathogens. The most common comorbidity for lower respiratory tract infections is cigarette smoking, which causes impaired removal of pathogens due to defective mucociliary clearance. Although smoking results in a significantly increased rate of both bronchitis and pneumonia, smokers are not normally described as immunosuppressed.

In AIDS patients, *Pneumocystis jirovecii*, *Cryptococcus neoformans*, *S. pneumoniae*, and multidrug-resistant *M. tuberculosis* are all seen more frequently than in other patient populations. Solid-organ transplant recipients have a greatly increased risk for pneumonia with cytomegalovirus, herpes simplex virus, *Legionella* spp., *P. jirovecii*, and *Nocardia* spp. Prophylactic antibiotics are frequently taken by these patients to prevent pulmonary infections with *P. jirovecii*. Prophylactic therapies are not as widely used for other agents for a variety of reasons, including expense, questionable efficacy of the prophylactic measures, or the rarity with which the organism is encountered. Profoundly neutropenic patients, especially those in whom the duration of neutropenia is prolonged, not only have a risk of infection with routine bacteria but have a very high risk of invasive aspergillosis and other invasive fungal infections.

TABLE II SELECTED RESPIRATORY TRACT PATHOGENS

ORGANISM	GENERAL CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Bacteria			
Acinetobacter baumannii	Glucose-nonfermenting, Gram-negative bacillus	Hospitalized adults	Ventilator-associated pneumonia
Actinomyces spp.	Branching, Gram-positive bacilli, usually anaerobic	Adults with aspiration	Lung abscess
Bacillus anthracis	Spore-forming, Gram-positive bacillus	Victims of bioterrorism due to exposure to spores; woolsorters in endemic areas	Inhalation anthrax with widened mediastinum, high-grade bacteremia
Bordetella pertussis	Fastidious, Gram-negative bacillus	Children, adults	Whooping cough, chronic cough
Chlamydia trachomatis	Obligate intracellular bacterium; does not Gram stain	Neonatal	Conjunctivitis, pneumonia
Chlamydiophila pneumoniae	Obligate intracellular bacterium; does not Gram stain	Children, adults	Pneumonia, bronchitis
Chlamydiophila psittaci	Obligate intracellular bacterium; does not Gram stain	Children and adults with exposure to birds	Pneumonia, ornithosis (psittacosis)
Corynebacterium diphtheriae	Catalase-positive, Gram-positive, club-shaped bacillus	Unvaccinated adults and children, improperly vaccinated adults	Diphtheria
Enterobacter spp., *Escherichia coli*	Lactose-fermenting, Gram-negative bacilli	Hospitalized adults	Health care-associated pneumonia
Fusobacterium necrophorum	Anaerobic, Gram-negative bacillus	Adolescents, adults	Pharyngitis, Lemierre's syndrome
Group A streptococci (*Streptococcus pyogenes*)	Catalase-negative, Gram-positive cocci in chains	Children >2 years, adults	Pharyngitis, pneumonia with empyema
Group B streptococci (*Streptococcus agalactiae*)	Catalase-negative, Gram-positive cocci in chains	Neonates	Pneumonia
Haemophilus influenzae	Pleomorphic, Gram-negative bacillus	Children; adults, especially with COPD[a]	Otitis media, conjunctivitis, epiglottitis, bronchitis, pneumonia
Klebsiella pneumoniae	Lactose-fermenting, Gram-negative bacillus	Adults	Community-acquired and health care-associated pneumonia

(continued next page)

TABLE II SELECTED RESPIRATORY TRACT PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Legionella pneumophila	Poorly staining, fastidious, Gram-negative bacillus	Adults, especially immunocompromised	Pneumonia
Moraxella catarrhalis	Oxidase-positive, Gram-negative diplococcus	Children; adults with COPD	Otitis media, conjunctivitis, bronchitis
Mycobacterium tuberculosis	Acid-fast bacillus	Children and adults, especially HIV-infected	Tuberculosis
Mycoplasma pneumoniae	Fastidious; does not Gram stain	Children, adolescents, adults	Walking pneumonia
Neisseria gonorrhoeae	Oxidase-positive, Gram-negative diplococcus	Individuals with oral-genital contact, neonates	Pharyngitis, conjunctivitis
Neisseria meningitidis	Oxidase-positive, Gram-negative diplococcus	Adults	Pneumonia
Nocardia spp.	Partially acid-fast, aerobic, branching, Gram-positive bacilli	Adults, especially with defects in cell-mediated immunity	Pneumonia with brain abscess
Nontuberculous mycobacteria (many species)	Acid-fast bacilli	Adults with chronic lung disease, CF[ψ] patients	Granulomatous lung disease
Prevotella spp., *Porphyromonas* spp.	Anaerobic, Gram-negative bacilli	Adults with aspiration	Lung abscess
Pseudomonas aeruginosa	Glucose-nonfermenting, Gram-negative bacillus	Adults and children, diabetic adults, hospitalized patients, CF patients (mucoid variant)	External otitis (swimmer's ear), malignant external otitis, ventilator-associated pneumonia, chronic bronchitis with mucoid strains
Staphylococcus aureus	Catalase-positive, Gram-positive cocci in clusters	Hospitalized patients	Pneumonia, pneumonia superinfections
Stenotrophomonas maltophilia	Glucose-nonfermenting, Gram-negative bacillus	Hospitalized patients	Ventilator-associated pneumonia
Streptococcus pneumoniae	Catalase-negative, Gram-positive diplococcus	Children, adults	Otitis media, sinusitis, conjunctivitis, pneumonia

Fungi			
Aspergillus spp.	Acute-angle-branching, septate hyphae in tissue; molds	Children and adults with chronic lung disease, adults with cavitary lung lesions, neutropenic individuals	Allergic bronchopulmonary aspergillosis, aspergilloma (fungus ball), invasive pneumonia
Blastomyces dermatitidis	Broad-based budding yeast; dimorphic	Adults	Pneumonia
Coccidioides posadasii/immitis	Spherules in tissue; mold with arthroconidia at 30°C	Children and adults, especially in desert southwest of United States and northern Mexico	Flu-like illness with pneumonia; can disseminate
Cryptococcus neoformans	Encapsulated, round yeast	Adults with defects in cell-mediated immunity, especially with AIDS	Pneumonia, often asymptomatic, preceding meningitis
Histoplasma capsulatum	Very small, intracellular yeast; dimorphic	Adults, primarily with AIDS, especially in Missouri and Ohio River Valleys and Caribbean	Pneumonia, mediastinal fibrosis
Pneumocystis jirovecii	Clusters of 4- to 6-μm cysts in tissue and secretions	Immunocompromised individuals, especially with AIDS	Pneumonia
Rhizopus spp., *Mucor* spp.	Ribbon-like, nonseptate hyphae in tissue; rapidly growing molds	Diabetics, neutropenic individuals	Rhinocerebral zygomycosis, invasive pneumonia
Parasites			
Ascaris lumbricoides	Larvae	Children, adults	Usually asymptomatic, incidental finding
Echinococcus granulosus	Tapeworm (cestode)	Exposure to dogs in areas with sheep	Cyst in lung growing over the course of years; rupture from liver may lead to pleural space
Entamoeba histolytica	Ameba	Children and adults with amebic liver abscess	Empyema, hepatobronchial fistula, lung abscess
Hookworm (*Necator americanus*, *Ancylostoma duodenale*)	Larvae	Children, adults	Usually asymptomatic, incidental finding
Paragonimus westermani	Fluke (trematode)	Children and adults in endemic areas	Hemoptysis, chronic bronchitis, bronchiectasis
Schistosoma spp.	Fluke (trematode); granulomas form around eggs	Children and adults in endemic areas	Pulmonary hypertension due to trapping of eggs in pulmonary capillaries
Strongyloides stercoralis	Rhabditiform larvae	Immunocompromised individuals	Wheezing, cough, pneumonia

(continued next page)

TABLE II SELECTED RESPIRATORY TRACT PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Viruses			
Adenovirus	Enveloped, dsDNA[c]	Children, adults	Pharyngitis, bronchiolitis, pneumonia, conjunctivitis ("pink eye")
Coronaviruses (229E, HKU1, NL63, OC43)	Enveloped, ssRNA[d]	Children, adults	Common cold; pneumonia in immunocompromised individuals
Coronaviruses, novel (SARS-CoV,[e] MERS-CoV[f])	Enveloped, ssRNA	Primarily adults	Acute respiratory distress syndrome
Cytomegalovirus	Enveloped, dsDNA	Immunocompromised individuals	Pneumonia
Enteroviruses	Nonenveloped, ssRNA	Children	Common cold, hand-foot-and-mouth disease, herpangina, pharyngitis, bronchiolitis, pneumonia
Hantaviruses	Enveloped, ssRNA	Children, adults	Acute respiratory distress syndrome, pneumonia
Herpes simplex virus	Enveloped, dsDNA	Immunocompromised individuals	Pneumonia
Influenza viruses	Enveloped, ssRNA	Children and adults, particularly elderly	Influenza, pneumonia
Metapneumovirus	Enveloped, ssRNA	Infants, young children, adults, immunocompromised individuals	Common cold, croup, bronchiolitis, pneumonia
Parainfluenza viruses (types 1, 2, 3, and 4)	Enveloped, ssRNA	Infants, young children	Croup, bronchiolitis, pneumonia, laryngitis
Respiratory syncytial virus	Enveloped, ssRNA	Infants, young children, elderly	Cough, wheezing, bronchiolitis, pneumonia
Rhinoviruses	Nonenveloped, ssRNA	Children, adults	Common cold; pneumonia in immunocompromised individuals
Varicella-zoster virus	Enveloped, dsDNA	Immunocompromised individuals, pregnant women	Pneumonia

[a]COPD, chronic obstructive pulmonary disease.
[b]CF, cystic fibrosis.
[c]dsDNA, double-stranded DNA.
[d]ssRNA, single-stranded RNA.
[e]SARS-CoV, severe acute respiratory syndrome coronavirus.
[f]MERS-CoV, Middle East respiratory syndrome coronavirus.



The patient was a 5-year-old male who awoke on the day prior to evaluation with a sore throat and fever. His mother had him stay home from kindergarten and treated him symptomatically with Tylenol. He slept well but the next day awoke still complaining of sore throat and fever, as well as headache and abdominal pain. He was an only child and neither parent was ill.

On physical examination, he was noted to have a fever of 38.4°C. His physical examination was significant for a 2+ (on a scale of 1 to 4+) red anterior pharynx, tonsillar region, and soft palate. His anterior cervical lymph nodes at the angle of the mandible were slightly enlarged and tender. No skin lesions or rashes were seen. He did not have a cough, runny nose, or conjunctivitis. A rapid antigen test for group A streptococci (GAS) and a positive and negative control of the assay are seen in Fig. 7.1. When the results of the rapid antigen test were known, the patient was given a 10-day course of oral amoxicillin.

1. Based on his clinical presentation, what organism was most likely causing this patient's infection? What does the rapid strep antigen test tell you?
2. Was antimicrobial therapy necessary in this patient? Explain.
3. This patient was at risk for two noninfectious sequelae. What are they? Briefly describe our current understanding of the pathogenesis of these two disease processes.
4. What antimicrobial resistance problems have been observed with this organism?
5. Sore throat associated with a maculopapular rash is frequently seen with this organism. What is this usually benign condition called? What virulence factor is believed to be responsible for production of this rash?
6. What is the current status of vaccine development for this organism?

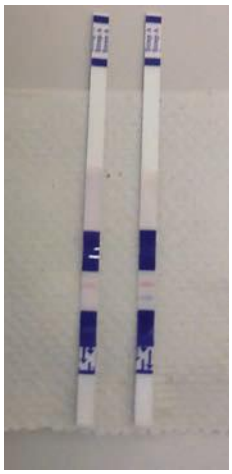


Figure 7.1



CASE DISCUSSION

1. Based on a GAS clinical prediction scoring system developed at the University of Virginia and validated in both adults and children, this patient scored positive for all the criteria: temperature of >38°C, no cough, tender anterior cervical lymphadenopathy, tonsillar swelling and exudates, and age 3 to 14 years. Patients with this score are estimated to have a risk of ~50% of having GAS pharyngitis. Although not part of the prediction rule, abdominal pain, nausea, and vomiting are frequently seen in patients with GAS pharyngitis, though only abdominal pain was seen in this patient. What if the patient had presented with low-grade fever (<38°C), cough, sore throat without exudates, conjunctivitis, and coryza? Such a patient would have a score of 1 (positive only by virtue of age). Patients with a score of 1 have only a 5 to 10% risk of GAS pharyngitis. Viruses including rhinovirus, coronavirus, adenovirus, and influenza virus can all cause a syndrome of sore throat, cough, coryza, and conjunctivitis. This constellation of symptoms by and large is self-limited. Viral pharyngitis should be treated only symptomatically with analgesics and warm saltwater gargles.

A decision was made to confirm the clinical impression by determining if GAS was present. There are two ways to detect GAS: by direct detection of group A polysaccharide antigen in throat swabs, as was done in this case (Fig. 7.1), and by culture using a blood-containing agar plate. Direct antigen detection is accomplished by extracting the group A polysaccharide antigen from the throat swab and then performing an immunoassay on the extract. The test is very rapid, taking 10 to 15 minutes, and is highly specific (>95%), but when compared with culture it has a sensitivity of 80 to 90%, meaning that GAS will not be detected by this test in 10 to 20% of patients with GAS in their throats. The advantage of the "rapid strep test," as it is called, is that a swab can be obtained in the office or clinic and a result can be obtained while the patient waits, i.e., a "real-time" microbiology test. For patients with a high pretest probability of disease, such as this patient, and a positive rapid GAS antigen test, antibiotics can be prescribed on the spot if that is the clinical decision that is reached. See answer 2 for further discussion of this issue.

Most guidelines no longer recommend performing culture in patients with negative rapid GAS antigen tests. For further explanation of why, see answer 2.

2. There are several benefits of antibiotics in the treatment of GAS pharyngitis. Of greatest significance is that treatment prevents nonsuppurative poststreptococcal sequelae (see answer to question 3 for further explanation). Further, if given early in the disease course (first 24 to 48 hours), they may also shorten the length of time the patient is symptomatic. Additionally, antibiotic therapy will prevent suppurative complications of GAS pharyngitis, such as peritonsillar and retropharyngeal abscesses, and decrease the infectivity of the infected individual. In school-age children, this is important so that they are less likely to infect their classmates and siblings, both at-risk populations. Because both suppurative and nonsuppurative poststreptococcal sequelae are now rare in the industrialized

world, the importance of antimicrobial therapy in treatment of GAS is limited to the benefits of shortening disease course and decreasing transmissibility. This must be balanced with the risks of antimicrobial therapy. These include allergic reactions, especially since this infection is treated with penicillin; changes in the microbiota that may put the patient at risk for other infections; and increasing antimicrobial resistance among respiratory pathogens such as *Streptococcus pneumoniae*.

The problem is even more complex with patients who have a negative rapid GAS antigen test. Often, physician practice, especially in pediatrics, is to "back up" negative rapid GAS antigen tests with culture. There are at least four possible outcomes of bacterial culture. (i) It can be negative for all potential bacterial agents of pharyngitis. (ii) It can be positive for GAS with a clinical score supporting the GAS diagnosis. The physician will need to decide whether to treat or not. (iii) It can be positive for GAS but represent asymptomatic carriage. During the winter and early spring months, when GAS pharyngitis is most common, carriage rates of between 10 and 20% may be present in children. Antimicrobial treatment in this group is controversial but may be done if recurrent GAS infections are being seen in other family members. (iv) It can be positive for other bacterial agents associated with pharyngitis, including groups C and G streptococci or *Arcanobacterium haemolyticum*. There is no evidence that these agents cause nonsuppurative poststreptococcal sequelae. Nor is there good evidence that antimicrobials will reduce the length of their disease course. Given the limited benefit, there is no evidence that culture should be used to support treatment of pharyngitis.

3. The patient was at risk for two nonsuppurative poststreptococcal sequelae, rheumatic fever and glomerulonephritis. Because he received antimicrobial therapy, his risk of rheumatic fever was essentially zero. The likelihood of an untreated, infected person developing either one of these complications is low in the industrialized world but is dependent on the serotype of the organism with which he is infected. Typing of GAS, called *emm* typing, is based on sequence analysis of the gene encoding the M protein, a surface protein that is anchored in the organism's cell wall. There are >150 different *emm* types of this antiphagocytic protein. Certain M types, such as M1 and M3, are associated with rheumatic fever and are said to be "rheumatogenic." Other strains, such as M12 and M49, are considered "nephritogenic" and are associated with glomerulonephritis. Glomerulonephritis is seen following both pharyngitis and skin infections (pyoderma or impetigo), whereas rheumatic fever is believed to occur only following pharyngitis.

These noninfectious poststreptococcal sequelae occur after an acute GAS infection. Rheumatic fever occurs 1 to 5 weeks after infection, while glomerulonephritis following pharyngitis occurs at 1 to 2 weeks and 3 to 6 weeks following pyoderma. Both sequelae are believed to be immune-mediated diseases whereby antibodies made in response to GAS react with tissues in the target organ.

In rheumatic fever, antibodies directed against the M protein are believed to cross-react with a variety of tissue components in the heart, including myosin, laminin,

and tropomyosin. This can result in damage to heart valves and muscle and produce the carditis and heart murmurs that are manifestations of this syndrome.

In glomerulonephritis, streptococcal antibodies that cross-react with the glomerular basement membrane are believed to be important in the disease process as well as the deposition in the glomeruli of circulating immune complexes containing streptococcal antigens. Clinically, individuals present with edema, hypertension, and hematuria.

4. Despite the use of penicillin to treat GAS infections for more than 50 years, this organism continues to be uniformly sensitive to this antimicrobial. In penicillin-allergic patients, erythromycin and the newer macrolide antimicrobials clarithromycin and azithromycin are recommended therapeutic agents for GAS pharyngitis. A study in Finland showed that GAS resistance to erythromycin was associated with increasing use of this antimicrobial. In 1993, almost 20% of GAS isolates were resistant to erythromycin. Following a national education effort, use of erythromycin and related antimicrobials declined. By 1996, the percentage of erythromycin-resistant strains of GAS declined to 8.6%, a level still much higher than that seen in the United States. The important lesson here is that once resistance is present in an organism, reducing specific antimicrobial pressure will only result in a reduction in the number of resistant strains, not an elimination of them. A 2011-2012 survey at a U.S. university teaching hospital of GAS isolates from patients with pharyngitis indicated that resistance is still modest, with 5% of isolates resistant to both erythromycin and clindamycin.

5. Streptococcal pyogenic exotoxins (Spe) A through C were once referred to as erythrogenic or scarlet fever toxins. Scarlet fever is considered to be a benign complication of pharyngitis caused by a pyrogenic exotoxin-producing strain of GAS. The skin rash seen in scarlet fever is believed to be superantigen mediated.

6. Given the frequency and the potential seriousness of GAS infections, they would seem a logical candidate for the development of a vaccine. Vaccine development strategies for GAS are targeting the M protein and a variety of other virulence factors, including the C5 peptidase (important in the organism evading phagocytes), cysteine protease, and hyaluronic acid capsule. The molecule that has been the most attractive target for the development of a GAS vaccine is the M protein. This protein is known to play an important role in evasion of the immune system; it is located on the cell surface, and with modern biochemical techniques it is fairly easy to purify. However, epitopes of M protein have been shown to share antigenic properties with several human tissue components, including myosin and sarcolemmal membrane proteins. Therefore, vaccines against M proteins have the potential to induce antibodies that could bind and damage a variety of tissues.

The challenge of making a vaccine against the M protein component of GAS is to identify epitopes that will induce the production of protective antibodies against as

many different M types as possible while at the same time ensuring that the antibodies raised against these epitopes will not react with human tissues. It is also important to have a vaccine strategy that will elicit mucosal immunity, as that is likely to be important in protecting against this respiratory tract pathogen. The most advanced GAS candidate vaccine is 26-valent, targeting small N-terminal peptides on the M protein. Based on an epidemiologic survey of invasive GAS disease, it should cover ~80% of those isolates. In phase 1 and 2 trials, the vaccine was found to be safe and to have good immunogenicity. A phase 3 trial is needed to judge efficacy. However, with the ever expanding repertoire of *emm* types in GAS, the individual M protein approach is likely flawed. Identification of antigens that are shared across *emm* types and can induce protective immunity without producing molecular mimicry is the holy grail of GAS vaccinology.

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The patient was a 64-year-old retired postal worker with a medical history of extensive facial reconstruction for squamous cell carcinoma of the head and neck. He had a 30-year history of smoking. The patient presented with progressive shortness of breath, a persistent, productive cough, purulent sputum, and fever to 39.0°C 2 days prior to admission.

On physical examination he had a temperature of 37.3°C, respiratory rate of 18 per minute, pulse rate of 103 beats/min, blood pressure of 154/107 mm Hg, and pO_2 of 92 mm Hg. Chest auscultation revealed coarse breath sounds at the left lower base with bibasilar fine crackles. He was found to have a left lower lobe infiltrate on chest radiograph. His admission white blood cell count was 10,600/μl with 70% neutrophils, and his hemoglobin was 9.4 g/dl. Sputum Gram stain at admission revealed >25 polymorphonuclear cells and >25 squamous epithelial cells per low-power field. Because of the high numbers of squamous epithelial cells, the specimen was not processed further. Two blood cultures obtained at admission were positive for the organism seen in Fig. 8.1. The Gram stain from the blood culture bottle is shown in Fig. 8.2.

The patient was admitted to the hospital and treated with ceftriaxone intravenously. Upon defervescence, he was discharged on a regimen of oral azithromycin based on the organism's identification and antimicrobial susceptibility results. Of note: this was the patient's third episode of this illness in the past month. Isolates from all three episodes belonged to the same serotype, type 23.

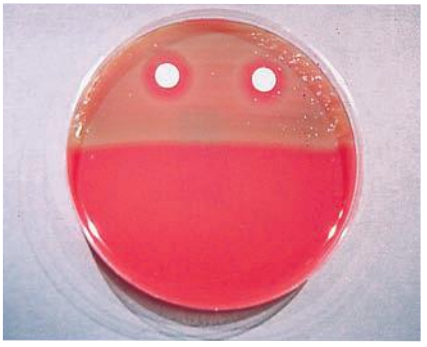


Figure 8.1

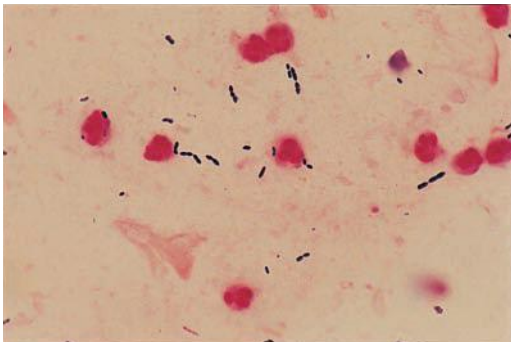


Figure 8.2

1. What disease process was ongoing in this patient? What clinical prediction rules could be applied to this patient in determining whether he should be hospitalized? Why do you think the decision was made to hospitalize him?
2. What organism was causing this individual's infection?

3. What other patient populations are at risk for infection with this organism?
4. Two different virulence factors produced by the organism infecting this patient are important in disease pathogenesis. What are they, and what role do they have in the pathogenicity of this organism?
5. What strategies are available to prevent infections with this organism? Why are preventive strategies becoming of greater importance with this organism?
6. How do you explain the patient's having repeated episodes of infection with the same serotype of this organism? There are at least two and possibly more explanations.



CASE DISCUSSION

1. Based on his physical findings of productive cough with purulent sputum, shortness of breath, fever, and bibasilar fine crackles on chest auscultation in the left lower lung, coupled with left lower lobe infiltrates on radiographic imaging, this patient had a lower respiratory tract infection most consistent with bacterial pneumonia. Because this patient was at home at the time of disease onset, he would be considered to have community-acquired pneumonia.

Two clinical prediction models are widely used to determine if patients with community-acquired pneumonia should be admitted to the hospital. Having metrics for this purpose is valuable because patients do not wish to be hospitalized. There are several reasons for this: they get better faster at home; they are not exposed to nosocomial risks, including infections; and it is more cost-efficient. These two models allow for a rational approach to this process. The pneumonia prediction rule is a scoring system based on demographics; coexisting conditions; and physical, laboratory, and radiographic findings. Because of its complexity, it is more of a research tool with limited practical application. The second system is CRB-65, a modification of CURB-65. CRB-65 is simple to use, as it has four criteria that can be easily determined: C, presence or absence of confusion; R, respiratory rate of >30 per minute; B, low systolic (≤90 mm Hg) or diastolic (≤60 mm Hg) blood pressure; and age >65 years. Patients are ranked on a scale of 0 to 4; those with a score of 3 or 4 are judged to have severe disease, with frequent admission to the intensive care unit and 30-day mortality of >40%. This patient had a CRB-65 score of 0. Patients with that score are usually not admitted to the hospital, as their 30-day mortality is 0%. However, CRB-65 is a simple system that does not take into account certain complexities in this patient. This patient was immunocompromised due to his history of head and neck carcinoma. He also had a long-term smoking history, which put him at increased risk for respiratory infections. Finally, he had previous episodes of respiratory infection, which were concerning to his physician; thus the decision to admit him.

2. In patients who are suspected of having bacterial pneumonia, attempts are made to determine the etiologic agent so that management can be directed toward a specific agent. In lobar pneumonia, as was seen on physical and radiographic examination of this patient, the most common etiologic agent is *Streptococcus pneumoniae*. Three approaches are widely used to determine if a patient is infected with this organism: sputum examination, blood culture, and pneumococcal urinary antigen detection. The organism isolated from this patient's positive blood culture was a catalase-negative, Gram-positive diplococcus (Fig. 8.2). It was alpha-hemolytic on sheep blood agar and was susceptible to the copper-containing compound optochin (ethylhydrocupreine hydrochloride). These phenotypic characteristics are consistent with *S. pneumoniae*. Approximately one-third of patients with pneumococcal pneumonia will have a positive blood culture, so the finding in this patient was consistent with this diagnosis. Pneumococcal pneumonia can often be diagnosed by its characteristic Gram stain, in which stained sputum demonstrates numerous polymorpho-

nuclear cells and the presence of many lancet-shaped, Gram-positive diplococci. However, it requires a high-quality specimen, which is defined as one where there are ≥25 neutrophils and <10 squamous epithelial cells per low-power field. In patients with high-quality specimens who have not received antimicrobials prior to specimen collection and have characteristic Gram-positive diplococci, Gram stain has a sensitivity of 80%. However, in the clinical setting, it is not uncommon to receive poor-quality sputum specimens that are unable to be analyzed, as was the case for this patient. Poor-quality specimens typically have high numbers of squamous epithelial cells because of contamination of the specimen with oropharyngeal secretions. Oropharyngeal secretions contain high numbers of squamous epithelial cells. Because the pneumococcus can be part of the resident microbiota of the oropharynx, the finding of this organism in a poor-quality sputum specimen cannot be reliably associated with the diagnosis of pneumococcal pneumonia. Such a finding may be a false positive.

Another test for invasive pneumococcal disease is a urinary antigen test. This test is most likely to be positive in patients with bacteremic pneumococcal pneumonia, the exact clinical situation seen in this patient. This test is most useful in a setting where antimicrobials have already been given, making it much less likely that organisms will be detected either by blood or sputum culture. Urinary antigen tests should not be used in children, especially in the winter months, since false positives due to high colonization rates may occur.

3. Many different patient populations are at increased risk for invasive pneumococcal disease—pneumonia, bacteremia, and meningitis. Patient populations in whom rates of pneumococcal invasive disease are increased include AIDS patients; patients who are anatomically or functionally asplenic (including patients with sickle-cell disease); patients with cardiovascular, liver, or kidney diseases; individuals with diabetes or malignancies; and individuals who are receiving immunosuppressive agents because of connective tissue disease or organ transplantation. Prevention strategies that target these populations are discussed in the answer to question 5.

4. The polysaccharide capsule is the major virulence factor of *S. pneumoniae*. More than 90 antigenically different capsular polysaccharides have been recognized, with 7 types—4, 6B, 9V, 14, 18C, 19F, and 23F—being responsible for 80 to 90% of cases of invasive pneumococcal disease. Animal experiments done in the first part of the 20th century established the importance of capsule in the organism's ability to cause disease. It is well recognized that the capsular polysaccharide allows the pneumococcus to evade phagocytosis.

The second virulence factor is the cholesterol-dependent cytolysin, pneumolysin. Pneumolysin acts on both alveolar epithelial cells and pulmonary endothelial cells. Pneumolysin may contribute to fluid accumulation and hemorrhage by directly damaging these two cell types. Animal studies of pneumococcal pneumonia indicate that pneumolysin plays a primary role in the inflammation, fluid accumulation, and hemorrhage that

occurs in the alveoli during lobar pneumococcal pneumonia. The inflammatory response is due at least in part to pneumolysin upregulating the synthesis of both tumor necrosis factor-α and interleukin-1 in the airways.

5. Currently, there are two vaccines licensed for prevention of pneumococcal disease, a 23-valent polysaccharide vaccine and a 13-valent conjugate vaccine. The 23-valent vaccine is used in adults, while the 13-valent conjugated vaccine was developed for use in children <2 years of age. Young children are not able to reliably mount a T-cell-independent immune response, the type of immune response necessary to produce antibodies against polysaccharide antigens. However, they are able to mount a T-cell-dependent immune response.

The 13-valent pneumococcal vaccine is also recommended for adults, especially immunocompromised individuals. Currently, many clinicians are still using the 23-valent vaccine in adults >60 years. In adults, the 23-valent polysaccharide vaccine has been used successfully for many years. The efficacy of the 23-valent vaccine in adults is not as high (efficacy ranges from 50 to 90% in different populations) as that of the 13-valent conjugate vaccine in children.

A conjugate vaccine is one in which a polysaccharide antigen is coupled to a carrier protein. The coupling of a polysaccharide antigen to a protein creates a "new" antigen. This new antigen stimulates a T-cell-dependent immune response (see case 45 for further details). Therefore, the conjugated pneumococcal vaccine results in a protective immune response to capsular types present in the vaccine and perhaps to other related serotypes in children <2 years old. It has been shown to be highly efficacious (>95%) in preventing invasive pneumococcal disease in this age group. It has been less effective in preventing a common pneumococcal infection in this age group, otitis media. The conjugated pneumococcal vaccine is now recommended for use in all children <2 years of age.

The widespread use of the 13-valent conjugated pneumococcal vaccine in children has resulted in declines in the two major populations with invasive pneumococcal disease: those <5 and those >65 years of age. Herd immunity clearly is playing a role in this decline and is discussed in greater detail in case 45.

An additional vaccine strategy that might be helpful in protecting this patient from pneumococcal disease would be to vaccinate him against influenza virus. Influenza infection has been recognized as being an important predisposing factor for the development of pneumococcal pneumonia.

Alternatively, prophylactic antimicrobials have been used in selected populations, such as sickle-cell patients with a history of recurrent invasive pneumococcal infections. Given the problem of emerging drug resistance in the pneumococci (see below), this is probably a preventive strategy that is becoming less efficacious.

The intense interest in pneumococcal vaccine is being driven to a significant degree by an alarming increase in the numbers of multidrug-resistant pneumococcal isolates being recovered from patients with invasive disease. Prior to 1990, pneumococcal isolates that were resistant to penicillin were quite unusual in the United States, as was the recovery of

isolates that were resistant to other classes of antimicrobials. Beginning in the 1990s, pneumococcal isolates resistant to multiple antibiotics, including penicillin, macrolides, and trimethoprim-sulfamethoxazole, became increasingly common. Rates of resistance accelerated in the late 1990s. Some of this increase was due to the dissemination of selected clones of multidrug-resistant pneumococci, including the international dissemination of a multidrug-resistant type 23 strain. However, a common theme in the increasing drug resistance in this organism is the inappropriate use of antimicrobial agents. Several studies have been able to link increased use of specific antimicrobials, such as the macrolides and fluoroquinolones, with increased resistance. Because multidrug-resistant organisms are being seen with increasing frequency in invasive pneumococcal disease, it is clear that these multidrug-resistant strains have maintained their virulence, unlike some drug-resistant strains of other organisms that appear to be less virulent than nonresistant ones. Prevention of invasive infection with multidrug-resistant organisms by the two vaccines may be possible because >90% of multidrug-resistant pneumococcal serotypes are either present in the vaccines or likely to cross-react with antibodies to the vaccine serotypes. It should be noted that in the pre-antibiotic era, mortality from invasive pneumococcal disease was 80%. It now stands at between 10 and 20%. With increasing resistance limiting the efficacy of antimicrobials, will mortality due to invasive pneumococcal disease begin to increase?

6. There are four potential explanations for why patients can have repeated episodes of infection with the same serotype. The first three fall under the category of inadequate treatment; the fourth involves reinfection.

In terms of inadequate treatment, the patient may have been treated with an antimicrobial to which the infecting organism was not susceptible. Given the increasing trend of multidrug resistance in pneumococci, this is a reasonable explanation. Susceptibility testing of this organism revealed it to be pan-sensitive, meaning it was susceptible to all antimicrobials against which it was tested, including the antimicrobial with which he was treated. The second explanation is that the patient did not receive antimicrobials for a sufficient period of time to eliminate the organism. If hospitalized, it is likely that the patient would receive appropriate antimicrobial therapy during his stay. However, in the managed care era, hospital stays are becoming shorter and shorter. Our patient received 4 days of intravenous antimicrobials in the hospital and then oral antibiotics prescribed for use after discharge. If he failed to take his oral antibiotics, i.e., was noncompliant, his infection may have been inadequately treated, contributing to a relapse. A third possibility is that he had an undrained focus of infection that the antimicrobials did not adequately penetrate. In pneumococcal pneumonia, highly viscous pleural exudates may form that antimicrobials cannot penetrate. Removal of these exudates by drainage may be required for treatment of severe infections. Occasionally, drainage of exudates is not possible percutaneously. In these cases, a surgical procedure may be necessary to remove this focus of infection.

The fourth possible explanation is reinfection with the same serotype. Serotype 23 is one of the most common serotypes of *S. pneumoniae*, being responsible for 7% of invasive

pneumococcal infections in a recent U.S. survey. It is possible that he was carrying the organism in his nasopharynx and became reinfected in that manner, since it has been shown that antimicrobial therapy does not reliably eliminate nasopharyngeal colonization of pneumococci. What is more difficult to understand is why his original infection did not result in his mounting a protective immune response to this organism. A possible explanation is that his immunosuppressed state due to the carcinoma blunted his immune response. It is uncertain if vaccination would be an effective preventive strategy in this patient given the observation that he had three infections in a month with *S. pneumoniae* serotype 23, which is present in the vaccine.

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The patient was an 18-day-old female who at initial presentation was brought to the emergency department by her mother after a 3-day bout of coughing. Her mother also reported that her daughter had been "spitting up" more than usual and had episodes of tachypnea. During the initial exam, a rapid respiratory syncytial virus test was obtained with negative results. A review of systems was notable only for a nonproductive cough. Her pulse was 168 beats/min, her respiratory rate was 32 inspirations per minute, and oxygen saturation was 92 to 95% on room air. Her complete blood count was significant for a white blood cell count of 15,300 cells/μl with an absolute lymphocyte count of 10,900 cells/μl. The mother had a chronic cough of 4 weeks' duration but had been afebrile. Six weeks before this patient's admission, her 10-year-old brother also had a prolonged coughing illness that responded to breathing treatments and inhaled steroids.

After initial examination, the child was admitted to the hospital. Her initial hospital course was uneventful, and she was discharged after 2 days. However, she was readmitted the following day with worsening respiratory symptoms. Over the next several days she had increasing difficulty breathing, tachypnea up to 100 breaths per minute, and oxygen saturations in the low 80s during coughing episodes. She was admitted to the pediatric intensive care unit for respiratory support. She had an extremely complicated and prolonged intensive care unit course that included pulmonary hypertension, acute respiratory distress syndrome, and health care-associated pneumonia. After a 10-week hospital stay, she was eventually discharged to return home, where her recovery was uneventful.

1. Nucleic acid amplification testing (NAAT) was performed on a nasopharyngeal swab. The amplified DNA was screened for a particular agent with positive results, and the patient was begun on azithromycin. What was the etiologic agent infecting this patient? What findings in this case support this conclusion?
2. Why is a nasopharyngeal specimen superior to any other clinical specimen for diagnosing this infection? Why has NAAT replaced culture for the diagnosis of this pathogen?
3. Describe the clinical course of this disease. Why didn't the patient respond to the antimicrobial she was given?
4. What about the pathogenesis of this disease puts this patient at increased risk for health care-associated pneumonia?
5. A vaccine exists to prevent infections such as the one in this patient. Explain why and how this patient was infected. What does this case tell you about the vaccine? Vaccine strategies for preventing infections with this organism have recently changed. What changes in the vaccine are

making better prevention possible? In particular, what groups of individuals should receive this new vaccine?

6. What type of isolation precautions should have been used while this patient was in the hospital? What therapy (if any) should have been provided to health care workers in close contact with this patient prior to institution of appropriate precautions?

CASE DISCUSSION



1. The patient was infected with *Bordetella pertussis*, the causative agent of whooping cough. With classic whooping cough, children have paroxysmal coughing, which is a series of coughs during a single expiration. Paroxysms are often accompanied by a "whoop" sound in children due to rapid inspiration through a narrow trachea. (An audio file of a child with pertussis can be found at www.immunizationed.org.) Because of repetitive coughing and resulting disruption of breathing, children will have abnormal oxygen exchange and will often turn red and sometimes blue. The repetitive coughing may also result in vomiting or choking on respiratory secretions. Although the classic "whooping" sound was not described for this child, she did have bouts of coughing leading to increased respiration, decreased oxygenation, and posttussive vomiting. In infants <6 months old, apnea is more common than whooping inspirations. Further, the patient had a lymphocytosis, which is commonly seen in pertussis. Although the etiologic agent is a bacterium, the disease is toxin mediated, explaining the rise in lymphocyte count. Historically, the pertussis toxin (a key virulence factor of *B. pertussis*) has also been described as the "lymphocytosis-promoting factor." Clinically, lymphocytosis, often as high as 70 to 80%, is routinely seen in patients with pertussis and is a distinguishing characteristic of this infection.

2. *B. pertussis* specifically binds to ciliated epithelial cells. This binding is mediated primarily by filamentous hemagglutinin, an important virulence factor of this organism. Since the nasopharynx is lined with ciliated epithelial cells, it is the most sensitive site for the detection of *B. pertussis*.

Culture has long been the gold standard for the laboratory diagnosis of pertussis owing to its superior specificity (~100%). However, there are many disadvantages to *B. pertussis* culture. First, the organism is very labile outside of the host. Second, it must be cultivated on specialized media such as Bordet-Gengou or Regan-Lowe agar. These attributes make the bacterium difficult to isolate. Third, it generally takes 7 to 10 days to isolate and identify *B. pertussis* from culture. In Fig. 9.1, we see an isolate of *B. pertussis* that grew after 7 days of incubation on a charcoal-containing medium, Regan-Lowe agar. In outbreak settings where *B. pertussis* can be rapidly spread from person to person, culture is too slow. Lastly, the clinical course of pertussis is complex (see answer 3), and the organism is generally only recovered during the first 2 weeks of illness. Sensitivity of culture during the first 2 weeks of pertussis is 30 to 60%, and it drops dramatically (1 to 3%) by the third week of illness. Sensitivity of culture is also

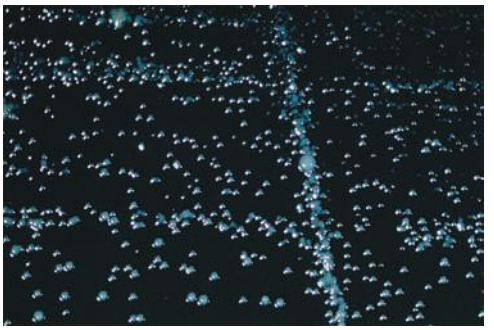


Figure 9.1 Organism infecting this patient.

negatively affected by antibiotic administration and prior vaccination. Nonetheless, the Centers for Disease Control and Prevention (CDC) recommends culturing of nasopharyngeal specimens during an outbreak so that specificity is preserved and isolates are obtained for susceptibility testing and epidemiologic studies.

For many years, direct fluorescent-antibody assay (DFA) for *B. pertussis* was done. This assay takes ~2 hours, versus 7 to 10 days for culture, but it has a sensitivity of only 50 to 65%, and false-positive results may occur, especially when laboratorians are unaccustomed to reading these DFA smears. DFA is no longer in the CDC's diagnostic algorithm for pertussis because of these limitations.

NAAT, and in particular PCR, has become the method of choice for diagnosing pertussis. Because PCR does not require that the organisms be alive, it is useful when specimens must be transported long distances. PCR is more rapid than culture, with results often available the same day the specimen was collected. PCR is more sensitive than culture and has a high negative predictive value. There are two FDA-cleared molecular products for the detection of B. pertussis. One is a 20-plex test that detects a number of respiratory viruses and bacteria simultaneously, while the other is a stand-alone test. Many laboratories use laboratory-developed NAATs for the detection of *B. pertussis*. The performance of these tests varies widely. Sensitivity and specificity are dependent on the target used for amplification, with the most sensitive tests targeting multicopy sequences and the most specific tests detecting multiple targets. The primary concern for PCR-based diagnosis of pertussis is the risk of false-positive results. False-positive PCR results have been the subject of "pseudo" outbreaks of pertussis that have been linked to cross-reacting *Bordetella* spp. (e.g., *B. holmesii*), laboratory contamination, and environmental contamination at collection. Interestingly, it has been reported that false-positive results can occur when specimens are collected in the same clinic room where pertussis vaccines (some of which contain genomic DNA) are administered. Since there is no perfect test for the diagnosis of pertussis, the CDC recommends that both culture and PCR be used diagnostically.

3. The clinical course of pertussis is defined by three stages: catarrhal, paroxysmal, and convalescent. The catarrhal phase lasts 1 to 2 weeks, but symptoms are often nonspecific and are similar to those of many respiratory viral illnesses (malaise, low-grade fever, rhinorrhea, and mild cough). Laboratory diagnosis is most sensitive at this phase, but laboratory testing (particularly in adolescents and adults) is often not performed. Even though pertussis is a toxin-mediated disease, appropriate antimicrobial therapy during the catarrhal stage decreases the organism load, thereby reducing the infectiousness of the patient, the duration and severity of symptoms, and the transmission rate. The paroxysmal phase is characterized by the paroxysmal cough, excessive mucus production, posttussive vomiting, and lymphocytosis that may last up to 6 weeks. This is the stage at which most children, adolescents, and adults are likely to seek medical attention and receive antimicrobial therapy. The damage that the *B. pertussis* cytotoxin causes—ciliostasis and death of the tracheal

epithelial cells—is not reversed by the administration of an antibiotic. Thus, the cough persists. The last phase is the convalescent phase, characterized by a chronic cough that may last weeks to months. As with the paroxysmal phase, therapy given at this stage is not effective, with the exception of therapy for secondary bacterial pneumonia that develops as a complication.

Macrolide antibiotics (e.g., azithromycin, clarithromycin, and erythromycin) are the drugs of choice for treating pertussis. In addition to delay in administering antibiotics (as was the case with this child), reasons for a lack of response to therapy might include patient noncompliance. Erythromycin, in particular, is often associated with gastrointestinal intolerance. Secondary bacterial pneumonia, an occasional complication of pertussis, must also be considered in patients with persistent cough, particularly if the patient worsens clinically. Finally, the possibility that the organism is resistant to macrolides must be considered. Although macrolide-resistant *B. pertussis* isolates have been described, susceptibility surveys suggest resistance is still rare.

4. *B. pertussis* has many virulence factors that are responsible for mediating attachment to host cells and causing tissue damage. Pertussis toxin acts as both a secreted toxin and an adhesin working synergistically with filamentous hemagglutinin. Pertussis toxin belongs to the classic A-B family of ADP-ribosylating toxins (like cholera toxin and Shiga toxin). Additional toxins include adenylate cyclase-hemolysin, a cytotoxin that inhibits chemotaxis and induces apoptosis of macrophages; tracheal cytotoxin, which eliminates mucociliary clearance by ciliostasis and extrusion of ciliated cells and inhibits DNA synthesis; dermonecrotic toxin, which causes dermal necrosis and vasoconstriction; and lipopolysaccharide endotoxin, which has proinflammatory activity. Taken together, these pathogenic properties result in a grossly damaged respiratory epithelium with decreased mucociliary clearance, which puts patients at increased risk for secondary pneumonia. Also, this patient required intubation for respiratory support, which further increases the risk of health care-associated pneumonia due to organisms such as methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

5. Historically, vaccination against pertussis was recommended at ages 2 months, 4 months, 6 months, 15 to 18 months, and 4 to 6 years. This patient was too young to have received any pertussis vaccine. Based on laboratory testing, the mother was confirmed to have pertussis, but the brother could not be confirmed due to the extended time since his illness. Nonetheless, it is probable that the brother also had pertussis. The possibilities are that neither the mother nor the brother was vaccinated against pertussis in childhood, or the fact that the protection offered by the vaccine wanes within 5 to 10 years of administration. In fact, both the mother and brother had been vaccinated in childhood, so the latter possibility is a likely explanation. Another possibility is these two individuals, closely genetically related, could not mount an immune response to the pertussis vaccine antigens. Studies have shown that vaccine-induced immunity wanes

after the fifth dose of pertussis vaccine. In this case, the older sibling likely got pertussis from his peers and then infected the mother, who was infectious at the time of the infant's birth. The infant's lack of protective immunity, along with the high infectivity of pertussis, made it very likely that the infant would get pertussis.

Vaccination against pertussis using a whole-cell vaccine began in the 1940s. This vaccine was combined with diphtheria (D) and tetanus (T) toxoids to make the combination DTP vaccine that was given to infants and toddlers. With widespread immunization, the incidence of pertussis decreased from 157 cases per 100,000 people to <1 per 100,000 in the 1970s. However, the whole-cell vaccine was associated with increased mild side effects such as erythema, swelling, and tenderness at the injection site; fever; drowsiness; and anorexia; as well as severe side effects such as high fever and seizures. Whole-cell vaccines were considered too reactogenic for use in adolescents or adults. Acellular pertussis vaccines, which have fewer side effects, were introduced in the 1990s to replace whole-cell vaccines. These vaccines target the primary virulence factors of *B. pertussis* and contain purified proteins including detoxified pertussis toxins and adhesins. The acellular vaccine is combined with DT for the DTaP vaccine given to children in a five-dose series that is completed by age 4 to 6. Neither natural pertussis infection nor vaccine-induced protection provides long-term immunity. Several studies have since shown that the acellular pertussis vaccine is not as effective as the whole-cell vaccine, making children 7 to 10 years old particularly vulnerable as a reservoir of pertussis transmission. In 2012, there were >41,000 cases of pertussis reported in the United States and likely many more that were not diagnosed and/or reported. In addition, the number of outbreaks due to pertussis has increased. A well-described outbreak in California occurred in 2010 in which 89% of cases were among infants <6 months old, with the next highest incidences in those 7 to 9 years old and 10 to 18 years old. In 2012, Washington state had >2,500 pertussis cases in 6 months, with the highest incidence in infants <1 year and children aged 10, 13, and 14 years. In 2012, 49 states reported increases in pertussis cases relative to the previous year. Better detection methods (e.g., PCR) are partially responsible for this increase, but so is natural pertussis epidemiology. It has been estimated that 13 to 20% of adolescents and adults with prolonged cough have pertussis. Diagnosing older individuals with pertussis is problematic because they often have an atypical presentation consisting of nothing more than a chronic cough. However, these individuals are common sources of infant infections, particularly parents, primary caregivers, siblings, and health care workers. Since infants are at the greatest risk for serious illness and death due to pertussis, these sources of transmission are primary targets for new vaccination strategies.

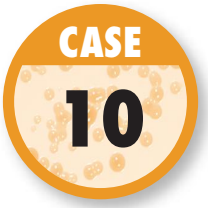
In 2005, two tetanus, diphtheria, and acellular pertussis vaccines, Tdap and DTaP, were approved for administration: DTaP for people 11 to 64 years old and Tdap for those 10 to 18 years old. Tdap vaccine has reduced antigen doses for diphtheria and pertussis compared to DTaP. The Advisory Committee on Immunization Practices now recommends Tdap vaccination for 11- to 12-year-olds, adults who have not previously received Tdap or with unknown vaccine status, and pregnant women during each pregnancy. In

addition, many health care institutions are requiring Tdap vaccination of all health care personnel. It is hoped that these new vaccination strategies will break the chain of transmission of a pathogen that only infects humans.

6. Hospitalized patients with pertussis should be on droplet precautions as pertussis is transmitted by large respiratory droplets produced when coughing, sneezing, or talking. Pertussis is highly communicable, with household attack rates of 80 to 100%. Droplet precautions should be maintained until the patient has received 5 days of appropriate antimicrobial therapy. There is no evidence of fomite transmission, which would require contact precautions as well. Close contacts of a person diagnosed with pertussis should be assessed for the infectiousness of the patient (e.g., which stage of disease), the intensity of the exposure, and the risks to the contact of getting pertussis or transmitting it to vulnerable populations (e.g., infants, pregnant women, and health care personnel). If warranted, postexposure prophylaxis with a macrolide should be administered to contacts within 21 days of onset of cough in the index patient. Alternatively, low-risk contacts can be monitored for pertussis symptoms for 21 days.

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In December 2009, a 45-year-old female presented to the emergency department (ED) 2 days following abrupt onset of sore throat, nonproductive cough, chills, and mild fever. A chest radiograph was performed, which was normal. She was diagnosed with bronchitis and asked to follow up with her primary care physician, who subsequently started her on levofloxacin and albuterol. Four days later she presented again to the ED with worsening cough, dyspnea, fever (38.3°C; 101°F), and generalized lethargy. Additionally, she reported new symptoms including a global headache, dizziness, myalgias, and arthralgias. She had no abdominal pain, but reported nausea and anorexia. Her chest radiograph showed diffuse reticulonodular opacities throughout the left lung, which were not present on her visit 4 days previously. The patient was admitted for further evaluation. Questioning revealed the following: she had a history of diabetes and hypertension, she smoked an average of a pack of cigarettes daily, and she had received the seasonal influenza vaccine. Her husband was recently ill with cough, but no other symptoms.

On day 2 of hospitalization the patient's respiratory rate increased from 22 to 46 and her oxygen saturation dropped while on oxygen administered by nasal cannula. She was transferred to an intensive care unit, where her respiratory status quickly deteriorated, necessitating emergency intubation. A new chest radiograph showed bilateral involvement, and she was begun on vancomycin, aztreonam, and azithromycin. Blood cultures drawn on her admission were negative, and an expectorated sputum sample taken at the same time was not processed due to poor specimen quality. A PCR test performed on a nasopharyngeal swab was positive for a viral agent, revealing the etiology of her infection (Fig. 10.1).

1. What is the agent causing her infection? What are the key virulence factors of this agent?
2. How does this virus change over time? What made this virus unique in 2009?

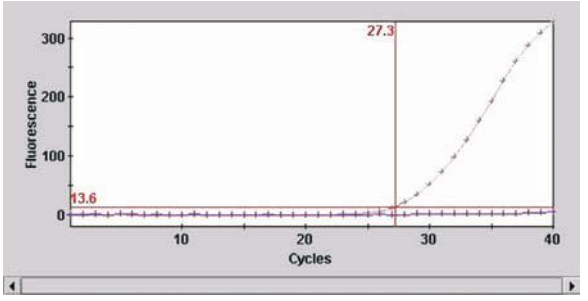


Figure 10.1 Amplification curves of a real-time PCR test.

3. Why was PCR used to diagnose this infection? What do the curves in Fig. 10.1 represent?
4. What is the usual outcome of this infection in this patient population? What groups of people are at greater risk of a poor outcome when they are infected with this virus? How did these groups differ in 2009?
5. What are the common complications associated with this infection that lead to increased morbidity and mortality? How are these complications diagnosed?
6. What antiviral drugs are available to treat this infection, and how do they work? Is there any concern for antiviral resistance?
7. Two types of licensed vaccines are available that can prevent this disease. Describe the nature of both of these vaccines and how they are used.



CASE DISCUSSION

1. Although a number of respiratory viruses could explain this patient's symptoms, influenza is the most common febrile respiratory illness in adults, particularly during the winter months, when influenza activity normally peaks. In pediatric patients (particularly <1 year old), respiratory syncytial virus should also be considered. The clinical clues of her influenza infection are the abrupt onset of fever and sore throat with nonproductive cough seen at her initial presentation to the ED. Clinically it is difficult to distinguish infections due to influenza A and influenza B, though influenza A tends to be associated with more severe disease, is generally the cause of annual epidemics, and has been responsible for all described pandemics.

Influenza virus has two major envelope proteins that contribute to its pathogenesis: neuraminidase and hemagglutinin. Neuraminidase likely has at least two functions. Its major function seems to be the cleavage of sialic acid from the cell surface and progeny virions, which facilitates the spread of new virions from infected respiratory cells. There is also evidence supporting the role of neuraminidase in viral entry to the cell. One mechanism that has been proposed is that neuraminidase cleaves decoy receptors on mucins, cilia, and cellular glycocalix so that the virus can have greater access to the functional receptors on the cell membrane. Once the virus penetrates to the cell surface, binding to specific sialic acid-rich receptors is mediated by hemagglutinin. Proteolytic cleavage of hemagglutinin by lung serine proteases is required for hemagglutinin activity. After the virus is endocytosed into the cell, hemagglutinin plays a role in the formation of channels through which viral RNA can enter the cytoplasm and initiate the viral replicative cycle.

2. In recent years only two hemagglutinin types (H1 and H3) and two neuraminidase types (N1 and N2) of influenza A virus have been circulating in humans (H1N1 and H3N2). However, due to antigenic variation, there are annual influenza epidemics and, in 2009, a pandemic. Why does this happen? There are two major evolutionary concepts related to influenza virus—antigenic drift and antigenic shift.

A unique property of influenza viruses is that they have single-stranded RNA genomes made of eight segments. Each influenza gene is found on a separate viral RNA segment. Since the mutation rate for RNA is higher than that of DNA (10^{-3} to 10^{-5} versus 10^{-6} to 10^{-8} per base per generation), point mutations readily accumulate in influenza viruses. Although mutations occur throughout the influenza genome, the accumulation of mutations (and corresponding amino acid changes) in surface antigens, such as hemagglutinin and neuraminidase, have the greatest impact. For influenza A virus, these changes will not necessarily result in the change of the classification of a viral strain (which is based on the subtypes of the H and N antigens), but they may be sufficient to render patients with antibodies to the parent strain susceptible to the new mutant strain. This is the basis for the decision to reevaluate and potentially change the formulation of

the influenza vaccine each year to include recent isolates, so that protective antibodies to the most recent isolates will be made in response to the vaccine. Both influenza A and influenza B are constantly changing by antigenic drift.

The more dramatic, and less common, antigenic shift is due to genetic reassortment of genes to form a novel human influenza virus, which typically has different hemagglutinin and/or neuraminidase proteins. Antigenic shift occurs during coinfection of a cell with two different influenza A viruses. Since the packaging of viral RNA segments occurs randomly, a coinfected cell could form a variety of different virions. The result could be a virus with a different classification (e.g., a shift from H1N1 to H5N1) or a virus of the same type but with divergent genomic sequences from nonhuman sources such as pigs or birds. The end result is a new virus that differs dramatically from parent strains.

The influenza A H1N1 pandemic of 2009 was a result of antigenic shift. Although an H1N1 influenza virus had circulated globally for years, a reassortant H1N1 virus was introduced and spread worldwide. The 2009 H1N1 virus was a result of the introduction of Eurasian swine segments (neuraminidase and matrix) into the classical swine influenza strain that previously had only caused swine-to-swine transmission and rare swine-to-human transmission. When an antigenic shift occurs, most of the world's population has little or no protection against the new virus, resulting in large epidemics or pandemics.

3. There are a variety of ways of diagnosing influenza in the laboratory, including rapid antigen tests, direct fluorescent-antibody assay (DFA), viral culture, and molecular detection. Rapid antigen tests are immunochromatographic assays that have been used for decades and have been favored due to their fast time to result (~15 minutes). However, as diagnostic methods have improved and circulating strains have changed, studies have shown that these tests suffer from lack of sensitivity. Sensitivities down to 10% were reported during the 2009 pandemic. Typical ranges of sensitivity reported are 20 to 90% depending on the strain circulating and the method used as the reference method. A further concern is the positive predictive value of rapid antigen tests when used outside of peak influenza season. Since positive predictive value is dependent on the prevalence of disease, using a test with imperfect specificities (90 to 95%) during times of low prevalence increases the chance that a positive result may actually be false positive rather than true positive. However, the times when laboratory testing for influenza is the most helpful clinically are at the beginning and end of the epidemic season, when the differential diagnosis is much broader. Another rapid method (~2 hours) is DFA testing. DFA uses a pool of monoclonal antibodies to influenza and other common respiratory viruses to directly detect infected cells obtained from the nasopharynx of patients. Although it is more sensitive than rapid antigen tests, DFA also had decreased sensitivity (~47%) for detecting the 2009 H1N1 pandemic strain. DFA sensitivity and specificity are also dependent on the skill of the personnel performing the test. Therefore, if rapid antigen tests or DFA must be used, alternative methods should be available to confirm the results, as needed.

Viral culture sensitivity is virus specific and ranges from 80 to 95%. Specificity approaches 100%. The disadvantage to culture is its longer turnaround time (up to 7 days). Rapid shell vial cultures have decreased the time to result to 24 to 48 hours, but this is still not adequate to aid in treatment decisions for influenza, which must occur in the first 48 hours of illness for the greatest benefit. Nonetheless, it is important for public health laboratories to maintain the capability of culturing influenza so that epidemiologic typing and resistance testing can be performed to inform next year's vaccine components and antiviral recommendations.

The increase in molecular testing for influenza has been largely due to the limitations outlined above for other methods. Several FDA-cleared assays exist for the molecular detection of influenza with turnaround times ranging from 20 minutes to 8 hours. Sensitivities of these tests are 90 to 99%, with specificities of 98 to 99%. Some of the tests can also type influenza (i.e., H1, H3, or 2009 H1N1), and others can detect other respiratory viruses simultaneously. However, the majority of these tests require significant laboratory expertise and are more expensive than the other diagnostic methods listed. Since influenza genomic sequences change rapidly, it is important to monitor the accuracy of molecular tests on an annual basis. The curves shown in Fig. 10.1 represent the increase in fluorescence during real-time detection of PCR amplification.

A fluorescent probe is incorporated into the PCR reaction to measure on a per-cycle basis the presence of amplicons. Once the level of fluorescence is higher than the background level, the sample is positive. A lower cycle number of positivity (the point at which the curve crosses the horizontal threshold line) indicates a greater amount of virus in the sample. The positive result for the patient is shown by the gray line. The cycle threshold (Ct value) for the positive result is displayed by the red vertical line (27.3) and represents the cycle at which the fluorescence from the real-time PCR detection exceeds background. An example of a negative result is depicted by the purple line. The horizontal red line represents the threshold required for positivity in the PCR.

4. Most cases of influenza in this age group are self-limited and do not require hospitalization. Influenza is a much greater threat to individuals >65 years of age and children <5 years old. During most epidemics the highest numbers of hospitalizations and deaths are in these age groups. Other individuals at risk for complications of influenza infection are those with underlying chronic pulmonary diseases, such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease; immunocompromised individuals; pregnant women, particularly in the second and third trimesters; and those with a variety of other chronic conditions such as cardiovascular disease and diabetes. This patient was a smoker and had diabetes, both of which put her at increased risk for severe influenza disease. In 2009, obesity was shown to be an independent risk factor for increased mortality due to H1N1. During the pandemic there was still significant disease and mortality in pediatric

patients, with more than double the number of pediatric patients dying than in the previous three influenza seasons. Interestingly, the death rate for those 25 to 49 years of age was greatly increased as well, but little disease was seen in those >55 years of age. This suggests that influenza strains circulating prior to 1955 provided some protection against the pandemic strain, which was confirmed by serologic surveillance studies.

5. The most common complication leading to increased morbidity and mortality is pneumonia. This could be primary influenza virus pneumonia, secondary bacterial pneumonia, or a combination of the two. The majority of reported influenza-associated deaths appear to be due to influenza with accompanying bacterial pneumonia, especially pneumonia caused by *Streptococcus pneumoniae* and *Staphylococcus aureus*. For this patient, we cannot determine whether she has influenza pneumonia or bacterial pneumonia. To differentiate these, we would need a lower respiratory specimen (preferably a bronchoalveolar lavage) obtained prior to antibiotic administration to culture for bacteria and test for influenza. The sputum specimen obtained from this patient was rejected as inadequate for culture because there were no neutrophils present, suggesting a poor specimen collection. Thus, she was treated empirically for bacterial pneumonia.

6. There are currently two classes of anti-influenza drugs. The first class of agents, M2 inhibitors, blocks formation of influenza-derived ion channels. The reason these virally derived ion channels are important is that they play an important role in the "uncoating" of the virus. This is a step in viral replication in which viral RNA is released from the viral particle and enters the cytoplasm of the cell. The two drugs in this class are the oral agents amantadine and rimantadine. The drugs must be administered in the first 2 days of illness to be effective. They have been shown to reduce the disease course by 1 day. In addition, these agents prevent influenza illness in approximately 70 to 90% of individuals who take these agents prophylactically. Unfortunately, resistance to these drugs increased rapidly in influenza A H3 and 2009 H1N1. They do not work on influenza B. Therefore, in practice, these drugs are no longer used.

The second group of agents is the neuraminidase inhibitors. Two agents belong to this class of drugs—zanamivir, which is an inhaled agent, and oseltamivir, which is an oral agent. These agents are most effective if given in the first 2 days of illness and, like the ion channel-blocking agents, reduce the disease course by 1 day. However, data suggest that giving neuraminidase inhibitors at any time to a seriously ill patient may have benefits. The advantage of the neuraminidase inhibitors is that they are active against both influenza A and B viruses. However, influenza A H1 (pre-pandemic strain) is resistant to oseltamivir, and sporadic cases of H3 and 2009 H1N1 resistance have been described. To date, the majority of circulating influenza strains maintain susceptibility to both neuraminidase inhibitors.

7. Both vaccines are trivalent vaccines containing the same three influenza strains. The strains present in the 2012 vaccine included two subtypes of influenza A, 2009 H1N1 and H3N2, and influenza B. For the first time, in 2013 the vaccine contained two antigenically distinct influenza B viruses. It is important to remember that the composition of the vaccine changes annually. This is determined by the types of viruses that circulated during the previous season in the Southern Hemisphere. Due to waning immunity and antigenic drift of the viruses, the vaccine must be given annually. The efficacy of the vaccine is dependent on the level of change that may occur from year to year in the circulating virus, but it is generally 60 to 70% effective. One vaccine is an inactivated vaccine and can be administered intramuscularly (to those 6 months or older) or intradermally (to those 18 to 64 years old). There is also a high-dose inactivated vaccine that is given to people older than 65. The other vaccine is a live attenuated vaccine given intranasally to individuals aged 2 to 49 years. The live attenuated vaccine should not be given to pregnant women, immunocompromised individuals, or those caring for immunocompromised individuals.

The Centers for Disease Control and Prevention recommends that influenza vaccines be given to at-risk populations (see the answer to question 4 for a listing of at-risk populations). This includes children aged 6 months to 4 years, people 50 years and older, and health care personnel who could transmit the virus to at-risk patients. The vaccine is not recommended for children <6 months of age, a population that would most likely benefit from influenza virus vaccination. Numerous studies have proven the efficacy of this vaccine strategy. Recent studies also show that immunocompetent children benefit from vaccination through reduction in hospitalizations, doctor office visits, antibiotic use, serious secondary bacterial infections, and spread to at-risk family members.

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A previously healthy 9-month-old infant presented in mid-February with a 2-day history of irritability, fever, and upper respiratory congestion. The mother reported that over the previous 24 hours the child had difficulty breathing with coughing and wheezing. The child's medical history included a normal delivery after a 9-month gestation without complications. She was up to date on all immunizations. At age 6 weeks the child was placed in a day care center so that the mother could return to work. Several of the infants at the center had been ill recently with colds, and one infant required hospitalization because of severe breathing problems.

On examination the child appeared agitated and had a temperature of 38.6°C. She had both tachypnea (respiratory rate of 70 per minute) and tachycardia (pulse, 200 beats/min). The ears, eyes, and throat were normal except that the oral mucous membranes and tongue were dry. The nasal mucosa was boggy with clear discharge. The lungs revealed diffuse inspiratory and expiratory wheezes. Findings from the rest of the examination were normal.

A chest radiograph revealed hyperexpansion of the lungs but no infiltrates. Arterial blood gases revealed hypoxemia and respiratory alkalosis. The child was admitted to the hospital because of moderate respiratory distress. Supplemental oxygen and intravenous fluids were administered along with bronchodilators and systemic corticosteroids. A rapid molecular test performed on a nasopharyngeal swab provided the diagnosis.

1. This child presented with bronchiolitis, an acute viral lower respiratory tract illness generally occurring in the first 2 years of life. What viruses can cause this syndrome? What are the epidemiologic clues in this case that makes one of the viruses most likely?
2. Describe the epidemiology of the agent causing her infection.
3. What characteristics of this virus are important in determining how the virus spreads in the respiratory epithelium? How does the pathogenesis of the virus contribute to the wheezing that often accompanies this infection?
4. Describe the diagnostic strategies available for the detection of this agent. Why is it important to establish this diagnosis quickly?
5. What prevention strategies exist to avoid initial infection with this virus and to keep it from spreading within the hospital?
6. Is specific therapy available to treat this virus?



CASE DISCUSSION

1. The differential diagnosis for this patient's bronchiolitis included respiratory viruses such as the parainfluenza viruses, adenovirus, influenza A and B viruses, coronavirus, rhinovirus, metapneumovirus, and respiratory syncytial virus (RSV). *Mycoplasma pneumoniae* or *Bordetella pertussis* also could have caused her illness. RSV causes ~70% of bronchiolitis cases in children <2 years of age, with more severe cases typically occurring in children <6 months of age and premature infants. In the day care setting, any of these agents could spread easily. However, the fact that another child had recently been hospitalized supports RSV or influenza as the most likely causes, as these viruses generally cause more severe disease. All of the potential viral causes circulate in the winter months, with RSV infections typically occurring between December and February. Increased RSV incidence often overlaps with both influenza and metapneumovirus, so those viruses cannot be excluded based on the time of the year the patient presented. To definitively diagnose this patient with RSV, a laboratory test must be performed, but this patient's clinical presentation and epidemiologic setting points to RSV as the most likely etiology.

2. RSV is the most important viral etiology of childhood respiratory illness in the industrialized world in terms of morbidity and mortality, particularly in children <1 year old. The World Health Organization estimates that ~160,000 deaths occur worldwide annually due to RSV. Approximately two-thirds of infants have an RSV infection during the first year of life, with nearly all children infected by the end of the second year. Clinical manifestations of RSV infection range from mild upper respiratory tract illness to severe lower respiratory tract illness, including bronchiolitis, croup, and pneumonia. Lower airway disease occurs in 15 to 50% of young children, with approximately 1 to 3% requiring hospitalization. This represents about 125,000 hospitalizations annually in the United States due to RSV. Premature infants, infants with chronic lung disease, and infants with significant congenital heart disease have hospitalization rates four to five times higher than healthy infants. Although deaths from RSV are uncommon outside of developing countries, premature infants and those with preexisting pulmonary or cardiovascular disease are at greatest risk. Incomplete protective immunity following RSV infection leads to reinfections throughout life. Reinfections in older children and adults generally result in minimal respiratory tract symptoms. However, immunocompromised individuals, patients with chronic cardiopulmonary disease, and the elderly who reside in long-term care facilities are at greater risk for developing severe lower respiratory tract disease. RSV is second only to influenza as a cause of death due to viral respiratory infections in elderly individuals.

RSV is spread by large droplets and on fomites. In hospitals and day care centers, it can be spread to the susceptible child on the hands of caregivers who do not use good hand-washing practices. Epidemics of RSV occur each winter in temperate climates. In the United States, peak disease incidence is seen from mid-December to early February.

Although there is only one serotype of RSV, it has two antigenically distinct subgroups, designated A and B. RSV-A and RSV-B cocirculate during epidemics, although one type tends to predominate. Both epidemiologic and *in vitro* data have suggested that RSV-A causes more severe disease. However, the antigenic heterogeneity that occurs within the subgroups makes this hypothesis difficult to confirm.

3. First, RSV must bind and enter the target cells, which are the apical ciliated epithelial cells of the airway lumen. The virus attaches to the cell membrane using electrostatic interactions and the viral G protein. Then the viral F protein, along with a cellular receptor, mediates fusion to the cell membrane and thereby viral entry. The fusion protein also causes neighboring cells to coalesce, resulting in multinucleated cells, or syncytia (where the virus gets its name). The end result of the infection is damage to the airway epithelium and loss of ciliated epithelial cells. Histopathologic evidence shows sloughed epithelial cells, fibrin, mucus, and inflammatory cells in the large airways. *In vivo* evidence of apoptosis and syncytia formation has also been noted. Only recently, with the use of the well-differentiated primary airway epithelial cell culture model, has RSV pathogenesis started to be understood. In this model, much of the RSV-infected epithelium remains intact; this has also been observed *in vivo*. These observations suggest that airway damage is not a direct effect of RSV but rather is caused by the immune response to RSV. RSV infection induces an innate immune response leading to the production of cytokines and chemokines by the epithelium, which recruits white blood cells and results in epithelial injury. The resulting necrosis and edema can lead to collapse and blockage of the small-diameter bronchioles, with air trapping distally causing the wheezing and stridulous cough that are often seen in infants with RSV infection.

4. There are several diagnostic approaches that offer a rapid result, each with varying sensitivities. Rapid enzyme immunoassays (EIAs), or rapid antigen tests, offer results in ~15 minutes. However, these tests can have sensitivities as low as 59% and have been shown to be a source of significant false-positive results, particularly at the beginning and end of RSV season, due to the decreased prevalence and positive predictive value. The advantage of the EIA method, and the reason it is widely used, is that it is easy to perform. A disadvantage of this technique is that specimen quality cannot be assessed. Another rapid method is the direct fluorescent-antibody assay (DFA), which offers results in 1 to 2 hours. Unlike the rapid EIA, DFA requires well-trained laboratory personnel to correctly perform and interpret the fluorescent results. The sensitivity and specificity of DFA can be directly correlated with laboratory expertise, but in general its performance is at least as good as culture. Specimen quality can be judged by the DFA technique, as the patient's nasopharyngeal cells can be quantified. Culture can be performed relatively rapidly using the rapid centrifugation, or shell vial, culture technique. The patient's specimen is gently centrifuged onto a permissive cell layer to promote virus-cell contact and decrease the time needed to detect infected cells if the virus is present in the patient's specimen. To

visualize viral infection, the laboratory uses an antibody pool consisting of antibodies specific for RSV, parainfluenza viruses, influenza A and B viruses, and adenovirus to fluorescently stain the shell vial monolayer after 24 to 48 hours of incubation. Depending on the antibody pool used, either the color of fluorescence can indicate which virus is present or antibodies specific for individual viruses can be used to detect the specific agent causing the infection. This method is typically used only if other rapid methods, such as EIA and DFA, are negative. Shell vial cultures are very specific, with 70 to 80% sensitivity, but take ~2 days to obtain results.

More recently, molecular methods have become commercially available to diagnose respiratory viral infections. Most of these products detect a panel of respiratory viruses, including RSV. Some detect only influenza A, influenza B, and RSV, while others detect 12 or more viruses. The time to result for these molecular platforms varies from 70 minutes to 8 hours. Some of the tests provide random access testing, while others are more efficiently performed in daily batches. The main advantage of molecular detection of RSV is increased sensitivity, but specimen quality is not determined. The primary obstacle in routinely performing these tests in the clinical laboratory is the cost of the equipment, reagents, and personnel needed to perform molecular testing. Independent of the method used to diagnose RSV in the laboratory, a rapid result is important for management decisions, including infection control and treatment. Although rapid detection of respiratory viruses has been hypothesized to decrease unnecessary use of antibiotics and decrease length of hospital stay, there are few supporting data in the literature.

5. Since there is not a vaccine available to prevent infection nor is there a broadly effective antiviral agent to treat RSV, infants who are at risk for severe RSV disease should receive passive immunoprophylaxis with a humanized mouse monoclonal antibody preparation against RSV called palivizumab. Although expensive, palivizumab has been shown to decrease hospitalization rates by 50% and total wheezing days in the first year of life by 61%. At-risk infants receive five monthly doses of palivizumab during RSV season, typically November through March. This includes infants/children <24 months old with hemodynamically significant congenital heart disease or chronic lung disease and infants <12 months old who were born prematurely or have congenital abnormality or neuromuscular condition of the airway.

Like all respiratory viruses, RSV can cause health care-associated infections; therefore, patients with RSV infections should be put on droplet and contact precautions to prevent spread to other patients via health care personnel. If patients are not isolated and stringent infection control practices are not followed, secondary infection rates of 20 to 50% can occur. Cohorting of RSV-positive children and their health care providers, plus the use of gloves and gowns during contact with infected children and consistent hand washing before and after patient contact, has been shown to significantly lower RSV health care-associated infection rates. Though positive patients may be cohorted when private rooms are not available, many centers do not consider the sensitivity of the EIA

high enough to place a child with a negative test in the same room as another child if there is a clinical suspicion of RSV. However, the negative predictive value of most of the molecular tests available would alleviate this concern. As with primary RSV infections, hospitalized patients with congenital heart disease, pulmonary disease, or an immunodeficiency are at greatest risk for life-threatening RSV infections.

6. No drugs that specifically target RSV are available for treatment. However, ribavirin (a nucleoside analog) has good *in vitro* activity against RSV and is FDA approved for RSV treatment. Ribavirin is generally delivered by aerosol since oral or intravenous administration may result in hepatic or bone marrow toxicity. The American Academy of Pediatrics recommends against the routine use of ribavirin in infants and children with lower respiratory tract illness because its efficacy in this population has not been proven. Randomized, controlled trials have demonstrated conflicting results. Further, ribavirin treatment is expensive. Treatment of RSV infection with ribavirin is reserved for immunosuppressed patients with severe RSV infection. Several reports have demonstrated that early administration of ribavirin significantly decreased lower respiratory tract illness, morbidity, and mortality in adult hematopoietic stem cell transplant recipients. Palivizumab cannot be used as a treatment for RSV; it only provides passive protection against infection.

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The patient was a 35-year-old previously healthy male who 1 week prior to admission began to feel ill with malaise, headache, fever, and chills. Over the next week his disease progressed. Three days prior to admission he developed nausea and vomiting. Over the next 3 days he developed a worsening cough, fever and chills, and shortness of breath. He presented to an outside hospital with a respiratory rate of 30 and an oxygen saturation of 87% on room air. He was intubated; begun on ceftriaxone, erythromycin, trimethoprim-sulfamethoxazole, and steroids; and transferred to our hospital, where he was admitted to the intensive care unit.

On physical examination, he had a temperature of 38.1°C, a pulse of 150 beats/min, and a respiratory rate of 16 on the ventilator. His physical examination was benign except for his pulmonary compromise. His blood gas was pH 7.13, pCO_2 of 69 mm Hg, and pO_2 of 89 mm Hg. His oxygen saturation on the ventilator on admission was 90%. A chest radiograph obtained in the intensive care unit showed multilobar infiltrates. Other significant laboratory findings included a white blood cell count of 18,500/μl with an absolute neutrophil count of 17,200/μl, a negative *Legionella* urinary antigen test, and a lactic acid dehydrogenase level of 1,300 IU/liter. Because the patient had a prior history of cocaine and ecstasy use, a toxicology screen and an HIV serology were performed. Both were negative. The patient was a manual laborer, had no recent travel history, was married, and had two young children.

A bronchoscopy was performed, with a bronchoalveolar lavage (BAL) Gram stain showing many white blood cells but no organisms. Calcofluor white stains for fungi and direct fluorescent-antibody assay (DFA) for *Pneumocystis* were also negative. The etiology of his illness was detected in his BAL and is seen in Fig. 12.1. Figure 12.2 shows a Gram stain of the colonies from Fig. 12.1.

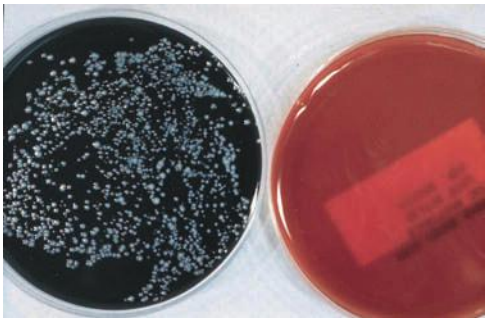


Figure 12.1 Colonies on buffered charcoal yeast extract (BCYE) agar (left) and 5% sheep blood agar (right).

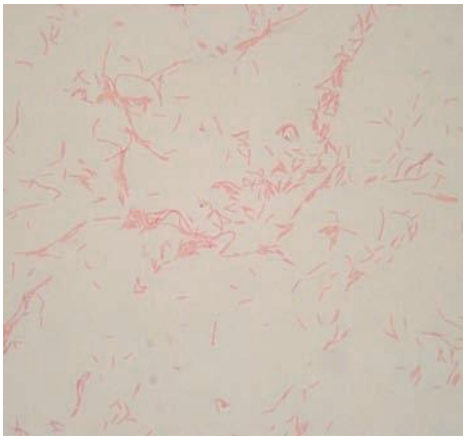


Figure 12.2 Gram stain of colonies seen in Fig. 12.1.

1. The organism was growing only on buffered charcoal-yeast extract (BCYE) agar and not on 5% sheep blood agar, and a Gram stain of the organism was also obtained. Based on these data, what organism is likely causing this patient's illness? Explain your reasoning. What other respiratory pathogens will grow in this medium?
2. This organism is considered an "environmental" organism because it comes from the patient's environment. What unique ecologic niche does this organism inhabit? How does its evolution to survive this niche affect its ability to cause human infection?
3. This organism has been associated with disease outbreaks in travelers. Name two venues where disease outbreaks have been associated with outbreaks. Why do you think these venues have been associated with these infections?
4. Culture may not be positive for this organism. Why is that? How do you explain it being positive in this patient? What alternative method is typically used to diagnose this infection? What are its strengths and weaknesses?
5. When considering antimicrobial therapy for this organism, what two factors should be considered? One is related to the answer to question 2; the other is an intrinsic characteristic that influences antimicrobial choice.
6. What infection control measures are needed to prevent spread of this organism from patient to patient? What steps would need to be taken if several health care-associated cases were discovered in a health care facility in a short time period?



CASE DISCUSSION

1. The organism causing this patient's infection is *Legionella pneumophila*. On Gram stain it tends to be a long, thin, poorly staining, Gram-negative rod. It does not grow on 5% sheep blood agar but will grow on BCYE agar. *L. pneumophila* is an important agent of community-acquired pneumonia, and the patient's respiratory symptoms are consistent with but not specific for infection with this organism. An interesting observation about *Legionella* epidemiology is that this is a disease that is more likely to be found in males. He does not have other risk factors that have been associated with *Legionella* infections, including older age, alcohol abuse, smoking, immunosuppression, and chronic obstructive pulmonary infections. Except for male sex, all of these risk factors could be associated with any number of agents causing pneumonia, including influenza virus, *Mycobacterium tuberculosis*, and *Streptococcus pneumoniae*. It also is a disease whose incidence is increasing in the United States, although that may be due primarily to improved case findings due to better diagnostic tests.

BCYE agar is frequently used to culture bronchoscopically obtained specimens, in part to ensure the recovery of *L. pneumophila* and other *Legionella* spp. However, other organisms such as *Francisella tularensis*, *Nocardia* spp., and a variety of *Bordetella* spp. may also grow on this medium, making it a useful tool for diagnosing atypical pneumonia.

2. *L. pneumophila* is found primarily in aquatic environments. Unlike many free-living environmental Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, *L. pneumophila* has evolved to live commensally within a variety of environmental amebae. It is speculated that *Legionella* parasitizes these amebae because they provide nutrients in this comparatively nutrient-poor environment. It is suggested that *L. pneumophila*, like *Chlamydia trachomatis*, has two stages, one an intracellular, reproductive stage occurring in the protozoan host and the other an extracellular stage that allows *L. pneumophila* to infect additional amebic hosts. Within the amebic host, *L. pneumophila* lives within a vacuole that protects it from environmental stresses that might kill the organism, such as pH and osmotic changes or toxic substances such as antibiotics produced by other environmental organisms.

The adaptation to survive in free-living amebae may also explain the organism's ability to survive within its main target cell in humans, the macrophage. It is postulated that in human infections, the organism is inhaled and taken up by macrophages found in the alveolar space. Ingestion of a microorganism by a macrophage results in the organism being present in a phagosome within the cytoplasm. Typically, this phagosome fuses with endosomes containing different digestive enzymes to form a phagolysosome. Within this intracellular body, most bacteria are killed. *Legionella* produces proteins that prevent the fusion of the phagosome with other endosomes, preventing its killing and allowing intracellular replication. Interestingly, *L. pneumophila* appears to contain many eukaryotic gene sequences, and it is postulated that these genes may be responsible for

proteins involved in the blockage of phagolysosome formation. This bacterial replication within macrophages may trigger the release of cytokines, resulting in the inflammatory response observed in the airways of these patients. Interferon-α release by macrophages is believed to be crucial to the innate immune clearance of these organisms. Interferon-α is believed to causes changes within the macrophage that result in the organism no longer being able to replicate. One possible explanation for this observation is that there is a reduction in intracellular iron, an essential nutrient for replication.

3. Hotels and cruise ships have been associated with both sporadic cases and outbreaks of *Legionella* infections. It is estimated that as many as 35% of *Legionella* cases are obtained while traveling. Exposure to *Legionella* is the result of inhalation of aerosols from contaminated water systems containing complex biofilms, including *Legionella*-infected amebae. Aerosols can be generated by showers, spas, or whirlpools. Contaminated air-conditioning cooling towers are another possible source of exposure and were the source of the initial outbreak of *Legionella* in Philadelphia in 1976.

4. Culture sensitivity ranging from 15 to 95% has been reported, with the higher sensitivities being seen in severely ill patients such as the patient presented here, making culture a less than reliable diagnostic tool. Patients with *Legionella* pneumonia often do not produce sputum, e.g., do not have a productive cough. Therefore, a sputum specimen may never be submitted for diagnostic testing. Second, laboratories may not routinely culture sputum for *Legionella* using BCYE agar (see the discussion of question 1). Because the organism infects macrophages in the alveolar spaces, the optimal specimen for culturing the organism is BAL fluid. Patients who are too ill may not be good candidates for bronchoscopy, while mildly ill patients are more likely to receive antimicrobial coverage for agents of "atypical pneumonia," including *Legionella*, rather than having diagnostic testing. This patient, who had a high organism burden based on his culture result (Fig. 12.1), got the optimal specimen, a BAL, for diagnosing this infection. His positive culture result was therefore not surprising.

There are two alternative tests that have been used for *Legionella* diagnosis. One is a DFA test that can detect the organism directly in clinical specimens. Although DFA was one of the first diagnostic tests developed for *Legionella*, its technical complexity and modest sensitivity have caused it to be abandoned. Currently, the diagnosis of *Legionella* infections is most frequently made by the urinary antigen test. This test detects *Legionella* soluble cell wall antigen that is excreted in urine. In a recent study, 97% of disease diagnoses were made using this test versus 5% by culture. Antigen detection is easy to perform, sensitive, and rapid; the test becomes positive earlier in the disease course than does culture; and obtaining clinical specimens is easily done. The shortcomings of this test are twofold. First, it only detects *L. pneumophila* serogroup 1. It is estimated that this organism is responsible for 80 to 90% of *Legionella* infections. Other serogroups and species will be missed with this test. Second, excretion of urinary antigen can persist for weeks to months

after the infection has resolved. Patients who initially have a *Legionella* infection might develop a second respiratory infection due to a completely different organism but be misdiagnosed due to the persistence of this antigen.

Nucleic acid amplification tests have been developed for *Legionella* using both rRNA and *mip* genes as target sequences. Currently there is only one commercially available nucleic acid amplification test for *Legionella*. Whether it will replace culture and urinary antigen as the major diagnostic tool for detecting *Legionella* is currently unknown.

5. *L. pneumophila* is an intracellular pathogen in humans. Therefore, antimicrobial selection should include antimicrobials that can penetrate into white blood cells. The most widely used antimicrobials to treat *L. pneumophila* infections are azithromycin and fluoroquinolones such as ciprofloxacin and levofloxacin. These agents have been shown to inhibit intracellular growth of this organism. β-Lactams have poor penetration into white blood cells. In addition, *L. pneumophila* produces a β-lactamase. The combination of these factors makes β-lactam antimicrobials a poor choice for treatment of *Legionella* infections. As a result, azithromycin or the fluoroquinolones are the recommended therapy for patients with community-acquired pneumonia. β-Lactam agents such as ceftriaxone may be added in severely ill, hospitalized patients.

The ability of *Legionella* to produce β-lactamase is likely an evolutionary adaptation. The organism is able to survive in microbial communities where other members may produce β-lactams. This adaptation is common in many environmental Gram-negative bacilli that are frequent causes of health care-associated infections, such as *P. aeruginosa*, *Acinetobacter* spp., and *S. maltophilia*.

6. There is no evidence that *Legionella* is spread from person to person, so once this diagnosis is established, respiratory infection control precautions are no longer needed. However, health care facilities have the same types of complex water systems that both hotels and cruise ships have. Outbreaks in health care facilities, although rare, have occurred and can be associated with contaminated water and air-conditioning systems. When such outbreaks occur, engineering controls in which water is either superheated or chemically treated to kill organisms within the pipes need to be undertaken. Another potential source of infection is contaminated air-conditioning cooling towers, which can produce *Legionella*-laden aerosols that may be introduced into building air-handling intakes. Finding the source of *Legionella* in health care-associated outbreaks can be a daunting task, as can eliminating this organism from its natural aquatic environment.

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The patient was a 34-year-old, HIV-seropositive woman who presented with a fever, productive cough, diarrhea, headache, dizziness, anorexia, nausea, sore throat, and dysphagia. She also noted a 10-lb (ca. 4.5-kg) weight loss over the previous 2 months. She had not received any treatment for her HIV infection or prophylaxis for opportunistic infections.

On physical examination, she was malnourished and in acute distress due to diarrhea. Her vital signs were normal. Her chest examination was abnormal with bibasilar crackles distributed up to the mid lung field. Her pO_2 was 77 mm Hg while breathing room air. Her chest radiograph showed diffuse interstitial infiltrates and a small pleural effusion in the right lung. Her white blood cell count was 3,700/μl with an absolute lymphocyte count of 600. Her HIV viral load was >750,000 copies/ml.

A bronchoscopy was performed. The organism detected in bronchoalveolar lavage (BAL) specimens from both lungs is seen in Fig. 13.1. A pathologic section from the lung of a patient infected with this organism is shown in Fig. 13.2.

1. What is the organism causing her pulmonary symptoms? Given her symptoms and her underlying disease, which other organisms would need to be included in your differential diagnosis?
2. A CD4 cell count was not done on this patient. What would you estimate her CD4 cell count to be, given the organism with which she was infected?
3. How might this infection have been prevented?
4. What technique is generally used in HIV patients to attempt to diagnose this infection prior to bronchoscopy? What type of bronchoscopically obtained specimen has the highest yield for detecting the organism

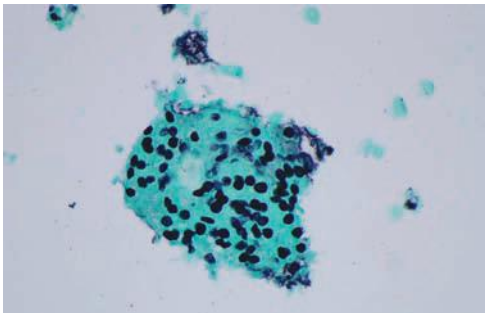


Figure 13.1

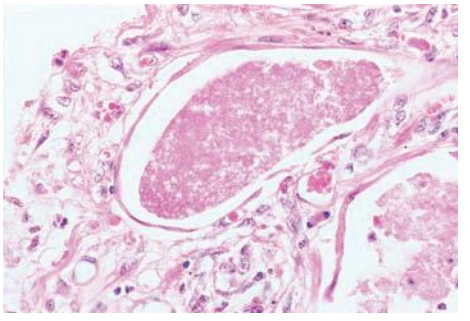


Figure 13.2

causing her infection? How would the diagnostic strategy for detecting this infectious agent differ in non-HIV-infected, immunocompromised patients?

5. Describe the epidemiology of this agent in HIV-infected individuals versus non-HIV-infected patients. How is this organism typically acquired?

6. Patients with infection with this organism often have poor oxygen exchange, as evidenced by low pO_2 levels. Based on the findings in Fig. 13.2, how do you explain this?

7. Are the dysphagia and sore throat this patient described due to the organism causing her lung infection? What organism is most likely to cause these symptoms in HIV-infected patients (Fig. 13.3)? How would it be diagnosed?

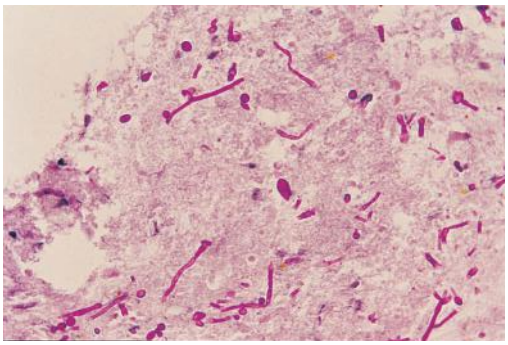


Figure 13.3



CASE DISCUSSION

1. Based on the silver stain in Fig. 13.1, which shows cyst-like structures approximately 5 μm in diameter, this woman has *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) pneumonia (PCP). *P. jirovecii* pneumonia continues to be a leading cause of pneumonia in HIV-infected individuals even though the incidence of this disease in HIV-infected patients has declined dramatically in the industrialized world with the introduction of highly active antiretroviral therapy (HAART). *P. jirovecii* pneumonia is now seen most commonly as an AIDS-presenting illness in an individual who was unaware that he or she was HIV infected. Alternatively, it might be seen in patients who are noncompliant with their antiretroviral therapy or who have never received antiretroviral therapy. Compliance problems with antiretroviral therapy are a major concern in part because of the complexity of many of the antiretroviral regimens and in part because of the side effects associated with these agents. Very rarely, *P. jirovecii* can cause infections in locations other than the lung.

Other organisms that might be infecting this woman include bacteria (such as *Streptococcus pneumoniae*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, and *Haemophilus influenzae*), viruses (such as influenza A virus and respiratory syncytial virus in HIV-infected children), and fungi (including *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans*). Other causes of pulmonary infiltrates in HIV-positive patients include Kaposi's sarcoma and lymphoma.

2. *P. jirovecii* pneumonia is typically seen in HIV-infected patients with CD4 counts <200 cells/μl. Her absolute lymphocyte count is low, so it would not be surprising if her CD4 count was <200 cells/μl. The lower the CD4 count is below 200, the greater the risk of developing *P. jirovecii* pneumonia.

3. Two approaches are important in preventing *P. jirovecii* pneumonia in HIV-infected individuals. The first approach is the use of prophylactic antimicrobial agents in patients when their CD4 count falls to <200 cells/μl. Trimethoprim-sulfamethoxazole is the prophylactic drug of choice for preventing *P. jirovecii* pneumonia because it is effective and inexpensive and it has activity against other infections, including certain agents of bacterial pneumonia and *Toxoplasma gondii*. In patients who cannot tolerate trimethoprim-sulfamethoxazole or who fail therapy, alternative prophylactic choices include dapsone, aerosolized pentamidine, and atovaquone.

The second approach is to effectively treat the HIV infection to either prevent damage to the immune system or "reconstitute" an HIV-damaged immune system. HAART has been shown to be effective both in delaying immune system damage by greatly slowing the decline in CD4 cell counts and in reconstituting the immune system. Studies have suggested that *P. jirovecii* prophylaxis can be stopped in patients receiving HAART whose CD4 cell counts increase to >200 cells/μl.

4. The least invasive and least expensive diagnostic approach is to examine an induced sputum sample for the presence of the typical *P. jirovecii* cysts (using silver stain) or cysts and trophozoites (using the more sensitive direct fluorescent-antibody stain). Conventional sputum examinations in AIDS patients give a low yield since sputum production in this disease is usually scanty. Sputum is induced by aerosolization of hypertonic (3%) saline into the airways, causing irritation, which results in coughing and expectoration of lower respiratory tract secretions. Because the number of organisms is so high in HIV-infected patients with *P. jirovecii* pneumonia, induced sputum has a sensitivity of 60 to 80% if properly collected.

BAL, as was used in this case, has a higher diagnostic yield than other types of bronchoscopic examinations and induced sputum. In BAL, large volumes of normal saline (25- to 50-ml aliquots) are introduced into a single lobe of the lung. This material lavages the bronchi and alveoli and is recovered by aspiration through the bronchoscope. This technique has a diagnostic yield of 90 to 95% for *P. jirovecii* detection in patients with AIDS who have not been treated or given prophylaxis with antimicrobial agents. In selected patients with a negative BAL examination, lung tissue can be obtained either by video-assisted thoracic surgery or by open lung biopsies. Pathologic examination of this tissue is considered definitive for detection of *P. jirovecii*. Biopsies are useful for detecting other agents of pneumonia as well, if appropriate cultures and stains are used.

In non-HIV-infected, immunocompromised patients, examination of induced sputum is not typically performed, in large part because there are few data on this test's reliability outside of HIV-infected patients. As a result, bronchoscopy with BAL would be the initial diagnostic step for detecting this organism in this patient population, followed by video-assisted thoracic surgery or open lung biopsy if deemed necessary.

In recent years, PCR has been used as a research tool for the detection of *P. jirovecii*. The performance of PCR in detecting *P. jirovecii* appears to be quite good in HIV-infected individuals, but it is not as sensitive in individuals who are immunocompromised because of organ transplantation or malignancy. The major drawback with PCR is that it is overly sensitive; i.e., it detects *P. jirovecii* when the organism is present in the lung in the absence of clinical illness. These patients are said to be colonized with *P. jirovecii*. Thus, quantitative PCR tests may be necessary to achieve acceptable clinical specificity.

5. Based on serologic surveys showing a high prevalence of *P. jirovecii* seropositivity early in childhood, it was long believed that these infections were due to reactivation of dormant organisms. Recent molecular epidemiology studies have shown clusters of infections due to the same strain of *P. jirovecii* and the presence of *P. jirovecii* DNA as detected by PCR in the respiratory tract of individuals exposed to *Pneumocystis*-infected patients. Since *P. jirovecii* only infects humans and cannot replicate outside of that host, these studies suggest that person-to-person spread of *P. jirovecii* is the usual manner in which this organism is disseminated. Current thinking is that both reactivation of dormant organisms and new acquisition from infected patients can result in infection.

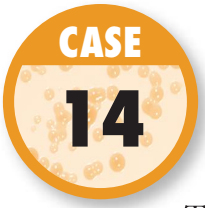
Immunologically intact individuals do not develop respiratory infections due to this organism but rather become colonized. Colonized individuals are a potential source for transmission to at-risk populations. Cell-mediated immunity (CMI) appears to be protective, and only when disruptions in CMI occur are patients at increased risk for *P. jirovecii* pneumonia. Because HIV disrupts CMI by causing a decline in the number of CD4-positive T-helper cells, *P. jirovecii* pneumonia is a common infection in this patient population. In fact, AIDS was first recognized when an unusual cluster of *P. jirovecii* cases was detected in homosexual men in southern California. In adults, the risk of becoming ill with *P. jirovecii* pneumonia increases when the CD4 count drops below 200/μl. *P. jirovecii* pneumonia incidence in HIV-infected patients has dropped dramatically in industrialized countries as a result of the introduction of HAART therapy, but it remains the leading serious opportunistic infection in this patient population. At the same time, studies have shown that the incidence of *P. jirovecii* pneumonia is increasing in solid-organ and bone marrow transplant recipients and in patients with hematologic malignancies.

6. The organism binds to type 1 pneumocytes in the alveoli. This causes diffuse injury to the alveoli with leakage of exudate into the air space. The presence of this exudate, which is pink on the hematoxylin-and-eosin section in Fig. 13.2, prevents proper oxygen exchange between the alveoli and the bloodstream. This results in hypoxemia as measured by a low pO_2.

7. *P. jirovecii* caused this patient's pulmonary signs and symptoms but is an unlikely cause of dysphagia and sore throat. On physical examination, this patient had white, plaque-like lesions in her throat. A KOH wet mount of scrapings of one of these lesions was examined microscopically and revealed the organism seen in Fig. 13.3. In this tissue section, note the yeast forms and pseudohyphae. This is consistent with the presence of *Candida* spp. *Candida albicans* is the leading cause of oral thrush, a common opportunistic infection in AIDS patients. This patient's dysphagia, sore throat, white, plaque-like lesions, and laboratory findings are consistent with oral thrush. She was treated with oral fluconazole, a triazole antifungal agent, and her symptoms resolved. Similarly to *P. jirovecii* pneumonia, thrush is generally seen only in patients with CD4 counts ≤200/μl.

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A 37-year-old man was admitted to the hospital with an increased white blood cell count and a peripheral smear consistent with acute leukemia. A bone marrow biopsy found 70 to 80% blast forms, diagnostic of acute myelomonocytic leukemia. The patient underwent induction chemotherapy. Following the chemotherapy, a repeat bone marrow biopsy again demonstrated blast forms. He therefore underwent a second round of induction chemotherapy, after which he became profoundly neutropenic (with <100 neutrophils/μl) and developed fevers without a clear source. Broad-spectrum antibacterial therapy was begun, but the fevers persisted. Empiric intravenous amphotericin B therapy was begun, and a subsequent chest radiograph revealed new bilateral fluffy pulmonary infiltrates. A bronchoscopy with biopsy was performed; the specimen demonstrated septate hyphae with acute-angle branching (Fig. 14.1). The organism recovered from the biopsy is seen in Fig. 14.2.

1. What is the differential diagnosis of pulmonary infiltrates in a leukemic patient?
2. Based on Fig. 14.1 and 14.2, what organism most likely caused this patient's infection? Why was biopsy and not lavage necessary to make this diagnosis? If direct examination was negative in this patient, what additional laboratory test could be done that would support the diagnosis of an invasive infection with the organism seen in Fig. 14.1 and 14.2?
3. What predisposed this patient to this infection?
4. Where in nature is this organism found?
5. This organism is associated with at least three different disease processes. What are they? What is the role of the immune system in these processes?



Figure 14.1 Calcofluor white stain of biopsy material from patient.

6. Patients who undergo hematopoietic stem cell transplants (HSCTs) are frequently given either echinocandins or azoles prophylactically when they are neutropenic. What potential infecting organisms is this therapy intended to prevent?

7. Would blood cultures have been useful in helping to make this diagnosis? Explain your answer.

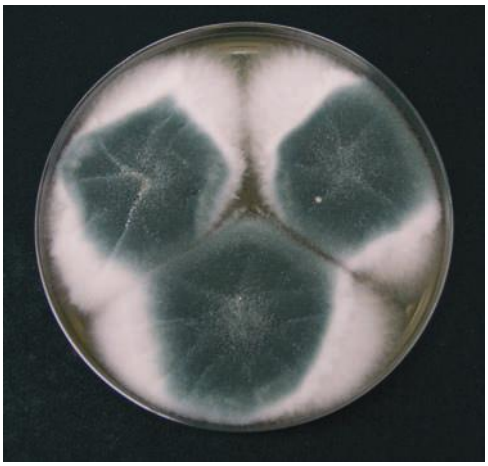


Figure 14.2 Organism growing from patient's transbronchial biopsy.



CASE DISCUSSION

1. Infectious causes of pulmonary infiltrates in leukemic patients include bacteria (especially Gram-negative rods and *Staphylococcus aureus*), fungi (including *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., zygomycetes, *Pneumocystis jirovecii*, *Candida* spp., and *Trichosporon* spp.), and viruses (particularly cytomegalovirus). Noninfectious causes of pulmonary infiltrates in these patients include bleeding into the lung, leukemic infiltrates, and drug toxicity, most often due to a chemotherapeutic agent.

2. The detection of fungal elements in a tissue biopsy by microscopic examination is a key criterion for confirming a patient as having an invasive fungal infection. The morphology of the fungal elements can give clues to the identification of the organism. In Fig. 14.1, the presence of septate hyphae (3 to 4 μm in diameter) with acute-angle branching is consistent with the presence of an *Aspergillus* species. However, other fungi with similar microscopic morphology, *Fusarium* spp. and *Scedosporium* spp., may also be seen in neutropenic patients. Therefore, definitive identification requires culture. Of the other fungi that often cause infections in neutropenic leukemia patients, *Candida* spp. and *Trichosporon* spp. are yeasts; *P. jirovecii* is a yeast-like organism. On microscopic examination the zygomcyetes are aseptate, typically branch at a right angle, and have a more ribbon-like appearance than do *Aspergillus* spp.

The organism that grew from the patient was *Aspergillus fumigatus* (Fig. 14.2), the most common cause of invasive fungal pulmonary infections in neutropenic hosts. Other clinically significant species of *Aspergillus* include *A. flavus* and *A. niger*. This patient had invasive aspergillosis, which produces a necrotizing pneumonia. In this disease, the organism actually grows into the tissue and often is not found superficially in the airway. Therefore, lavage is not sufficient and a tissue sample is needed to make this diagnosis.

An alternative approach to the diagnosis of invasive aspergillosis is the use of fungal antigen tests. Two are available, galactomannan and β-D-glucan, two distinct carbohydrates found in abundance in *Aspergillus* cell walls. Both are released during invasive infections and can be detected in blood or bronchoalveolar lavage fluid (BALF). Detection of both antigens is superior in BALF compared with serum or plasma. Galactomannan detection in BALF is preferred over β-D-glucan because it has superior positive and negative predictive value for detection of *Aspergillus*. In particular, *Candida* spp., the other common agents of invasive fungal disease, are detected by the β-D-glucan assay but not the galactomannan assay. Additionally, there are many more potential causes of false-positive β-D-glucan tests compared with galactomannan. When only serum or plasma is available, both tests have poorer performance, resulting in the need for testing multiple serum/plasma samples to obtain a reliable answer.

3. This patient had neutropenia, a dramatically decreased number of neutrophils in his peripheral blood. This condition predisposes to invasive infections not only by bacteria but also by fungi, including fungi of low virulence such as *Aspergillus* spp. The risk of infection by fungi is related to both the severity and the duration of the neutropenia. Individuals undergoing bone marrow transplantation are at particular risk for infection with *Aspergillus* spp. since they have to endure prolonged periods of neutropenia during the transplant process. This patient not only had very few neutrophils (sometimes none were detected) but also was neutropenic for a prolonged period. Despite treatment with intravenous amphotericin B, many leukemic patients succumb to invasive fungal infections. In this case, the patient's neutrophil count began to rise, and he survived the infection. The return of functioning neutrophils was central to this patient's ability to resolve this infection.

4. *Aspergillus* species can be isolated from grains, hay, decaying vegetable matter, soil, and plants. Aflatoxin, a potent carcinogen that has been linked to hepatocellular carcinoma, is produced by strains of *A. flavus* on improperly stored grains and nuts. *Aspergillus* spores are present in the air. Therefore, humans are constantly exposed to (and breathe) spores of these organisms. Because of the constant exposure to these spores, people may become colonized by *Aspergillus* species. Neutropenic patients who become colonized often will develop clinical disease. The finding of a positive respiratory tract culture in the neutropenic host should be managed aggressively (see answer to question 5 for more details). In contrast, the finding of this organism in the respiratory tract of an immunocompetent host is less likely to be clinically significant.

5. The type of disease process that patients develop from *Aspergillus* spp. is dependent on their immune status. These can be divided into three broad categories: invasive aspergillosis, pulmonary mycetoma (a "fungus ball" that often forms in a preexisting pulmonary cavity such as in patients with prior cavitary tuberculosis) due to *Aspergillus*, and allergic bronchopulmonary aspergillosis (in patients with preexisting chronic lung disease). Other less common processes are infections of the external ear, nasal sinuses, eyes (following corneal trauma), and heart valves.

The immune status of the host plays a central role in determining which of these three disease processes might develop. Humans are exposed daily to hundreds of *Aspergillus* conidia, which are the reproductive structures for molds. Typically when humans inhale fungal conidia, the conidia can be cleared either by mucociliary clearance or by phagocytosis by alveolar macrophages and neutrophils. Three factors make *Aspergillus* spp. particularly well adapted to infect humans if they can evade the human innate immunity. First, they can grow at human body temperature. Human core body temperature is too hot for the growth of most environmental, saprophytic mold species. Second, the conidia have a small enough diameter, 2 to 3 μm, to allow them to easily bypass mucociliary clearance and enter the alveoli. Third, if innate immunity is dysfunctional in the alveolar space, the

organism's adaptation to living in the natural environment means that it has a variety of enzymatic systems and the ability to scavenge iron, allowing it to invade tissue.

Invasive aspergillosis is the most severe type of *Aspergillus* infection. It is most likely to occur in four patient populations: (i) neutropenic hosts (secondary to ablation therapy for leukemia or HSCT); (ii) solid-organ transplant recipients with rejection; (iii) HSCT recipients with graft-versus-host disease whose neutrophil function is suppressed by immunosuppressive agents, especially corticosteroids; and (iv) patients such as those with chronic granulomatous disease whose neutrophils are dysfunctional. The severity of the disease in the neutropenic host is based on the length of the neutropenic state. In the solid-organ transplant recipient with rejection and in patients with graft-versus-host disease, disease severity is dependent on the length and degree of immunosuppression from immunosuppressive agents. Corticosteroids, in particular, are known to impair the killing by neutrophils of *Aspergillus* conidia. In patients with chronic granulomatous disease, the severity of the illness is based on the ability to successfully remove *Aspergillus*-infected lung tissue. Mortality rates are 40 to 100%, depending on the severity of the immunosuppressed state.

In patients with deficient innate immunity, germination of inhaled conidia may be unimpeded. Hyphal elements can invade endothelial cells, causing changes in these cells that may result in thrombosis and subsequent tissue infarction. The resulting necrotic tissue is an ideal environment for tissue destruction by the variety of degradative enzymes produced by this saprophyte. A particularly problematic form of invasive aspergillosis, tracheobronchitis, occurs following lung transplantation. In this disease manifestation, the anastomosis can be infected and, if the infection is severe, can result in graft dehiscence, endangering the transplant.

The pathogenic process in patients with pulmonary mycetoma is quite different. The conidia access a preexisting cavity secondary to diseases such as emphysema, sarcoidosis, or tuberculosis. In the cavity, the organism forms a mass of hyphae, or "fungus ball." Generally the patient remains asymptomatic. However, invasion of pulmonary parenchyma may occur, leading to hemoptysis and, in its most severe form, hemorrhage.

Allergic bronchopulmonary aspergillosis is the result of a hypersensitivity reaction to hyphal antigens found in the bronchi. This disease is most frequently found in patients with asthma and is especially common in patients with cystic fibrosis. The hyphal antigens stimulate a Th2 response, resulting in excessive mucus production, inflammation, and the presence of eosinophils in the bronchi. This can result in obstruction of the bronchi and atelectasis. This diagnosis may be particularly difficult to make in cystic fibrosis patients since the underlying chronic bacterial infection may cause similar pathogenic processes and ultimately may result in pulmonary fibrosis and loss of lung function. Paradoxically, corticosteroids, a known inducer of invasive aspergillosis, along with antifungal therapy are central therapeutic agents.

6. The major fungal pathogens in neutropenic patients are *Candida* spp. and *Aspergillus* spp. The goal of antifungal prophylaxis during neutropenia is to prevent invasive infec-

tions with these organisms. Unfortunately, this prophylaxis is ineffective against *Fusarium*, *Trichophyton*, and the zygomycetes. Although all of these agents are still much less frequent causes of invasive disease in neutropenic HSCT recipients, they may cause invasive disease despite the use of antifungal prophylaxis and cause devastating invasive disease with a high mortality rate.

7. Because they are almost never positive in patients with invasive aspergillosis, blood cultures have little diagnostic value. In patients with invasive aspergillosis, the organism has a predilection for invading endothelial cells and, as a result, is rarely detected in the bloodstream. Fungal agents that are recovered from blood cultures include *Candida* spp., *Cryptococcus neoformans*, *Trichosporon*, and the dimorphic fungi *Histoplasma capsulatum* and *Blastomyces dermatitidis*. *Fusarium* is the only mold that is recovered with any degree of frequency from routine broth blood cultures. See case 68 for additional details.

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This 62-year-old man presented with a 4-day history of left eye swelling and ptosis along with left frontal headache. He had an unremarkable medical history, although his family history was strongly positive for diabetes mellitus. On examination, the patient was febrile to 38.1°C (100.6°F) and had complete left ptosis. Laboratory studies were notable for an elevated white blood cell count of 17,900/μl with 14,400 neutrophils/μl, an elevated blood glucose level of 484 mg/dl, and urinalysis positive for the presence of ketones. A computed tomography scan of the sinuses and orbits was notable for fluid in both ethmoid sinuses and inflammatory changes lateral to the left medial rectus muscle. The patient underwent surgery (a left external ethmoidectomy). A calcofluor white preparation of the material from the left ethmoid sinus obtained at the time of surgery demonstrated broad, aseptate hyphae with right-angle branching (Fig. 15.1). The resulting culture is seen in Fig. 15.2.

1. Which organisms are consistent with these microscopic findings?
2. What is the natural habitat of this organism? How did this patient likely become infected? What is the explanation for this patient having ptosis?
3. What criteria are needed to demonstrate that a patient is infected with a fungal agent? Were they met in this patient? Explain.
4. Infections with this organism are frequently difficult to detect. Why is this? Are there alternative methods that can be used to detect this organism?
5. In what clinical conditions are these organisms most likely to be seen? Explain why each one of these conditions predisposes the patient to those infections.

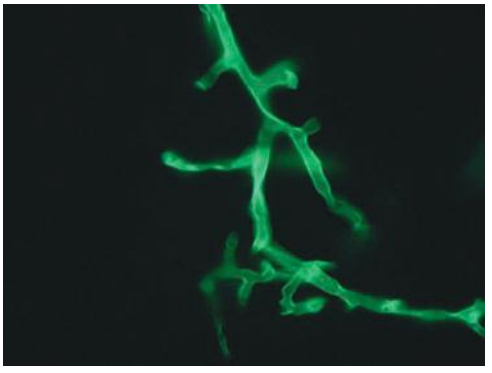


Figure 15.1 Calcofluor white examination of left ethmoid sinus biopsy.

6. How is this infection managed? What is the prognosis for this patient?
7. Infections with the group of organisms infecting this patient have been associated with natural disasters. Give an example of such a natural disaster and why this organism is associated with it.



Figure 15.2 Culture from sinus specimen.



CASE DISCUSSION

1. The presence of broad, aseptate, or sparsely septate hyphae with right-angle branching showing a ribbon-like, frequently folded appearance (Fig. 15.1) is diagnostic of an agent of the class *Mucormycetes*. The disease entity is called mucormycosis. The mucormycetes belong to the fungal order *Mucorales* and include molds from the genera *Mucor*, *Rhizopus*, *Rhizomucor*, and *Cunninghamella*. In surgical sections of clinical material, these genera are indistinguishable. It is important to determine the actual genus and species because of differential response to antifungal therapy. In clinical specimens including surgical sections, they can typically but not always be differentiated from the major cause of invasive fungal infection, *Aspergillus* species, because *Aspergillus* species have acute-angle branching (instead of right-angle branching as with agents of mucormycosis), frequent septations, and thinner hyphae.

Mucormycotic infections are extremely aggressive and frequently fatal. They represent a true medical (and surgical) emergency, and differentiation from *Aspergillus* species can be crucial. Culture results showed that this patient was infected with a *Rhizopus* species, which is responsible for approximately 70% of mucormycoses. As can be seen in Fig. 15.2, there is abundant growth of this organism after only a few days of incubation, with the mycelial elements "filling" the plate.

2. The agents of mucormycosis are commonly found in the environment, on fruit and bread, on other organic matter such as decaying wood, and in soil. In fact, sterile bread devoid of preservatives (which may prevent the growth of mucormycetes) can be used as a sporulation medium for these organisms.

In patients who develop rhinocerebral mucormycosis, the infection occurring in this patient, conidia of the fungus are inhaled from the environment. Fungi then begin to grow in the sinuses. Mucormycetes are angioinvasive. They invade blood vessels in tissue surrounding the sinuses. Angioinvasion can result in blood vessel thrombosis and infarction. Ptosis, seen in this patient, indicates necrosis of the orbital muscles secondary to thrombosis in the vessels supplying those muscles.

3. Because fungal conidia are ubiquitous environmentally, patient airways can frequently be transiently colonized with molds. To demonstrate invasive infections in patients such as the one seen here, specific criteria must be met. To make the definitive diagnosis of rhinocerebral mucormycosis, the organism must be observed in tissue section, seen in direct microscopic observation of sterile tissue such as biopsy material, or grown from normally sterile tissue. This biopsy tissue was considered sterile, so the observation of the organism microscopically and its growth from the biopsy was considered definitive evidence of invasive disease. If the organism had been observed or grown from sinus drainage or an aspirate of the sinus, it would be considered a probable cause of infection. The reason for this interpretation is that the sinuses might be colonized with the organism, and it

is possible that it was not causing the clinical disease being observed. To make the definitive diagnosis of a fungal infection, demonstration of the organism in tissue or normally sterile body fluids either by microscopic observation or culture is essential.

4. Only 50% of patients with invasive mucormycosis have the organism identified premortem either microscopically or by culture. Invasive molds such as the mucormycetes and *Aspergillus* are angioinvasive. As a result, these organisms are not found in blood cultures. The mucormycetes are quite delicate organisms and can be crushed and killed by grinding tissues. To isolate these organisms from tissue, it is important to mince the tissue using a scalpel or scissors. Many of the patients who have these infections have hematologic malignancies. One of the complications of these malignancies is low platelet counts that preclude obtaining tissue specimens for diagnosis because of concerns about bleeding.

Nonculture methods such as β-D-glucan and galactomannan have been used to support the diagnosis of invasive fungal infection, especially with *Aspergillus*. Unfortunately, these antigens are not made by the mucormycetes, so they have no value in the diagnosis of infections due to this group of organisms. PCR amplification and direct sequencing of mucormycete-specific genes from blood and tissue currently remain research tools. The insensitivity of culture and the lack of alternative diagnostic methods make the diagnosis of mucormycosis difficult. In profoundly immunocompromised patients in whom these infections are most common, failure to diagnose and thus treat these infections frequently results in a fatal outcome.

5. Mucormycosis is associated with several clinical conditions.

Patients with poorly controlled diabetes mellitus, particularly those with ketoacidosis (as was seen in this patient), are most likely to develop rhinocerebral mucormycosis. Interestingly, approximately half of patients diagnosed with rhinocerebral mucormycosis do not know that they are diabetic at the time of their infection. This was true for the patient presented in this case.

Two factors seem to be important. High glucose levels, as were seen in this patient, are recognized to inhibit phagocytic cell function, allowing evasion of immune clearance. Second, mucormycetes grow better in comparatively acidic conditions such as ketoacidosis seen in diabetics. The findings of highly elevated blood glucose and ketones in urine indicate that this patient had ketoacidosis.

Two other patient populations in which invasive mucormycosis disease is frequently seen are hematopoietic stem cell transplant (HSCT) recipients and burn patients.

In the early stages of HSCT, patients are neutropenic. They are given echinocandin prophylaxis to prevent invasive *Aspergillus* infection, a highly feared early complication of HSCT. However, mucormycetes are resistant to echinocandins, making these patients susceptible to invasive mucormycosis. In later stages of HSCT, graft-versus-host disease is common. This condition is treated with high-dose corticosteroids. This therapy, which suppresses cell-mediated immunity involved in this disease process, places these patients

at increased risk for mucormycosis since cell-mediated immunity is central to the immune control of these organisms. These patients typically present with sinopulmonary disease, with the organism arising in the sinuses and spreading to the lung. Single or multiple nodular lesions may be observed radiographically in the lungs of these patients.

Burn patients are also at increased risk for infection with the mucormycetes. The skin is the major organ in the immune system. When patients receive full-thickness burns, they are at risk for infections with a variety of environmental organisms including mucormycetes. If patients receive burns outdoors, they frequently roll on the ground, where they can introduce fungal conidia into the burn wound.

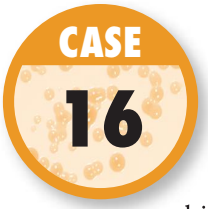
6. Since rhinocerebral mucormycosis, when untreated, runs a progressive and fatal course, early recognition and a high degree of suspicion are necessary. It was the suspicion of mucormycosis that caused the surgeon in this case to obtain a frozen section intraoperatively, a procedure in which the pathologist examines the tissue as rapidly as possible (without the standard techniques used to fix tissue) while the patient is still in the operating room. Often, multiple frozen sections are examined during surgery. Debridement of the infected area continues until a frozen section is obtained from the surgical site margin in which the organisms are no longer seen. The required surgical debridement is frequently extensive, resulting in disfigurement. In addition to aggressive surgical removal of infected and necrotic tissue, treatment includes antifungal therapy and medical management, including the correction of the underlying condition, such as diabetic ketoacidosis.

One of the major treatment challenges faced with mucormycetes is their high degree of antifungal resistance. Mucormycetes are resistant to the echinocandins and the azoles, with the possible exception of posaconazole. Liposomal amphotericin B is the antifungal treatment of choice because it is better tolerated by the recipient and is less nephrotoxic than amphotericin B. Interestingly, there have been small clinical studies that suggest that the combination of liposomal amphotericin B and echinocandins has better efficacy than liposomal amphotericin B alone in rhinocerebral mucormycosis.

7. A 2011 outbreak of necrotizing cutaneous mucormycosis occurred after a force 5 tornado in the central United States. This outbreak included 13 individuals, 5 of whom died. The etiology was an infrequently encountered mucormycete, *Apophysomyces trapeziformis*. Outbreaks due to mucormycetes have also occurred following tsunamis and volcanic eruptions. In these outbreaks, patients receive significant trauma whereby soil and organic debris such as pieces of wood are introduced into traumatic injuries. In the most recent outbreak, the patients had multiple traumatic, penetrating injuries. Death in at least some of the patients in the recent outbreak was likely the result of the traumatic injuries rather than infection.

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This 38-year-old North Carolina man was in good health until 2 months prior to admission, when he developed a low-grade fever, myalgias, and a nonproductive cough. He was given oral erythromycin by his local physician. After 2 weeks of therapy, his condition had not improved. A chest radiograph demonstrated "right middle lobe air space disease," and therapy with oral ampicillin was begun. Over the next month his condition worsened. He noted daily fevers, chills, night sweats, and a 15-lb (7-kg) weight loss. One month prior to admission a chest radiograph demonstrated consolidation of the right middle lobe. A purified protein derivative skin test was negative with positive controls, and an oral antibacterial agent was given. The patient's symptoms continued, and he was admitted to the hospital.

The patient had an unremarkable travel history and no animal exposure, was a nonsmoker, and had no HIV risk factors. He worked for the power company cutting tree limbs and tops. On physical examination he was febrile to 38.3°C. The skin examination was notable for a tender, raised, erythematous papule (1 by 1 cm) on the bridge of the nose (Fig. 16.1). A chest radiograph and subsequent computed tomogram scan were notable for a densely consolidated right middle lobe, a 3.5-cm subcarinal mass, and a small right hilar mass. Bronchoscopy was performed. Calcofluor-KOH, acid-fast, modified acid-fast, and Gram stains gave negative results. Examination of the skin lesion using a silver stain demonstrated a large, round budding yeast with a broad base connecting the mother cell to the daughter cell (Fig. 16.2).

1. What is the differential diagnosis for this patient's pulmonary disease?
2. Which organism was causing his illness? What are its epidemiology and culture characteristics?



Figure 16.1

3. This patient's lungs and skin were involved with this infection. Which other sites are commonly involved?
4. What in this patient's history might alert a physician to think of this organism?
5. Which organisms may be detected by a calcofluor-KOH examination? An acid-fast stain? A modified acid-fast stain? What other diagnostic tests are available for the diagnosis of infection by this organism?

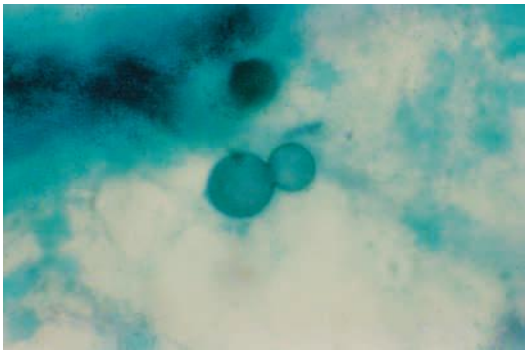


Figure 16.2



CASE DISCUSSION

1. The patient had a hilar mass and a densely consolidated right middle lobe. The differential diagnosis includes both noninfectious processes (such as malignant and benign tumors) and chronic infections with slowly growing organisms, such as fungi (including *Blastomyces dermatitidis*, *Coccidioides posadasii*, *Coccidioides immitis*, and, less likely, *Histoplasma capsulatum*), mycobacteria (especially *Mycobacterium tuberculosis*), and other slowly growing bacteria such as *Actinomyces* and *Nocardia* spp.

2. The etiologic agent of this individual's illness was *B. dermatitidis*. The morphology of the organism seen in Fig. 16.2, a fairly large, broad-based budding yeast, is typical of this organism. It is a dimorphic fungus, so at room or ambient temperature it grows as a mold and at body temperature (37°C) it grows as a yeast. It is the etiologic agent of North American blastomycosis and should not be confused with *Paracoccidioides brasiliensis*, the agent of South American blastomycosis. *B. dermatitidis* is endemic in much of the southeastern United States. Other regions where it is endemic include areas within the Mississippi and Ohio River basins, small areas in western New York State, and Canadian provinces bordering the Great Lakes and adjacent to the St. Lawrence Seaway. There have also been sporadic cases elsewhere, both in the United States and in other countries.

3. Other sites that are frequently infected are bone, joints, and the genitourinary tract. This patient subsequently returned with pain in his shins. A bone scan showed multiple lesions, especially in his long bones. An aspirate of a bone lesion that was sent for fungal culture grew *B. dermatitidis*. He also was at risk for infection of the prostate and epididymis, both of which are common sites in men with disseminated infection. Physical examination of his prostate was normal. Detection of a prostatic infection due to this organism is important because it requires prolonged antifungal therapy.

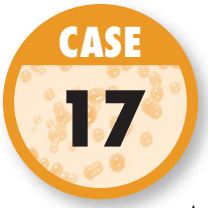
4. The patient's symptoms were quite nonspecific. However, he failed to respond to three different regimens of antimicrobial therapy designed to treat common bacterial agents of community-acquired pneumonia, such as *Mycoplasma pneumoniae* and *Streptococcus pneumoniae*, and agents of bronchitis, such as *Haemophilus influenzae* and *Moraxella catarrhalis*. The weight loss, low-grade fevers, and indolent clinical course are all suggestive of *M. tuberculosis* infection. However, tuberculosis usually presents with upper lobe involvement, and a negative purified protein derivative skin test with positive skin test controls also argues against this infection. Patients with an indolent disease course and a nonproductive cough over extended periods may have pulmonary mycoses. The finding of the skin lesion on the face, a frequent occurrence in blastomycosis, further supports this diagnosis. Though the isolation of the organism from the environment has been problematic, the patient's occupation probably increased his risk for this infection. This organism can be recovered from decomposing wood. He probably was infected by inhaling spores

while cutting down dead trees or branches. His skin infection was secondary to his primary pulmonary process.

5. On the basis of this patient's clinical presentation, a wide variety of microorganisms would be included in the differential diagnosis. Different techniques are required to best demonstrate the different organisms that need to be considered. An acid-fast stain was done to detect mycobacteria. Despite the negative skin test and atypical chest radiograph for tuberculosis, *M. tuberculosis* must still be considered, as must other mycobacteria. The modified acid-fast stain is used to detect *Nocardia* spp., which could cause infections with a case presentation similar to this patient's. Finally, calcofluor-KOH examination is a commonly used technique to demonstrate fungi in clinical specimens. Fungi are fairly refractory to the activity of KOH while human tissues are dissolved, clearing the specimen and making the microscopic demonstration by calcofluor staining of the fungi much easier. Calcofluor white nonspecifically binds chitin and cellulose and is particularly usefully for highlighting the cell walls of fungi in specimens. The sensitivity of calcofluor-KOH examination of sputum has ranged from 50 to 90% in patients with pulmonary blastomycosis. Other special stains (such as methenamine silver or periodic acid-Schiff) may demonstrate the presence of fungal elements in histologic specimens and are most often used to stain specimens from patients with extrapulmonary disease. Sputum culture has a high yield, and culture of bronchial washings in patients with pulmonary blastomycosis approaches 100%. Once there has been growth of the organism, the confirmation of the identification is often performed with a commercially available chemiluminescent DNA probe. Although a urinary antigen test specific for *B. dermatitidis* is not available, *Blastomyces* is known to cross-react with the *H. capsulatum* antigen test, which may prove useful in clinical care. Serologic testing for *B. dermatitidis* is both insensitive and lacking in specificity.

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This 26-year-old man presented for evaluation of a neck mass and a right axillary mass. The patient, who came to the United States from Vietnam 6½ years ago, noted a right axillary mass 1 month prior to admission. The mass gradually increased in size. Approximately 3 weeks prior to admission he noted a midline neck mass. These masses were incised and drained twice. Samples of purulent material were sent for routine bacterial culture at an outside hospital. The patient had lost 3 kg (ca. 7 lb) in the 2 months prior to admission but denied fever. Travel history was notable for his having lived in Arizona for 6 years prior to moving to Boston.

On examination, he was afebrile. An erythematous, fluctuant, nontender mass (8 cm by 6 cm) was present in the midline of the neck (Fig. 17.1; computed tomography [CT] scan in Fig. 17.2). The right axilla demonstrated incision sites that were draining and were tender to palpation. He had no adenopathy elsewhere. His PPD (purified protein derivative) skin test was reactive, and his chest X ray was notable for apical scarring in the right lung.

1. Because of the reactive PPD and apical scarring seen on the patient's chest X ray, the clinical suspicion of cervical tuberculosis (scrofula) was high, and the patient was begun on a four-drug anti-tuberculous regimen. Other than tuberculosis, what is in the differential diagnosis of the neck and axillary mass?

2. Several days after the neck mass was drained of several milliliters of purulent material for culture, a mold was found to grow on the blood agar plates in the routine bacteriology section of the microbiology laboratory. The laboratory technologist did not notice the presence of the mold and opened the plates to examine them for bacteria. Why is this of concern?

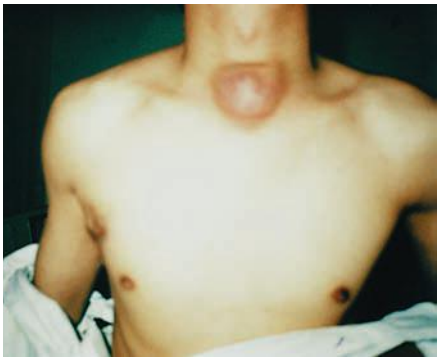


Figure 17.1



Figure 17.2

3. The mold was white (Fig. 17.3) and initially did not have any identifying characteristics when a lactophenol cotton blue preparation was examined under a phase-contrast microscope. It was subcultured at both room temperature and body temperature, and after subculture began to demonstrate the presence of arthroconidia microscopically (Fig. 17.4). The identification was confirmed by using a commercially available genetic probe. What is this organism?
4. What are the most common clinical manifestations following an infection by this organism? What in this patient's history makes him more likely than the U.S. population as a whole to have disseminated disease? What other body sites does this organism commonly involve?
5. The incidence of disease with the organism infecting this patient has been increasing. What are possible explanations for this?
6. This organism is being seen with apparent increasing frequency in patients who have received solid-organ transplants. What is the explanation for this observation?

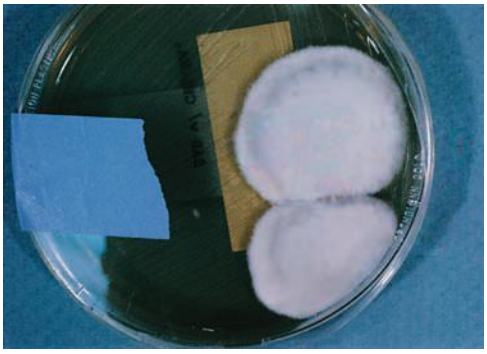


Figure 17.3

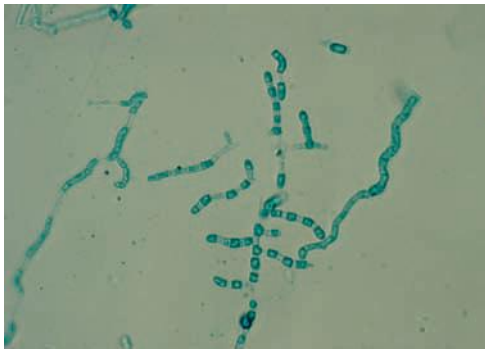


Figure 17.4



CASE DISCUSSION

1. The differential diagnosis includes several causes of subacute enlarging masses, both noninfectious and infectious. Of noninfectious causes, the most important are malignancies, such as lymphoma. Among the infectious causes, those etiologic agents that progress slowly typically include not only *Mycobacterium tuberculosis*, of which the reactive PPD and apical scarring on his chest X ray are supportive, but also other mycobacterial, fungal, and even some bacterial infections. Among the fungal causes, the patient's history of travel from Vietnam is suggestive of the thermally dimorphic fungus *Penicillium marneffei*, which is found in Southeast Asia and is the third most common opportunistic infection among AIDS patients there (behind tuberculosis and cryptococcal disease). Another fungus that must be considered, given the patient's history of recently living in Arizona, is *Coccidioides immitis* (or *Coccidioides posadasii*, which appears to be the more common of the two in Arizona and can only be distinguished from *C. immitis* via genetic tests). This dimorphic fungus is found in the soil in arid areas of the United States, Central America, and South America that correspond to the Lower Sonoran Life Zone. The patient does not have any relevant exposure history to the other dimorphic fungi (*Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and *Histoplasma capsulatum*) that are found in rather well-defined geographic regions in the Western Hemisphere. Among bacteria, the rather slowly growing *Actinomyces* spp. and *Nocardia* spp. can certainly cause involvement of the neck, chest wall, and contiguous tissues. *Actinomyces* spp. must be sought by obtaining appropriate anaerobic cultures.

2. Laboratory-acquired infections with dimorphic fungi are a real risk to the clinical microbiologist. During the process of opening and examining a petri dish, it is possible for the arthroconidia of *Coccidioides* spp., which are easily aerosolized, to become airborne and to infect a laboratory worker. This has been well documented and has actually resulted in the deaths of laboratory workers. As a result, clinical laboratories routinely require the use of biological safety cabinets for the isolation and identification of molds. In addition, all fungal cultures that are planted on petri dishes are routinely closed with either tape or a commercially available product such as Shrink Seal to prevent the plates from being inadvertently opened. This is not, however, the routine for bacterial cultures. Occasionally a mold grows on bacterial media. It is important that technologists be aware of this possibility and as a matter of good safety technique acquire the habit of looking at the plates before opening them, to minimize the risk of laboratory-acquired infection. The other, less important reason why fungal cultures should only be opened in a biological safety cabinet is to prevent cross-contamination of other cultures by the fungal conidial elements.

3. The presence of barrel-shaped arthroconidia is consistent with *Coccidioides* spp. (Fig. 17.4). Arthroconidia are formed by the fragmentation of hyphae during sporulation. Although there are several other fungi that produce arthroconidia, they are not likely to produce this clinical syndrome. The patient, as noted above in the answer to question 1,

lived in Arizona for 6 years, an area with the highest incidence of coccidioidomycosis in the United States. Generally, to definitively identify thermally dimorphic fungi, it is necessary to demonstrate that the fungus can convert from a mold form (at room temperature) to a yeast form (at body temperature), to demonstrate the presence of an antigen characteristic of the organism, or to identify the fungus with a genetic probe or by genetic sequence. Since the nonmold phase of *Coccidioides* is a spherule in tissue and does not grow in the laboratory, the mold was sent to a reference laboratory that had the commercially available genetic probe. This helped to speed its identification.

4. The majority of *Coccidioides* infections are asymptomatic or cause a flu-like illness/pneumonia characterized by fever, cough, dyspnea, and myalgias. The flu-like illness caused may be referred to as Valley fever because this infection is common in the San Joaquin Valley of California. Disseminated infection with *C. immitis* or *C. posadasii* is unusual (<1 to 5%) and is more common in nonwhites than it is in whites. Populations in which there is an increase in the risk of dissemination include Latinos, African-Americans, pregnant women, and immunocompromised patients, including those with solid-organ transplants, patients with AIDS, and people who receive chemotherapy. Studies have indicated that the risk of disseminated infection in Filipinos may range from 10 to 175 times the risk in Caucasians. Although there are no known data specifically on the risk of disseminated disease in persons of Vietnamese heritage, it is likely that there is an increased risk of dissemination among this population as well. In some patients (<5%), *C. immitis* or *C. posadasii* may spread to bone, meninges (which may be life-threatening), or skin.

5. Recent epidemiologic studies (1998 to 2011) have shown an 8-fold increase in coccidioidomycosis infections in the United States. Almost all the cases (>95%) were seen in individuals in Arizona (two-thirds of cases) and California (one-third), with a small number of cases in Nevada, New Mexico, and Utah. One of the problems with the observation of a dramatic rise in infection rates is that a change in the definition of what constitutes an infection or an improvement in diagnostic testing may increase the rate compared with historical norms. It is unlikely, however, that such a dramatic rise could be attributed to these two factors alone. It is far more likely that the rise in the infection rate is real. During the first decade of this century, Arizona, the state with the greatest number of cases, saw a 26% increase in population. Notably, this included a 73% increase in the number of African-Americans, who are known to have a much higher rate of invasive disease than the rate for Caucasians. Environmental disruption due to construction activities to accommodate the rising population likely resulted in increased aerosolization of arthroconidia from soil, leading to increased exposure to and infection with this organism.

6. There are an increasing number of case reports of invasive disease due to *Coccidioides* in solid-organ transplant patients. There are two possible explanations for this observation. One is that transplant recipients are latently infected with the organism prior to transplant and the immunosuppression results in reactivation of infection, leading to

clinical disease. Cell-mediated immunity is well recognized as playing a central role in the control of infection with this organism. Suppression of that arm of the immune system would put patients at increased risk for both reactivation and acquisition of many infectious agents, including *Coccidioides*. These patients would need to live in or have visited a region in which *Coccidioides* is endemic. Transplant recipients who have not visited such a region can also develop this infection posttransplant. We previously reported a case of a lung transplant recipient who had never left the state of North Carolina who received lungs from a patient who had visited northern Mexico 2 years prior to organ donation. He developed a rapidly progressive invasive infection soon after transplant that proved fatal. At autopsy, the patient had *Coccidioides* spherules (Fig. 17.5) in his lungs. We believe the organism was present in the transplanted lungs and caused invasive disease due to the immunosuppressive therapy he was receiving.

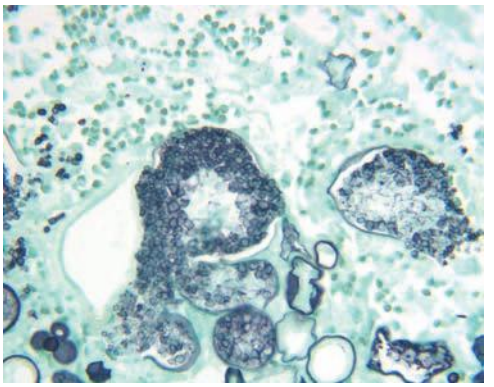


Figure 17.5 *C. immitis* spherules (from reference 5 with permission).

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The patient was a 59-year-old female who underwent a cardiac transplant 6 months earlier for an idiopathic cardiomyopathy. At the time of transplant she was seropositive for cytomegalovirus (CMV) and seronegative for HIV, hepatitis B, and hepatitis C. Her heart donor was CMV seropositive and HIV, hepatitis B, and hepatitis C negative. Since the transplant she had done reasonably well, with the exception of two episodes of acute rejection that required increased doses of immunosuppressive agents to control rejection. One week prior to this admission she complained of malaise, fatigue, a low-grade fever, and mild dyspnea on exertion. She was admitted to determine the etiology of her complaints. The physical examination was significant only for a temperature of 38.3°C and cushingoid body habitus (due to the steroids). Examination of her lungs revealed fine bibasilar rales. A stool specimen was guaiac positive. Her laboratory studies revealed a hematocrit of 24%, a white blood cell count of 2,300/μl (leukopenia), and a normal platelet count. She was transfused with 3 units of blood and underwent upper gastrointestinal endoscopy, which revealed nodular gastric erosions. Biopsies and brushings were taken and submitted to the pathology and microbiology laboratories. A chest radiograph revealed diffuse infiltrates. A bronchoscopy was done, and transbronchial biopsy and bronchoalveolar lavage specimens were sent for histopathologic and cytologic examination and bacterial, fungal, viral, and mycobacterial cultures. Gram stains were negative. The next day, Papanicolaou stains of the gastric lesion brushings, as well as the lung tissue, revealed the cause of her infection (Fig. 18.1). Additionally, molecular testing of her blood detected a virus at 2,800 copies/ml.

1. What was the most likely etiology of the patient's infection? How did she become infected?
2. What is the typical clinical presentation of this organism in patients such as this woman who have received solid-organ transplants?

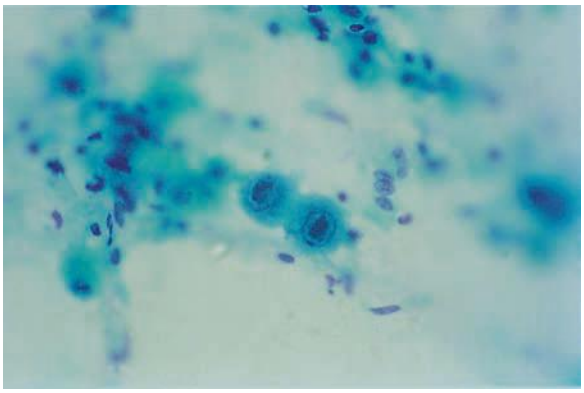


Figure 18.1 Papanicolaou staining of gastric brushing.

3. Which other two patient populations are subject to serious infections with this organism?
4. Which other opportunistic infections are seen with some degree of frequency in patients receiving cardiac transplants?
5. What diagnostic approaches are available to detect this organism?
6. What strategies are employed to attempt to prevent this infection in individuals receiving solid-organ transplants?
7. What agents are available for treatment of this infection? What drug resistance problems, if any, have been observed with these agents?

CASE DISCUSSION



1. The characteristic "owl's-eye" cell (containing large intranuclear inclusions) seen in Fig. 18.1 is characteristic of CMV, one of the human herpesviruses. CMV is an enveloped, double-stranded DNA virus and is the most common infectious agent complicating transplantation. This virus was the cause of the patient's gastritis and pneumonitis. In the absence of antiviral prophylaxis, CMV infections usually occur 1 to 3 months posttransplant. However, depending on the length of prophylactic therapy, the window for CMV infection can extend to beyond a year (see answer 7). CMV, like other herpesviruses, causes lifelong latent infection. In patients who are CMV positive pretransplant, i.e., latently infected, infections posttransplant are usually the result of reactivation of latent viral infection. The source of the patient's CMV infection could also have been either the CMV-positive organ or blood transfusions she received. Approximately 50 to 80% of blood donors are CMV positive. CMV infections are common in immunocompetent individuals, but clinical disease is rare. The immunosuppression of cell-mediated immunity necessary in transplantation greatly increases the likelihood of developing clinical disease with CMV, whether through reactivation of the patient's latent virus or viral superinfection from the transplanted organ or blood products. This is an important point conceptually. Infection does not necessarily mean that an individual will be sick. One may become "infected" with an infectious agent, as measured by an immune response to that agent, without developing any clinical manifestations of that infection; i.e., the patient does not have "clinical disease." If the organism can cause latent infection, immunosuppression of a latently infected patient may result in reactivation and the development of clinical disease.

2. CMV infections following solid-organ transplantation are common, occurring in 8 to 50% of recipients depending on the organ that was transplanted and the CMV serostatus of the recipient and donor. The spectrum of CMV disease in these individuals ranges from asymptomatic infections to life-threatening disease. Most clinical CMV disease is classified as mild to moderate, with more severe disease being seen primarily in seronegative recipients of seropositive organs. Most commonly, CMV disease presents nonspecifically as fever in the setting of neutropenia. However, it can also manifest with lymphadenopathy, hepatitis, thrombocytopenia, as well as a variety of gastrointestinal and pulmonary symptoms, as was seen in this case. Rarely, severe CMV disease will present with meningitis. CMV infection has a predilection for the transplanted organ. For example, it causes hepatitis in liver transplant recipients, pneumonitis in heart-lung transplant recipients, pancreatitis in pancreas transplant recipients, and nephritis in kidney transplant recipients. CMV-associated myocarditis does occur in heart transplant recipients, but it is rare. In addition to its direct effects, CMV infection may also have indirect effects due to secondary immunologic phenomena leading to allograft injury and/or rejection, other opportunistic infections, and Epstein-Barr virus-associated posttransplantation lymphoproliferative disorder.

3. In addition to transplant recipients, AIDS patients and newborns can develop severe CMV infections. AIDS patients may develop CMV retinitis, which can result in blindness. The incidence of this is low in patients receiving highly active antiretroviral therapy. Retinitis is rarely seen in transplant patients. AIDS patients may also present with CMV pneumonia, but it is typically less severe than that seen in posttransplant patients. The reasons for the different patterns of CMV disease in these high-risk populations are unclear.

Neonatal CMV infection can occur either *in utero* (congenital infection) or after contact with CMV-positive maternal genital secretions during birth or CMV-positive breast milk. Seronegative women who get primary, but not necessarily symptomatic, CMV infection during pregnancy are at greatest risk for fetal transmission. Approximately 30 to 50% of women of childbearing age are CMV seronegative, but only 1 to 4% will have primary CMV infection during pregnancy. Of these women with primary infections, approximately one-third will pass the virus to their fetus.

A minority of neonates with congenital CMV infection have symptoms at birth (~10%). Signs and symptoms include microcephaly, chorioretinitis, pneumonia, hepatitis, and rash (petechiae). About 90% of those born with symptoms and 5 to 10% of those asymptomatically infected will have long-term sequelae of CMV infection such as deafness and psychomotor retardation.

4. Other opportunistic infections in cardiac transplant recipients include those caused by pathogens common in patients with impaired cell-mediated immunity. These infections include toxoplasmosis, *Pneumocystis jirovecii* pneumonia, varicella-zoster, cryptococcal pneumonia or meningitis, *Listeria monocytogenes* bacteremia or meningitis, *Nocardia* spp. pneumonia, and invasive fungal infections. Data suggest that CMV is itself immunomodulatory, and therefore CMV infection increases the likelihood of other opportunistic infections.

5. As was seen with this case, cytopathologic examination of infected tissue often reveals the diagnosis. Both direct visualization of cytopathic effect (i.e., "owl's-eye" cells) and/or CMV-specific *in situ* hybridization performed on tissue are helpful in making the diagnosis of CMV-associated disease. Additional methods of detection include rapid shell vial cultures, direct antigenemia, and nucleic acid amplification tests (NAATs). Although some laboratories may still use conventional cell cultures for CMV detection, this method is much slower and less sensitive than any of the methods above.

For shell vial cultures, fibroblast cells are grown as monolayers on a glass coverslip in a shell vial. Clinical specimens, including urine, respiratory secretions, tissue specimens, and/or white blood cells, obtained from patients suspected of being infected with CMV are slowly centrifuged onto the cell monolayer. After 1 to 2 days of incubation, the monolayer is stained with a fluorescent monoclonal antibody specific for a CMV early antigen. This technique is more sensitive and much more rapid than conventional culture (1 to 2 days versus 2 to 3 weeks) but lacks sensitivity compared with either antigenemia detection

or NAAT. Therefore, it is of limited value in monitoring solid-organ transplants for CMV infection. Viral culture and/or molecular testing of tissue specimens, particularly in conjunction with histopathology, can still be valuable in invasive gastrointestinal disease when peripheral blood testing is not always positive for CMV.

In the direct antigenemia assay, leukocytes are harvested from the patient's peripheral blood and directly stained with a fluorescently labeled monoclonal antibody that detects the CMV structural protein pp65. The number of infected cells is quantified. The higher the number of infected cells, the more likely the patient will have clinical disease, though detection of a low number of infected cells in an asymptomatic patient may indicate a patient in the early stages of active infection. Historically, this method was preferred to culture for detection of CMV in peripheral blood in posttransplant patients, but was very labor-intensive and time-sensitive owing to the lability of blood cells. Additionally, the test cannot be accurately performed on patients with absolute neutrophil counts below 1,000/μl. Thus, CMV antigenemia has largely been replaced with molecular amplification methods for detecting CMV in peripheral blood.

NAAT, and particularly quantitative real-time PCR, has become the standard for diagnosing CMV disease, assisting in making decisions regarding preemptive therapy, and monitoring response to therapy in posttransplant patients. There are currently no guidelines detailing the level of viremia that should trigger therapeutic intervention, but data in the literature suggest it is ~2,000 copies/ml. Recently, an international standard has become available to normalize interlaboratory variability of CMV viral load results, which will be reflected in the change of reporting from copies/ml to IU/ml. The application of the universal standard and reporting to laboratory-developed tests and future FDA-approved tests should allow the determination of clinically relevant quantitative thresholds. For now, an absolute viral quantity may not be as important as the trend of a patient's viral load over time. The presence of higher amounts of CMV DNA in the blood correlates with a higher likelihood of clinical disease. Studies comparing CMV antigenemia with quantitative PCR show them to have similar performance in the detection of both CMV infection and clinical disease. One of the concerns of using NAAT for detecting CMV in the peripheral blood is the inability to differentiate latent virus from actively replicating virus. Although quantitative CMV NAAT can be performed on both whole blood and plasma, plasma is generally preferred to decrease the number of latently infected cells that are amplified. In theory, primarily "free," and therefore actively replicating, virus would be present in plasma, as opposed to the cell-associated virus present latently in peripheral white cells. This theory is, of course, not absolute, so the need for clinically relevant viral thresholds for therapeutic decisions is critical.

6. The ideal approach for preventing CMV infection posttransplant is to transplant organs from seronegative donors into seronegative recipients. If these individuals require blood products, they should come from seronegative donors or should be leukocyte depleted to remove as much CMV as possible. However, given the high rates of CMV

seropositivity and a shortage of donor organs, this approach is the exception rather than the rule. Since these interventions cannot always occur, and a significant number of seropositive recipients get CMV disease, alternate strategies have been developed to help prevent CMV disease in transplant patients. These strategies include prophylactic therapy and preemptive therapy.

The patient population with the highest rate of developing CMV disease posttransplant is CMV-negative recipients who receive organs from CMV-positive donors. However, CMV-positive recipients receiving a CMV-positive transplant are also at significant risk. In prophylactic therapy, all transplant patients at risk for CMV disease are treated with a prophylactic regimen for 3 to 6 months. Recent guidelines outline the preferred prophylactic regimen depending on the type of transplant, but it usually consists of anti-CMV agents (e.g., ganciclovir) with or without CMV immunoglobulin. The second approach is to use preemptive therapy. The strategy here is to screen patients for the presence of CMV in peripheral blood with a highly sensitive technique such as antigenemia testing or quantitative PCR to detect an early stage of active infection. At that point, the patients would be treated with anti-CMV agents with or without CMV immunoglobulin to prevent them from developing clinical disease. Unlike prophylactic therapy, which is used on all at-risk patients, preemptive therapy is used only on those patients with evidence of CMV in their bloodstream. The rationale for preemptive therapy is that it is more cost-effective than prophylaxis and prevents at least some transplant patients from being exposed to potentially toxic antiviral agents. However, the consensus recommendations are to use prophylactic therapy in the most at-risk patients. One outcome of the implementation of prophylactic therapy is the increase in late-onset CMV disease, which occurs after prophylactic therapy has ended. Late-onset disease has been associated with higher mortality and graft rejection and is not seen with preemptive therapy.

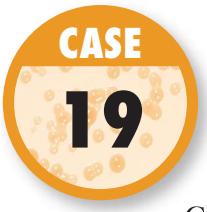
7. Three main drugs are currently available for treatment of CMV infections: ganciclovir (and its oral version, valganciclovir), foscarnet, and cidofovir. Valganciclovir is a prodrug of ganciclovir that is converted to the parent compound by intestinal and hepatic esterases. All of the drugs inhibit viral replication by inhibiting the activity of CMV DNA polymerase. Ganciclovir must be phosphorylated three times to be active. The first phosphorylation occurs by the CMV-derived serine/threonine protein kinase encoded by UL97, while the last two phosphorylations occur by cellular enzymes. Cidofovir requires two phosphorylation steps, both performed by cellular enzymes. Phosphorylated forms of ganciclovir and cidofovir slow and then stop CMV DNA chain elongation by competing with dGTP. Foscarnet is a noncompetitive inhibitor of CMV DNA polymerase that acts by blocking the cleavage of pyrophosphate from the deoxynucleotide triphosphates, preventing chain elongation. Mutations in the UL97 sequence can result in resistance to ganciclovir but not cidofovir or foscarnet. However, mutations in the CMV DNA polymerase (UL54) can result in resistance to ganciclovir, cidofovir, and/or foscarnet, including mutations that confer multidrug resistance. A few studies suggest that as with HIV and

herpes simplex virus, CMV drug-resistant mutants may prove to be less fit and therefore less virulent than wild-type CMV. Patients who fail or cannot tolerate ganciclovir or valganciclovir therapy are usually treated with foscarnet. Cidofovir is used primarily in CMV-infected AIDS patients, especially those with retinitis who have failed alternative therapies. CMV drug resistance is rare in solid-organ recipients, making ganciclovir and valganciclovir the drugs of choice in this patient population. However, with the prophylactic strategies discussed above, resistance is becoming more of a concern.

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This 83-year-old man with metastatic adenocarcinoma of the prostate and end-stage chronic obstructive pulmonary disease (COPD) was in his usual state of poor health (requiring home oxygen and corticosteroids) until he had an exacerbation of his COPD. He was seen by his home health nurse, who noted shortness of breath, and trimethoprim-sulfamethoxazole was prescribed by his physician, with subsequent improvement. Five days after discontinuing his antibiotic, he had another exacerbation of his COPD, this one requiring hospitalization, an increase in his dose of corticosteroids, and empiric intravenous antibiotics. After discharge from the hospital, the patient began to have nausea and vomiting, as well as shortness of breath and purulent sputum. A wet mount of his sputum is shown in Fig. 19.1. This organism was initially seen on Gram stain of his sputum.

1. List the nematodes that have a lung phase. Which one do you think is most likely in this patient? Would it help you decide if you learned on further history-taking that this patient was a Vietnam War veteran who spent significant time in the Mekong Delta region?
2. Describe the life cycle of this parasite. How long can this parasite persist within the gut? How is it able to persist for this period of time?
3. In what way does corticosteroid therapy alter the host-parasite relationship in infections with this nematode? Organ transplant candidates are typically screened for the organism found in the patient's sputum. How and why is this screening done?
4. Which of the white blood cells is frequently increased in number in infections with this parasite? Would you expect them to be increased in this patient? Explain.
5. If this organism were to invade the bloodstream or the central nervous system, how might this manifest clinically?



Figure 19.1



CASE DISCUSSION

1. Three common types of nematode larvae pass through the lung as part of their life cycle: *Ascaris lumbricoides*, hookworm (*Necator americanus* and *Ancylostoma duodenale*), and *Strongyloides stercoralis*. The findings of filariform larvae in sputum and the development of gastrointestinal symptoms after an increase in the patient's corticosteroid dose are consistent with hyperinfection with *S. stercoralis* (see answer to question 3 for further information). Interestingly, strongyloidiasis has not been a significant problem in Vietnam War veterans, although the parasite is endemic in that country, especially in the rice-growing regions of the Mekong Delta. This patient was not a Vietnam War veteran, but as these veterans age, they may begin to manifest latent infections potentially obtained in Vietnam, such as *Stronglyloides*, *Burkholderia pseudomallei*, or *Mycobacterium tuberculosis*.

2. This parasite has a highly complex life cycle, with autoinfection being a prominent feature. Two larval forms are central to its life cycle: the filariform larvae (the infective form) and the rhabditiform larvae (the initial form of the worm, which develops into the filariform larvae). The life cycle begins with filariform larvae penetrating the skin from fecally contaminated soil. The larvae migrate via the bloodstream to the lung, where they break through the wall of the alveoli, crawl out of the bronchus and up the trachea, are swallowed, and reach the duodenum. There the parasite develops into an adult worm that invades and lives beneath the intestinal wall mucosa. Only female worms are present, and they reproduce by parthenogenesis. Eggs hatch as rhabditiform larvae. Most of these larvae are passed in feces. If feces are passed onto soil, the rhabditiform larvae either develop into filariform (infectious) larvae, and the cycle begins anew, or they develop into adult worms. These adult worms lay eggs in the soil that hatch into rhabditiform larvae and develop into filariform larvae. An important phase of the *Strongyloides* life cycle in hyperinfection states is the autoinfective stage. In this stage, rhabditiform larvae develop into filariform larvae in the intestinal tract. The filariform larvae then penetrate either the intestinal wall or the perianal skin, enter the bloodstream, migrate to the lung, and begin the infectious process again.

It is estimated that the parasite can live for 30 or 40 years in the human gastrointestinal tract. The ability of *S. stercoralis* to complete its entire life cycle within the human host is very unusual for a nematode, and it is this autoinfective cycle, plus the adult worm living within the intestinal mucosa rather than at the mucosal surface, that contributes to this parasite's unusual longevity in the human host.

3. Although the parasite may persist for many years, the parasite-host relationship appears to be kept in balance by the immune system. Infected, immunocompetent individuals frequently are asymptomatic, or they may have intermittent symptoms, which usually are gastrointestinal, including abdominal pain, diarrhea, nausea, or vomiting. A few

patients might have intermittent "larva currens" (racing larvae), characterized by itching and skin rash. However, when patients receive immunosuppressive therapy, the balance between host and parasite is tilted in favor of the parasite. The worm burden increases dramatically, aided by autoinfection. This sharp increase in the number of parasites, with corresponding severe clinical disease due to tissue invasion by *S. stercoralis* larvae, is called the hyperinfection syndrome. Patients with the hyperinfection syndrome frequently begin with worsening gastrointestinal symptoms similar to but more severe than those in symptomatic, immunocompetent individuals. These patients also may have cough, shortness of breath, wheezing, and an abnormal chest radiograph, and they can rapidly progress to respiratory failure. In addition, the larvae can migrate to other organs, including the central nervous system.

Hyperinfection due to *Strongyloides* is of concern in patients who are organ transplant candidates because they will receive long-term immunosuppressive therapy. This therapy suppresses cell-mediated immunity, which is essential for immunoregulation of *Strongyloides* infection. Therefore, in patients who have lived in areas in which the nematode is endemic, such as tropical and subtropical regions of Asia and South America and the southeastern United States, screening for antibodies to *Stronglyloides* is routinely done prior to transplantation. If positive, the patient is treated even though the test may give false positives due to the cross-reaction with other helminths. It is important to note that serology cannot differentiate between current and prior infection, so treatment of a seropositive transplant candidate is done strictly as a precautionary measure. By comparison, stool exam in all but hyperinfection states such as occurred in this patient is insensitive and does not reliably detect low-grade infections. The use of repeated stool examination has been shown to increase the sensitivity of *Strongyloides* detection but not to the level of serology. However, stool examination is the only way to detect current infection. The use of the agar plate culture technique has been shown to be more sensitive for the detection of *S. stercoralis* than routine stool examination techniques. In this method, stool is placed on sterile agar plates, and the presence of characteristic furrows due to the migration of the parasites on the surface of the agar is sought.

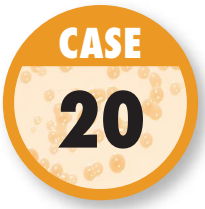
4. As with many tissue-invasive parasites, eosinophil counts are increased in patients with strongyloidiasis. Although the eosinophil count was not available in this patient, the absence of eosinophilia in hyperinfection is not unusual. Patients presenting with hyperinfection are frequently receiving immunosuppressive drugs that may reduce the numbers of white cells (including eosinophils) in the blood.

5. As mentioned previously, larvae can migrate to the central nervous system during hyperinfection. Individuals with invasion of the blood or of the central nervous system during the hyperinfection syndrome may have polymicrobial bacteremia or meningitis from which multiple species of enteric bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, or *Enterococcus* spp., are simultaneously recovered. It is postulated that the migrating

Strongyloides larvae are transporting enteric bacteria during their migration. Whenever enteric bacteria are recovered from cerebrospinal fluid or blood of an immunocompromised host, especially if more than one species is found, hyperinfection syndrome due to *Strongyloides* should be considered.

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The patient was a middle-aged male who was in a high-speed motor vehicle accident. He was trapped for 30 minutes in his car, which had caught fire. After extraction from the vehicle, he was sedated, put on a backboard with cervical collar, intubated, and transported to a level 1 trauma center. In the emergency department, he was hypothermic with a temperature documented to be as low as 34.8°C; he had a pulse of 98 beats/min, a blood pressure of 102/69 mm Hg, and a respiratory rate of 14 breaths per minute while being ventilated. His physical examination was notable for 20% body surface third-degree burns involving the scalp, face, neck, and chest. He was noted to have inhalational burn injuries; multiple fractures on chest, pelvic, and extremity radiographs; and a subarachnoid hemorrhage on head computed tomography. He was admitted to the burn unit.

The patient had a complicated hospital course including documented ventilator-associated pneumonia (VAP), which developed on the 9th hospital day. On the 26th hospital day he continued to have declining pulmonary function secondary to his VAP, for which he was receiving voriconazole, levofloxacin, linezolid, and piperacillin-tazobactam. Despite these antimicrobials, he developed a fever to 38.1°C with a blood pressure of 107/50 mm Hg and a heart rate of 140 beats/min. His white blood cell count at the time was 800/μl, his platelet count was 86,000/μl, and he was anemic. Other significant laboratory findings included an arterial blood gas with a pH of 7.25, pO_2 of 65 mm Hg, and lactate of 3.1 mmol/liter (normal, 0.5 to 1.8 mmol/liter). Two sets of blood cultures were obtained. A Gram stain of the organism from a positive blood culture is seen in Fig. 20.1. A subculture of the organism growing in the blood culture is seen in Fig. 20.2. The organism was found to be oxidase negative and glucose nonfermenting. The susceptibility testing results for this organism are seen Fig. 20.3. The patient became hemodynamically unstable and was given intravenous epinephrine, norepinephrine, and vasopressin. Despite this, the patient's cardiovascular condition deteriorated, and he died of a cardiopulmonary arrest on the 28th hospital day.

1. This patient's initial infection was likely ventilator-associated pneumonia (VAP). Why do patients on ventilators have a significantly higher rate of pneumonia than do patients who are not intubated? Why do burn patients have a higher rate of VAP than do non-burn patients?
2. The organism causing this infection is a member of the "ESKAPE" organisms. List the ESKAPE organisms and determine which organism was most likely infecting this patient. What do ESKAPE organisms have in common?

3. The organism causing this infection has recently become of global concern. Why?
4. The organism infecting this patient has intrinsic resistance to β-lactam antimicrobials. Explain why. Why were the drugs with which he was being treated ineffective in treating his bacteremia? What antimicrobial therapy is typically used to treat the infection?
5. How did he become infected with the organism that caused his bacteremia?

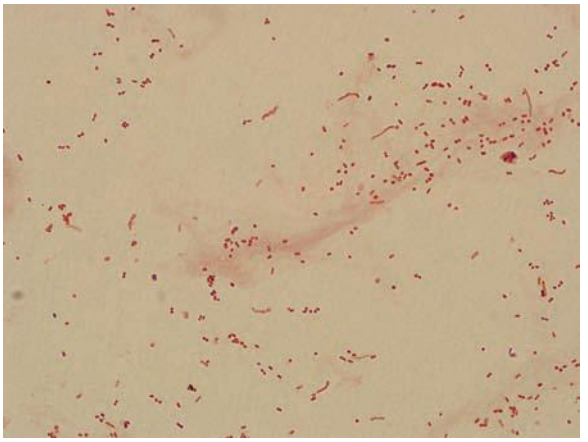


Figure 20.1 Gram stain of the organism that was recovered from a positive blood culture.

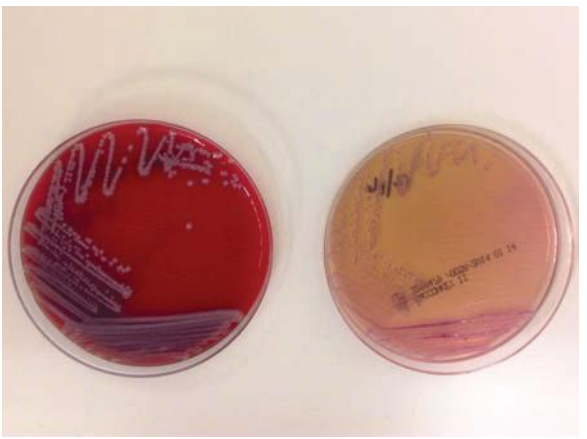


Figure 20.2 A subculture of the organism from the positive blood culture demonstrating growth on both sheep blood agar and MacConkey agar plates.

6. The organism causing his bacteremia was the cause of a serious outbreak in the burn unit caring for him. Several patients died as result of sepsis caused by this organism. Explain how an outbreak of this type of infection is detected and outline steps that are taken to attempt to control such outbreaks.



Figure 20.3 Susceptibility test for the organism that was isolated from the patient's positive blood culture.



CASE DISCUSSION

1. There are three important factors that play a role in patients developing VAP. (i) Patients who are ventilated do not have a gag reflex and can easily aspirate secretions from the upper airways. (ii) A biofilm forms on the endotracheal tube with which the patient is intubated. The tube can become colonized with organisms from the upper airway. The upper airways of hospitalized patients in general and intubated patients in particular are frequently colonized with Gram-negative bacilli (GNB). If there is not frequent, adequate suctioning, these GNB-contaminated secretions can be aspirated into the lung. (iii) Organisms from the upper airways can "leak" around the endotracheal tube and be aspirated into the lung in that manner.

The longer the patient is intubated, the greater the likelihood that the patient will develop pneumonia. The risk of VAP increases with each day of ventilation. If VAP occurs early in the patient's hospital course (<5 days), it is typically due to organisms associated with community-acquired pneumonia such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. The longer the patient is hospitalized, the greater the likelihood that the upper airways will become colonized with GNB and *Staphylococcus aureus*. Starting at about 5 days of hospitalization, these organisms become the predominant etiologic agents of VAP.

Burn patients have one of the highest rates of VAP. This is due in part to the fact that 10 to 20% of burn patients have inhalational injuries. The likelihood of inhalational injuries is proportional to the surface area burned. Inhalational injuries predispose the patient to pneumonia in three ways. First, thermal injury to the upper airway damages ciliated epithelial cells, compromising organism clearance. Second, inhalation of smoke particles causes injury to the lower respiratory epithelium. This injury results in inflammatory response with increased blood flow to the lung and leakage of plasma proteins into alveolar spaces. Third, mechanical ventilation is frequently required because of the damage done to the lung by the inhalational injury. The aspiration of microorganisms into a nutritionally rich environment leads to the high rates of VAP seen in these patients.

2. Over the past decade there has been a growing concern about the emergence of a number of multidrug-resistant (MDR) organisms. The term "ESKAPE" has been coined to describe these organisms because they are thought to "escape" the activity of many antimicrobial agents that are used empirically to treat health care-associated infections.

Enterococcus faecium (vancomycin-resistant *Enterococcus*)
Staphylococcus aureus (methicillin-resistant *S. aureus*)
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter species

ESKAPE organisms have three things in common. (i) They are highly resistant to antimicrobial agents. An increasing number of GNB ESKAPE organisms may only be susceptible to colistin. *E. faecium* is resistant to all first-line antimicrobials including vancomycin, ampicillin, and synergy with aminoglycosides. The only first-line antimicrobial to which MDR *S. aureus* is reliably susceptible is vancomycin. Organisms with reduced susceptibility to vancomycin, i.e., vancomycin-intermediate *S. aureus* isolates, are becoming more common. (ii) They are common causes of health care-associated infections, especially pneumonia, urinary tract infections, wound infections, and bacteremia. (iii) When causing infections, they result in increased length of stay and higher mortality than do non-MDR strains of the same species.

The organism that was infecting this patient is *A. baumannii*. This is a GNB that is oxidase negative, distinguishing it from *P. aeruginosa*. It is a glucose nonfermenter, which distinguishes it from *Klebsiella* and *Enterobacter* species. Examination of Fig. 20.1 shows that the organism can be best described as a coccobacillus, a group in which many of the bacteria appear coccoid. Additionally, but not seen here, the organism may stain as Gram positive and be confused as either streptococci or staphylococci. *Acinetobacter* may also produce pink to purple colonies on MacConkey agar because strains can be strong lactose oxidizers. As a result, though they do not ferment lactose, as do the ESKAPE organisms *Klebsiella* and *Enterobacter*, they may appear to be lactose fermenters on MacConkey agar.

MDR strains of *A. baumannii* have emerged in the last decade. Most problematic is the emergence of carbapenemase-producing *Acinetobacter* strains. These are frequently resistant to all antimicrobial classes except colistin (see Fig. 20.3 and answer 4 for additional details).

3. One of the major concerns about ESKAPE organisms is their ability to rapidly spread globally. *Acinetobacter* is a prime example of the rapid global spread of an MDR organism. A carefully studied example of the global spread of MDR *Acinetobacter* involved U.S. military personnel wounded in Iraq during the first decade of this century. These soldiers were infected in field hospitals in Iraq, airlifted to a military hospital in Germany, and then airlifted to military hospitals in the United States. This organism spread from Iraq to Germany to the United States, with secondary spread at each locale. With the increase in medical tourism, in which patients from the more affluent industrialized world go to the developing world for medical procedures such as inexpensive cosmetic and joint replacement surgery, the specter of the rapid global spread of MDR organisms in patients who acquire postsurgical infections is quite real. The potential for the emergence and spread of extensively resistant or pan-resistant organisms (resistant to all available antimicrobials), in an era when very few new antimicrobials are being developed, is a cause for alarm.

4. Many GNB are intrinsically resistant to β-lactam antimicrobials. *Acinetobacter* contains chromosomal genes for an inducible β-lactamase. The reason for this is evolutionary. It is now believed that β-lactam antimicrobials were first produced not by fungi such as *Penicillium* but by environmental bacteria. The β-lactamases would have had two functions.

The first was to protect the organism from the activity of β-lactams the organism itself was producing as well as any β-lactams produced by other microorganisms in the environment. Second, the β-lactams could be degraded under starvation conditions to be used as a carbon source. Experiments have been conducted with an environmental GNB, *Burkholderia cepacia*, which demonstrated that it could use penicillin G as a sole source of carbon. In the last 2 decades, environmental bacteria that not only have intrinsic resistance to antimicrobials, but also are adept at obtaining antimicrobial resistance genes from other organisms, have begun to emerge as important causes of health care-associated infections. Most prominent are *Acinetobacter*, *P. aeruginosa*, and MDR *Enterobacteriaceae*. Other MDR environmental organisms that also are playing an increasingly important role in health care-associated infections are rapidly growing mycobacteria and members of the *B. cepacia* complex. All of these organisms have large genomes, which give them great metabolic flexibility. One of the most important features of this metabolic flexibility is the ability of these organisms to survive in hostile environments for long periods of time. This ability to survive is an important feature of health care-associated pathogens. Additionally, *Acinetobacter* has been shown to be able to obtain antimicrobial resistance genes from bacteria that are genetically distinct. As a result, *Acinetobacter* may have a broad repertoire of antimicrobial resistance genes that allow it to be successful in resisting a variety of antimicrobials and antimicrobial combinations.

At the time of his development of bacteremia, this patient was receiving extremely broad antimicrobial coverage including two agents, levofloxacin and piperacillin-tazobactam, with activity against Gram-negative organisms (including, in the case of piperacillin-tazobactam, anaerobes); linezolid, for Gram-positive organisms including vancomycin-resistant *Enterococcus* and methicillin-resistant *S. aureus*; and voriconazole, which has broad coverage of fungi. Despite this extremely broad antimicrobial coverage, he developed bacteremia with *Acinetobacter* that was resistant to all of the antimicrobials he was receiving. By the time the organism was identified 2 days later, the patient was close to death and his condition was irreversible. Figure 20.3 shows that the organism was susceptible only to colistin. Colistin is a somewhat toxic antimicrobial that acts on the outer membrane of GNB. It is not active against Gram-positive organisms. There are some GNB such as *Serratia* and *B. cepacia* complex that are intrinsically colistin resistant. While all of the ESKAPE GNB are initially susceptible to colistin, resistance may develop during colistin therapy, creating pan-resistant strains. Fortunately, there is no current evidence of widespread transmission of pan-resistant *Acinetobacter*.

5. There are four potential sources of bacteremia in a burn patient. These include the burn wound, intravenous lines and catheters, urinary tract catheters, and VAP. *Acinetobacter* organisms, along with *P. aeruginosa* and *S. aureus*, are the most common cause of infections in these four sites in burn patients. This patient had a burn wound, a central venous catheter and peripheral intravenous lines, a urinary catheter, and VAP. Thus, he had all four potential sources of bacteremia. An endotracheal aspirate taken near the time of the devel-

opment of his VAP was positive for growth of MDR *Acinetobacter*, suggesting that this was the likely source of his bacteremia.

6. The first step in controlling an infectious disease outbreak is to recognize it. Laboratories and infection control departments within hospitals routinely monitor infection rates of hospitalized patients, including rates of bacteremia, both institutionally and by specific inpatient unit. Burn patients are especially vulnerable to infections. Monitoring infection rates in a burn unit, especially for organisms known to have the potential for patient-to-patient spread, either directly or indirectly, is important. This patient was cared for during a period of weeks during which multiple patients developed bacteremia with MDR *Acinetobacter*. The finding of more than one patient with MDR *Acinetobacter* bacteremia in this hospital was unusual. The finding of several cases temporally related in a single hospital unit called for a full-scale investigation.

The next important point is to detect the organism responsible for the outbreak. The blood culture isolates from the burn patients were analyzed using pulsed-field gel electrophoresis (PFGE). PFGE is used to "fingerprint" organisms to determine how closely they are related. By PFGE, these organisms were essentially identical. This indicated that they were either from a common source or were spread from person to person.

The next step was to identify all patients either infected or colonized with the organism. Screening cultures were obtained from all burn patients; additional patients were identified who were colonized with this organism.

Next, infection control measures were put into place to prevent further transmission. Patients with the MDR *Acinetobacter* were cohorted and had a dedicated staff. Infection control efforts that were intensified included the monitoring of the hand washing of all health care providers entering and leaving the rooms, cleaning of rooms and equipment, the use of dedicated equipment, and MDR *Acinetobacter* culture surveillance to detect newly infected and newly colonized individuals. Spread to other units within the hospital was detected. One policy that was implemented to prevent further spread of the organism outside the burn unit was to eliminate transfer of patients from the burn unit to other units. After many months the outbreak was controlled, and eventually the burn unit was determined to be free of MDR *Acinetobacter*.

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SECTION THREE

GASTROINTESTINAL TRACT INFECTIONS

INTRODUCTION TO SECTION III

The major clinical manifestation of infections affecting the gastrointestinal tract is diarrhea. Diarrheal pathogens have two basic mechanisms by which they produce diarrhea. One is by the production of toxins called enterotoxins. Enterotoxins cause physiologic changes in the intestinal epithelium resulting in fluid and electrolyte secretion. *Vibrio cholerae*, which produces the enterotoxin cholera toxin, is a classic example of a diarrheal pathogen that produces a secretory diarrhea due to the action of an enterotoxin. Microscopically, the intestinal epithelium appears normal in patients with enterotoxin-induced diarrhea.

The other major mechanism of diarrheal disease is by damage to the intestinal epithelium. Organisms may also produce toxins that directly damage the intestinal epithelium. The protozoan *Entamoeba histolytica* produces such a cytotoxin. This cytotoxin is responsible for the characteristic ulcerative lesions that can be seen in individuals with amebic dysentery. Damage to intestinal epithelium can also occur as a result of direct invasion of the intestinal epithelium. A number of gastrointestinal pathogens, including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and *Yersinia enterocolitica*, are capable of invading the intestinal epithelium. Inflammation frequently occurs in response to these pathogens. Patients with diarrhea due to organisms that damage the epithelium frequently will have white blood cells visible in their feces. However, these cells may also be present in feces of patients with noninfectious inflammatory bowel disease, so results of examination of feces for white blood cells should be interpreted cautiously.

Diarrheal diseases are almost always spread by the **fecal-oral route**. This means that individuals who become infected with diarrheal pathogens ingest either food or water that has been contaminated with human or animal feces. Improper handling or preparation of food and contamination of water due to poor sanitation are major means by which diarrheal pathogens are spread. In the industrialized world, the spread of diarrheal disease is particularly problematic in day care centers for children. In addition to spread by contaminated food and water, infected children can pass the organisms directly by placing contaminated hands in the mouths of other children, or indirectly by using contaminated hands to handle toys that are then mouthed by other children. The infectious dose of diarrheal pathogens varies greatly, from hundreds of thousands to millions in *Salmonella* spp. and *V. cholerae* to less than 100 organisms in *Shigella* spp.

Because the major pathophysiologic effect of diarrhea is dehydration due to fluid and electrolyte loss, the most important treatment is **rehydration**. In recent years, simple solutions of glucose, salts, and water given orally have been developed, which are proven to be highly effective in treating patients with even the most severe forms of diarrhea. The widespread use of **oral rehydration** in the past 2 decades, especially in the developing world, has been credited with saving literally millions of lives, primarily young children, in whom diarrheal disease takes the greatest toll. Despite improvements in the treatment of diarrheal disease, one of the major infectious disease catastrophes of this century, the ongoing cholera epidemic in Hispaniola, has resulted in more than 8,500 deaths as of early

2014, with most of the deaths occurring in Haiti. This outbreak was an unintended consequence of bringing in United Nations peacekeepers from Nepal who carried the deadly organism, which found its way into the water supply in rural Haiti and spread throughout Haiti, neighboring Dominican Republic, and on into Cuba.

Industrialization of the food supply, whereby cattle are raised in feedlots and chicken houses with 10,000 to 100,000 birds, has resulted in outbreaks caused by *Salmonella* or Shiga toxin-producing *Escherichia coli* (STEC). Packaging of large lots of leaf vegetables, sprouts, and fruits has been associated with STEC and *Listeria monocytogenes* outbreaks. Industrialization of the food supply makes food less expensive but carries the risk of large-scale food outbreaks affecting hundreds to thousands of people.

In addition to diarrheal disease, hepatitis is an important infection in the gastrointestinal system. The epidemiology of hepatitis A and E viruses is the same as that of diarrheal pathogens. They are usually obtained by ingestion of raw shellfish taken from water contaminated by human sewage or ingestion of food handled by infected people with poor personal hygiene, i.e., individuals who fail to wash their hands after a bowel movement. Hepatitis B, C, and D are spread by contaminated blood. Contracting hepatitis used to be a major concern in individuals receiving blood transfusions. With the recognition of these agents and the development of screening tests for them, the epidemiology of hepatitis due to hepatitis B and C viruses has changed. Hepatitis B, C, and D infections (as well as HIV infections) are frequent in individuals who share needles while using illicit intravenous drugs. Hepatitis B virus (HBV) is also spread sexually, especially in populations that practice anal intercourse. The frequency of sexual spread of hepatitis C virus (HCV) is not as well understood. Unlike hepatitis A virus, which causes a relatively mild, self-limited disease, HBV can cause fulminant, sometimes fatal disease. HBV and HCV can also cause chronic infections culminating in liver failure. Vaccines are available for hepatitis A virus and HBV but not for HCV.

Other important types of gastrointestinal infection are those in which the resident intestinal microbiota or a pathogen escapes from the bowel and enters "sterile" tissues. One example is *E. histolytica* trophozoites that enter the liver and cause an amebic abscess. Another is when there is penetrating trauma to the intestines, as might occur with a gunshot wound to the abdomen or during bowel surgery. In either situation, microbes can escape from the intestines into the peritoneum, where they can cause peritonitis or form an abscess. The organisms causing these infections are typically a mixture of both facultative and anaerobic bacteria that reside in the colon.

TABLE III SELECTED GASTROINTESTINAL TRACT PATHOGENS

ORGANISM	GENERAL CHARACTERISTICS	USUAL SOURCE OF INFECTION	DISEASE MANIFESTATION
Bacteria			
Bacteroides fragilis	Anaerobic, Gram-negative bacillus	Endogenous	Abdominal abscess
Campylobacter spp.	Microaerophilic, curved, Gram-negative bacilli	Poultry	Invasive diarrhea; sepsis in AIDS patients
Clostridium difficile	Anaerobic, toxin-producing, Gram-positive bacillus	Endogenous; health care-associated	Antibiotic-associated diarrhea, pseudomembranous colitis
Clostridium perfringens	Anaerobic, Gram-positive bacillus	Endogenous; high-protein foods	Gangrenous lesions of bowel or gall bladder, food poisoning
Enterotoxigenic *Escherichia coli*	Lactose-fermenting, Gram-negative bacillus	Fresh fruit and vegetables	Traveler's diarrhea, watery diarrhea
Salmonella spp.	Lactose-nonfermenting, Gram-negative bacilli	Animal products; typhoid (human to human)	Invasive diarrhea, typhoid fever
Shiga toxin-producing *Escherichia coli* (STEC)	Sorbitol-nonfermenting (*E. coli* O157:H7), Gram-negative bacillus	Improperly cooked ground beef	Enterohemorrhagic colitis, hemolytic-uremic syndrome
Shigella spp.	Lactose-nonfermenting, Gram-negative bacilli	Human to human; day care centers	Invasive diarrhea, dysentery
Staphylococcus aureus	Catalase-positive, Gram-positive coccus	High-protein foods	Food poisoning
Vibrio spp.	Oxidase-positive, Gram-negative bacilli	Fecally contaminated water, raw fish and shellfish	Large-volume watery diarrhea
Yersinia enterocolitica	Lactose-nonfermenting, Gram-negative bacillus	Meat and dairy products	Watery or invasive diarrhea
Parasites			
Ascaris lumbricoides	Roundworm	Food, soil	Diarrhea, abdominal discomfort, intestinal obstruction
Cryptosporidium parvum	Coccidian parasite	Fecally contaminated water; day care centers; farm animals	Malabsorptive diarrhea (chronic in AIDS)
Cyclospora spp.	Coccidian parasites	Water, fresh fruits and vegetables	Malabsorptive diarrhea
Diphyllobothrium latum	Fish tapeworm	Consumption of rare or undercooked freshwater fish	Minimal symptoms, possible vitamin B_{12} deficiency with heavy worm burden, passage of proglottids in stools

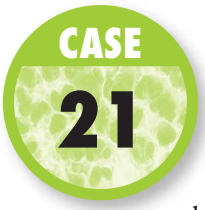
TABLE III SELECTED GASTROINTESTINAL TRACT PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	USUAL SOURCE OF INFECTION	DISEASE MANIFESTATION
Echinococcus spp.	Dog tapeworms	Ingestion of tapeworm eggs from infected dog	Hydatid cyst of liver
Entamoeba histolytica	Ameba	Water, fresh fruits and vegetables	Diarrhea, amebic dysentery, liver abscess
Giardia lamblia	Flagellated trophozoite, cyst	Fecally contaminated water; day care centers	Malabsorptive diarrhea (acute, chronic)
Necator americanus, *Ancylostoma duodenale*	Hookworms	Skin contact with larvae in soil	Anemia, gastrointestinal discomfort
Strongyloides stercoralis	Threadworm	Skin contact with larvae in soil; autoinfective cycle	Gastrointestinal discomfort, diarrhea, rash; larval invasion of lungs and other organs in immunosuppressed patients
Viruses			
Adenoviruses	Nonenveloped DNA virus types 40 and 41	Fecal-oral	Diarrhea (types 40 and 41)
Enteroviruses	Nonenveloped RNA viruses	Fecal-oral	Diarrhea
Hepatitis A virus	Nonenveloped RNA virus	Shellfish; infected food handlers via fecal-oral	Acute, self-limited hepatitis
Hepatitis B virus	Enveloped DNA virus	Blood, secretions, direct sexual contact	Acute and chronic hepatitis, fulminant hepatitis, hepatic carcinoma
Hepatitis C virus	Enveloped RNA virus	Blood	Acute and chronic hepatitis, fulminant hepatitis, hepatic carcinoma
Hepatitis D virus	Enveloped RNA virus; requires coinfection with hepatitis B virus	Blood, secretions, direct sexual contact; can occur as superinfection of hepatitis B chronic carrier or coinfection with hepatitis B	Acute and chronic hepatitis, fulminant hepatitis, hepatic carcinoma; worse prognosis than in hepatitis B infection without hepatitis D
Hepatitis E virus	Nonenveloped RNA virus	Fecal-oral; contaminated water; shellfish; possibly zoonotic (pigs, rats)	Acute, self-limited hepatitis; may be fulminant in pregnant women

(continued next page)

TABLE III SELECTED GASTROINTESTINAL TRACT PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	USUAL SOURCE OF INFECTION	DISEASE MANIFESTATION
Norovirus	Nonenveloped RNA virus	Shellfish; common-source food outbreaks	Vomiting, diarrhea
Rotavirus	Wheel-like, nonenveloped RNA virus	Human to human; day care centers	Diarrhea, vomiting



This 18-year-old male presented to the outpatient medical clinic for evaluation of diarrhea and abdominal discomfort. The patient first noted mild abdominal discomfort and three loose bowel movements per day 1 week prior to evaluation. Two days prior to evaluation he noted intermittent, crampy periumbilical abdominal pain. He denied drinking well water, fever, blood in the stool, relation of the pain to meals, dysuria, or hematuria.

On examination, the patient was afebrile and had normal vital signs. The abdominal examination was notable for mild lower abdominal tenderness. The fecal examination demonstrated a greenish, watery stool that was negative for occult blood.

Laboratory evaluation included a normal white blood cell count, hematocrit, and platelet count. Examination of the feces microscopically was remarkable for the presence of white blood cells. The organism causing his illness is shown in Fig. 21.1 (Gram stain) and Fig. 21.2 (growth on special medium).

1. On the basis of the laboratory findings, what is the likely etiology of this patient's diarrhea? Is the finding of white cells in the feces consistent with the recovery of this organism? Explain your answer.
2. What special laboratory conditions are necessary to recover this organism?
3. What is the epidemiology of this organism? What simple precautions can be taken to prevent its spread?
4. How have modern means of food production contributed to an increasing incidence of infections with this organism?
5. What is the current status of drug resistance in this organism? What factors are believed to play an important role in this status?
6. Although the patient has evidence of local invasion in the intestinal tract with this organism, bacteremia due to this organism is unusual. Explain this observation.
7. What is the most important postinfectious sequela associated with this organism? Briefly describe the pathogenesis of this pathologic process.

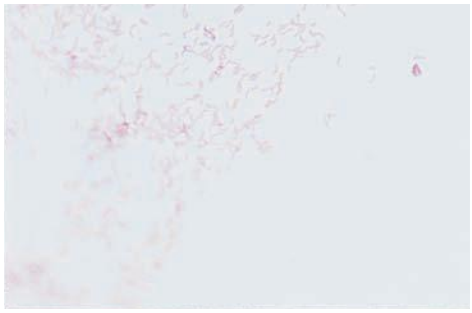


Figure 21.1

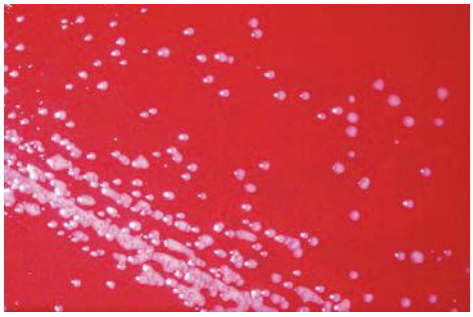
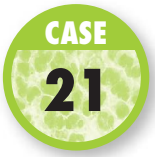


Figure 21.2



CASE DISCUSSION

1. Both *Vibrio* and *Campylobacter* spp. are slightly curved, Gram-negative rods that cause diarrhea (Fig. 21.1). The pathogenesis of the most important *Vibrio* species, *V. cholerae*, is due primarily to the production of an exotoxin, cholera toxin, that causes a secretory diarrhea. The stools of patients with severe cases of cholera have a "rice water" appearance. Because of the secretory, noninflammatory nature of the diarrhea, white blood cells are rarely seen in the feces of patients with cholera. *Campylobacter* spp. cause an invasive diarrhea distinguished by the presence of white blood cells in the stool. Up to 50% of patients with *Campylobacter* diarrhea may have bloody stools due to the presence of red blood cells. The diarrhea seen in this patient is consistent with a *Campylobacter* infection, and *Campylobacter jejuni* was isolated from his stool (Fig. 21.2). Abdominal pain, as was seen in this patient, is an important although nonspecific feature of the clinical presentation of *Campylobacter* gastroenteritis.

2. It is important to remember that the facultative aerobic fecal flora consists of approximately 10^7 to 10^9 CFU/g of feces and that finding an enteric pathogen, which may represent only a small fraction of this flora, is akin to trying to find a needle in a haystack. Selective media, such as Hektoen and MacConkey agars, used for the isolation of *Salmonella* and *Shigella* spp. from feces do not support the growth of *Campylobacter* spp. Therefore, several selective media have been developed for the isolation of *Campylobacter* spp. To further complicate matters, *Campylobacter* spp. are microaerophilic organisms, and so culture conditions that will support their growth must be used when attempting to isolate them. Finally, *C. jejuni*, the most frequently recovered *Campylobacter* species, grows optimally at 42°C, the body temperature of chickens, a natural host of this organism. Many laboratories inoculate fecal specimens onto campylobacter selective agar and incubate these plates at 42°C under microaerophilic conditions in an attempt to isolate *C. jejuni*. This approach is problematic since other *Campylobacter* spp. either fail to grow on certain types of campylobacter selective agar or cannot grow at 42°C. Alternative methods are available for the isolation of these species.

Recently nonculture methods have been developed for the detection of *C. jejuni* and *C. coli*, the two species most likely to cause diarrheal infections. The most widely used one detects *Campylobacter*-specific antigens directly in stool specimens by enzyme immunoassay. The accuracy of these tests approaches that of culture, and they can be done in <2 hours, versus a minimum of 2 days for culture. Alternatively, multiplex PCR techniques which detect multiple enteric pathogens, including the *Campylobacter* spp. *C. jejuni*, *C. coli*, and *C. lari*, are also being used with increasing frequency, replacing culture and antigen testing. One such multiplex PCR technique has the potential for same-day detection of common bacterial (*Campylobacter*, toxigenic *Escherichia coli*, *Salmonella*, and *Shigella*), parasitic (*Giardia* and *Cryptosporidium*), and viral (norovirus and rotavirus) pathogens.

3. *C. jejuni* along with *Salmonella* spp. are the two most frequently recovered bacterial causes of gastroenteritis in the United States. The incidence of *Campylobacter* infections in the United States is 15 to 20 per 100,000. *C. jejuni*, like all enteric pathogens, is spread by the fecal-oral route. Improperly cooked poultry or cross-contamination of foods by raw poultry is postulated to be the most important source of infection. Studies have shown that ~50% of chicken carcasses in food markets are contaminated with *C. jejuni*. By contrast, 5% of ground beef packages have been shown to be contaminated with this organism. Outbreaks of *Campylobacter* infection have also followed the consumption of nonpasteurized milk, but large-scale outbreaks such as occur with two other food-borne organisms, *Salmonella* and Shiga toxin-producing *E. coli*, have not been reported for *Campylobacter*. Contaminated water is an infrequent vehicle for this infection. There is no evidence of person-to-person spread of this organism. Adequate cooking of poultry and avoidance of cross-contamination of other foods will result in prevention of most *Campylobacter* cases. The infectious dose for this organism appears to be intermediate between those for *Shigella* spp. (low) and *Salmonella* spp. (high). Like *Salmonella* and *Shigella* spp., it is an organism that causes disease mainly during the warm-weather months, with a peak incidence in July. One of the interesting observations concerning this organism is that the highest incidence of infection is in infants (<1 year old) and adolescents and young adults (15 to 29 years old). It is the most frequent cause of bacterial gastroenteritis in college students in the United States, with isolation rates on certain campuses as high as 15% in individuals with diarrhea. In the adolescent and young adult age group, infections are more common in males. It is speculated that these individuals are preparing the bulk of their meals for the first time in their lives and may not practice the best food preparation hygiene.

4. Animals and animal products are the primary source of infection for *Campylobacter*. "Factory farming," in which large numbers of animals are grown in close quarters, results in high rates of colonization with this organism. For example, cattle "finished" on feedlots have a much higher rate of colonization than grazing animals. Essentially all chickens raised in commercial chicken barns, which can hold as many as 100,000 animals, are colonized with *Campylobacter* by the fourth week of life. Studies have shown that contamination of poultry carcasses can increase significantly during automated processing. Agricultural research is focusing on ways to reduce or prevent contamination during processing.

5. Two classes of antimicrobials are used to treat *Campylobacter* infections. In children, macrolides are widely used, while in adults, ciprofloxacin is the treatment of choice. Resistance to ciprofloxacin is much more common than resistance to macrolides. The reasons for this difference are multifactorial.

First, a single mutation in the DNA gyrase, the target protein for the fluoroquinolones, can result in high-level resistance, while macrolide resistance usually requires multiple

mutations to reach clinical significance. Second, fluoroquinolone-resistant mutants appear to be more "fit" than macrolide-resistant ones. Why this is significant is that in the early part of this century, "animal use only" fluoroquinolones such as enrofloxacin were widely used to prevent bacterial respiratory infections in large chicken houses that contained as many as 30,000 birds. The use of fluoroquinolones in these birds results in the selection of mutant strains of *Campylobacter* that are not only resistant to the "animal use only" fluoroquinolones but also have cross-resistance to the "human" ones. The overcrowded chicken houses also resulted in the colonization of large percentages of animals with these resistant organisms. Although enrofloxacin has been banned for use in the United States in poultry, the fluoroquinolone-resistant organisms have persisted because the drug-resistant strains are as genetically fit as the susceptible ones. In the United States, between 20 and 50% of isolates are fluoroquinolone resistant, while in Europe, resistance rates are even higher. Macrolide resistance is much less common in animals (<10%) and even less so in humans. Research has shown that macrolide-resistant organisms are not as genetically fit as susceptible ones.

Ciprofloxacin is frequently prescribed for travelers to the developing world, to be taken if they develop diarrhea. This is done because ciprofloxacin is active against many of the bacterial pathogens that they may encounter. Unfortunately, ciprofloxacin resistance is increasing not only in *Campylobacter* but also in *Salmonella* and *Shigella*, calling into question the durability of this approach.

6. *C. jejuni* was locally invasive in this patient, as evidenced by the presence of white blood cells in his feces. Like *Shigella* spp., this organism rarely causes bacteremia in the immunocompetent host. The most likely reason for this is that this organism, unlike *Salmonella* spp., does not survive within phagocytic cells. It is either locally ingested and killed by phagocytes in the intestinal wall or carried by lymphatic drainage to the Peyer's patches, where it is killed. Occasional cases of *C. jejuni* bacteremia occur, but most are transient because the reticuloendothelial system is able to eliminate this organism from the bloodstream.

Campylobacter-associated diarrhea and bacteremia are much more common in HIV-infected individuals who have CD4 counts of <200/μl. However, with the widespread use of highly active antiretroviral therapy in the industrialized world, *Campylobacter* bacteremia has declined dramatically in this patient population. *C. fetus* rather than *C. jejuni* is the species of *Campylobacter* most likely to cause bacteremia.

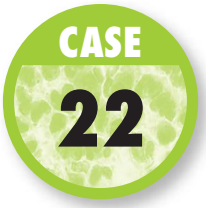
7. Guillain-Barré syndrome is characterized by acute flaccid paralysis in which patients become either hyporeflexic or areflexic. Patients require intubation in ~25% of cases, and secondary pneumonia is an important contributor to its 5% mortality rate. Guillain-Barré syndrome can have multiple antecedents that typically occur 3 to 6 weeks prior to the onset of symptoms. It is estimated that 30% of these episodes are associated with *C. jejuni* diarrhea and that it occurs after 1 in 1,000 to 1 in 2,000 *C. jejuni* infections.

The disease is thought to be a result of molecular mimicry, in which antibodies targeted against the lipooligosaccharide of *C. jejuni* cross-react with peripheral nerve ganglioside, resulting in an acute inflammatory polyneuropathy. Two treatment strategies have been developed. One uses plasma exchange to remove antibodies and complement, which mediates this inflammatory neuropathy. The other uses immunoglobulin to bind the autoantibodies produced in response to the *Campylobacter* infection or inhibit the activation of complement by autoantibody.

Other autoimmune syndromes associated with *Campylobacter* are reactive arthritis (Reiter syndrome), the major manifestation being inflammation of either the urethra or conjunctiva, and hemolytic-uremic syndrome. *Campylobacter* may also be responsible for flares of inflammatory bowel disease. This organism, as well as other diarrheal pathogens, has also been associated with irritable bowel syndrome. As we unravel the role of the intestinal microbiota in inflammatory and irritable bowel disease, we are likely to develop a better understanding of the role of these bacteria in these disease processes.

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The patient was a 5-year-old male who presented to his pediatrician with a 5-day history of increased stool frequency, with stools occurring 1 to 2 hours after meals. The patient also had increased stool incontinence with soiling of his underwear on each of the past 3 days. The child's father described his son's stools as being fatty appearing and foul smelling. The child's social history was significant for having been adopted from an orphanage in Russia 6 months previously. He was currently being cared for in the home by a nanny. He had no siblings, but there were two dogs in the home with which he frequently played. He had visited a llama farm approximately 3 months previously. He lived in a rural area and the home had well water. Neither the parents nor the nanny had any abdominal symptoms. Figure 22.1 shows an organism found in the patient's feces at the time of his arrival in the United States. Figure 22.2 shows the parasite found in his feces from a stool obtained on the day of his visit to the pediatrician.

1. What is the organism that was found in the feces of this child on his arrival in the United States?
2. Why is this child at increased risk for infection with this organism? What characteristic makes this organism particularly problematic in an orphanage in Russia?
3. Briefly describe the life cycle and pathogenesis of this organism.
4. How would this initial infection be treated?
5. What is the organism that is currently infecting him?
6. How did he likely obtain this agent?
7. What is the natural history of infections with this organism in a child like this? What would be the natural history in an HIV-positive child with a CD4 count of <10 cells/μl?



Figure 22.1

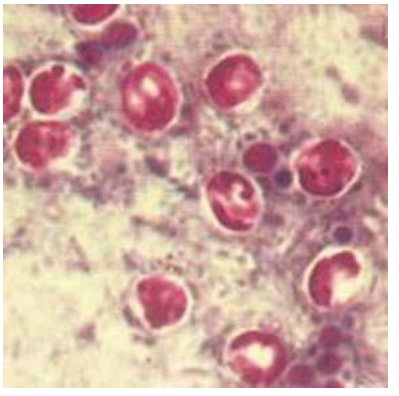


Figure 22.2

8. How would you treat this illness in this child?
9. Widespread outbreaks resulting in tens of thousands of infections with both of these agents have occurred in the United States. Explain the circumstances under which outbreaks of infection due to either one of these organisms might occur.
10. Foreign adoption is becoming increasingly common. What infectious disease issues should be addressed in this patient population on their immigration to the United States?



CASE DISCUSSION

1. On arrival in the United States, the patient was infected by *Giardia lamblia*, a common intestinal parasite. Figure 22.1 shows the cyst form of the organism stained with Lugol's iodine.

2. Approximately 15 to 30% of all children adopted in Russia are found to be infected with *Giardia* on arrival in the United States. Studies have shown that not only adopted children but other populations who have traveled to Russia have increased rates of *Giardia* infection. Poor water sanitation is an important contributing factor to the increased rates of *Giardia* infection in Russia.

Three factors are important for the development of chronic infection in children in orphanages. (i) The cysts that are the infective form of the parasite are resistant to halides, chlorine, and iodine, the active ingredients in a variety of disinfectants. (ii) The infectious dose of *Giardia* cysts is small, likely <100 cysts. (iii) The crowded, understaffed orphanages create an ideal environment for the spread of any number of infectious agents. In the case of *Giardia*, failure to wash hands between changing diapers and preparing food, plus the poor hygiene of children who might place fecally soiled hands on toys that are then placed in the mouths of other children, may contribute to the spread of this organism in this institutional setting. This is not just true in Russia but might also contribute to *Giardia* outbreaks in day care settings in the industrialized world, including the United States.

3. The cyst form of the parasite is swallowed either in contaminated food or water or following placement of a fecally soiled object in the mouth. The cysts excyst in the small intestine in response to stomach acidity and pancreatic enzymes, releasing the trophozoite. Sucking disks on the ventral surface of the trophozoite allow adherence to the intestinal mucosa. The trophozoite damages the intestinal brush border, resulting in a malabsorptive diarrhea. Malabsorptive diarrhea is characterized by frothy, foul-smelling stools 1 to 2 hours after a meal.

4. *Giardia* infection is typically treated with the antimicrobial metronidazole. Metronidazole is an interesting agent because it has activity against both protozoans and most anaerobic bacteria. Other agents have been found to have anti-*Giardia* activity, but the greatest clinical experience for treatment of *Giardia* infections is with metronidazole. Oral rehydration will also have value in the management of this patient, especially once malabsorption is brought under control by metronidazole.

5. The acid-fast structure seen in the fecal specimen obtained during his current clinic visit is consistent with *Cryptosporidium* spp.

6. Like *Giardia*, *Cryptosporidium* is resistant to halides, has a small inoculum size, and is a common cause of diarrheal disease in young children, especially those who are in institutionalized settings. Unlike *Giardia*, it is unusual in foreign adoptees. The reason for this disparity is unclear.

There are two possible explanations for how this child became infected. First, the child may have been chronically infected with both *Giardia* and *Cryptosporidium* on immigrating to the United States. Because diagnostic tests for *Cryptosporidium* are probably no more than 90% sensitive and more likely to be in the 70 to 80% range, it is possible that the organism was missed on the original fecal examination in which *Giardia* was observed, but was subsequently found on his second clinic visit. Alternatively, he may have been infected after he came to the United States. Contact with farm animals, swimming in freshwater or swimming pools, and contact with infected individuals all are risk factors for developing *Cryptosporidium* infection. He had not been swimming nor was anyone sick in his home environment. Interestingly, contact with dogs, which this child did have, is not widely associated with *Cryptosporidium* infections. During his visit to the llama farm, he was exposed to a variety of different farm animals, making this a potential source of his infection.

7. Sporadic *Cryptosporidium* infections are typically self-limited in an immunologically normal child, lasting 5 to 10 days. In children in the developing world—and this child must initially be considered a member of that population—diarrhea can persist for longer than 14 days, with associated weight loss and developmental delay. With this child's improving nutritional status, he transitioned from being a developing-world child nutritionally to a developed-world child. This improved nutritional status was likely associated with improved immunocompetence, allowing the child to clear his infection.

In profoundly immunocompromised patients such as those with a CD4 count of <10 cells/μl, *Cryptosporidium* typically presents as a chronic, malabsorptive diarrhea with frequent, foul-smelling stools. Weight loss can be profound, with an associated high mortality. This disease in AIDS patients in Africa has been referred to as "slim disease" because of the extreme cachexia seen in these patients.

8. There are no clearly effective anti-*Cryptosporidium* agents, although nitazoxanide has been approved in the United States for treatment of both *Cryptosporidium* and *Giardia*. It is rarely used in the clinical setting presented here, as it is usually reserved for use in patients with particularly protracted diarrheal disease courses. The main focus of treatment in patients such as the one presented here would be oral replacement therapy. A lactose-free diet is recommended since lactose intolerance in the short term may be observed in patients with *Cryptosporidium* infections.

9. Because both of these agents are refractory to disinfection by halides, municipal water supplies that use surface water depend on flocculation and filtration to eliminate these organisms. Outbreaks with both of these agents have occurred when there has been failure or disabling of these steps in water purification. In Pittsfield, MA, an outbreak with >700 confirmed *Giardia* cases occurred when the filtration plant was taken offline for upgrading and the only treatment available for a secondary water supply was chlorination but not flocculation/filtration. Just prior to the beginning of the outbreak, residents of this city complained that their tap water was turbid.

In one of the largest waterborne outbreaks ever recorded, an estimated 400,000 individuals had *Cryptosporidium*-associated diarrhea in Milwaukee, WI. In that outbreak, there was a malfunction in the flocculation/filtration of the water in one of the two city water plants, both of which obtained "raw" water from Lake Michigan. The treated water from the implicated plant had increased levels of turbidity in the period proximal to the outbreak. In addition, illness was much more common in individuals who lived in areas supplied by the implicated water plant, and ice made from water from this plant during the time of the outbreak contained *Cryptosporidium* oocysts, all of which point to contaminated water produced by this water plant as the source of the outbreak.

10. Over the past 10 years, approximately 200,000 children have been adopted to the United States primarily from China, Russia, former Soviet republics, South Korea, and Guatemala. Sadly, Russia passed a law in 2013 prohibiting foreign adoption to the United States. An increasing number of children from sub-Saharan Africa, in particular Ethiopia, are being adopted. These children have very specific infectious disease issues that need to be considered. Childhood vaccinations may not be up-to-date, because certain vaccines such as conjugate pneumococcal and varicella-zoster vaccines were not available in their birth country or the vaccines that were used had inadequate potency because of manufacturing difficulties or failure to maintain a vaccine cold chain. Because of this, revaccination with all the childhood vaccines may be recommended for these children.

These children are frequently in countries with high rates of *Mycobacterium tuberculosis* endemicity and may have had exposure to this organism. Management of these children may further be complicated by their having received the tuberculosis vaccine, BCG. BCG vaccination can complicate the interpretation of tuberculin skin tests, an important screening test for tuberculosis. Children with positive skin tests should nevertheless have chest radiographs and be carefully examined for clinical signs of tuberculosis.

HIV infection can be a concern especially in children adopted from countries with high rates of endemic infection such as Russia, the former Soviet republics, and sub-Saharan African countries. All children should be screened for HIV on arrival in the United States, although the number of cases of HIV in foreign adoptees is very small, <1 in 1,000 based on two surveys.

Both hepatitis B and C infection are of concern in foreign adoptees. Children are typically screened for hepatitis B virus and are typically not placed for foreign adoption if

they have evidence of chronic infection. Nevertheless, newly arrived children should be tested for hepatitis B and those without evidence of immunity should be vaccinated. Those with evidence of infection such as increased liver enzymes with positive surface antigen or hepatitis B viral load should be considered for antiviral therapy.

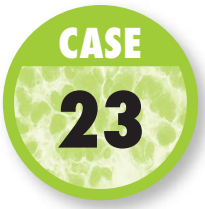
All international adoptees should be tested for hepatitis C virus antibodies. As with HIV, infection rates are low, estimated to be <1%. Those positive for antibodies should have hepatitis C viral load determined. Patients with positive viral loads may benefit from antiviral therapy; patients with negative viral loads either have maternal antibodies or resolved infections.

Children with chronic hepatitis B and C infections can be a source of infection for caregivers. Caregivers need to be educated in how to prevent acquisition of these viruses. In particular, caregivers need to know how to avoid blood and body fluid exposures, which can transmit both of these viruses.

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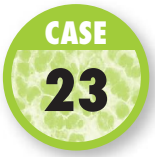
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The patient was a 4-year-old who presented to her pediatrician in early summer with a 3-day history of vomiting and bloody, mucus-flecked diarrhea. She had several episodes of vomiting and approximately 10 bowel movements/day. Her bowel movements were described as painful, and the parents reported that the patient had moderate abdominal pain. She also had fevers to 40°C with chills. The patient had decreased fluid input and urine output. She had no recent travel or camping history, no recent change in diet, no one at home with a similar illness, and no history of exposure to "bad" food; she was cared for at home.

On physical examination she was alert with vital signs within normal limits. She had normal skin color and turgor, and her skin was warm and dry. There was no lymphadenopathy. Her abdominal examination was within normal limits, with normal bowel sounds and no organomegaly, guarding, or rebound. A stool was guaiac positive and was sent for culture. She tolerated a "popsicle" challenge and was tolerating oral liquids when she was discharged home. Three days later the laboratory reported that a non-lactose-fermenting, nonmotile, H_2S-negative organism was growing on MacConkey agar from the patient's stool specimen. When the father was called, he reported that the diarrhea had completely resolved.

1. What is your clinical impression of this child?
2. What is a stool guaiac test? Do you think it had any influence on the clinician's decision to perform a stool culture? Explain.
3. What organism do you think caused her diarrhea? Was her clinical presentation consistent with this organism? What virulence factor does this organism produce that could explain her clinical symptoms? Explain.
4. This child was treated with an oral cephalosporin. Was this a good idea? Explain.
5. What was the significance of the "popsicle challenge"?
6. The organism that infected this patient is a frequent cause of child care center outbreaks of diarrhea. What characteristic does this organism have that makes it easily spread in a day care setting? Name two interventions that you would institute to try to end an outbreak of this organism in a child care center.



CASE DISCUSSION

1. This patient had bacterial dysentery. Bacterial dysentery is caused by invasive bacterial pathogens. Patients with bacterial dysentery typically have grossly bloody stools and fever and appear ill. They generally also have mucus in their stool. The disease course of bacterial dysentery is typically <14 days and is consistent with the patient's clinical course.

2. The stool guaiac test is a simple, point-of-care spot test for the detection of blood in stools. It has several uses. In a patient such as the one here, it can confirm the presence of bloody diarrhea. It can also be used as a screening test for colon carcinoma or upper gastrointestinal bleeds due to peptic ulcer disease. Patients with inflammatory bowel disease may also have a positive test, as well as patients with hemorrhoids. Given the nonspecific nature of this test, it cannot readily predict the clinical condition of the patient without taking into account the clinical presentation. In a patient with the type of presentation seen here, it would help narrow the differential of potential agents (see answer 3 for further information). Since it is important to establish the etiology of bacterial dysentery for appropriate patient management, an enteric pathogen culture was obtained.

3. The major agents of bloody diarrhea in order of frequency in the industrialized world are *Shigella*, *Campylobacter*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC). The description of the organism plus the clinical presentation is most consistent with *Shigella*. *Shigella* is a nonmotile, non-lactose-fermenting, Gram-negative organism that does not produce H_2S. Other phenotypic tests would have been done to establish the identity of this organism. The other organisms on this list are not consistent with the characteristics listed in the case. *Campylobacter* will not grow on MacConkey agar, *Salmonella* organisms are both motile and H_2S producers, and STEC is a lactose fermenter and generally motile.

The clinical presentation is also consistent with *Shigella*, which causes invasive diarrheal disease characterized by abdominal pain, pain on defecation, and the presence of blood and mucus in stool.

The key feature of this organism is a 31-kb pathogenicity island-like region on a large virulence plasmid. This pathogenicity island encodes a type 3 secretion system that plays a critical role in the invasion of the intestinal epithelium, which is central to the pathogenicity of this organism as it invades the basolateral side of the intestinal epithelium. *Shigella*, although nonmotile, can move from cell to cell via actin polymers. *Shigella* is translocated across the colonic epithelium via M cells and subsequently ingested by macrophages. The organism produces molecules that induce apoptotic cell death of the macrophages, which leads to the escape of the organism and the release of a variety of cytokines that cause a massive inflammatory response. Neutrophils destroy the integrity of the epithelial cell lining while at the same time killing the intracellular *Shigella*. The

severe tissue destruction results in impaired water, solute, and nutrient absorption and also leads to the presence of blood and mucus is the stool, which is characteristic of *Shigella* infections.

4. Treating diarrheal disease with antimicrobials is controversial. Some physicians treat dysentery using the belief system (and support from the literature) that it will shorten the course of illness and the period of infectiousness. This is an important consideration when deciding whether to treat shigellosis since it is only spread from person to person either directly or indirectly via food, water, or fomites. Cefixime is a good choice in a child because it is likely to be tolerated, and there is little resistance reported.

The reason for the controversy is that shigellosis is almost always a self-limited disease that will resolve within a few days in the absence of antimicrobial therapy. Antimicrobials alter the intestinal microbiota, negating some of its benefits while putting patients at risk for other infections, including *Clostridium difficile*. Any antimicrobial pressure increases the likelihood of selecting for drug-resistant organisms. Multidrug-resistant *Shigella* is relatively common in the United States; as many as 30% of isolates are resistant to three or more antimicrobials. It is important to note that international travelers are more likely to have multidrug-resistant *Shigella*. A final consideration is the problem of hemolytic-uremic syndrome (HUS) and bloody diarrhea. Five to 10% of patients with bloody diarrhea due to STEC develop HUS. One of the important predisposing factors for STEC-induced HUS is the prior use of antimicrobials, especially trimethoprim-sulfamethoxazole. As a result, antimicrobials are contraindicated in patients with STEC. Pediatricians should use caution when considering antimicrobial therapy in patients with bloody diarrhea, especially if the diarrhea began as watery and turned bloody, since this clinical course is much more consistent with STEC. (For further details, see case 30.)

5. The hallmark of treatment for all diarrheal diseases is oral rehydration therapy. This child had a history of vomiting, so the nurse caring for her wanted to make sure she could tolerate oral fluids to treat the dehydration associated with this infection; thus the "popsicle challenge." If the child "failed" this challenge, that is, could not tolerate oral fluids, she would have needed intravenous fluids, which might require a brief admission to the hospital until her electrolytes had been stabilized and she could tolerate oral fluids. Both parents and children would much prefer to be able to return home as soon as possible, and this is best accomplished by treating with oral rehydration.

6. *Shigella* has a low infectious dose, probably ≤100 organisms. Transmission in a child care setting is facilitated by poor personal hygiene by infected children. Children may not wash their hands after defecating, getting organisms on their hands. They may then place their hands either directly in other children's mouths or on toys or utensils, contaminating them. These contaminated items can then be placed in the mouths of well individuals, resulting in acquisition of the organism. In addition, care providers may get organisms on

their hands during diaper changes. If these caregivers do not wash their hands and then prepare food, they can spread the organisms from their hands to food.

At least four strategies could be used to try to stop the spread of *Shigella* in a child care setting:

1. Exclude all infected individuals, including children and child care providers, from attendance.
2. Have one group of workers prepare food and another group change diapers.
3. Remind workers about the importance of strict hand-washing practices.
4. Regularly disinfect toys that are handled by the children.

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CASE 24

A 1-year-old male was admitted to the hospital in December because of fever and dehydration. His parents reported that he had a 1-day history of fever, diarrhea, emesis, and decreased urine output. On admission, his vital signs revealed a temperature of 39.5°C, slight tachycardia with a pulse rate of 126 beats/min, and a respiratory rate of 32 breaths/min. He was very somnolent. His general physical examination was remarkable only for hyperactive bowel sounds. Laboratory tests showed a leukocytosis with a white blood cell (WBC) count of 14,200/μl with 80% polymorphonuclear leukocytes (PMNs). Urinalysis was significant for a high specific gravity and ketones (consistent with the patient's dehydration). Stool, blood, and urine samples were sent for bacterial culture. A stool sample was also checked for ova and parasites. There were no fecal leukocytes. The patient was given intravenous normal saline and had nothing by mouth. Over the next 48 hours his emesis abated. Once he was rehydrated and was tolerating oral feedings, he was discharged home. All cultures for bacterial pathogens gave negative results, but a rapid viral diagnostic test was positive (Fig. 24.1).

1. What is the differential diagnosis?
2. What is the most common cause of pediatric gastroenteritis? Briefly outline the pathophysiology seen with the organism causing this patient's infection.
3. Briefly describe the epidemiology of this agent.
4. What rapid diagnostic test was used? Why are rapid diagnostic tests useful in this setting? What other laboratory tests are available to diagnose this infection?
5. What treatment is effective?
6. What special infection control precautions are necessary in the hospital setting when caring for a patient with gastroenteritis?
7. A vaccine for this infection was removed from the market in 1999. Why? Discuss the risks and benefits of the vaccines currently available.

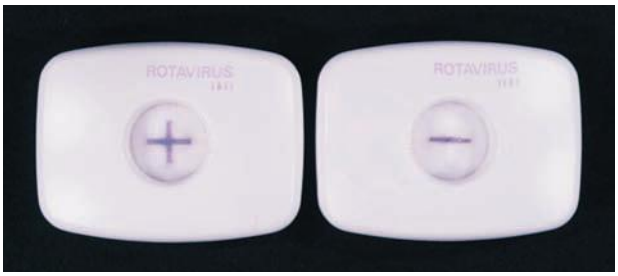


Figure 24.1



CASE DISCUSSION

1. The differential diagnosis for acute diarrhea includes bacterial, parasitic, and viral etiologies of gastroenteritis. Because of the absence of fecal leukocytes, agents of invasive diarrhea such as *Salmonella* spp., *Shigella* spp., Shiga toxin-producing *Escherichia coli*, *Campylobacter* spp., and *Yersinia enterocolitica* are less likely, although certainly possible. *Entamoeba histolytica* also causes invasive diarrhea, but is more unlikely given the patient does not have a travel history. The leading parasitic possibilities include *Giardia* and *Cryptosporidium* spp., especially if this child was in a day care center. The viruses that can cause gastroenteritis include rotavirus, norovirus, adenovirus, astrovirus, sapovirus, coronaviruses, and enteroviruses. A recent study determined the etiologies of acute gasteroenteritis in children less than 5 years of age in the United States. Rotavirus and norovirus caused 40% of illness, while adenovirus (12%), astrovirus (5%), and sapovirus (5%) were the other major causes. Notably, rates of bocavirus and parechovirus (an enterovirus) were not different between sick children and healthy controls. Vomiting is seen frequently in viral gastroenteritis and less frequently in infections with the other agents listed, making a viral agent much more likely in this case.

2. Rotavirus, which is a common cause of viral gastroenteritis in children of this age, was the etiologic agent. An enzyme immunoassay (EIA) performed on the patient's stool was positive for rotavirus antigen. In Fig. 24.1, the left EIA is the patient's positive test, and the right EIA is a negative control. The clinical disease spectrum caused by this virus varies from asymptomatic infection to severe vomiting and dehydration. The disease is usually self-limited, lasting approximately 1 week. This duration of illness is much longer than that seen with most other viral agents of gastroenteritis, which usually resolve in 24 to 48 hours and rarely cause the type of severe symptoms that can be seen with rotavirus. Patients with rotavirus infections have watery diarrhea and frequent vomiting. These symptoms can be severe, resulting in dehydration which may require hospitalization.

The pathophysiology of disease caused by rotavirus is due to three interrelated mechanisms. First, there is malabsorption due to viral destruction of mature enterocytes (the site of rotavirus infection). Second, there is a decrease in epithelial permeability due to the disruption of tight junctions. Third, an enterotoxin-like molecule, NSP4, produced by rotavirus is thought to stimulate the enteric nervous system, which increases the concentration of calcium and results in secretion of fluids and electrolytes into the lumen of the intestine. The cumulative effect of these mechanisms is the watery diarrhea typical of rotavirus and other enterotoxin-mediated diarrheal diseases.

3. Rotavirus causes diarrheal disease primarily in children less than 5 years of age, with the most severe disease seen in children less than 2 years of age. As with all diarrheal diseases, it is spread primarily by the fecal-oral route. Common-source outbreaks outside of day care centers and health care settings are not well documented. Adults who become

infected usually are caregivers of an infected child. The disease is seen primarily during winter months in temperate zones and, along with norovirus, has been referred to as "winter vomiting disease." Disease seasonality is not as obvious in tropical areas or in areas with high vaccination rates. Prior to the vaccine introduced in 2006, it is estimated that 55,000 to 70,000 pediatric hospitalizations and 20 to 60 pediatric deaths were attributed to rotavirus annually in the United States. Fatalities are seen primarily in those who are malnourished and immunocompromised. In the developing world, rotavirus is a major cause of death in children less than 5 years of age, with more than 500,000 deaths occurring annually.

4. The EIA test is most commonly used to detect rotavirus. The virus was first discovered in the stools of children with vomiting and diarrhea by using electron microscopy. It was named for its characteristic wheel-like ("rota") morphologic appearance seen by electron microscopy. However, this technique is not routinely used because of the ease of EIA. Although latex agglutination tests were once commonly used, the sensitivity of EIA has been shown to be superior. Likewise, the application of molecular methods has shown that rotavirus EIA tests have decreased sensitivity compared to PCR. Compared to EIA, various PCR assays have detected 10 to 28% more positives without an apparent decrease in clinical specificity. Until recently, these PCR tests were largely used only in research and surveillance studies. There is now a Food and Drug Administration (FDA)-cleared molecular multiplex test for the detection of multiple agents of acute gastroenteritis, including rotavirus. However, this test is more expensive than the EIA test, is not random access, and takes about 5.5 hours. EIA tests take 10 to 75 minutes to perform, and some EIAs can be performed as the stool arrives in the lab.

Rapid testing for the detection of rotavirus is valuable for three reasons. First, children known to have rotavirus will not need any other expensive tests to determine the etiology of their disease. Second, appropriate rehydration therapy can be begun and the use of antibacterial agents can be avoided. Third, children who are infected with rotavirus can be cohorted. Because pediatric hospital beds are often at a premium during the winter months when rotavirus infection typically is seen, the ability to cohort children with the same illness allows the hospital to save isolation rooms for other children who need them.

5. No specific antiviral therapy is available for rotavirus. Thus, supportive care with a focus on rehydration is key. Both oral and intravenous rehydration therapy are effective. Which therapy is used is based on the severity of the patient's vomiting. If the patient can tolerate it, oral rehydration is performed because of its low cost and ease of administration.

6. Hospitalized patients with diarrhea should be placed on enteric precautions even prior to knowing the etiology. Enteric precautions are similar to contact precautions, except hand washing when entering and leaving the room is required as opposed to alcohol-based hand rub. For both enteric and contact precautions, gloves and a gown

must be worn when caring for the patient. During acute infection, there are extremely high quantities of rotavirus in the stool (over 1 billion viruses per gram of stool); thus, it is easy to contaminate the environment. In addition, as a nonenveloped virus, rotavirus can remain infectious on inanimate objects for days and on hands for as long as 4 hours. Strict adherence to infection control policies is critical to prevent health care-associated diarrheal disease outbreaks, which have occurred when health care personnel have transmitted the virus from one patient to another.

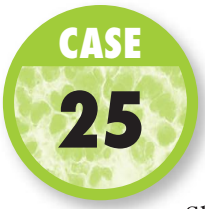
7. Since rotavirus was recognized as the most clinically significant cause of infantile diarrheal disease, the development of a protective vaccine became an important public health goal. An attenuated, recombinant rhesus rotavirus vaccine was developed. In clinical trials, the vaccine was found to prevent 50% of cases compared with the control group, and it reduced the severity of disease in those patients who developed disease postvaccination. Given its apparent efficacy, the vaccine was licensed in the United States and began to be used in September 1998. Over the next 10 months, there were several reports to the Vaccine Adverse Event Reporting System (VAERS) of intussusception (blockage of the intestines when the bowel folds over on itself) in the immediate postvaccine period. The majority of these individuals required surgical intervention. Two case-control studies confirmed that intussusception was increased in the immediate postvaccine period. These findings resulted in a recommendation by the Advisory Committee on Immunization Practices that this vaccine be withdrawn from use in 1999. The obvious question was why this adverse event was not noted during clinical trials. Intussusception was observed in vaccinated children during the clinical trial; however, the rate was not statistically higher than that seen in the general population. Only when large numbers of patients were vaccinated was the association between vaccine and intussusception clearly established.

In 2006, two new rotavirus vaccines were licensed in the U.S. Both are orally administered live attenuated vaccines, but one is made from five human/bovine reassortant viruses and the other from one human strain. The clinical trials of these vaccines showed 74 to 79% efficacy in preventing any rotavirus disease and 96 to 98% efficacy in preventing severe disease. Randomized, placebo-controlled studies did not show an increase in intussusception with these two vaccines. However, VAERS data and other safety studies indicate there may be 1 to 3 excess cases of intussusceptions per 100,000 infants receiving a rotavirus vaccine. Other safety studies showed no increased risk or were inconclusive. An additional setback was the discovery of porcine circovirus DNA in one of the vaccines, leading to a temporary FDA advisory against the use of this vaccine. Porcine circovirus is not known to infect humans. Although the risk of intussuception and contaminating viral DNA must be considered, the benefit of rotavirus vaccination is far greater than the risk. Data from 2009–2011 show the vaccines to be 70 to 84% effective against emergency department visits and hospitalizations, with no waning immunity in the first 2 to 4 years of life. It is estimated that vaccination prevents more than 65,000 hospitalizations annually.

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A 62-year-old female presented to the emergency department with abdominal pain and vomiting. The day prior to presentation she had acute onset of diffuse midline abdominal pain around 3 p.m., and by 6 p.m. she began having copious emesis. She had approximately 20 episodes of emesis overnight. She tried taking activated charcoal and loperamide, with no improvement in her symptoms. The patient also reported a fever (38.8°C), generalized weakness, abdominal bloating, and headache. Although the bloating and headache improved, she had three episodes of watery stools after arriving in the emergency department. The patient had not been able to eat or drink since the previous afternoon. She lived at home alone with two dogs and worked at a preschool.

The patient's past medical history included hypertension, hyperlipidemia, and bronchitis. She had received azithromycin for her bronchitis 3 weeks ago. She stated that her bronchitis symptoms had mostly improved. Her physical exam revealed that she was febrile (38.3°C) and tachycardic (heart rate, 144 beats/min). A complete blood count was within normal limits except for a slightly elevated hemoglobin level of 16.9 g/dl and hematocrit of 49%. Her electrolyte studies were within normal limits. She was given ondansetron (Zofran) for her nausea and vomiting along with 4 liters of intravenous normal saline.

The patient had negative blood cultures, and her stool was negative for *Clostridium difficile*. She had a positive stool lactoferrin test, and a multiplex PCR test performed on her stool revealed the etiologic agent.

1. Given the patient's clinical presentation, what organism was most likely causing her nausea, vomiting, and diarrhea? What is the treatment for her infection? Given her history, what other organism should be considered?
2. Describe the epidemiology of this agent. Where might the patient have been infected with this organism?
3. Is the patient's positive lactoferrin test consistent with her infection? What are other causes of a positive lactoferrin test?
4. How are infections with this organism different in immunocompromised patients?
5. What are the challenges associated with the laboratory diagnosis of this agent?



CASE DISCUSSION

1. The patient's rapid onset of "gastrointestinal flu," which includes nausea, vomiting and/or diarrhea, abdominal pain, and low-grade fever, suggests viral gastroenteritis. Her headache and malaise are also consistent with viral gastroenteritis. The most common causes of viral gastroenteritis are rotavirus, caliciviruses (e.g., norovirus and sapovirus), astrovirus, and enteric adenoviruses (serotypes 40 and 41). Although this clinical presentation could be any of these viruses, the most common cause in adult patients is norovirus (previously known as Norwalk-like virus). Norovirus is a highly infectious nonenveloped RNA virus that has also been called "small round structured virus" based on its appearance visualized by electron microscopy.

There are no specific therapies that target norovirus. Because viral gastroenteritis due to norovirus can last 1 to 3 days, dehydration is the main complication. Replenishing fluids and electrolytes lost from vomiting and diarrhea is critical to prevent hospitalization due to viral gastroenteritis. This patient's increased hemoglobin, hematocrit, and heart rate indicated that she was dehydrated; therefore, she received intravenous saline. Viral gastroenteritis was most likely in this patient. However, if symptoms persisted, *C. difficile* infection would need to be considered. First, antimicrobial therapy, which she received 3 weeks prior to her presentation, is a key risk factor for the development of *C. difficile*-associated diarrhea. Second, community-acquired *C. difficile* infections do occur. This patient was tested for *C. difficile* and was found to be negative.

2. Norovirus causes both sporadic and epidemic gastroenteritis and is considered the primary cause of endemic diarrhea in children. Worldwide, rotavirus is the most important cause of severe gastroenteritis in children (see case 24), but in countries that have rotavirus vaccines, norovirus has become the most common cause of severe gastroenteritis in children. Norovirus causes ~90% of nonbacterial and ~50% of all-cause epidemic gastroenteritis. In the United States, this translates to ~20 million cases of acute gastroenteritis annually, leading to ~65,000 hospitalizations and up to 800 deaths.

Transmission of norovirus primarily occurs by the fecal-oral route, but contaminated environmental surfaces and aerosolization during emesis are other sources of transmission. Norovirus can survive at a wide range of temperatures (0 to 60°C) and is relatively resistant to detergents and common disinfectants (ethanol-based and quaternary ammonium compounds), but it is effectively killed by bleach. It is estimated that the infectious dose is <100 viral particles. An individual infected with norovirus sheds billions of viral particles; shedding begins prior to symptoms and can last for weeks after symptoms resolve. Other factors that contribute to the communicability of norovirus include strain diversity and lack of long-term immunity. There are six genogroups of norovirus (I to VI), with genogroups I, II, and IV causing human disease, while genogroups III, V, and VI cause disease in cattle, mice, and dogs, respectively. Within each genogroup, there are antigenically distinct genotypes. This antigenic diversity is attributed to the accumulation

of point mutations during RNA replication (i.e., antigenic shift) as well as to recombination events between antigenically distinct noroviruses. However, despite this diversity, norovirus GII.4 (genogroup II, genotype 4) has emerged as the primary strain responsible for outbreaks. Human challenge studies have suggested that immunity is short-lived, as reinfection occurred when volunteers were challenged with an identical strain 2 to 3 years later. It should be noted that 13 to 40% of volunteers were never infected, which appears to be related to specific histo-blood group antigen receptors in the human gastrointestinal tract. The combination of viral antigenic diversity and lack of long-lasting immunity is a significant challenge to developing a norovirus vaccine.

Norovirus outbreaks can occur year-round but are more predominant in the colder months in temperate climates; thus the term "winter vomiting disease." The incubation period is 12 to 48 hours, and illness generally begins abruptly with vomiting followed by abdominal cramps, fever, and nonbloody diarrhea. Outbreaks have been described in hospitals, schools (particularly child care centers and preschools), long-term care facilities, military camps, restaurants, and cruise ships. Although this patient could have been exposed to norovirus anywhere in the community, it is likely that she was exposed at the preschool where she worked. Food-borne outbreaks due to norovirus represent ~25% of produce-associated outbreaks; fruits, vegetables, and lettuce have been sources of outbreaks. Shellfish are another source of food-borne outbreaks, particularly oysters because they are often consumed raw or undercooked. Because shellfish feed by filtration, oysters harvested from norovirus-contaminated water have high concentrations of norovirus. A major source of food-borne outbreaks in restaurants and catered events is symptomatic food handlers. It is unclear whether asymptomatic shedding of norovirus by food handlers leads to transmission. It is currently recommended that food handlers not work while symptomatic and for 72 hours following illness.

3. Diarrhea can be categorized as inflammatory or noninflammatory. Infectious agents that invade tissue or produce toxins cause inflammatory diarrhea, which is characterized by the presence of neutrophils in the stool. Fecal leukocytes can be visualized directly with a methylene blue stain if the stool is promptly transported to the laboratory, but this is often not possible. Lactoferrin is an iron-binding glycoprotein found in neutrophilic granules that is important for mucosal immunity. Inflammatory processes in the bowel that result in the recruitment and turnover of mucosal neutrophils will result in lactoferrin being released into the intestinal lumen, which can be detected in the stool by an enzyme-linked immunosorbent assay or immunochromatographic test. Studies have shown lactoferrin to be a more sensitive indicator of intestinal inflammation than direct observation of leukocytes because its detection is not dependent on intact cells. Lactoferrin is also found in breast milk; therefore, children who are breast-fed should not be tested for fecal leukocytes by lactoferrin detection. The challenge with interpreting a positive lactoferrin result resides in its lack of specificity for a specific disease process. For example, patients with inflammatory infectious diarrhea, inflammatory bowel disease, ulcerative colitis,

diverticulitis, and colon cancer all have lactoferrin-positive stools. However, in the appropriate clinical context, lactoferrin testing can be beneficial. This is particularly true for differentiating patients with active inflammatory bowel disease from those with noninflammatory irritable bowel syndrome.

Bacterial pathogens associated with inflammatory diarrhea include *C. difficile*, *Shigella*, *Salmonella*, *Campylobacter*, enteroinvasive *Escherichia coli*, and *Yersinia enterocolitica*. Parasitic causes of diarrhea rarely result in a positive lactoferrin test, with the exception of *Entamoeba histolytica*. Interestingly, viral gastroenteritis is not usually inflammatory. Occasionally, rotavirus and norovirus can cause mild inflammation, but usually the stool is negative for leukocytes. The positive fecal lactoferrin in this patient is not consistent with most norovirus infections. However, host differences may contribute to the amount of inflammation seen with viral gastroenteritis. This patient also had a history of diverticulitis, which can cause a positive lactoferrin test, so the positive may not have been a direct result of her norovirus infection. It is worth noting that both direct fecal leukocyte and lactoferrin tests can be falsely negative in immunocompromised patients who are neutropenic.

4. Although norovirus infection is usually self-limiting, it can cause greater morbidity and mortality in immunocompromised patients. Because patients who are immunosuppressed cannot efficiently clear the virus, norovirus often causes chronic gastroenteritis. Protracted norovirus illness has been reported in individuals with congenital immunodeficiency, persons receiving immunosuppressive therapy posttransplant, patients with cancer undergoing chemotherapy, and HIV-positive patients. Approximately 15 to 20% of hematopoietic stem cell and renal transplant recipients reportedly have chronic infection with norovirus often lasting for months to years. This can lead to weight loss, malnutrition, dehydration, and altered mucosal barrier, which may complicate the outcome of the underlying disease. Gastroenteritis is a common complication in posttransplant recipients, with causes including therapy, graft-versus-host disease (GvHD), and a wide variety of infections. When a diagnosis of GvHD is being considered, it is critical to obtain testing for norovirus and other infectious agents as immunosuppression is increased in the management of GvHD but potentially decreased in infections.

5. Routine diagnostic tests are not currently available for most physicians. However, a number of commercial tests are beginning to make their way into the clinical laboratory. Since methods to culture norovirus have yet to be described, laboratory diagnosis relies on detecting either viral antigen or RNA or visualizing the virus by electron microscopy. A commercially available enzyme immunoassay detecting norovirus GI and GII antigens has been shown to have a sensitivity of 58% and specificity of 92%. Due to the low sensitivity of norovirus antigen tests, the Centers for Disease Control and Prevention does not recommend their routine use for the diagnosis of sporadic norovirus cases, but they have been used in outbreak settings when multiple specimens are being tested. Reverse transcription-PCR (RT-PCR) is the preferred method for detect-

ing norovirus in stool specimens. RT-PCR-based tests are very sensitive and can differentiate the most common norovirus genogroups (GI and GII). While there is currently no commercially available molecular test to detect only norovirus, there are several products in development. There is an FDA-cleared molecular multiplex gastrointestinal panel that includes norovirus among other common agents of gastroenteritis, which is what was used to diagnose this patient. There are several challenges to using molecular methods for norovirus detection. For example, feces and vomitus can be difficult to extract and may be inhibitory to RT-PCR. Therefore, it is critical to have controls that measure extraction and RT-PCR efficiency so that false-negative results are not reported. Also, the exquisite analytical sensitivity of molecular methods can decrease the clinical specificity. This is readily apparent in immunocompromised patients, who may shed norovirus for extended periods of time. Although symptomatic patients have high fecal viral loads, asymptomatic persons and chronically infected immunocompromised patients typically have low viral loads. It can be difficult to determine if a low-level positive by a molecular test is indicative of the current disease process. Quantitative RT-PCR may prove to be valuable in distinguishing acute gastroenteritis from chronic shedding, particularly in immunosuppressed patients.

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CASE 26

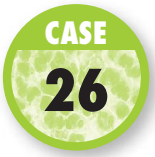
The patient was a female in her 80s with pulmonary hypertension requiring chronic oxygen by nasal cannula. She had a hospital admission 1 month previously for worsening shortness of breath, dyspnea on exertion, and chest pain. Chest radiograph was consistent with right middle lobe pneumonia. She was given a 14-day course of levofloxacin and discharged to a skilled nursing facility. Three weeks later she had the onset of loose, watery stools, which increased in frequency to >10 times/day. She had generalized weakness and diffuse abdominal pain and was unable to walk. She also was unable to eat, with several episodes of nausea and vomiting. Her family and social history was significant only for her having recently moved to a nursing home. On readmission, approximately 3 weeks into her diarrheal disease course, she had fever, chills, malaise, myalgias, and dizziness. She had had a 5-lb weight loss since her prior admission. She continued to have shortness of breath but no cough. Physical examination was significant for a pulse of 120 beats/min, respiratory rate of 24 per minute, and blood pressure of 82/45 mm Hg. On examination her abdomen was soft, diffusely tender, and mildly distended with no rebound or guarding. She had decreased bowel sounds. Otherwise her physical examination was consistent with her underlying disease. Laboratory data were significant for a white blood cell count (WBC) of 29,000/μl. Her stool was heme negative. A test for the detection of glutamate dehydrogenase (GDH) and toxins of *Clostridium difficile* is seen in Fig. 26.1. An abdominal radiograph showed grossly dilated loops of bowels. Abdominal computed tomography revealed colonic wall thickening consistent with pancolitis. Over the next 4 hospital days her WBC counts progressively increased from 29,000/μl to 127,000/μl. She had increasing abdominal girth and decreasing bowel sounds. She was judged to be a poor surgical risk. On the fourth hospital day the patient expired.

1. What bacterial toxin(s) can cause the constellation of symptoms that this patient had and was responsible for her death? Describe these toxins and explain how they cause the disease seen in this patient. What pathologic lesions are pathognomonic for this disease?
2. Most bacterial agents of infections are detected by culture. Why isn't this approach used for detection of this pathogen?



Figure 26.1 Immunochromatographic test for GDH and toxins A and B from *C. difficile*.

3. Describe three factors that predisposed this patient to infection with this organism.
4. Describe the spectrum of disease seen with this organism.
5. Explain what structure this organism produces that is important in the spread of this organism among patients. What other potential roles might it have in the disease course observed with this organism?
6. The epidemiology of infections with this organism indicates that the disease it causes is more severe than previously. What data support this observation? What are possible explanations for this increased virulence?
7. What special problems have been encountered in treatment of this organism?



CASE DISCUSSION

1. *C. difficile* toxins A and B were detected in the feces of this patient by immunochromatography ("Tox" line in Fig. 26.1) during her fatal disease course. These large protein exotoxins, which have very similar structures and amino acid sequences, are responsible for the constellation of symptoms associated with *C. difficile*-induced disease. Other toxins, such as the Shiga toxin produced by enterohemorrhagic *Escherichia coli*, can be detected in the feces of patients with diarrheal disease, but not as frequently as *C. difficile* toxins A and B. In addition, certain features of her disease presentation (see answer 3 for more details) are most consistent with this organism.

C. difficile grows and produces these two toxins at the colonic epithelial surface, where both toxins are taken into the cell by a process called receptor-mediated endocytosis. Once inside the colonic epithelium, they inactivate small GTPases. This inactivation results in a number of pathologic events within the cell leading to the pathophysiologic changes seen in tissue.

An important initial event is stimulation of proinflammatory cytokine production. These cytokines cause increased vascular permeability in the colon and influx of phagocytic cells, resulting in inflammation of the bowel, or colitis. Increased colonic permeability also contributes to the diarrhea seen in these patients. Other changes observed include apoptosis and cell death and the loss of tight junctions, which results in neutrophil migration into the gut and contributes to the watery diarrhea seen with this organism.

The most severe manifestation of this disease is the development of pseudomembranous colitis and toxic megacolon (which likely occurred in this patient, based on her abdominal radiographic and physical findings such as increasing abdominal girth and decreasing bowel sounds). Pseudomembranes are areas of viable tissue overlaid with dead cells, neutrophils, fibrin, and bacteria (Fig. 26.2). This lesion is pathognomonic of *C. difficile* infections.

Interestingly, this woman had a leukemoid reaction, with her peripheral WBC count exceeding 125,000/μl. Such a finding is associated with a poor prognosis in patients infected with *C. difficile*.

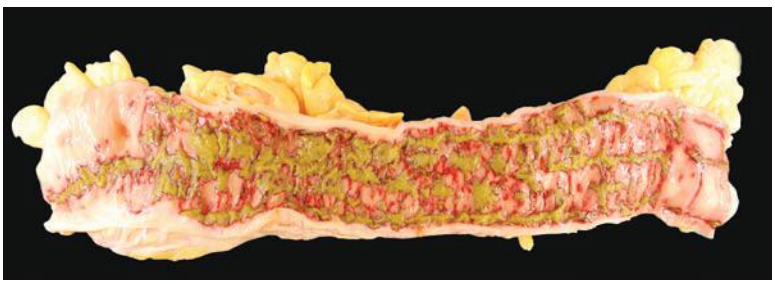


Figure 26.2 The pseudomembranous lesions are the yellow-green areas on the surface of the colon.

2. The organism's name, "difficile," gives a clue as to why culture is not done. Recovery of the organism is difficult and time-consuming. In addition, non-toxin-producing *C. difficile* strains can be frequently recovered from patients. Nontoxigenic organisms are nonpathogenic, so when cultures are done, the ability to produce toxin must be demonstrated, further delaying the establishment of the etiologic agent. Culture done to detect toxigenic organisms is of value for epidemiologic reasons. It is also believed to be the most sensitive means of establishing the diagnosis of *C. difficile*-associated disease.

Currently two approaches are widely used for the detection of *C. difficile* infections. The most widely used approach is the detection of toxin genes by using nucleic acid amplification tests (NAATs). Several different formats for NAAT are available in the industrialized world. A specific problem with NAAT is that it can detect both live and dead organisms. As a result, this testing approach should only be used in patients with documented diarrheal disease to establish the diagnosis of *C. difficile* infection. The best way for the laboratory to determine whether the patient has diarrhea is if the stool specimen takes the form of the specimen container. Because the organism can persist once the diarrhea resolves, this test should not be used in patients as a test of cure.

A second diagnostic approach, seen in Fig. 26.1, is to use an antibody-based method that detects both a cell wall antigen of *C. difficile*, GDH ("Ag" in the figure), and toxins A and B ("Tox" in the figure). The strategy for using this test is to report it as negative for *C. difficile* if the test is negative for both antigens and positive if it is positive for both antigens. The problem with this assay is that ~10 to 15% of specimens are positive for GDH but negative for toxin A plus B because of the relative insensitivity of the toxin A and B portion of this assay compared with toxigenic culture. Since nontoxigenic strains of *C. difficile* along with other bacteria may give a positive GDH result, it is necessary to perform confirmatory testing for GDH-positive, toxin-negative specimens to establish that the patient has a strain that can produce toxin. Confirmatory testing is typically performed by NAAT. The major reason to use the algorithmic approach is that the immunochromatographic test is 25 to 50% less expensive than primary testing by NAAT.

3. The most important of the predisposing factors is the patient having received the fluoroquinolone antimicrobial levofloxacin prior to the development of her diarrhea. It has been shown in animal models that antimicrobials—especially those with superior activity against gut anaerobes, such as clindamycin, cephalosporins, and certain fluoroquinolones—alter the gut microbiota. This alteration provides an ideal ecologic niche in which *C. difficile* may grow, produce toxin, and induce disease. Almost all cases of *C. difficile* occur either concurrently or up to 8 weeks after antimicrobial administration. In this case, disease developed 1 week after her antibiotic course was completed. It should be noted that any agent that can alter the gut microbiota can induce *C. difficile* disease. Other agents not normally considered antibacterial agents that have been shown to induce *C. difficile*-associated disease include methotrexate and azidothymidine.

The second factor is her stays in both a hospital and a long-term care facility. Stays in both of these types of health care facilities have been associated with increased likelihood of developing *C. difficile*-associated disease.

The third factor is the patient's age. There is a growing literature that demonstrates that this disease is more common and more severe in those over 65, with ever-increasing morbidity and mortality as aging continues. The reason for the increase in disease as individuals age is multifactorial, due to a combination of immunosenescence, loss of protective microbiota, increased antimicrobial use, and increased stays in health care institutions including hospitals and long-term care facilities.

4. *C. difficile* causes a broad spectrum of disease. The most common manifestation of infection appears to be asymptomatic carriage of the organism. Infected patients can have mild diarrhea, often associated with concurrent antimicrobial therapy. They can have more severe diarrhea accompanied by nonspecific inflammatory changes in the intestinal tract. The most severe manifestation of *C. difficile*-associated diarrhea is pseudomembranous colitis. Pseudomembranous colitis is a life-threatening condition that can be complicated by perforation and toxic megacolon. It must be aggressively treated, including removal of diseased portions of the colon. Because of the patient's tenuous medical condition, it was decided that she was not a good surgical risk, and she died as a result of her *C. difficile* disease.

5. *C. difficile* is a spore-forming bacterium. Spores can remain viable for months in the environment and are much more resistant to disinfectants than vegetative cells. These spores are frequently found throughout the rooms of infected individuals. They may remain capable of infecting other patients weeks after the infected patient has left the room. Spores are resistant to many commonly used disinfectants including alcohol. This requires two courses of action. First, health care providers should use soap and water, rather than alcohol hand gels, to wash their hands following encounters with *C. difficile*-infected individuals. Second, upon discharge or transfer, rooms of infected patients should be washed with high concentrations of hypochlorite (bleach). The concentration of bleach necessary to kill spores is caustic and may damage the surface of furniture, equipment, and other fixtures in patient rooms.

In addition, spores have proven to be resistant to all antimicrobials. As a result, spores are likely to play an important role in relapses of *C. difficile* disease (see answer 7 for further details).

6. Beginning in 2001, the number of cases of *C. difficile* increased significantly in the United States (2-fold increase), Canada (8-fold increase), and Great Britain. In Canada, there was a significant increase in mortality due to this organism (attributed mortality, 7%; overall mortality, 25%), especially in individuals who were >60 years of age. In addition, the rates of therapeutic non-response and relapse also increased significantly. The

increased rates of infection in Great Britain resulted in the National Health System taking drastic action, including fining hospitals that did not meet specific targets for *C. difficile* infection rates. This action has resulted in reduced numbers of infections with this organism. In the United States, hospitals are now required to report their *C. difficile* infection rates, with the intended consequence that hospitals will strive to reduce infections with this deadly organism.

The most likely explanation for this increase in virulence is the observation that a specific *C. difficile* strain designated NAP1/027 is being found with increasing frequencies in all three of these countries. This organism has been shown to produce ~20 times more toxin A and B than other strains of *C. difficile in vitro.* The reason for this increased toxin production is a lethal mutation in a gene designated *tcdC* that downregulates toxin production. When this suppressor is not produced, toxin production is increased.

A second strain of *C. difficile*, 078, which also has mutations in *tcdC* and produces increased levels of toxin, has recently been recognized in humans. This strain is particularly interesting because it is also found widely in food animals such as cattle and pigs. With the increasing recognition of community-acquired *C. difficile* infections, there is some thought that this organism has been transferred to humans from meat, but an absolute link has proven elusive.

7. The problem of increasing frequency and severity of infections with *C. difficile* has been compounded by two additional problems related to treatment of this infection: poor response to therapy and recurrences after treatment.

Prior to the emergence of the NAP1/027 strain, response to therapy with either metronidazole or vancomycin was ~90%. The recurrence rate was between 5 and 12%. Since the emergence of NAP1/027, studies have found a response rate as low as 50% with failure rates of 20% and recurrence rates of 30%. In those >65 years of age, recurrence rates of as high as 60% have been reported. The failure rate does not appear to be due to the development of resistance to either vancomycin or metronidazole.

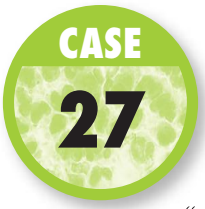
Recurrences can be due to either relapse or reinfection. Recurrences are believed to be due to the patient's inability to develop colonization resistance and/or to mount a humoral immune response to the *C. difficile* toxins following discontinuation of antimicrobial therapy. In relapse, when antimicrobial levels decline or disappear, *C. difficile* spores present in the gut can vegetate and begin producing toxin and disease. In reinfection, patients obtain organisms from their environment. Again because of the absence of suppressive microbiota, the organism can grow in the colon and produce toxin.

A novel strategy to treat recurrences is to use a "fecal microbiota transplant" in patients with a history of recurrence, to repopulate the gut with suppressive microbiota. Stool is obtained from healthy individuals and is transplanted into the gastrointestinal tract of the ill individual by using a nasogastric tube, enema, or colonoscope. A recent case-controlled clinical trial has shown this to be highly effective in the treatment of recurrences. More conventional approaches with probiotic preparations have been of

more limited effectiveness in recurrence prevention. Additionally, a new antimicrobial agent, fidaxomicin, has been developed and used in the treatment of recurrence of infection due to non-NAP1/027 strains. Because of its expense, this agent is not generally used to treat initial *C. difficile* infections. A final strategy to prevent recurrences is to use intermittent dosing of antimicrobial agents, the idea being to discontinue antimicrobials for a brief time to allow vegetation of spores in the gut and to then kill the newly vegetated cells with a "pulse" of antimicrobials.

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The patient was a 15-year-old boy who presented to his local pediatrician with acute onset of rectal pain. On the morning of his presentation he noticed that he passed what he described as a yellow "string" of about 4 feet in length in his stool. This "string" was not associated with either blood or pus. Residual "string" was stuck in his anus, and when the patient tried to pull it, it caused severe lower left quadrant abdominal pain. With his mother out of the room, the patient denied ingesting anything unusual and adamantly denied inserting anything in his anus or any kind of sexual contact.

On physical examination, vital signs were within normal limits. An anal examination revealed the presence of a 50-cm-long rubbery, segmented string extruding from his anus. The patient reported pain when the object was pulled. It was extracted from the anus and sent for microbiologic examination, the results of which are seen in Fig. 27.1 and 27.2.

1. What was the organism found in this patient?
2. Describe the segment that was "crushed" for Fig. 27.2. What was the structure found in it that was used to identify the organism?
3. When the identity of the organism was known, the father stated that the patient had been on a wilderness trip to Canada. While he was there, what did he likely do to become infected with this organism?
4. Briefly describe the life cycle of this organism. What is the definitive host of this organism? What is the secondary host? What is this organism called in common parlance? Name two organisms belonging to the same group of organisms that can infect humans. Are humans secondary or definitive hosts for these organisms?



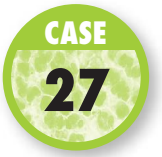
Figure 27.1 Material extracted from anus.



Figure 27.2 Material obtained from crushing segment of material in Fig. 27.1.

5. People who eat sashimi, sushi, ceviche, carpaccio di persico, or smoked whitefish all are at risk for infection with this organism. Why? Name two fish that are commonly associated with infection with this organism. What can be done to prevent the organism from being transmitted when any of the above foods are ingested?
6. What is the major complication of this infection when a patient has a significant organism burden?

CASE DISCUSSION



1. The organism extracted from the patient's anus was a flat, wide worm most consistent with a tapeworm. In addition, the segments of the worm were quite wide, which make this most consistent with the tapeworm *Diphyllobothrium latum*. *Latum* is the Latin word for "broad" or "wide."

2. The structure of a tapeworm is fairly simple, with a head, the scolex, attached to many segments that appear rectangular, called proglottids. Proglottids contain both sexual organs and are egg-producing machines with hundreds of thousands of eggs produced per worm per day. This species of tapeworm grows rapidly in humans and may grow by more than a meter in a week. Worms can grow to be as long as 15 meters in the human intestine, but most are much shorter than that. The segments may also break off and be passed, appearing like rice grains in feces. The segment that was "crushed" was a proglottid. When a gravid proglottid is crushed, eggs are released. The size (45 µm by 65 µm) and shape of the egg seen in Fig. 27.2 are consistent with *D. latum*. A key feature of the eggs of this worm is the operculum, which unfortunately is difficult to appreciate in Fig. 27.2. The operculum is on the end of the egg and is the site where the initial larval stage emerges from the egg.

3. The boy went camping during the summer months in northern Ontario. While there, he caught some type of whitefish in a freshwater lake. He cleaned and ate the fish, which he cooked over a campfire. It is likely that he did not cook the fish thoroughly and became infected with the larval form of *D. latum* present in the fish flesh.

4. Humans are the definitive host for *D. latum*, which means a human is the host in which reproduction of the parasite takes place. The life cycle of *D. latum* is complex. Eggs are produced and fertilized in the proglottids. These eggs are released from the worm and passed in feces. The eggs hatch in freshwater and the larvae are ingested by copepods (small crustaceans), which are the first intermediate host of this parasite. In the crustaceans, the parasite develops into a stage called the procercoid. When the infected crustacean is ingested by a freshwater or anadromous fish, the procercoid enters the tissue of the fish and develops into the plerocercoid phase. This phase, when ingested by humans in uncooked or undercooked fish flesh, develops into the adult tapeworm, where the cycle can begin again.

There are two other tapeworms that are common in humans, the pig tapeworm, *Taenia solium*, and the bovine tapeworm, *Taenia saginata*. Humans can be both the definitive and intermediate host for *T. solium* but only the definitive host for *T. saginata*. The determining factor in whether humans are an intermediate or definitive host for *T. solium* is the stage of the parasite that is ingested. If eggs excreted by humans are ingested, the patient is an intermediate host, and the disease process that develops is called cysticercosis.

If *T. solium* cysts are ingested by eating raw or undercooked pork, then the individual is the definitive host and an adult tapeworm results. It is somewhat ironic that the common parlance for all three tapeworms is dependent upon the name of the intermediate host from whom the human host becomes infected.

5. All these forms of fish are eaten uncooked, which allows the plerocercoid larval stage to survive and infect the human. The larval stage can be killed in one of two ways. One is by cooking it to 55°C for 5 minutes; the other is to freeze the fish at –20°C for 1 week or –35°C for 1 day. Unfortunately, most individuals who eat sushi, sashimi, or other types of raw fish prefer the texture of unfrozen fish.

A large variety of freshwater lake fish have been found to be infected with this parasite, including pike, perch, burbot, and walleye. In certain European locales as many as 15% of the lake fish are infected with this organism. *D. latum* is also common in anadromous fish, which are fish that swim upriver in order to spawn in freshwater. The key anadromous fish that are infected with *D. latum* and can cause human infection are the different species of salmon. Both trout and whitefish also can be a source of this infection.

6. Most individuals do not realize they are infected with *D. latum* unless they see passed proglottids in the feces. In the individual in this case, it is likely that he had the worm in his large bowel and that explained the pain he felt when the physician tried to extract the worm. A single dose of praziquantel is sufficient to purge the worm. Most patients are asymptomatic, but those with a high worm burden may experience gastrointestinal symptoms including diarrhea, abdominal pain, and discomfort. Probably the most severe complication of *D. latum* infection in patients with a high worm burden is pernicious anemia. Anemia is a result of a parasite-induced vitamin B_{12} deficiency. It is estimated that the worms absorb 100 times more B_{12} than the host, and if worm burden is high enough, this can result in the B_{12} deficiency.

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CASE 28

The patient was a 21-year-old civil engineering student in the United States with expertise in water sanitation, who had traveled to Ethiopia to work on a water project at a school in Addis Ababa. He presented with a 2-week history of nausea and vomiting that had increased in severity and frequency over the previous 3 days. He had been unable to keep anything "down" for the past 3 days and had occasional loose stools with cramping. He had not taken his malaria prophylaxis, nor had he taken any ciprofloxacin that was given to him by his physician in case he developed diarrhea. He worked at a school in an urban slum where he ate food and drank water even though the school did not have running water. He had never been tested for HIV but denied having had any sexual activity in Ethiopia.

On physical examination, he had a temperature of 38°C, blood pressure of 110/70 mm Hg, and pulse of 120 beats/min. He appeared pale, exhausted, but alert and oriented. His examination was notable for his extremities being cool to touch. He had a peripheral white blood cell count of 15,000/μl with 13,500/μl neutrophils. He was not anemic. A fecal specimen was obtained for ova and parasite examination and stool culture. The stool was guaiac positive and had white blood cells observed on methylene blue stain of the feces. Bacterial stool cultures were negative, but the parasite exam showed the organism in Fig. 28.1.

1. What organisms should always be in the differential diagnosis of a patient who returns from Ethiopia with fever and chills? You should be able to name four. Which is the most important, and why?
2. Based on the picture and clinical presentation, what organism was likely causing his infection? How did he likely become infected?
3. Briefly describe the virulence factors produced by this organism that are responsible for the tissue damage it causes.
4. One of the observations made about intestinal tract infections with this agent is that up to 90% are asymptomatic. What information concerning this genus may explain that finding?
5. Approximately 1% of patients infected with this organism have a specific extraintestinal manifestation. What is it? How is it best diagnosed?

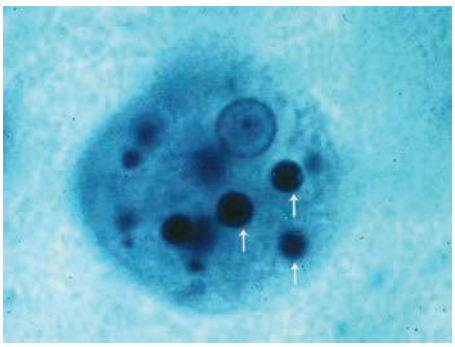
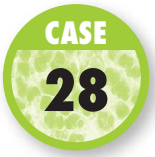


Figure 28.1



CASE DISCUSSION

1. The most common reason an individual consults a physician after traveling to sub-Saharan Africa is a systemic febrile illness with or without diarrhea, the exact scenario seen in this case. As travel from North America and Europe to sub-Saharan Africa for business, educational, or social reasons becomes more frequent, understanding the potential agents with which such individuals are frequently infected is taking on greater import. A patient returning from sub-Saharan Africa with fever is assumed to have malaria until proven otherwise. This is particularly true for individuals who did not take malaria prophylaxis, as was the case here. Individuals who have been in Ethiopia are most likely to be infected with *Plasmodium falciparum*, which causes the most severe form of malaria (see case 61 for further details). It is important to note that patients with malaria can have diarrhea, so its presence as part of the clinical presentation should not be used to exclude malaria from the differential diagnosis. In our experience, malaria-infected patients who have the poorest outcomes are those who do not take malaria prophylaxis and do not have malaria considered in the differential diagnosis at initial presentation with a febrile illness. This patient had multiple peripheral blood smears stained and examined for malaria, all of which were negative for this parasite.

The second most common cause of systemic illness with fever in an individual from sub-Saharan Africa would be tick-borne rickettsial diseases with *Rickettsia africae*. *R. africae* typically causes a much milder disease than *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever.

Typhoid fever caused by *Salmonella typhi* should also be considered in light of his fever, diarrheal disease, and likely exposure to contaminated food and water. Typhoid fever typically is a more systemic illness, with diarrhea occurring in a minority of patients.

Finally, agents of diarrheal disease must be considered, especially those that cause invasive disease such as *Entamoeba histolytica*, *Campylobacter*, and *Shigella*.

Both dengue and *Neisseria meningitidis* should be mentioned in the context of febrile illness in travelers returning from sub-Saharan Africa. Individuals who travel to Ethiopia as well as many other countries in sub-Saharan Africa are at risk for yellow fever and should be vaccinated against that virus, although this vaccination is not required for entry. Dengue is endemic in Ethiopia but is infrequent in travelers there. By contrast, it is an important agent in the febrile traveler who has recently returned from the Caribbean, Central America, South America, Southeast Asia, and the Indian subcontinent. Ethiopia is located in the "meningitis belt" that traverses Central Africa. Despite the increased rate of *N. meningitidis* among the native population in this region, cases of *N. meningitidis* infection are rarely reported in travelers. Since only half of individuals who travel to that region of the world consult a physician prior to travel, it is unlikely that special precautions such as meningococcal vaccination are widely practiced in this group.

2. This patient was infected with *Entamoeba histolytica*. This is based on a clinical presentation of dysentery, of which *E. histolytica* is a common etiologic agent in travelers to sub-Saharan Africa. In fact, it is more common than bacterial agents of dysentery in this patient population. Additionally, the parasite seen in stool is morphologically consistent with *E. histolytica*, although this species cannot always be distinguished from either *Entamoeba dispar* or *Entamoeba moshkovskii* microscopically. However, an important additional piece of information seen in Fig. 28.1, phagocytized red blood cells (white arrows), is indicative of *E. histolytica*. *E. histolytica* readily ingests red blood cells, while neither *E. dispar* nor *E. moshkovshii* does.

E. histolytica infections are acquired by ingestion of water or food that has been contaminated with human feces. Given the patient's consumption of food and water of questionable quality during his stay in Ethiopia, it is likely that he obtained the organism in this manner. We found it interesting that someone with expertise in providing clean water would be so careless about the water he consumed! Alternatively, these infections can be acquired by direct oral-anal contact. Transmission of the disease is via the cyst form excreted in the stool of infected individuals.

3. As the name of this parasite implies, this organism is histotoxic and is capable of producing ulcerative-type lesions in the intestinal tract as well as liver abscesses. It has been well documented that for this organism to cause its cytotoxic effect, it must directly adhere to its target cell.

Three virulence factors have been established as playing a role in the pathogenesis of *E. histolytica*. First, the Gal-GalNAc adhesin is important in the organism's ability to penetrate the thick mucus layer that overlays and protects the colonic epithelium, the target site for the parasite. This adhesin also plays a role in the parasite's direct adherence to the colonic epithelium. It is also speculated to be involved in stimulating colonic epithelial cells to produce the cytokine interleukin-8. This cytokine recruits and activates neutrophils, resulting in the inflammatory response that is so characteristic of this disease.

The second virulence factor is a polypeptide called the amebapore. It is well recognized that this polypeptide can form channels in the lipid bilayers of bacterial and cultured eukaryotic cells, causing them to lyse. It is less clear what role this virulence factor has in cytolysis of colonic epithelial cells, which is a central feature of the disease process.

The third virulence factor is the cysteine proteinases. *E. histolytica* produces several different types of cysteine proteinase. These enzymes appear to have two roles in the pathogenesis of *E. histolytica* infection. First, they play a role in the invasiveness of the parasite by degrading the extracellular matrix of the colonic mucosa. Second, they can degrade a variety of proteins that may contribute to the cytolytic process produced by this protozoan.

Together, these three virulence factors allow the trophozoite to ingest not just bacteria but also apoptotic epithelial cells and red blood cells. Phagocytosis is a central feature in the formation of ulcerative colonic lesions and liver abscesses.

4. Recent studies have shown that there are three closely related species of *Entamoeba*: *E. histolytica*, *E. dispar*, and *E. moshkovskii*. These studies have shown that *E. histolytica* is pathogenic while *E. dispar* and *E. moshkovskii* are not. Although *E. dispar* produces several molecules that are similar to the virulence factors described for *E. histolytica* in the answer to question 3, these molecules do not have the same level of biologic activity or, in some cases, have different biologic activity than their *E. histolytica* homologs. The diagnosis of *E. histolytica* infections is typically made by microscopically examining feces for organisms with distinct morphologic features characteristic of the parasite. Because these three parasites are morphologically indistinguishable in the absence of phagocytized red blood cells, microscopic examination is nonspecific. As a result, many of the clinical and epidemiologic data that are published about *E. histolytica* are misleading. Until recently, asymptomatic infection with *E. histolytica* was believed to be the most common clinical presentation of infection with this organism. It is now recognized that asymptomatic infections with *E. histolytica* are actually infrequent. Many of the patients who were previously reported to be asymptomatically infected with *E. histolytica* were most likely infected with *E. dispar* or *E. moshkovskii*.

These parasites can be distinguished from each other by a variety of molecular methods, including PCR.

5. The major extraintestinal manifestation of *E. histolytica* is liver abscess. Patients who develop liver abscesses do not necessarily have a prodrome of intestinal infection. The parasite is infrequently found in the feces, so two alternative diagnostic approaches are useful to diagnose liver abscesses due to *E. histolytica*. One approach that is valuable in a patient who does not live in an area where *E. histolytica* is endemic is the detection of antibodies against soluble somatic antigen of trophozoites. Serology is highly sensitive in the diagnosis of amebic liver abscess, being positive in >90% of patients.

A second approach is to detect adherence lectin of *E. histolytica* in either serum or aspirated material from patients with *E. histolytica* liver abscess, using an antigen detection test. This test is highly sensitive (95%) in the serum of patients with *E. histolytica* liver abscess, provided they have not received anti-*Entamoeba* therapy such as metronidazole. This test is very insensitive in patients who have received such therapy. The antigen can also be detected in material aspirated from the liver abscess, but the sensitivity does not appear to be as high as for that found in serum.

It would seem that the most effective strategy for diagnosing amebic liver abscesses would be to use the antigen assay in patients who have not received appropriate antimicrobial therapy and the antibody assay in those who have.

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CASE 29

The patient was a 5-year-old male who presented to the emergency department (ED) with a 5-day history of fevers to 39.3°C with some vomiting. Over the last 3 days he had developed abdominal pain and nonbloody diarrhea. Three days prior to admission a blood culture was drawn, which grew Gram-negative bacilli 1 day prior to admission. After the report of the positive blood culture, he was evaluated by his primary care pediatrician the morning of his admission. At that time he was afebrile and appeared well. He was not given antimicrobials. The physician advised the parents to call if there was any change in his condition. When he became febrile to 38.5°C that evening, the pediatrician instructed the parents to bring their son to the ED.

On admission he was febrile to 38.0°C. His blood pressure was 111/67 mm Hg, his heart rate was 126 beats/min, his respiratory rate was 24 per minute, and he had normal oxygen saturation. On presentation he was alert and in no apparent distress. His physical examination was unremarkable, including no abdominal pain and normal bowel sounds. He had a normal white blood cell count, blood glucose, liver enzymes, electrolytes, blood urea nitrogen, and creatinine. Blood cultures were drawn, and he was begun on intravenous ceftriaxone.

His past medical history was significant for him having helped with the neighbor's chickens for the past several weeks. He had also been exposed to a variety of reptiles in the area near his home. The next day, blood cultures obtained in the ED grew a Gram-negative rod (Fig. 29.1 and 29.2). His hospital course was uneventful. His fever resolved, and he was discharged on an antimicrobial regimen of 12 days of oral ciprofloxacin.

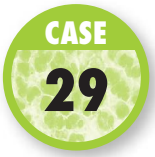


Figure 29.1 Organisms recovered from blood on Hektoen agar (black colonies indicating H_2S production) (left) and lactose nonfermenters on MacConkey agar (right).



Figure 29.2 TSI slant of organism recovered from blood (courtesy L. M. de la Maza).

1. What Gram-negative bacilli are likely to cause the type of presentation seen in this patient? The images in Fig. 29.1 and 29.2 should allow you to narrow your choice to one genus of bacteria. Explain why.
2. How did this individual likely get infected?
3. Patients who have achlorhydria or have received antimicrobials are at increased risk for infection with this organism. Why?
4. Explain the pathogenesis of bacteremia caused by the organism recovered in a patient such as the one described here.
5. The patient was discharged on ciprofloxacin despite the fact that susceptibility testing was not performed for that antimicrobial. Rather, antimicrobial resistance testing was done for nalidixic acid. Why? What antimicrobial resistance problems are common with this organism?
6. Many widespread outbreaks affecting hundreds to thousands of people are associated with the organism causing this infection. Why? How are these outbreaks frequently discovered?



CASE DISCUSSION

1. One of the most common reasons children are brought to their pediatrician is fever. In this particular case the patient was judged not to be particularly ill on his initial visit, at least based on his pediatrician's offering no intervention beyond performing a blood culture. He had some localizing symptoms—vomiting, abdominal pain, and nonbloody diarrhea—suggestive of gastroenteritis. When the initial blood culture became positive, the patient was again seen by his pediatrician. The child was well at that time, and again no intervention was taken. However, when the child again had a high fever, the decision was made to have him admitted to the hospital to receive intravenous antimicrobials.

The reality is, the pediatrician had a very good idea of what was causing the patient's illness when she received notification of a blood culture growing Gram-negative rods. The Gram stain of an organism growing from a blood culture can give much useful information to the physician. In a 5-year-old child with symptoms of gastroenteritis, the findings of lactose-nonfermenting, H_2S-producing, Gram-negative bacilli in the blood—as evidenced by nonpigmented colonies on MacConkey agar, indicating a lactose-nonfermenting organism, and black colonies on Hektoen agar and black butt on triple sugar iron (TSI) slant, indicating H_2S production (Fig. 29.1 and 29.2)—are highly suggestive of nontyphoidal *Salmonella* (NTS) infection. In the developing world, typhoid forms of *Salmonella* would also need to be considered. However, the TSI slant would give a different H_2S reaction.

Salmonella is divided into only two species, *S. enterica* and *S. bongori*. There are >2,500 serotypes of *S. enterica*, with *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteriditis being the most commonly recovered. This patient was infected with *S. enterica* serotype Braenderup, an infrequently recovered serotype. Other Gram-negative rods that could cause bacteremia secondary to gastroenteritis might include *Campylobacter*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*, but all three of these organisms would be highly unusual.

If the patient did not have localizing symptoms but only fever, several other Gram-negative organisms would need to be considered. *Escherichia coli* is the second most common Gram-negative bacillus recovered from the bloodstream of children between 1 and 5 years of age. It usually is associated with urinary tract infections or bowel catastrophes and not gastroenteritis. Other members of the *Enterobacteriaceae* would also need to be considered. Other Gram-negative bacilli that might occur in this setting are *Brucella* and *Francisella*, but most physicians will not see cases of these organisms in their professional careers.

Another Gram-negative rod, which once was the most common cause of Gram-negative bacilli bacteremia in this age group in the industrialized world and is now vanishingly rare, is *Haemophilus influenzae* type b. The reason for this organism's disappearance was the development and widespread distribution of a conjugated capsular polysaccharide vaccine that has proven to be highly efficacious.

2. NTS infections are zoonotic infections, meaning that humans obtain the infection from animals either directly or indirectly. The asymptomatic carriage rate in chickens is very high, reaching as high as 90% in some studies but usually around 60%. Studies have shown that chicks sold by commercial hatcheries have high *Salmonella* carriage rates despite attempts to control this organism. Eggs also are frequently contaminated with NTS. In addition to chickens, this patient was also exposed to amphibians and reptiles. Both snakes and turtles are well-recognized sources of NTS. Reports of outbreaks of *S.* Braenderup have been associated primarily with produce, with recent outbreaks being due to both Roma tomatoes and mangoes. The actual source of the child's isolate is unknown, but his contact with chickens and cold-blooded animals gave him ample opportunity to become infected with NTS.

3. A very high NTS inoculum, 10^6 to 10^8 organisms, is needed to cause infections in humans with functioning innate immunity. One of the key components of innate immunity in the gastrointestinal tract is the pH of the stomach. When the pH of the stomach is low, many of the organisms that are ingested will be killed. Only when the inoculum is high can this effect of the innate immunity be overcome. In patients with achlorhydria, the inoculum size is much reduced because the higher pH in the stomach allows organism survival and increased risk for infection.

The microbiota of the intestinal tract also plays a role in innate immunity to NTS by conferring colonization resistance. There are at least three different mechanisms of colonization resistance. Members of the microbiota may produce substances such as certain metabolites and bacteriocins that inhibit the growth of NTS. Second, these microbes may physically block NTS from binding to the mucosa, preventing this organism from causing disease. Finally, the microbiota may stimulate the ongoing production of mucins, secretory IgA, and antimicrobial-like molecules called defensins that may play a role in protection. The importance of this third factor is unclear since NTS is known to be resistant to human defensins. When antimicrobials are given to individuals, this alters the microbiota, reducing colonization resistance. In NTS epidemiologic outbreak investigations, prior antimicrobial therapy has been recognized as an important risk factor for NTS.

4. NTS can cause bacteremia secondary to gastroenteritis. In the initial stage of NTS gastroenteritis, the organism is taken into mucosal epithelial cells, preferentially M cells, by a process called bacteria-mediated endocytosis. The *Salmonella* pathogenicity island-1 (SPI-1) type 3 secretion system (T3SS) plays a key role in this process. The SPI-1 T3SS injects effector molecules into the epithelial cells, causing ruffling, a change in the cells' cytoskeleton that results in engulfment of the bacteria. Other effector cells of the T3SS induce an inflammatory response to the organism. The end result is colitis and diarrhea with neutrophil transmigration in the gut and loss of tight junctions between mucosal epithelial cells. This loss of tight junctions allows paracellular fluid leakage, leading to the diarrhea that is observed. Another result of this inflammatory process is the production of

tetrathionate at the epithelial surface. This molecule can be used as an electron acceptor by NTS but not by other members of the microbiota, giving NTS a fitness advantage.

In patients who develop bacteremia, a second T3SS encoded by SPI-2 plays an important role. Once the organism has translocated across epithelial cells, it can be ingested by macrophages either by an SPI-1 T3SS-mediated process or by other processes. The SPI-2 T3SS is expressed within the phagosome and plays a central role in the organism's survival and replication within a structure called the *Salmonella*-containing vacuole. These infected macrophages can be disseminated throughout the body, resulting in bacteremia, as was seen in this patient.

5. Antimicrobial therapy is used to treat NTS bacteremia but rarely NTS gastroenteritis, which is generally self-limiting. Fluoroquinolones have been an attractive choice to treat NTS because they can be given orally and resistance is still <5% in the United States. However, treatment failures have occurred with ciprofloxacin even with organisms that were reported to be sensitive to it. Ciprofloxacin clinical failure was first reported in *S.* Typhi, with subsequent cases being observed with NTS. The most common fluoroquinolone resistance mechanism in Gram-negative organisms is modification in binding to the antimicrobial target, DNA gyrase. The quinolone resistance determining region (QRDR) is the specific region in the *gyrA* gene in which resistance mutations most frequently occur. A single mutation in the QRDR results in high-level resistance to nalidixic acid but not to ciprofloxacin. Two mutations in the QRDR are needed to result in the detection of ciprofloxacin resistance by standard antimicrobial susceptibility testing. Because fluoroquinolone treatment failures can be associated with nalidixic acid-resistant isolates, it is recommended that nalidixic acid be used to screen for fluoroquinolone-resistant organisms. Changes in fluoroquinolone susceptibility testing standards have recently been made, but not every laboratory can perform the recommended testing, so nalidixic acid testing remains a reasonable screening test for fluoroquinolone resistance.

As with all *Enterobacteriaceae*, antimicrobial resistance is increasing in *Salmonella*. Part of the reason for this is the industrialization of chicken, egg, pork, and beef production. Highest efficiency in the husbandry of these animals is achieved by keeping them in close quarters and feeding them vast quantities of food. When animals are kept in close quarters and are overfed, infectious disease spreads. One strategy is to use antimicrobials to "treat" the herds and flocks. It is estimated that antimicrobial use is 10 times higher in animals than in humans, creating tremendous selective pressure for antimicrobial-resistant organisms. As a result, the finding of multidrug-resistant (MDR) *Salmonella* is common. The good news is that the trend in MDR *Salmonella* is an encouraging one, as the number of these organisms has declined in the United States over the past decade, although 1 in 10 *Salmonella* organisms is MDR. Unfortunately, for the two mainstays of therapy, ceftriaxone and ciprofloxacin, the news is less encouraging, as over the past decade resistance rates have increased 15-fold for ceftriaxone and nonsusceptibility rates (nalidixic acid resistance) have increased 6-fold for ciprofloxacin; however, overall rates remain low, 3.4% for ceftriaxone and 2.4% for ciprofloxacin.

6. The industrialization and globalization of the food supply is thought to be responsible for large outbreaks of NTS disease. Two large outbreaks of NTS have occurred in the United States during the past 5 years. One, due to contaminated peanut butter and paste, resulted in >700 NTS cases. In this outbreak, >3,900 products containing either contaminated peanut butter or paste were recalled. The actual source of the peanut contamination was not known, although breaks in proper peanut processing were found. This outbreak shows how a raw ingredient, peanuts, can find its way into myriad products, including dog treats, breakfast cereals, and snack foods as well as peanut butter and peanut butter crackers, putting large populations at risk. A second outbreak of NTS in 2010, due to contaminated shell eggs, caused >1,900 NTS cases. It was estimated that >350 million potentially contaminated eggs were shipped during the outbreak and 550 million eggs were recalled. The source of the outbreak was two large commercial egg producers that kept >1 million birds in cages and fed them by conveyor-belt systems. These operations produce hundreds of thousands of eggs per day.

One of the important surveillance systems for the detection of national food outbreaks in the United States is PulseNet. PulseNet is a national network of public health laboratories that "fingerprint" enteric pathogens such as NTS from infected patients, using a technique called pulsed-field gel electrophoresis, to look for disease outbreaks. Individuals infected with organisms with the same fingerprint suggest that an outbreak might be occurring. In Fig. 29.3, organisms in lanes 2, 3, and 5 have a similar "fingerprint," indicating that those organisms are genetically related to each other but not to the organisms in lanes 1 and 4.

Detecting a potential outbreak strain takes 2 to 3 weeks. From there, local, state, and federal public health authorities will investigate whether an outbreak is occurring and what the potential source of the outbreak might be. As a result, recognition and determination of the source of large-scale food-borne outbreaks may take weeks to months.

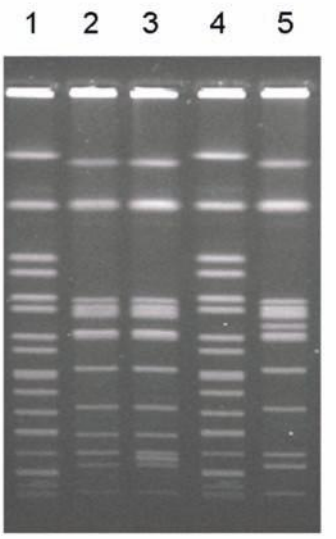


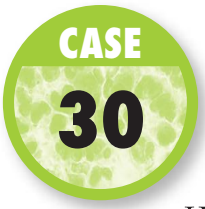
Figure 29.3 Pulsed-field gel electrophoresis fingerprints of *Salmonella* (from U.S. Department of Agriculture).

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The patient was a previously healthy 11-year-old female who came to the emergency department (ED) in mid-September with a 2-day history of bloody diarrhea. Three days previously she had the onset of fever, headache, and lower abdominal pain. Her diarrhea began as watery and became increasingly bloody. She denied any recent travel but reported that her brother also had bloody diarrhea. In her history, she said she had eaten a hamburger at a school picnic prior to the onset of disease, as well as having consumed spinach. There was no family history of inflammatory bowel disease or bloody stools.

On physical examination, the patient's vital signs were normal and the physical findings were unremarkable except for severe abdominal pain. Her stool was hemoccult positive and showed 2+ white blood cells (WBCs). A complete blood count was within normal limits except for a WBC of 14,900/μl, with an absolute neutrophil count of 13,500/μl. She was given morphine in the ED for her abdominal pain. An abdominal ultrasound ruled out acute appendicitis but revealed thickened bowel loops consistent with colitis.

During the first week of her hospital course she continued to have bloody diarrhea and severe abdominal pain. Her final stool submitted to the laboratory on hospital day 7 was consistent with a blood clot. During her hospital course she developed low urine output and hematuria, with a serum creatinine of 2.1 mg/dl on hospital day 5. Her renal symptoms were treated with fluids and her renal function was closely monitored. In addition, on hospital day 6 she had a platelet count of 16,000/μl and a hemoglobin level of 7.2 mg/dl. She received a unit of packed red blood cells on the 6th, 7th, and 11th hospital days. By discharge on the 13th hospital day her serum creatinine, blood urea nitrogen, and platelet count had returned to normal and her hemoglobin had stabilized at 10.2 mg/dl.

Culture of her stool on sorbitol MacConkey agar is seen in Fig. 30.1.

1. What organism is infecting this patient?
2. What two virulence factors does this organism produce, and what are their roles in the gastrointestinal disease seen in this patient? Explain why multiple serotypes of this organism can produce these virulence factors.
3. Was the clinical course of her illness consistent with infection caused by this organism?

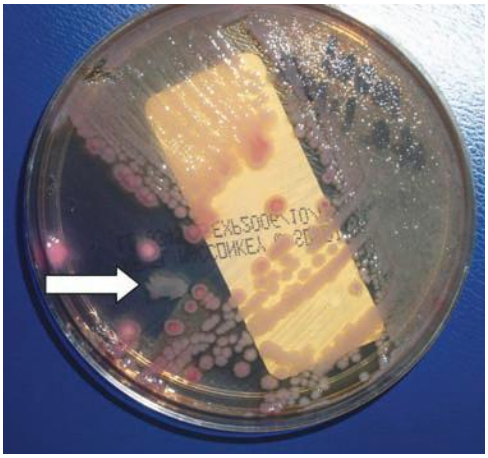


Figure 30.1

Explain. Were her renal findings consistent with infection with this organism? Explain. What strategy has proven useful in preventing renal sequelae of this disease? Why are antimicrobials contraindicated in the treatment of this infection?

4. How do you think this patient became infected? How could you prove a specific source was responsible for infection with this organism?
5. In 2011, a large outbreak of disease with a variant of the organism seen in this patient occurred in Germany. What was the variant of this organism? What was the transmission vehicle for this outbreak? What was unusual about this outbreak?
6. Give at least two reasons why large outbreaks caused by this organism are being recognized with increasing frequency.
7. In the laboratory, three different techniques are used to detect this organism. What are they, and what are their strengths and weaknesses?

CASE DISCUSSION



1. This patient had one colony of a sorbitol-negative organism (see the arrow pointing to the colorless colony surrounded by pink [sorbitol-fermenting] colonies on sorbitol MacConkey agar in Fig. 30.1). Isolates that fail to ferment sorbitol are unusual among *Escherichia coli*. One of the most common sorbitol-negative serotypes of *E. coli* is O157:H7, an organism associated with bloody diarrhea. Subsequent serotyping of this sorbitol-negative colony confirmed it as *E. coli* O157:H7. An alternative culture approach is to use chromogenic agar, which can detect sorbitol-negative *E. coli*. *E. coli* O157:H7 is also referred to as Shiga toxin-producing *E. coli* (STEC) because of its ability to produce Shiga toxin, the major virulence factor in the development of enterohemorrhagic colitis (inflammation of colon with bloody diarrhea). Other serotypes of *E. coli* can produce Shiga toxin and thus cause enterohemorrhagic colitis (see answer 2 for further details), but O157:H7 appears to be the most common cause of this illness in the Northern Hemisphere, although perhaps not in the Southern Hemisphere.

2. All STEC strains produce Shiga toxin, and most strains produce intimin. There are two forms of Shiga toxin, Stx1 and Stx2. STEC strains can produce both or either toxin. Biochemically the two toxins are both AB_5 toxins with the B subunits binding to a specific glycosphingolipid receptor, GB_3, found on both epithelial and renal endothelial cells. The A subunit is taken into the endoplasmic reticulum, where it inhibits protein synthesis at the ribosomal level, causes apoptosis, and promotes expression of proinflammatory cytokines. In animal models, these toxins have been shown to induce the pathophysiology associated with STEC. Stx1 and Stx2 are distinct toxins both sequentially and immunologically. Stx2 represents a family of toxins with seven subtypes. Stx2 is associated with the most severe manifestation of STEC infection, hemolytic-uremic syndrome (HUS) (see answer 3 for more details). Two subtypes, Stx2a and Stx2c, either alone or in combination, are most frequently associated with HUS.

A second virulence factor frequently found in STEC is an outer membrane protein, intimin. *E. coli* strains that express intimin use a type 3 secretion system to inject a receptor, translocation intimin receptor (Tir), into the host cell, to which intimin binds. Intimin-producing *E. coli* strains form a specific lesion referred to as an attaching-and-effacing lesion. This lesion is characterized by the formation of a pedestal on the surface of the epithelial cell on which the intimin-containing bacterium sits.

Multiple serotypes of *E. coli* are able to cause enterohemorrhagic colitis because the genes that code for Shiga toxin are carried on a phage that can be transferred to a number of different serotypes of *E. coli*. More than 100 different *E. coli* serotypes causing Shiga toxin-induced disease have been found in humans.

3. Much of what is seen in this patient's history is typical of the clinical course of STEC infection. It often begins with watery diarrhea, which becomes increasingly bloody over the next 48 to 72 hours of illness. Severe abdominal pain, which was documented in this patient, is a key feature of this illness. Approximately half of patients have WBCs in feces.

On the 5th day of illness this patient began to experience signs of renal failure. The major complication of STEC infection is HUS, which ordinarily occurs between the 5th and 14th days of illness. HUS is characterized by a triad of thrombocytopenia, elevated serum creatinine levels, and anemia. The patient had all three of these findings, and both her thrombocytopenia and anemia were severe. Approximately 5 to 15% of patients with STEC infection progress to HUS. HUS is found primarily in children <5 years of age and is the most common cause of renal failure in childhood. The mortality rate is ~5%. All strains associated with HUS have Stx2 but not necessarily initimin or Stx1. The pathophysiology of disease is due to Stx2 toxin variants binding to and damaging endothelial cells in the glomeruli and epithelial cells in the renal tubules. This damage is associated with deposition of fibrin in the renal microvasculature, leading to hemolysis and renal failure.

A recent study indicates that intravenous volume expansion with isotonic saline protects patients with bloody diarrhea from developing HUS. It is suggested that all patients with bloody diarrhea be treated intravenously with isotonic saline as early as possible to prevent or ameliorate the HUS disease course since renal damage begins prior to the development of HUS manifestations. This individual had three risk factors—bloody diarrhea, female sex, and WBC count of >13,000/μl—that have been associated with HUS development, so intravascular volume expansion earlier rather than later in her disease course would likely have been beneficial.

Antimicrobial administration to patients with STEC has been found to be a significant risk factor for the development of HUS in children. Thus, antimicrobial agents are contraindicated for the treatment of STEC infections. Antimicrobials should be avoided in patients with bloody diarrhea and then used only if STEC has not been detected and the infection is severe.

4. The patient's illness was temporally associated with a large national outbreak of *E. coli* O157:H7 due to the consumption of contaminated bagged spinach from a specific supplier. During the outbreak, approximately 200 individuals had confirmed infections with a specific *E. coli* O157:H7 genotype that was found in several bags of implicated spinach. Sixteen percent developed HUS.

The patient's isolate was analyzed using a microbial forensics technique called pulsed-field gel electrophoresis (PFGE) (see "A Primer on the Laboratory Diagnosis of Infectious Diseases" in the front of this book for more details on this technique). PFGE is used to determine how closely related different bacterial isolates are genetically. The best way to explain PFGE is to say it is a way of "fingerprinting" the organism, with the fingerprint type being referred to as a pulsotype. Although this patient consumed bagged spinach, her

isolate was found by PFGE to be a fairly common pulsotype and not the specific outbreak pulsotype. However, it is possible that more than one genotype was involved in the outbreak. Alternatively, she may have had what is called a "sporadic" case.

STEC infections can be obtained in a variety of ways. It is believed that most cases can be ultimately linked to cattle that carry this organism asymptomatically as part of their gastrointestinal microbiota. The most common manner in which individuals become infected is by the consumption of undercooked ground beef that is contaminated with STEC from cattle gastrointestinal tracts or hides during processing. The patient also ate a hamburger prior to her illness, so it is possible that that was the source of her infection. Carriage rates in cattle are higher in the summer months, the time when vegetables may be growing in adjacent fields. The organism can contaminate surface waters through runoff from cattle pastures or feedlots. Individuals can swim in these contaminated surface waters and become infected. Leaf vegetables such as spinach, lettuce, and sprouts can be contaminated by field runoff of STEC-tainted water. These vegetables are particularly problematic because it is impossible to wash them sufficiently to remove these organisms. In addition, these vegetables are generally consumed without cooking, which could kill the organism. Leaf vegetables have been implicated in as many as 25% of U.S. STEC outbreaks. Petting zoos have also been the source of STEC infections, and as a result, either they have been discontinued or hand disinfectants are made available for use upon exiting the animal enclosures.

Human-to-human spread via defecation into swimming pools or on the hands of caregivers in day care centers has also been reported as a mode of STEC spread.

STEC strains are relatively acid stable, which enhances their infectivity. STEC has an inoculum size similar to that of *Shigella* (~1 to 100 organisms), which makes dissemination fairly efficient and increases the likelihood of both sporadic cases and outbreaks.

5. The largest outbreak to date of non-O157:H7 STEC infections occurred in the summer of 2011 in Germany. It was due to a rarely encountered STEC strain, *E. coli* O104:H4. There were ~3,800 reported cases and 54 deaths. The transmission vehicle was sprouts used in salads and as garnishes. Interestingly, the largest *E. coli* O157:H7 outbreak, which occurred in Japan in 1996 and caused almost 2,800 culture-confirmed cases, was also associated with the consumption of sprouts. One of the unusual findings in the German outbreak was the high rate of HUS (22% of O104:H4-infected individuals). In other large outbreaks, the HUS rate is usually 5 to 10%. Further, 88% of the cases were seen in adults, with a predominance (68%) in women. This predominance was attributed to their preference for consuming sprouts. In the Japanese outbreak, all HUS cases were in children. HUS secondary to STEC is generally believed to be a disease primarily of children, making the HUS case distribution in female adults highly unusual. Like *E. coli* O157:H7 associated with HUS, this strain carried the *stx2* gene, with the specific toxin variant being Stx2a. It is worth noting that outbreaks of *Salmonella* have also been linked to consumption of sprouts.

6. The manner in which food is processed in much of the industrialized world has changed dramatically over the past few decades. Fast-food chains consume large quantities of both ground beef and leaf vegetables. To ensure product consistency and adequate supply, lots of many tons of ground beef containing the carcasses of hundreds of animals are purchased. During production of these multi-animal beef lots, the entire lot may become contaminated by a single STEC-tainted animal. These large lots are then distributed over many states. If the lot is contaminated and the meat is improperly cooked (i.e., it does not reach an internal temperature of 160°F), STEC infection may follow. Chopped leaf vegetables containing hundreds of heads may also be packaged in large lots for commercial customers. If these vegetables are contaminated during growing or processing, outbreaks may result. Multiple outbreaks consistent with both scenarios have been documented.

Another reason these outbreaks are more readily detected is that actual case findings and associated epidemiologic investigations have improved. During the last 2 decades, the Centers for Disease Control and Prevention (CDC), along with local and state health departments, has developed a laboratory-based surveillance system (PulseNet) for the detection of food-borne outbreaks. PulseNet uses PFGE to track bacterial food-borne illnesses. PulseNet data have shown that cases of food-borne illness in the United States are declining, which suggests that the food supply is safer than ever. The rapid detection of outbreaks via PulseNet may also be contributing to this decline.

A third reason is that many clinical microbiology laboratories now test stool directly for the two Shiga toxins (see answer 7). These assays can detect not only *E. coli* O157:H7 strains but other toxin-producing serotypes.

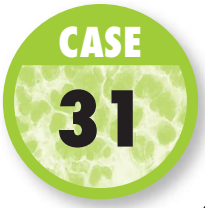
7. The three techniques for detection are culture on selective medium (discussed in answer 1), Shiga toxin detection, and PCR detection of Shiga toxin genes. It should be emphasized that enterohemorrhagic colitis and HUS are both Shiga toxin-mediated diseases and that the detection of either this toxin or the genes that encode it is a superior means of detecting STEC isolates. More than 100 serotypes of *E. coli* shown to produce Shiga toxin have been recovered from humans with STEC-associated diseases. Why then is culture on sorbitol MacConkey agar or *E. coli* O157:H7 chromogenic agar, which can only be used to detect a single serotype, ever used?

Culture is easy to do, is inexpensive, and as a result is much more widely available than PCR or Shiga toxin enzyme immunoassay (EIA). Sorbitol MacConkey or *E. coli* O157:H7 chromogenic agar culture is valuable because the approximately two-thirds of isolates associated with both enterohemorrhagic colitis and HUS in the United States are serotype O157:H7. Because STEC disease is uncommon and frankly bloody diarrhea is a key feature of this illness, culture of bloody stools is likely to be the most cost-effective means of detecting *E. coli* strains causing enterohemorrhagic colitis or HUS. Strains of O157:H7 are almost always toxigenic, so demonstrating the ability to produce toxin in this serotype is probably not important.

The value of both toxin and toxin gene detection is much greater for detecting other serotypes of STEC believed to be responsible for one-third of STEC infections in the United States. One of the keys for successful detection of either Shiga toxin by EIA or Shiga toxin genes by PCR is to use an enrichment broth rather than direct detection from feces. Feces is a complex matrix from which to detect toxin or toxin genes and may interfere with the assays. Growth in an enrichment broth amplifies both the amount of toxin and the number of toxin genes that may be present, while reducing stool matrix components. Even with a 12- to 24-hour broth enrichment step, PCR and EIA are more rapid and likely more sensitive for detecting O157:H7 than culture and have the added advantage of detecting multiple serotypes. False positives can occur with both methods. A major drawback of nonculture methods for the detection of STEC is that the organism is not readily available for epidemiologic investigation. Isolation of toxin-producing, non-O157:H7 strains from enrichment broth is laborious because non-O157:H7 strains lack a clearly identifiable phenotypic characteristic like being sorbitol negative, which makes identification of O157:H7 strains comparatively straightforward. In 2009, the CDC recommended that both culture and Shiga toxin detection methods should be used on all stools submitted for detection of enteric pathogens. This recommendation has been controversial in the clinical microbiology community because of the expense involved in performing both tests, especially in a clinical setting, where this is likely to be a low-yield effort.

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The patient was a 46-year-old man with a history of alcohol abuse who presented to the emergency department (ED) with acute abdominal pain, fever, chills, and leg cramping. On physical examination he had a temperature of 37.1°C, blood pressure of 93/34 mm Hg, heart rate of 125 beats/min, respiratory rate of 30 breaths/min, and O_2 saturation of 100% on room air. He was in acute distress, toxic appearing, dyspneic, and moaning in pain. His abdomen was mildly diffusely tender with guarding; he had hepatomegaly, but his lungs were clear and his neck was supple. Venous blood gas showed a pH of 7.1, pCO_2 of 33 mm Hg (normal, 40 to 60 mm Hg) and pO_2 of 68 mm Hg (normal, 30 to 55 mm Hg), blood lactate of 10.5 mmol/liter (normal, 0.5 to 1.8 mmol/liter), creatinine of 3.37 mg/dl (elevated), bilirubin of 2.1 mg/dl (elevated), elevated liver enzymes and lipase, creatine kinase of 955 U/liter (highly elevated), and lactate dehydrogenase of 951 U/liter (elevated). His white blood cell count of 4,100/μl was within normal limits, but he was mildly anemic (hemoglobin of 11.7 g/dl) and thrombocytopenic (platelet count of 53×10^9/liter). Abdominal computed tomography demonstrated cirrhosis of the liver and extensive varices of the spleen, stomach, and esophagus that were likely secondary to alcohol abuse. Blood cultures were obtained in the ED. He was given 8 liters of fluid, started on ceftriaxone and doxycycline, and transferred to the medical intensive care unit (MICU), where he was intubated because of acute respiratory distress and metabolic acidosis. His blood cultures grew the organism seen in Fig. 31.1. Further information gathered on the organism indicated that it was oxidase positive, halophilic, and a lactose fermenter (Fig. 31.2). When the blood culture results became available, he was switched from ceftriaxone to piperacillin-tazobactam and tobramycin. On arrival in the MICU, the patient's white blood cell count was 1,700/μl, his platelet count had dropped to 14×10^9/liter, his activated partial thromboplastin time was greatly elevated at >225 seconds, his lactate had increased to >16 mmol/liter, and his blood pH was 6.95. He also developed a dusky, nonblanching purpuric rash with some bullae. Despite the use of three vasopressors, aggressive fluid resuscitation (>10 liters), and antimicrobials, the patient developed refractory shock and died of cardiopulmonary failure approximately 24 hours after admission to the MICU.

1. The physician noted in the chart that the patient had SIRS. What is SIRS and what characteristics of SIRS did this patient have? This patient was treated with a combination of a β-lactam/β-lactamase inhibitor and an aminoglycoside. Why is this combination frequently used in patients with SIRS?
2. The patient's systemic infection was complicated by disseminated intravascular coagulation (DIC). Briefly describe what DIC is. What bacterial

virulence factor produced by the organism infecting this patient can cause DIC?

3. What is the organism that caused this infection? Name two virulence factors that are believed to contribute to the virulence of this organism.
4. How did the patient likely become infected with this organism? Given the organism that infected this patient, is the outcome in this case surprising?
5. Another organism that is oxidase positive and a glucose fermenter (but not a lactose fermenter) can cause the same type of syndrome that was observed in the patient in this case. The difference is that this organism belongs to a different genus than does the organism infecting this patient and does not grow in salt water. What is this organism?
6. The organism infecting this patient belongs to the genus in which the concept of quorum sensing was first described. What is quorum sensing? What is our current understanding of its role in bacterial infections?
7. A different species within the genus of the organism infecting this patient was associated with a major epidemic following the 2010 earthquake in Haiti. What is the organism? How is it thought that this organism was introduced into Haiti and was spread? Briefly explain the pathogenesis of disease and how this infection is treated.

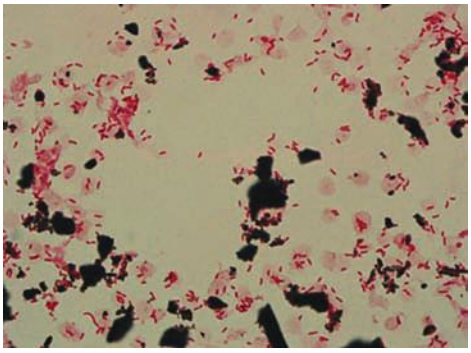


Figure 31.1 Gram stain of organism growing from blood culture bottle.



Figure 31.2 Organism growth on MacConkey agar after 24 hours' incubation at 35°C.



CASE DISCUSSION

1. SIRS, which stands for the systemic inflammatory response syndrome, was initially defined in 1992 by the American College of Chest Physicians and the Society of Critical Care Medicine. These definitions are the ones that are used here.

The body's response to any of numerous different stressors may result in an increase in the production of a variety of inflammatory mediators, such as cytokines, resulting in a clinical syndrome that is designated SIRS. This designation is independent of its cause. As a result, there are many causes of SIRS, both infectious and noninfectious (including pancreatitis, ischemia, hemorrhagic shock, multiple trauma and tissue injury, burns, and others). When the underlying cause of SIRS is a confirmed infectious process (most often bacterial, but also fungal, parasitic, or viral), it is termed sepsis. The term "septicemia" is imprecise and is no longer in use in the medical literature.

As initially defined in the published 1992 criteria, in order for a patient to be classified as having SIRS, at least two of the following four criteria must be present:

1. Fever (temperature of >38°C) or hypothermia (temperature of <36°C)
2. Tachycardia (heart rate of >90 beats/min)
3. Tachypnea (respiratory rate of >20 per minute) or hyperventilation (pCO_2 of <32 mm Hg)
4. Abnormal white blood cell count (>12,000/μl or <4,000/μl) or >10% immature neutrophils (band forms)

The criteria for SIRS have been subsequently modified and are now more complex. In addition, they are defined differently in children.

There is a continuum of clinical illness within the broader definition of sepsis. As a result, additional terms have been defined to establish the degree of severity of a patient's condition, which affects prognosis, rather than simply using the term "sepsis." Thus, severe sepsis, which has a mortality rate of 25 to 30%, is defined as sepsis associated with organ dysfunction of at least one organ system, hypoperfusion abnormality, or sepsis-induced hypotension. Sepsis-induced hypotension is defined by a systolic blood pressure of <90 mm Hg or its reduction by 40 mm Hg or more from baseline in the absence of other causes for hypotension. Septic shock, with a mortality rate of 40 to 70%, is a subset of severe sepsis in which severe sepsis occurs with hypotension and the need for vasopressors despite adequate fluid resuscitation.

With respect to the administration of antibiotics in those cases in which SIRS is thought to be infectious in origin, and hence is sepsis, unless the pathogen is established and narrow-spectrum anti-infective agents can be used, empiric, broad-spectrum antibiotics are typically administered intravenously. It is very important that the causative microbe (or microbes), which is most often bacterial, is treated with antibiotics that are appropriate. The selection of antibiotics in such cases will depend on many factors: any history of a specific prior infection; prior antibiotic use; whether the patient was an out-

patient or an inpatient at the time he or she developed signs and symptoms of infection; if the patient has been recently hospitalized or in a facility that is known to have antibiotic-resistant organisms; if the patient is or has been in an intensive care unit; whether there is a specific, likely site of infection (skin and soft tissue, pneumonia, intra-abdominal, catheter-associated, etc.) that may help target the antibiotic selection; whether there is a specific site, such as the central nervous system, for which some antibiotics will not achieve therapeutic levels; and knowledge of the hospital's and the community's antibiotic resistance patterns.

If the initial selection of antibiotics is inappropriate (that is, it doesn't "cover" the infectious agent causing the patient's illness), the patient has an increased rate of mortality compared with patients who receive appropriate antibiotics as initial therapy. This has been seen most clearly in patients who are treated with antibiotics for septic shock. In one study, patients with septic shock who received inappropriate initial antibiotic therapy had a 5-fold increase in mortality compared with those patients who received initial therapy that was appropriate (50% versus 10%). As a result of the need to select appropriate antibiotic therapy, a combination of a broad-spectrum antibiotic such as piperacillin-tazobactam or one of the carbapenems and an aminoglycoside (such as tobramycin or gentamicin) is often used as initial therapy with the idea that once the identification of the infectious agent and its antibiotic susceptibility results are known, the antimicrobial therapy can be modified. An antibiotic that is effective for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus*, such as vancomycin, is typically added to initial therapy if methicillin-resistant *S. aureus* is suspected, such as in infections of skin and soft tissue and in catheter-associated infections. There has also been an increase in sepsis due to fungemia (typically due to *Candida* spp.) in recent years, especially in recently hospitalized patients who are immunocompromised and who have received antibiotics; in such cases, appropriate antifungal therapy needs to be considered in the anti-infective regimen.

2. DIC is characterized by the systemic activation of the coagulation cascade in an unregulated manner. This causes intravascular deposition of fibrin and, as a result, occlusive thrombi in the microvasculature and the consumption of coagulation factors and platelets. The occlusive thrombi trigger ischemic events that impair the perfusion of vital organs. The consumption and subsequent depletion of clotting factors and platelets, or "consumptive coagulopathy," often leads to bleeding. This may be noted in the skin as petechiae, purpura, ecchymoses, or bleeding from venipuncture or injection sites. In addition, DIC may bring about gastrointestinal or vaginal bleeding. DIC is life-threatening and is associated with a high mortality.

The initiating event for DIC is the pathologic activation of coagulation in response to any of a variety of processes. These include both noninfectious and infectious causes. Noninfectious causes include severe tissue injury secondary to trauma or burns, the presence of any of a number of solid malignancies, acute promyelocytic leukemia, retained intrauterine fetal demise, and amniotic fluid embolism. One common factor in these processes is that there is the release of significant amounts of tissue factor, which produce the

activation of the extrinsic clotting cascade via the binding of tissue factor to and activation of factor VII.

The bacterial virulence factor made by the organism infecting this patient is endotoxin. Infectious causes of DIC, which are also characterized by the release of tissue factor, are most commonly due to endotoxemia from the release of lipopolysaccharide or lipooligosaccharide from the outer membrane of Gram-negative bacteria during bacteremia. This is especially prominent in bacteremic infections with *Neisseria meningitidis*, which may progress in just hours from nonspecific symptoms to fever and a nonblanching papular rash, to fatal septic shock with DIC and bleeding into the adrenal glands (Waterhouse-Friderichsen syndrome) demonstrated on autopsy.

Other systemic infections known to initiate DIC include those caused by *Streptococcus pneumoniae*, infections due to a number of the agents of viral hemorrhagic fever, and severe cases of malaria.

The treatment of DIC is aimed at the underlying cause of the process, be it infectious or noninfectious.

3. The patient had septic shock due to *Vibrio vulnificus*. As can be seen in Fig. 31.1 and 31.2, this organism is a slightly curved, Gram-negative bacillus that is a lactose fermenter on MacConkey agar. The organism grows relatively poorly on MacConkey, likely because it is a halophile (requires increased salt for growth). Halophiles associated with human disease are typically found in sea or estuarine waters. *V. vulnificus* is endemic in warm salt-water environments. For example, it can be found in high concentrations in waters of the Gulf of Mexico during warm-weather months.

V. vulnificus produces a polysaccharide capsule that allows it to evade phagocytosis. It also produces a hemolysin and an extracellular protease that contribute to its cytotoxic activity. Additionally, it produces an RTX toxin that can form pores in a variety of cell membranes, which are believed to be important in tissue damage and the dissemination of this organism. Mutational studies suggest that both RTX toxin and hemolysin are essential for maximal virulence. The organism also has sophisticated iron acquisition mechanisms that include the production of the siderophore vulnibactin, which allows it to compete for iron, an essential nutrient, in the human host.

4. In this case, we can only speculate because the patient lived alone and was in extremis when he was seen at the hospital. He was admitted during the summer months, the time during which most disseminated cases of *V. vulnificus* occur. More than 95% of disseminated infections are associated with the consumption of raw oysters. This is because filter feeders such as oysters can concentrate microorganisms in their flesh. During the summer months, *V. vulnificus* counts in seawater are increased in oyster-raising areas such as the Gulf of Mexico, from which 60 to 70% of the oysters are harvested in the United States. As a result, the concentration of *V. vulnificus* in these filter feeders is increased. It is believed that these high concentrations are needed to overcome the gastric acid that plays

an important role in the innate immunity against this organism. The assumption in this case is that the patient consumed raw oysters prior to becoming ill.

Hepatitis A is another pathogen that is found in increased numbers in filter feeders and can be contracted by consumption of raw oysters.

Patients with cirrhosis of the liver, as was seen in this patient, are 80 times more likely to be infected by *V. vulnificus* than the general population. It is believed that this increase in susceptibility to disseminated *V. vulnificus* infection is due to decreased levels of complement produced in these patients, which adversely affects both chemotaxis to and phagocytosis of these organisms.

The outcome in this case was not surprising. Patients with hepatic cirrhosis who consume raw oysters and develop *V. vulnificus* septicemia have a 200-fold-increased likelihood of dying compared with the general population. Early institution of appropriate antimicrobial therapy is essential for survival. Even when antimicrobials are administered within 24 hours of the onset of clinical disease, the mortality rate is >50%. If they are administered after 72 hours of illness, the mortality is 100%. An interesting observation concerning *V. vulnificus* is that infection is more common in males than in females, with approximately 90% of septic events occurring in males.

5. Another organism causing a waterborne illness that is clinically indistinguishable from *V. vulnificus* septicemia is *Aeromonas hydrophila*. Unlike *V. vulnificus*, *A. hydrophila* is more likely to be associated with freshwater and estuarine sources. The manner by which *Aeromonas* is obtained in patients with sepsis is not as definitive as in cases that occur with *V. vulnificus*, although seafood has been implicated in some studies from Southeast Asia. However, patients who develop sepsis typically have contact with freshwater or estuarine sources, and the majority of cases occur in patients with cirrhosis of the liver. As with *V. vulnificus*, disease is more common in males and during summer months, and has a significant mortality rate of 33%. Both *V. vulnificus* and *A. hydrophila* can cause necrotizing fasciitis with secondary bacteremia following trauma involving waterborne sources such as injuries while fishing. As with primary bacteremia, these infections are more common in males and during warm-weather months and result in a mortality rate of >50%.

6. Quorum sensing is the bacterial phenomenon of differential gene expression based on bacterial cell population density. One set of quorum sensing-regulated genes is expressed at low bacterial concentrations, whereas another set of genes is expressed at high concentrations. Cell density-dependent gene regulation is achieved by the production and extracellular accumulation of chemical signal molecules called autoinducers. The autoinducers increase in concentration as a function of cell density and act to induce or repress specific genes. Quorum sensing was first discovered in the luminescent marine bacterium *Vibrio fischeri*, which is a symbiont in the light-producing organ of the Hawaiian bobtail squid. At low bacterial densities, *V. fischeri* does not produce light, but at high cell density (in the squid light organ), it is luminescent. It is now appreciated that a variety of Gram-negative

and Gram-positive organisms use quorum sensing to regulate a diverse array of physiologic activities including symbiosis, virulence, antibiotic production, motility, and biofilm formation. A classic example of the impact of quorum sensing on the disease process is the quorum sensing system found in *Pseudomonas aeruginosa*. There are three quorum sensing systems found in *P. aeruginosa* that contribute to the activation of virulence factors such as elastase, proteases, pyocyanin, swarming motility, and toxins. Further, biofilm formation is partially controlled by quorum sensing in *P. aeruginosa*, as well as in other organisms. The production of biofilms protects organisms from antimicrobial activity and host defenses, which contributes to the pathogenesis of chronic infections such as cystic fibrosis airway disease and chronic wound infections. Since quorum sensing regulates virulence and/or biofilm production in many pathogenic organisms, "quorum quenching" mechanisms are being investigated and may provide a novel approach to inhibiting microbial infections.

7. The largest outbreak of *Vibrio cholerae* in the Western Hemisphere in the last 100 years is ongoing on the island of Hispaniola, with the vast majority of cases occurring in Haiti (>600,000 cases with 7,500 deaths) and a much smaller number in the bordering Dominican Republic (~20,000 cases with >100 deaths). This epidemic is due to a variant strain of *V. cholerae* 01 biotype El Tor, which has been associated with more severe disease. It was likely introduced onto the island by Nepalese United Nations peacekeepers who carried this organism. The particular epidemic strain is genetically identical to strains that are endemic in Nepal. It has been postulated that poor sanitation at the soldiers' base camp along the River Meille/Meye resulted in raw sewage from the camp being discharged into the river, contaminating it. Because of destruction of the drinking water infrastructure caused by the 2010 earthquake, people living downstream from the camp used untreated water from this river as a drinking water source. It is along this river that the epidemic began and soon spread throughout the island. Because water infrastructure was superior in the Dominican Republic and had not suffered significant damage, the magnitude of the epidemic there has been limited.

V. cholerae causes the disease cholera. Cholera is a disease characterized by a voluminous, watery diarrhea in which the infected individual can lose many liters of fluid in a day. If this fluid is not replaced, it can result in multiorgan failure and death. Cholera has one of the best-understood pathophysiologies of any infectious disease. The understanding has resulted in a simple, life-saving treatment that was widely applied during the recent Haitian epidemic.

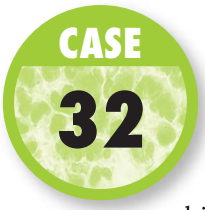
Cholera toxin is an A+B toxin produced by *V. cholerae*. The B subunits bind to specific receptors on intestinal cells, the GM1 gangliosides. The B subunit produces a pore-like structure through which the A subunit enters intestinal cells, where it activates adenylate cyclase, producing increased cAMP levels. Increases in cAMP result in the blockage of Na^+ and Cl^- uptake in intestinal epithelium and promote the secretion of Cl^- and water from crypt cells, resulting in the characteristic watery diarrhea. The recognition that glucose could reverse the blockage of Na^+ and water uptake by microvilli induced by cholera toxin led to the development of a novel, simple oral rehydration therapy (ORT) in which

table salt (NaCl), table salt supplement (KCl), baking soda ($NaHCO_3$), glucose, and water are mixed together and drunk by the patient with diarrhea. When initially used, ORT was shown to reduce cholera mortality from 30 to 3%. Today the World Health Organization target for the mortality rate in cholera outbreaks is <1%, a goal that was reached by late 2012 in the Haiti epidemic, although the overall mortality rate of the epidemic was above that rate. ORT has played a significant role in making this reduced mortality rate feasible.

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The patient was a 38-year-old Caucasian man who was referred to the digestive diseases clinic for unexplained elevated liver enzymes. The patient originally presented at a primary care clinic for chronic fatigue and muscle pain. He had a 10-year history of alcohol abuse but had been sober for 8 months. His serum alanine aminotransferase (ALT) level was 554 U/liter (normal, <35), and his aspartate transaminase (AST) level was 292 U/liter (normal, <40). He previously had normal liver function tests 2 years ago.

The patient reported feeling tired with muscle aches in his calves and other areas and stated that he had had sharp right upper quadrant (RUQ) pain for the past month. This RUQ pain was intermittent with no association with food and only lasted for a few seconds. During the physical exam, his RUQ was tender to palpation. Review of his social history revealed that he had unprotected sex with a known hepatitis C virus (HCV)-positive partner but that the partner's HCV RNA was negative.

The patient's hepatitis virus serologic testing results were as follows.

Hepatitis A virus (HAV) IgM antibody	negative
HBsAg (surface antigen)	positive
HBs (surface) antibody	negative
HBc (core) total antibody	positive
HBc (core) IgM antibody	positive
HCV antibody	negative

1. How do you interpret the patient's hepatitis serologic tests? Are the serologic test results consistent with his symptoms?
2. How did the patient likely acquire his infection? How does the epidemiology of the infection differ between patients from the United States and Asia?
3. What is the natural course of untreated infection with the organism causing his infection? What viral agent can, in the setting of coinfection, be associated with severe disease? What complications of this infection occur?
4. The patient's viral load was determined to be $>28 \times 10^6$ IU/ml, yet antiviral therapy was not initiated. When should therapy be started? What therapeutic options are available?
5. What is the role of HBeAg testing, and how does it affect treatment and outcomes?
6. An effective vaccine is available for preventing infection with this agent. Describe the nature of the vaccine, its efficacy, and who should receive the vaccine.



CASE DISCUSSION

1. The patient was not infected by HAV (indicated by negative HAV IgM) nor by HCV (negative HCV antibody). The interpretation of the serologic testing for HBV infection is of great clinical importance. Knowledge of the time course of the typical serologic patterns seen in acute infection (Fig. 32.1) and in patients who become chronic carriers of HBV after the initial infection (Fig. 32.2) is needed to interpret the serology. The positive HBsAg test, in combination with the positive HBc IgM antibody test, indicates that this patient had acute HBV infection (Table 32.1). The HBs antibody usually does not develop until at least 3 months postinfection, when there is a concomitant disappearance of HBsAg. The HBc total antibody test remains positive throughout the disease course because it detects both IgM and IgG antibodies. The presence of HBsAg indicates that the virus is actively replicating, which was supported in this case by HBV DNA testing. The patient's viral load was >28 million IU/ml.

The presentation of acute HBV infection ranges from subclinical to fulminant hepatitis. Approximately 70% of patients present with anicteric hepatitis, with the majority being asymptomatic. However, ~30 to 50% of patients may have a variety of other symptoms including anorexia, nausea, vomiting, low-grade fever, myalgia, fatigue, aversion to

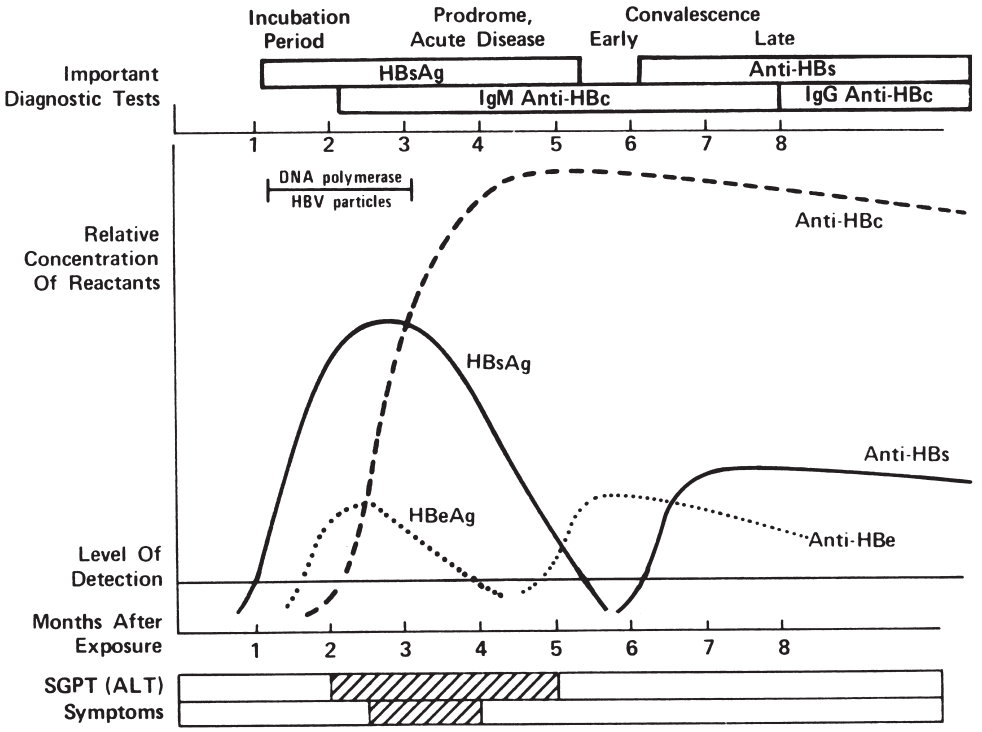


Figure 32.1 Serologic and clinical patterns observed during acute HBV infection. SGPT, serum glutamic pyruvic transaminase. (Reprinted from *Manual of Clinical Microbiology*, 7th ed., ©1999 ASM Press, with permission.)

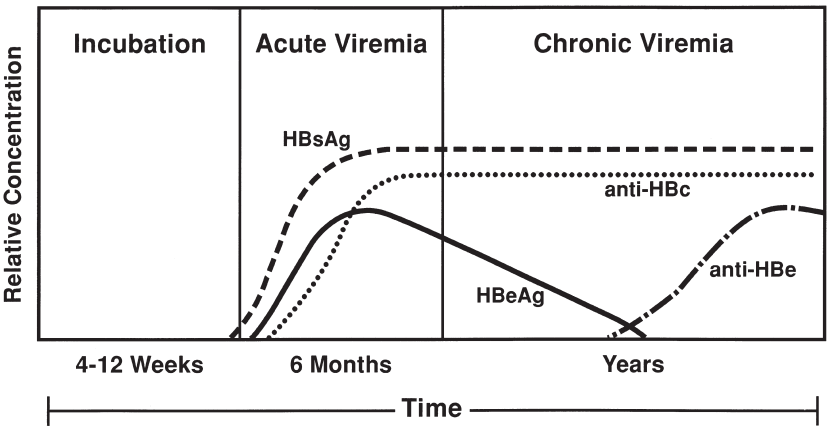


Figure 32.2 Typical sequence of serologic markers in patients with acute hepatitis B who develop persistent infection after exposure to HBV. (Reprinted from *Manual of Clinical Microbiology*, 7th ed, ©1999 ASM Press, with permission.)

food and cigarettes, and RUQ and epigastric pain. Acute HBV presentation may be more severe in patients with underlying immune dysfunction, coinfection with other hepatitis viruses, and underlying liver disease. This patient had several symptoms consistent with his diagnosis of acute HBV infection, including anicteric hepatitis, RUQ pain, and myalgia. Although this patient had a risk factor for severe acute disease (potential liver disease from alcohol abuse), his presentation was relatively mild.

2. The patient presumably acquired his infection from an infected sexual partner. Approximately 9 to 30% of individuals with chronic HBV are also infected with HCV. Thus, his HCV-positive sexual partner could also have been coinfected with HBV, or perhaps the patient had additional risk factors that were not disclosed. HBV is more transmissible than HCV or HIV. Following percutaneous exposure to a known positive source, the risk of acquisition for HBV is approximately 1 in 3, while the risk for HCV is 1 in 30 and that for HIV-1 is 1 in 300. Of course, the risk is dependent on the viral load of the source as well as the type of body fluid exposure (e.g., mucocutaneous exposure carries a much lower risk of transmission than percutaneous exposure for all three viruses). HBV is found in the body fluids of infected individuals, including, most notably, blood and semen. In addition to sexual transmission, HBV can be acquired perinatally from an infected mother to the infant and via blood either directly (such as by transfusions of blood products prior to effective donor screening) or indirectly (such as by sharing contaminated needles). Needle-stick injuries in health care workers are of particular concern, as HBV is transmitted more efficiently than HIV.

There are an estimated 350 million carriers of HBV worldwide, many of whom live in Asia. In Asian-Pacific countries, HBV is usually acquired either perinatally or by horizontal

TABLE 32.1 INTERPRETATION OF HEPATITIS B SEROLOGIC STUDIES

TEST	RESULT	INTERPRETATION
HBsAg	Negative	Susceptible
Anti-HBc	Negative	
Anti-HBs	Negative	
HBsAg	Negative	Immune due to natural infection
Anti-HBc	Positive	
Anti-HBs	Positive	
HBsAg	Negative	Immune due to hepatitis B vaccination
Anti-HBc	Negative	
Anti-HBs	Positive	
HBsAg	Positive	Acutely infected
Anti-HBc	Positive	
IgM anti-HBc	Positive	
Anti-HBs	Negative	
HBsAg	Positive	Chronically infected
Anti-HBc	Positive	
IgM anti-HBc	Negative	
Anti-HBs	Negative	
HBsAg	Negative	Four interpretations possible[a]
Anti-HBc	Positive	
Anti-HBs	Negative	

[a]Possible interpretations: (i) the patient may be recovering from acute HBV infection; (ii) the patient may be distantly immune, and the test is not sensitive enough to detect very low levels of anti-HBs in serum; (iii) the patient may be susceptible with a false-positive anti-HBc; and (iv) there may be an undetectable level of HBsAg present in the serum, and the patient is actually a carrier.

transmission in early childhood. This is in contrast to the United States and Western Europe, where most individuals are infected as adults. Perinatal infection is not typically associated with an acute hepatitis clinical syndrome, but rather chronic HBV infection.

3. Approximately 30% of adult patients who are infected with HBV have an acute illness characterized by jaundice due to elevated bilirubin, fatigue, and abnormal liver function tests (i.e., icteric hepatitis). Clinically, liver function tests indicative of hepatocellular injury (AST and ALT) are elevated in patients with acute hepatitis. Other tests of hepatic function, such as coagulation times, are typically abnormal because of a decrease in the hepatic synthesis of clotting factors. However, as noted in the answer to question 1, many patients with acute hepatitis B are asymptomatic or have anicteric hepatitis with a variety

of other symptoms. While the majority of infected patients will have a self-limiting illness, a small subset of acutely infected patients will develop fulminant hepatitis, which can be fatal. This is more likely to occur in those patients who are coinfected with hepatitis D virus (previously called delta hepatitis), an RNA virus that requires the presence of HBV to replicate and cause an infection. HBV genotype and environmental factors (acetaminophen, alcohol, and methamphetamine use) may also play a role in the development of fulminant hepatitis.

The risk of developing chronic HBV infection is dependent on the age at initial infection. Fewer than 5% of adults become chronically infected. This contrasts with infected neonates, 90% of whom will become chronic asymptomatic carriers, and children 1 to 5 years old, 20 to 50% of whom will become chronically infected. Chronic infection, associated with a significant rate of cirrhosis, may lead to end-stage liver disease and to the development of hepatocellular carcinoma. Other complications, due to immune complex formation, may result in extrahepatic manifestations, such as polyarteritis nodosa and other vasculitides. These can result in injury to the kidney, lung, and other organs.

4. Only supportive treatment is recommended for acute HBV infection, since most patients will clear the infection naturally. Patients with a more severe initial presentation (severe jaundice, encephalopathy, or coagulopathy) may require hospitalization. Although there are not randomized, placebo-controlled trials to support the use of antivirals in acute HBV, many physicians choose to treat patients with prolonged severe initial infection, fulminant hepatitis, immunodeficiency, coinfection with HCV or hepatitis D, or preexisting liver disease. Nucleoside/nucleotide monotherapy is preferred to interferon and should be discontinued when the patient's HBsAg test is negative.

HBV antiviral therapy is generally reserved for the treatment of chronic HBV to reduce the risk of cirrhosis and hepatocellular carcinoma. Successful treatment is indicated by the loss of HBsAg from the serum, but this occurs in a minority of patients. More commonly, sequential liver biopsies are used to monitor the progression of chronic liver disease. Histologic improvement does occur in patients who remain HBV carriers (indicated by a positive HBsAg test). This is likely due to decreased viral replication associated with nucleoside/nucleotide antiviral therapy, which is supported by the decreased levels of HBV DNA detected in the serum of patients successfully treated.

There are seven drugs approved by the FDA for HBV treatment: pegylated alpha interferon and standard alpha interferon; the nucleoside analogs lamivudine, entacavir, and telbivudine; and the nucleotide analogs adefovir and tenofovir. HBV is unique in that its genome is composed of partially double-stranded DNA that is transcribed from covalently closed circular DNA to form pregenomic RNA. The nucleoside/nucleotide analogs are viral DNA polymerase inhibitors that act as chain terminators to prevent the reverse transcription of the HBV pregenomic RNA to DNA. Interferon therapy, which acts as an immunomodulator, is not known to be associated with the emergence of HBV mutations, whereas significant resistance rates have been demonstrated with lamivudine monotherapy

(16% at year 1, 61% at year 3) and also, to a lesser extent, with the other nucleoside/nucleotide drugs. Therefore, combination therapy will likely become the most effective treatment for chronic HBV infection. Unfortunately, interferon therapy has a high rate of side effects and is more difficult to administer than the specific antiviral drugs, which are administered orally.

5. HBeAg is a protein that is secreted by HBV-infected cells and is a marker of active viral replication. HBeAg generally appears within a week of HBsAg. In the natural progression of HBV infection, HBeAg is present for 3 to 6 weeks and then disappears before HBsAg. The disappearance of HBeAg is followed by the appearance of anti-HBeAg (seroconversion), which is associated with greatly reduced viral replication. Persistence of HBeAg indicates chronic HBV infection with active viral replication. However, mutations occur in the precore region and core promoter that render HBeAg negative or reduce its expression. Core promoter mutations, in particular, have an association with more severe liver disease, including hepatocellular carcinoma. Therefore, treatment guidelines have a lower viral load threshold for treating HBeAg-negative disease relative to HBeAg-positive disease (i.e., 2,000 IU/ml versus 20,000 IU/ml, respectively). In patients with HBeAg-positive disease who are treated with interferon, sustained reductions in HBV DNA occur primarily in those who demonstrate seroconversion with anti-HBeAg. In patients treated with lamivudine, the reduction in HBV DNA occurs even in those who do not seroconvert.

6. The most important method used to prevent HBV infection is an immunization given as a series of three intramuscular injections. Effective vaccines against HBV have been available since 1982. The initial vaccine was derived from individuals who were chronic carriers of HBV. The vaccine that is now in use is a recombinant vaccine. A subset of people do not respond to the vaccine (i.e., do not produce detectable antibodies to HBsAg). With repeat vaccinations, some of these individuals will respond, as demonstrated by the production of an adequate antibody level to HBsAg.

The neutralizing antibodies that are induced by vaccination are directed to a hydrophilic region of the HBsAg. One concern is the development of vaccine-escape mutants of HBV. HBV mutations in the *S* gene that confer amino acid substitutions within the hydrophilic region of the surface antigen can allow the replication of HBV in people who have received vaccination. Fortunately, these events are rare to date, and there has been no evidence of epidemiologic spread of vaccine-escape mutants even among close contacts. Nonetheless, if the frequency of these mutations increases, there could be a decrease in the efficacy of the vaccine. Therefore, surveillance for vaccine-escape mutants is of great importance to assess the need for modification of the current vaccine.

An additional method of protection, which is used in the setting of a known exposure to HBV in a nonimmune individual, is the use of hepatitis B immunoglobulin (HBIg). For example, HBIg is given following a needle-stick injury in a nonimmune individual. Both vaccination and HBIg have been shown to decrease the rate of chronic infection due to HBV in infants born to mothers who are infected with HBV.

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The patient was a 49-year-old woman who presented to the emergency department with a 3- to 4-week history of nausea, vomiting, and periodic fevers. She had recently returned from a visit to rural areas of India. She admitted to swimming in the Ganges River, eating in restaurants, drinking water without boiling or filtration, and having "raw" milk in her tea. She was exposed to rats, cattle, and mosquitoes. She did not take malaria or gamma globulin prophylaxis and received no pretravel vaccinations. Two days prior to admission she began feeling tired and "started sleeping all day." She complained of vomiting after meals and 2 to 3 days of watery diarrhea at the onset of the illness, which had since resolved. She had fevers every 5 days with associated arthralgias and myalgias. Her fevers were documented to 38°C.

On physical examination she was a thin woman in no apparent distress. She had a temperature of 36.9°C, pulse rate of 70 beats/min, and blood pressure of 134/80 mm Hg reclining and 104/76 mm Hg standing. Her physical examination was significant for scleral icterus and a liver palpable at the costal margin. Laboratory tests were significant for the following values: aspartate aminotransferase (AST), 4,872 U/liter; alanine aminotransferase (ALT), 3,682 U/liter; γ-glutamyltransferase (GGT), 174 U/liter; and bilirubin, 6.0 mg/dl. The results of her hepatitis serologic tests were as follows: hepatitis B virus (HBV) surface antigen, negative; HBV surface antibody, positive; anti-HBV core antibody, positive; anti-HBV core immunoglobulin M (IgM) antibody, negative; anti-hepatitis A virus (HAV) antibody, positive; and anti-HAV IgM antibody, positive.

1. Given the patient's travel history and exposures, name three infectious agents with which she had an increased likelihood of being infected. What were her risk factors for each of these agents?
2. Minimally, what organisms should have been ruled out in this patient, and how would that have been done?
3. What agent was causing her present illness? Explain the results of her serologic tests and how they helped you come to the conclusions that you did. What results from her physical examination and laboratory tests other than serologic tests are consistent with this illness?
4. How do you think she obtained her infection? What feature of this agent allows it to be spread in this way?
5. What is the usual outcome of infection with this organism?
6. What prophylactic strategies are available for this agent? (Note: on previous trips to India, she employed one of them.)



CASE DISCUSSION

1. Her travel history put her at increased risk for a number of infectious diseases. In particular, this woman appeared to have taken none of the precautions that would lessen the likelihood of her getting the most common infectious diseases that travelers to India encounter. These include a variety of diarrheal diseases, such as typhoid fever, nontyphoidal salmonellosis, shigellosis, amebic and shigella dysentery, cholera, and traveler's diarrhea due to enterotoxigenic *Escherichia coli* (risk factors: bathing in Ganges, eating in restaurants, drinking nonboiled or nonfiltered water); hepatitis A (same risk factors as for the diarrheal pathogens plus failure to be vaccinated against HAV or to take gamma globulin); hepatitis E (risk factor: ingestion of contaminated water); malaria (risk factors: failure to take malarial prophylaxis; failure to use protection against mosquito bites, such as insect repellent, netting, and protective clothing); brucellosis (risk factors: drinking "raw," i.e., unpasteurized, milk); tuberculosis (risk factor: high rate of endemic infection in India); plague (risk factor: exposure to rats); and leptospirosis (risk factor: cutaneous exposure to water that was potentially contaminated with infectious animal urine). For further information on agents for which travelers are at increased risk in the Indian subcontinent and how they can be avoided, visit the website http://wwwnc.cdc.gov/travel/destinations/india.htm.

2. Given her history of fever, two things should have been done for this woman. First, blood smears should have been done to rule out *Plasmodium* infection. Patients who visit regions where malaria is endemic and fail to take steps to prevent malaria and present with fever have malaria until proven otherwise. Onset of disease is usually within the first 4 weeks of returning from such a region, but it can be much longer with *Plasmodium ovale* and *Plasmodium vivax*, both of which can have persistent forms within the liver (hypnozoites). Symptoms of nausea, vomiting, diarrhea, and icterus can all occur in malaria. *Plasmodium falciparum* infections can be life-threatening, so ruling out malaria infection is important. Second, this patient should have had blood cultures performed due to her risk of typhoid fever and enteric fever due to nontyphoidal serotypes of *Salmonella* spp., as well as brucellosis.

In addition, given her symptoms and her travel history, she was at increased risk for HAV infection. Appropriate serologic tests should be done to rule out this infection. Since all hepatitis infections have similar clinical presentations acutely, hepatitis B and C viruses should also be considered. Both hepatitis B and C are typically spread parenterally or via sexual contact (hepatitis B); thus, these latter two viruses were less likely because there was nothing in her history to suggest that she had exposures putting her at risk for these infections. Hepatitis E virus (HEV) is transmitted by fecally contaminated water in endemic areas and should also be considered. In India, HEV is the most common cause of acute hepatitis and has caused several epidemics there. Similar to HAV (though not genetically related), HEV generally causes a self-limited acute infection but can present with fulmi-

nant hepatitis along with prolonged cholestasis, malaise, nausea, vomiting, and fever. Interestingly, fulminant hepatic failure occurs more often in pregnant women, particularly when they are infected during the third trimester, when mortality can be as high as 25%. HEV does not cause chronic hepatitis except in solid-organ transplant recipients, of whom ~50% may develop chronic hepatitis due to HEV. Serologic studies, though not routinely performed in most U.S. laboratories, should be obtained to rule out HEV infection.

3. The patient had an acute illness consistent with hepatitis with positive serologic tests for antibodies to HBV surface antigen, HBV core antigen, and IgM and total antibodies to HAV. Clinical and laboratory findings consistent with hepatitis include markedly increased liver enzymes (ALT, AST, and GGT), markedly increased bilirubin levels, hepatomegaly, and scleral icterus. Symptoms of nausea, vomiting, malaise, fever, arthralgias, and myalgias are much less specific and could be associated with a wide array of agents to which she was potentially exposed.

Her serologic profile is consistent with an acute hepatitis A infection and prior infection with hepatitis B. In patients with acute hepatitis A infection, IgM antibody is almost always present at the time of acute illness. Anti-HAV IgM antibodies can persist for months after the resolution of the acute illness. In acute hepatitis B infections, patients typically have HBV surface antigen in their blood, with an absence of antibodies to either the surface or core antigen. IgM antibodies to core antigen would be expected to be present in patients with acute hepatitis B infection. Her core IgM antibodies were negative. Her serologic profile is not consistent with her having received HBV vaccine because the vaccine contains only the surface antigen. Thus, vaccine-derived immunity should consist of only antibodies to the surface antigen. Vaccinated individuals do not have antibodies to core antigen.

4. Hepatitis A is spread by the fecal-oral route, usually by ingestion of fecally contaminated water or food, especially shellfish. It is a disease controlled by good sanitation practices and a public health infrastructure. Where these do not exist, the disease is common. In many parts of the developing world, including India, essentially all adults have serologic evidence of prior hepatitis A infection. Infections are common in children, who probably serve as the source of much of the fecal contamination of water sources in those locales. This patient was likely exposed to the virus while bathing in the Ganges, which is known to be highly contaminated with human fecal material. Approximately 5% of hepatitis A infections in the United States occur in individuals traveling to the developing world who are exposed to HAV-contaminated food and water. Infections in the industrialized world are most common in intravenous drug users, in children who attend child care centers, and in adults who work there. In addition, shellfish taken from water contaminated with human feces has been shown to be a source of hepatitis A, both in the industrialized world and in the developing world. Shellfish are filter feeders; as part of the filter-feeding process, they may ingest HAV-contaminated human feces, and the virus persists in their flesh.

In locales in the United States where sanitation is poor, the incidence of hepatitis A infection is high.

HAV belongs to the family *Picornaviridae*. They are nonenveloped, single-stranded RNA viruses and are similar to other groups of *Picornaviridae* viruses, the rhinoviruses ("common cold" viruses) and enteroviruses (polioviruses and coxsackieviruses, among others), which infect humans and are known to be stable in the environment. The pathogenesis of hepatitis A infection begins by ingestion of viral particles. The virus can survive pH extremes found in the stomach. It is absorbed into the bloodstream in either the stomach or small intestine and specifically infects and replicates in the liver, where it has its pathologic effect by inducing a robust immune response. T cells and natural killer cells, along with gamma interferon, clear virally infected hepatocytes but also cause tissue damage leading to hepatitis. Viral particles are excreted in bile in large numbers (10^8 viral particles/ml) and can then be passed into the environment in feces. Once in the environment, the virus can survive for weeks both in fresh- and salt water.

5. Hepatitis A is almost always an acute, self-limited disease. In children, the infection is frequently subclinical. Severe manifestations of the disease, although rare, are typically seen in adults. Fulminant hepatitis due to hepatitis A is also rare, with a mortality rate of <1.5% in patients hospitalized with hepatitis A infection. Unlike hepatitis B and C viruses, and occasionally hepatitis E, chronic infection with HAV does not occur.

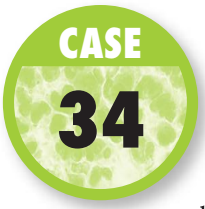
6. The first formalin-inactivated hepatitis A vaccine was approved for use in the United States in 1995. The vaccine is safe and highly efficacious, with seroconversion occurring in >99% of vaccinated individuals and a low rate of minor side effects. The vaccine is recommended for adults who travel to areas outside the industrialized world. In 2006, the Centers for Disease Control and Prevention began recommending HAV vaccination of all children with a two-dose series between 12 and 23 months of age.

Prior to the availability of the vaccine, immunoprophylaxis using human gamma globulin was used for individuals traveling to the developing world. (This patient had used it on previous visits to India.) The protection is relatively short-lived, but can last up to 6 months depending on the dosage. For an individual who will immediately embark on a trip to a developing country and who, therefore, will not seroconvert to the hepatitis vaccine during the trip, gamma globulin can be given to afford immediate protection. Gamma globulin has been shown to be protective for exposed individuals during HAV outbreaks, but it must be given within 2 weeks of exposure.

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A 59-year-old man with a history of cirrhosis secondary to hepatitis C and alcohol abuse was referred to a hepatology clinic for a consultation on two new liver masses. The patient was diagnosed 10 years ago with hepatitis C during a life insurance physical examination. At that time he was treated for 6 months with ribavirin and interferon-α2b (IFN-α2b) combination therapy, but did not respond. Three years ago, a liver biopsy was performed which showed stage III fibrosis with grade 2-3 inflammation. Since then he received ultrasounds every 6 months to screen for hepatic lesions. His last ultrasound showed two small lesions, and magnetic resonance imaging (MRI) confirmed two hepatic masses of approximately 2 cm each.

The patient had a history of alcohol abuse, consuming 12 to 18 beers per week. At the time of referral he drank about 10 beers per week despite being counseled not to drink alcohol due to his hepatitis C. In addition, he had a 2-year history in the 1970s of intravenous drug use (IVDU), but currently denied IVDU. He had multiple tattoos and body piercings, all of which were placed in the 1970s.

Liver function tests performed at his clinic visit showed elevated results for aspartate aminotransferase (AST) (1,718 U/liter), alanine transaminase (ALT) (597 U/liter), gamma-glutamyl transpeptidase (GGT) (110 U/liter; normal, 13 to 68 U/liter), and bilirubin (4.6 mg/dl). His hepatitis C virus (HCV) viral load was 186,319 IU/ml, and his HCV genotype was determined to be 1b. He had a liver biopsy the following day from which histopathology confirmed his diagnosis.

1. What is the incidence and natural history of HCV infection? Given the patient's history, what is the likely diagnosis of his liver lesions?
2. How is HCV transmitted? How did this patient likely get infected?
3. Describe how HCV infection is diagnosed. Who should be tested for hepatitis C?
4. What laboratory test is used to monitor response to therapy? How is this test used in practice?
5. Name the therapeutic options available for the treatment of HCV infection and their respective mechanisms of action.
6. There are two genotype tests that are important for therapeutic considerations; one is a viral genotype and the other a human genotype. Explain the importance of these tests. What does this patient's viral genotype (1b) tell us?
7. Despite this patient's diagnosis, he was not initially considered for liver transplantation. Why not?



CASE DISCUSSION

1. It is estimated that 3.9 million persons in the U.S. are infected with HCV, with ~70% of them chronically infected (i.e., HCV RNA detected in blood). Globally, the incidence of HCV is higher in parts of Asia, Africa, and South America. Over 200 million persons worldwide are infected; this represents 3% of the world's population. Although the annual incidence of new HCV cases in the U.S. is decreasing, chronic HCV-associated liver disease is on the rise. HCV infection is the leading indication for liver transplantation in the U.S.

Approximately 15 to 30% of patients with HCV have an asymptomatic or mild infection that resolves spontaneously. Rarely, fulminant acute hepatitis with liver failure and death occurs with primary HCV infection, and chronic hepatitis B virus (HBV) carriage has been shown to be a risk factor. The majority of individuals (70 to 85%) progress to develop chronic infection that may evolve over decades from persistent chronic hepatitis to hepatic fibrosis, leading to cirrhosis and potentially to liver failure and/or hepatocellular carcinoma. Both chronic alcohol abuse and HIV infection may accelerate the natural history of liver disease. This patient presented with a history of alcohol abuse and chronic hepatitis C infection with hepatic fibrosis, increasing the likelihood that these lesions represent hepatocellular carcinoma, which was confirmed by histopathology.

2. There are four major modes of transmission recognized for HCV: (i) sharing of needles, syringes, or drug-preparing utensils during the injection of illicit drugs; (ii) blood transfusions, including the use of clotting factors in hemophiliacs; (iii) sexually; and (iv) needle-stick injuries or mucous membrane exposure of blood from an HCV-infected individual. In addition, HCV has been transmitted by tattooing and body piercing performed in unregulated settings. The major mode of HCV transmission in the U.S. and the industrialized world is IVDU. Acute infection occurs most commonly in the 20- to 39-year age group. Up to 90% of intravenous drug users are HCV positive. Given that this patient was an intravenous drug user in the 1970s, he was mostly likely infected by sharing drug paraphernalia with an HCV-infected individual. Prior to 1992 and the development of serologic screening tests for HCV, blood transfusion was a major mode of transmission. With the introduction of nucleic acid testing of blood products in 1999, the incidence of HCV transmission by blood transfusion in the U.S. has dropped to ~1 in 2 million. Patients with hemophilia who received clotting factor preparations prior to 1987 (when heat treatment of clotting factor preparations began) are at increased risk of HCV infection. Although HCV can be spread sexually, its transmission is inefficient compared with HIV and other sexually transmitted infections, including HBV. As with HIV, sexual transmission is more efficient from males to females, and individuals with multiple partners are at greater risk of infection. Exposure of health care personnel to the blood of HCV-infected individuals via needle-stick or sharps injuries or mucous membrane exposures carries a 3% risk of infection, which is 10-fold less than the risk for acquiring HBV

infection but 10-fold greater than the risk for acquiring HIV infection. Vertical transmission of HCV from an infected mother occurs infrequently and is more likely from mothers who are concurrently infected with HIV. About 4% of babies born to HCV-positive mothers become infected. The virus does not appear to be transmitted via breast milk.

3. The strategy for diagnosing HCV is to detect HCV-specific antibodies by enzyme immunoassay (EIA) followed by a confirmatory test (Fig. 34.1). Newer-generation EIA tests are highly sensitive, detecting ca. 97% of HCV-infected patients and becoming positive by 8 weeks postinfection. Studies comparing seroversion to HCV viremia suggest that the false-negative serologic "window" following primary infection may be much longer than 8 weeks. Since serologic tests detect total anti-HCV (IgM and IgG), they cannot distinguish between acute, chronic, or resolved infections. Specificities for serologic tests range from 94 to 100%; therefore, confirmatory testing is recommended. A recombinant protein immunoblot assay (RIBA) was previously used to differentiate between past resolved infection and false-positive EIA results, but it has been discontinued. The Centers for Disease Control and Prevention (CDC) currently recommends that EIA-positive specimens be tested for HCV RNA to determine the current status of infection (Fig. 34.1). Further, RNA testing can be useful in acute infections before the patient develops an antibody response. However, since the vast majority of patients present for medical care in the chronic phase of HCV infection, when they are seropositive, detection of HCV RNA is rarely used as a primary diagnostic tool.

Since many chronically infected individuals are viremic and asymptomatic for years, screening for HCV infection in at-risk populations has become an important tool to prevent transmission. Many HCV-infected persons are not aware of their infection, and 45%

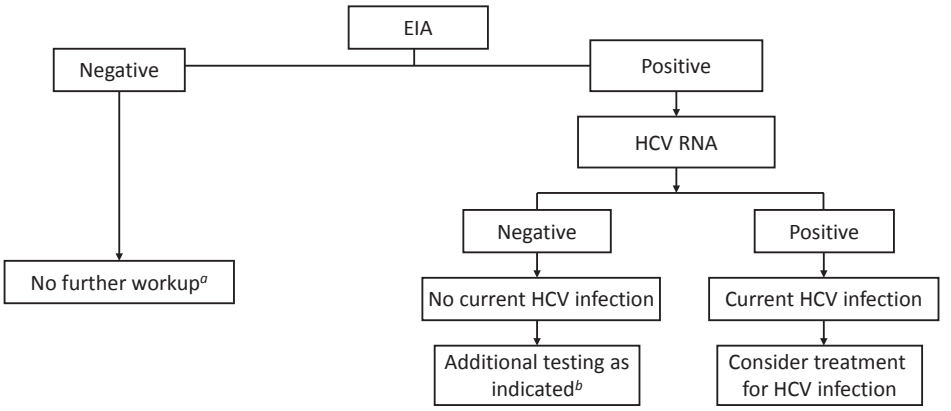


Figure 34.1 Testing algorithm for the diagnosis of hepatitis C infection. [a]If acute HCV infection is suspected, HCV RNA or follow-up serologic testing is recommended. Consider HCV RNA for immunocompromised patients. [b]To differentiate past, resolved HCV infection from a false-positive result, consider testing with another serologic assay. Repeat HCV RNA testing if person has been exposed in the past 6 months or has clinical evidence of hepatitis. (Adapted from reference 3.)

do not recall a potential exposure. In 2012, the CDC recommended that all adults born from 1945 to 1965 be tested for HCV independent of risk factors. This population has a disproportionately high prevalence of HCV infection and related disease. The at-risk populations that should be tested routinely were outlined by the CDC in 1998. This includes current injection drug users and anyone who has ever injected drugs, even if only once or many years ago (such as this patient). Persons who received clotting factor concentrates before 1987, recipients of blood or blood components or an organ transplant prior to 1992, and anyone notified that they had received blood from a donor who later tested positive for HCV should be tested. Any individual who has been on long-term hemodialysis and anyone with persistently abnormal aspartate aminotransferase levels (AST) should receive HCV serologic testing. Further, individuals whose blood was the source in a needle stick or sharps or mucosal exposure should be tested for HCV. If the source person is HCV positive, the exposed health care or public safety worker should be tested and should have follow-up testing at 4 to 6 months. Children born to HCV-positive women should be tested for HCV RNA until 12 months of age, when maternal antibody declines below detectable levels. HCV testing can be considered for the following persons, though the need to do so is uncertain: recipients of transplanted tissue, non-injecting illegal drug users, persons with tattoos or body piercings, persons with a history of multiple sexual partners or sexually transmitted infections, and long-term steady sexual partners of HCV-positive persons.

4. Quantitative RNA nucleic acid amplification tests, or viral loads, are used to monitor response to therapy. Historically, both qualitative and quantitative tests have been used because the qualitative tests had lower limits of detection than the quantitative tests. However, with real-time PCR, quantitative tests are as sensitive as, or more sensitive than, qualitative tests, detecting as few as 12 IU/ml. Therefore, most laboratories use highly sensitive quantitative real-time PCR for viral load monitoring. HCV is treated using response-guided therapy. This means that the reduction in viral load, or lack thereof, informs the length of therapy and/or whether therapy should continue. The primary time points when HCV viral load testing is performed are: (i) at initial determination of baseline viremia; (ii) 4 weeks into treatment, to assess rapid virologic response (RVR); (iii) 12 weeks into treatment, to assess early virologic response (EVR); (iv) at 24 or 48 weeks, to determine end-of-treatment response (ETR); and (v) 24 weeks after completion of therapy, to determine sustained virologic response (SVR). The goal of HCV therapy is to achieve an SVR (no detectable HCV RNA at 24 weeks after completion of therapy), which has been associated with resolution of liver injury, reduction in fibrosis, low risk of relapse, and improved survival. Patients who achieve an RVR (HCV undetectable at 4 weeks) or EVR (HCV RNA undetectable or ≥ 2 $\log_{10}$ reduction at 12 weeks) have a high likelihood of achieving an SVR. Patients with an RVR may be able to have shorter duration of treatment.

5. The standard therapy for HCV for many years has been combination therapy with pegylated IFN-α2a or 2b (PegIFN) and ribavirin. However, in 2011 directly acting antivirals (DAAs) were approved by the Food and Drug Administration for combination therapy with PegIFN and ribavirin for genotype 1 HCV (see answer 6 for more genotype-specific information). There are currently additional DAAs in the pipeline, including second-generation protease inhibitors, NS5B polymerase inhibitors, NS5A-complex inhibitors, and microRNA inhibitors. PegIFN-free regimens are also being investigated.

IFN-α is a cytokine that is produced in response to viral infections. When a synthetic version is administered, it boosts the immune system to stimulate a nonspecific antiviral response. The downside to IFN-α therapy is that it has significant side effects, such that many individuals cannot complete a full therapeutic course for HCV. PegIFN has a polyethylene glycol (PEG) attached to the IFN-α, which increases the half-life of the drug, allowing for increased viral activity and response rate. Long known for its nonspecific antiviral properties, ribavirin significantly increases SVR rates when used in combination with PegIFN. Ribavirin acts as a nucleoside inhibitor of viral RNA synthesis, though the exact mechanism has not been proven. Ribavirin cannot be used as monotherapy due to the rapid rate at which resistance develops in HCV. The DAAs teleprevir and boceprivir are protease inhibitors. Boceprevir is an NS3/4A protease inhibitor, and teleprevir is an NS3 inhibitor. Both drugs must be used in combination with PegIFN and ribavirin, due to rapid development of resistance when used as monotherapy. In clinical trials, both DAAs showed significantly improved SVR rates in treatment-naïve patients and treatment nonresponders.

6. HCV genotyping is important for disease prognosis and to predict response to therapy. Six different genotypes, 1 through 6, have been described for HCV. There are also subtypes of the six different genotypes. The nucleic acid sequence of the six genotypes may vary by as much as 33%. High mutation rates resulting in variation in the viral envelope proteins allow HCV to escape the immune system and establish a chronic infection. In the U.S. and Europe, genotype 1 is most common, followed by types 2 and 3. Genotype 1b (which this patient had) appears to be the most pathogenic of the genotypes, with more rapid progression of chronic active hepatitis to cirrhosis and a greater likelihood of requiring liver transplantation compared with patients infected with other genotypes. HCV patients who develop hepatocellular carcinoma are often infected either with genotype 1b or with a combination of genotypes including 1b. HCV genotypes also inform treatment duration with PegIFN and ribavirin. Patients with genotype 1 HCV are treated for 48 weeks, compared to patients with genotypes 2 and 3, who are treated for 24 weeks. Approximately 74 to 78% of patients with genotype 2 or 3 achieve an SVR with 24 weeks of therapy, whereas only 26 to 36% of genotype 1-positive patients achieve an SVR. SVR rates increase to 41 to 57% for genotype 1 with 48 weeks of therapy. However, if patients with genotype 1 still have detectable HCV RNA at 24 weeks, they are null responders, and

treatment can be discontinued. Based on this patient's history of being genotype 1 and only receiving 6 months of therapy, he was a null responder, and therapy was discontinued early. Genotypes are geographically distributed, with genotypes 1, 2, and 3 being found worldwide. Genotype 4 is found in the Middle East and North Africa, genotype 5 is found in South Africa, and genotype 6 is found in Asia with a high prevalence in Hong Kong. Less is known about treatment duration need for genotypes 4, 5, and 6, so they tend to be treated as a genotype 1. Viral genotyping is performed by analyzing the sequence in the 5'-UTR (untranslated region) of HCV. Accurate subtyping, particularly for genotype 1, depends on sequence analysis of the NS5B (polymerase) region.

Pharmacogenomics is a burgeoning field in medicine that uses host genetic data to predict response to a specific drug. Host genotyping has been demonstrated to be important for predicting response to PegIFN/ribavirin therapy, particularly for patients infected with genotype 1. Interleukin 28B (IL28B; also called IFN-λ3) is a cytokine involved in the immune defense against viruses. Polymorphisms near the IL28B gene are associated with response to PegIFN/ribavirin therapy. Specifically, a C/T polymorphism at allele position *rs12979860* has been shown to be important. A C/C genotype at this position is found more than twice as frequently in persons who spontaneously cleared HCV infection as in those who progressed to chronic hepatitis. A T/T genotype is associated with the lowest rates of SVR, while a C/T genotype shows intermediate rates. Interestingly, the natural C/C allele frequency is highest in East Asians (>90%), who have a >70% SVR rate, compared to African-Americans, who have a C/C frequency of ~40% and an SVR rate of <30%. The predictive value of IL28B genotyping for achieving an SVR is superior to that of pretreatment HCV RNA level and fibrosis stage.

7. The concern for the patient's alcohol use in spite of being counseled against it makes him a poor candidate for successful transplantation. Alcohol abuse has been associated with poor compliance post-transplant and increased risk of graft loss. Further, continued alcohol use after transplant causes liver damage and reduces survival. This patient received radiofrequency ablation for his hepatocellular carcinoma while he sought help for his alcohol abuse. After not drinking for 12 months, he received a liver transplant.

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This 60-year-old woman with a medical history of a gastric ulcer had recently noted symptoms of dyspepsia. She characterized her discomfort as a pressure in the upper abdominal area that radiated to her chest and neck. She underwent an upper gastrointestinal series that showed radiologic findings compatible with a thickened fold within the stomach.

An outpatient esophagogastroduodenoscopy (EGD) was performed. A biopsy of the antral portion of the stomach was consistent with moderate gastritis. No tumor was seen. In addition, the biopsy demonstrated significant growth (3+ to 4+) of a bacterial organism (Fig. 35.1).

1. What bacterium has been associated with chronic gastritis?
2. What clinical syndromes, other than chronic gastritis, have been linked to this organism?
3. Other than by histopathologic examination of a biopsy specimen, how can infection with this organism be diagnosed?
4. What special properties of this organism allow it to live in the rather inhospitable environment of the human stomach?
5. What is the epidemiology of infection with this organism?

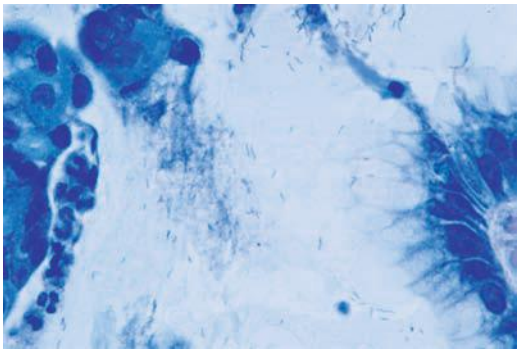


Figure 35.1



CASE DISCUSSION

1. The curved or helical, Gram-negative rod *Helicobacter* (formerly *Campylobacter*) *pylori* has been associated with chronic gastritis. The photomicrograph (Fig. 35.1) is consistent with *H. pylori* in an area of chronic gastritis.

2. There are data to support links between *H. pylori* and chronic gastritis and peptic ulcer disease, including both gastric and duodenal ulcers. In addition, there is epidemiologic evidence that both adenocarcinoma of the stomach that is distal to the gastroesophageal junction and gastric mucosa-associated lymphoid-tissue lymphoma are associated with *H. pylori* infection. As you might imagine, it would be difficult to employ Koch's postulates in demonstrating that *H. pylori* is an etiologic agent of these two malignancies. Of interest, studies on adenocarcinoma of the stomach have demonstrated that a diet that is high in salt is a cofactor for the development of this malignancy. The data for cigarette smoking as a cofactor are less compelling. Published, peer-reviewed medical literature on extragastric associations with infection by *H. pylori* has suggested that the infection is associated with idiopathic thrombocytopenic purpura and that the use of *H. pylori* eradication in patients with this condition correlates with a favorable response in the subsequent platelet count. Iron deficiency anemia has also been associated with *H. pylori* infection. While a number of studies evaluated whether or not *H. pylori* infection is associated with an increased risk of coronary artery disease, the results have been conflicting. Other studies have, to a limited degree, supported an association between *H. pylori* infection and Graves' disease, an autoimmune thyroid disease, and with the development of Henoch-Schönlein purpura in children.

3. The isolation of *H. pylori* in a culture of a gastric biopsy specimen is the gold standard for establishing the diagnosis of infection with this organism. Unfortunately, this method, which requires endoscopic biopsy and gastric biopsy culture, is not routinely performed by all clinical microbiology laboratories. Culture is estimated to be relatively insensitive, with ~70% of cultures being positive in patients with *H. pylori*-associated disease. Another method, also requiring endoscopic biopsy, is the *Campylobacter*-like organism test, which relies on the presence of the bacterial enzyme urease (see answer to question 4). In this test, gastric biopsy tissue is added to a tube that contains urea agar. The urease activity of tissue containing *H. pylori* is demonstrated by the change in color of the indicator present in the urea agar (Fig. 35.2). Noninvasive tests include the demonstration of

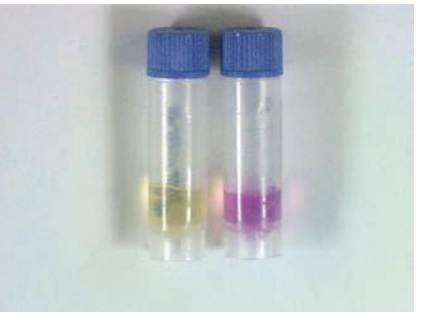


Figure 35.2 The tube on the left (yellow color) is a negative control; the tube on the right (pink color) holds a biopsy containing *H. pylori* that has degraded the urea present in the tube, resulting in the color change observed.

urease production following the ingestion of ^{13}C- or ^{14}C-labeled urea by the detection of labeled carbon dioxide in expired air (the urea breath test), serologic tests to demonstrate the presence of IgG antibody to *H. pylori*, and the use of an *H. pylori* stool antigen enzyme immunoassay. Because of its noninvasive nature, *H. pylori* serology is being used with increasing frequency in an attempt to diagnose this infection. Serologic results must be interpreted cautiously as both the sensitivity and the specificity are <90%. Seropositivity increases with age, presumably because of increased exposure to the organism. Patients can be seropositive without clinical evidence of gastritis or peptic ulcer disease. In addition, the monitoring of patients who have received treatment for *H. pylori* cannot be done with the serologic assays. By contrast, the urea breath test and the stool antigen test both have sensitivities and specificities of ~95% and are useful in the monitoring of patients after treatment.

4. While the ability of *H. pylori* to survive and multiply in the ecologic niche of the acidic stomach is noteworthy, we now know that the stomach contains on the order of 200 different species of bacteria. There are a number of interesting adaptations that are important in allowing the colonization and subsequent multiplication of *H. pylori* within the stomach.

The enzyme urease, which may represent as much as 6% of the protein synthesis of *H. pylori*, is active at the low pH of gastric juice. It has been established by studies with mutant *H. pylori* that lack urease that this enzyme is essential for gastric colonization. Urease catalyzes the hydrolysis of urea, resulting in the production of ammonia, which raises the pH of the microenvironment, resulting in improved bacterial survival. Another factor of importance is the presence of flagella, which confer motility to *H. pylori*. The flagella enable *H. pylori* to move through the thick mucus coat that is present in the lining of the stomach. The urease activity of *H. pylori* enhances the motility of the flagella to move through the mucus layer. At a low gastric pH (typically 1 to 2), gastric mucins are able to trap the organism. Following the urease-catalyzed production of ammonium ions, the pH of the mucin increases. As a result, there is a transition of the mucus gel to a viscoelastic solution that does not trap the bacteria, but is one through which *H. pylori* can swim.

To persist in this microenvironment, *H. pylori* encodes proteins that detoxify reactive oxygen species such as catalase and superoxide dismutase. In addition, the *H. pylori* arginase limits nitric oxide production by inhibiting the nitric oxide synthase of macrophages, neutrophils, and epithelial cells.

A number of protein toxins are made by *H. pylori*. These include vacuolating cytotoxin A, which is a pore-forming toxin that disrupts cell polarity, promotes apoptosis of epithelial cells, and inhibits T-cell function. Another important toxin is cytotoxin-associated gene A. *H. pylori* persists due to a combination of immune evasion and virulence factors. For example, on a structural basis, the lipopolysaccharide (LPS) of *H. pylori*, which is predominantly tetra-acylated, is 1,000-fold less bioactive in inducing Toll-like receptor 4 (TLR4) than is the LPS of *Escherichia coli*, which is hexa-acylated. The inflammatory

cytokine response to *H. pylori* is able, however, to occur via the activation of TLR2, which may occur in the setting of granulocytes and monocytes. Given that these cells are not typically in the stomach in high numbers and the *H. pylori* LPS only weakly activates TLR4, the immune response that occurs in the stomach due to the presence of *H. pylori* may be less than would otherwise be expected.

5. Studies on the time of acquisition of *H. pylori* infection have generally reported seroprevalence data. The prevalence of infection due to *H. pylori* varies by geographic area, age, race, ethnicity, and socioeconomic status. *H. pylori* is typically acquired during early childhood in developing countries. In developed countries, such as the United States, the infection is not typically acquired during childhood, and the incidence is lower. It is important to recognize that although ~50% of Americans have been infected with this organism by age 60, most infections are asymptomatic. In the United States, whites have a lower seroprevalence rate than do either African-Americans or Latinos. Although other routes of transmission may occur, it is likely that the most common means of transmission are via either the oral-oral route or the fecal-oral route. Given the high rate of infection globally, it was estimated that in 2008 the number of new cases of gastric cancer attributable to *H. pylori* infections was 470,000 in less developed regions and 190,000 in the more developed regions of the world. As a result, the eradication of *H. pylori* infection has been addressed with the hope that this would prevent gastric cancers. There has been an increased rate of resistance of *H. pylori* to antibiotics that have been a part of many eradication regimens, including metronidazole and clarithromycin, and simultaneous resistance to both antibiotics has been well described.

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SECTION FOUR



SKIN AND SOFT TISSUE INFECTIONS

INTRODUCTION TO SECTION IV

The resistance of skin to infection is due to the integrity of the keratinized skin, the presence of inhibitory fatty acids produced by sebaceous glands, the dryness of the skin, and the inhibitory effect of the resident normal skin microbiota. Skin and soft tissue infections can be caused by either direct penetration of a pathogen through the skin or hematogenous spread of the pathogen to the site. The normal skin microbiota includes organisms that may cause infection in the setting of a disruption in the integrity of the skin (such as the presence of a surgical suture or an insect bite). In the setting of severe damage to the skin, as occurs with burns, even normally innocuous organisms, including endogenous bacteria, can cause severe disease. Similarly, when the skin is no longer dry, as may occur in moist intertriginous spaces or when occlusive dressings are present, the patient is at increased risk of infection.

Cutaneous manifestations of systemic disease are common. Rocky Mountain spotted fever, meningococcemia, enteroviral infection, and toxic shock syndrome can all present with fever and a diffuse erythematous macular rash. Other systemic infections that can present with a diffuse rash include scarlet fever, measles, and German measles. The characteristic rash of Lyme disease, erythema migrans, is specific enough to establish the diagnosis. The nature of the lesion (macular, papular, vesicular, pustular, or bullous) may help to narrow the differential diagnosis. For example, varicella-zoster virus infection typically results in vesicular skin lesions. The rash of secondary syphilis, on the other hand, may present clinically as macular, papular, maculopapular, or pustular skin lesions but does not present as a vesicular rash.

Skin and soft tissue infections can be classified on the basis of the anatomic level at which infection occurs. The more superficial infections, such as folliculitis caused by *Staphylococcus aureus* or cellulitis caused by *Streptococcus pyogenes*, are important to treat at an early stage. Delay in treatment may result in invasion of the deeper structures, as in necrotizing fasciitis, which has a high mortality rate.

Damage to the skin and soft tissues, as occurs in traumatic injuries, may allow the entry into the wound of soil organisms such as *Clostridium perfringens*, an anaerobic, Gram-positive rod. Under favorable conditions, potentially fatal soft tissue infections (myositis, gas gangrene) may occur.

New technologies such as 16S rRNA gene sequencing and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allow us to identify to the species level Gram-positive bacilli recovered from soft tissue infections. Rapidly growing mycobacteria including *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum* have been found to cause infection secondary to cosmetic surgery and pedicures. Other environmental mycobacteria such as *Mycobacterium marinum* have been associated with soft tissue infection following traumatic injury involving water exposure. *Cornyebacterium kroppenstedtii* has been associated with mastitis. Three species of *Actinomyces*—*A. neuii*, *A. radingae*, and *A. turicensis*—are now recognized to cause skin and soft tissue infections. These three organisms are also indigenous flora on skin, so the find-

ing of these organisms in clinical specimens should be accompanied by evidence of inflammation such as the presence of white blood cells on direct Gram stain of the patient's specimen.

Important agents of skin and soft tissue infection are listed in Table 4. The presence of ectoparasites, such as lice and bedbugs, is not designated an infection but rather an infestation. Ectoparasites are, however, included for completeness.

TABLE IV SELECTED SKIN AND SOFT TISSUE PATHOGENS

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Bacteria			
Actinomyces neuii	Short, Gram-positive bacillus	Endogenous (skin flora)	Cellulitis, skin abscess, superficial wound infections
Actinomyces radingae	Short, Gram-positive bacillus	Endogenous (skin flora)	Cellulitis, skin abscess, superficial wound infections
Actinomyces turicensis	Short, Gram-positive bacillus	Endogenous (skin flora)	Cellulitis, skin abscess, superficial wound infections
Bacillus anthracis	Spore-forming, aerobic, Gram-positive bacillus	Exogenous; livestock or animal products; bioterrorism agent	Cutaneous, gastrointestinal, and inhalation anthrax; meningitis; bacteremia
Bartonella henselae	Fastidious, Gram-negative bacillus	Exogenous; cats appear to be primary host	Cat scratch disease, bacillary angiomatosis (in immunocompromised individuals)
Borrelia burgdorferi	Spirochete	Tick-borne	Lyme disease; rash, arthritis, nervous system and cardiac manifestations
Clostridium perfringens	Anaerobic, Gram-positive bacillus	Exogenous (wounds); endogenous (bowel flora)	Gas gangrene, bacteremia, food poisoning, emphysematous cholecystitis
Clostridium tetani	Anaerobic, Gram-positive bacillus	Exogenous (wounds)	Tetanus
Corynebacterium diphtheriae	Aerobic, Gram-positive bacillus	Exogenous	Diphtheria (pharyngeal) and wound diphtheria
Corynebacterium kroppenstedtii	Aerobic, Gram-positive bacillus	Endogenous (skin flora)	Mastitis, breast abscess
Group A streptococci (*Streptococcus pyogenes*)	Catalase-negative, Gram-positive cocci	Endogenous; exogenous	Cellulitis, bacteremia, scarlet fever, necrotizing fasciitis, pharyngitis, pneumonia, rheumatic fever, poststreptococcal glomerulonephritis

(continued next page)

TABLE IV SELECTED SKIN AND SOFT TISSUE PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Group B streptococci (*Streptococcus agalactiae*)	Catalase-negative, Gram-positive cocci	Endogenous	Cellulitis, sepsis, meningitis, UTI[a] (diabetics)
Mycobacterium abscessus	Acid-fast bacillus, environmental	Exogenous (water)	Surgical wounds, chronic lung infections in cystic fibrosis patients, line-related sepsis
Mycobacterium chelonae	Acid-fast bacillus, environmental	Exogenous (water)	Surgical wounds, line-related sepsis, post-LASIK keratitis
Mycobacterium fortuitum	Acid-fast bacillus, environmental	Exogenous (water)	Surgical wounds; line-related sepsis; traumatic, chronic wounds
Mycobacterium marinum	Acid-fast bacillus, environmental	Exogenous (fresh, brackish, and salt water)	Traumatic wounds, septic arthritis, cellulitis
Neisseria gonorrhoeae	Oxidase-positive, Gram-negative diplococcus	Direct sexual contact; vertical, mother to child	Genital tract involvement, pharyngeal infection, ocular infection, bacteremia, arthritis with dermatitis
Neisseria meningitidis	Oxidase-positive, Gram-negative diplococcus	Endogenous (from colonization)	Meningitis, bacteremia, pneumonia
Pasteurella multocida	Oxidase-positive, Gram-negative bacillus	Zoonosis (often animal bite or scratch)	Cellulitis, bacteremia, osteomyelitis, meningitis, pneumonia
Pseudomonas aeruginosa	Lactose-nonfermenting, oxidase-positive, Gram-negative bacillus	Exogenous	Skin infections in burn patients, community and health care-associated UTI, health care-associated pneumonia, health care-associated bacteremia, ecthyma gangrenosum
Staphylococcus aureus	Catalase-positive, coagulase-positive, Gram-positive coccus	Endogenous	Cellulitis, bacteremia, endocarditis, septic arthritis, abscesses, pneumonia
Treponema pallidum	Spirochete (does not Gram stain)	Direct sexual contact; vertical, mother to child	Primary (painless chancre), secondary (diffuse rash), latent, and late syphilis; can affect any organ

TABLE IV SELECTED SKIN AND SOFT TISSUE PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Fungi			
Blastomyces dermatitidis	Dimorphic mold	Exogenous	Cutaneous infection, pneumonia, meningitis, bone infection
Candida albicans	Yeast, often germ tube positive	Endogenous	Thrush, vaginal yeast infection, diaper rash, esophagitis, health care-associated UTI, health care-associated bloodstream infection
Candida spp., non-*albicans*	Yeasts, germ tube negative	Endogenous	Thrush, vaginal yeast infection, health care-associated UTI, health care-associated bloodstream infection
Cryptococcus neoformans	Encapsulated yeast	Exogenous (environmental, rarely zoonotic)	Meningitis, pneumonia, bloodstream infection, cellulitis
Epidermophyton floccosum	KOH-positive skin lesions; club-shaped macroconidia, absent microconidia	Anthropophilic	Dermatophyte infection of keratinized tissue (rarely nails)
Microsporum spp.	KOH-positive skin lesions; fluoresce yellow-green under Wood's light	May be zoophilic (e.g., *M. canis*), geophilic (e.g., *M. gypseum*), or anthropophilic (e.g., *M. audouinii*)	Dermatophyte infection of keratinized tissue (rarely nails)
Trichophyton spp.	KOH-positive skin lesions	May be zoophilic (e.g., *T. mentagrophytes*) or anthropophilic (e.g., *T. schoenleinii*)	Dermatophyte infection of keratinized tissue (including nails)
Parasites			
Ancylostoma braziliense	Hookworm of dog	Exogenous	Cutaneous larva migrans
Ancylostoma caninum	Hookworm of dog	Exogenous	Cutaneous larva migrans
Cimex lectularius	Ectoparasite	Exogenous	Bedbug; itching skin lesions
Leishmania tropica, *Leishmania braziliensis*	Protozoans	Exogenous (sand fly)	Ulcerative skin lesions
Pediculus spp.	Ectoparasites	Exogenous	Body lice
Phthirus pubis	Ectoparasite	Exogenous	Crab louse

(continued next page)

TABLE IV SELECTED SKIN AND SOFT TISSUE PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Sarcoptes scabiei	Ectoparasite	Exogenous; zoonotic varieties less common than human varieties	Scabies infestation
Viruses			
Enteroviruses	Nonenveloped, ssRNA[b]	Usually fecal-oral	Aseptic meningitis, rash, myocarditis
Herpes simplex virus 1 and 2	Enveloped, dsDNA[c]	Person to person; reactivation of latent infection; during passage of the neonate through the birth canal	Genital ulcers; oral, ocular infections; encephalitis; neonatal infection; esophagitis (immunocompromised individuals)
HIV	Enveloped RNA retrovirus	Blood-borne and sexual transmission; vertical, mother to child	AIDS, mononucleosis-like syndrome with rash in primary infection
Human herpesvirus 6	Enveloped, dsDNA	Person to person	Exanthema subitum (roseola)
Rubella virus (German measles)	Enveloped, ssRNA	Vertical, mother to child	Inapparent or subclinical infection in adults; birth defects in infants
Rubeola virus (measles)	Enveloped, ssRNA	Respiratory spread	Measles, pneumonia, encephalomyelitis, subacute sclerosing panencephalitis
Papillomavirus	Nonenveloped, dsDNA	Person to person	Warts
Varicella-zoster virus	Enveloped, dsDNA	Respiratory spread	Chicken pox; zoster (may disseminate)
Variola virus	Enveloped, dsDNA	Person to person, respiratory spread; bioterrorism agent	Smallpox; vesicular, pustular, hemorrhagic rash

[a] UTI, urinary tract infection.

[b] ssRNA, single-stranded RNA.

[c] dsDNA, double-stranded DNA.

CASE 36

The patient was a 45-year-old male who was in his usual state of good health when he awoke at 3 a.m. with pain in the lateral aspect of his left calf. He looked at his calf and thought that the pain was due to an ingrown hair and went back to sleep. At 10 a.m., he expressed a small amount of pus from the ingrown hair. Over the next 8 hours, the patient developed an area of cellulitis on the lateral aspect of the calf of approximately 5 by 10 cm. At that time, a small amount of pus was again expressed from the area of the ingrown hair. The next morning, the area of cellulitis extended from just below the knee to just above the ankle. The patient visited his physician. His vital signs at that visit, including pulse, respirations, blood pressure, and temperature, were all within normal limits. Physical exam was significant for an area of cellulitis as described that was red and warm to the touch but with no area of obvious fluctuance. No lymphadenopathy was observed. The central area of the cellulitis, near the area that the patient described as where the ingrown hair had been, was punctured three times with a 20-gauge needle but no pus was drained. The patient was referred to the surgery service. The surgeons examined the patient and said they would follow him. The patient was given 2 g of ceftriaxone intramuscularly and begun on oral cephalexin.

The patient returned to the surgical clinic 48 hours later with an obvious area of fluctuance in the center of the area of cellulitis. Over the preceding 48 hours, the patient reported low-grade fevers. Approximately 1 ml of pus was aspirated and was sent for Gram stain and culture (Fig. 36.1 and 36.2). When pus was aspirated from the lesion, the surgeon decided to excise and drain the lesion (Fig. 36.3).

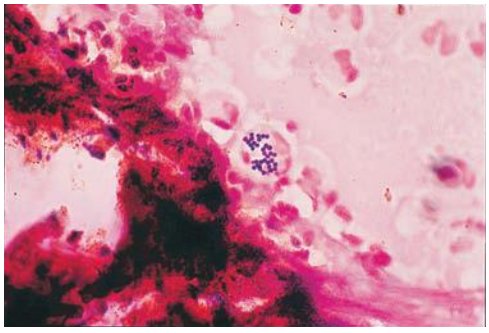


Figure 36.1



Figure 36.2

1. Describe what you observed in Fig. 36.1 and 36.2. The organism is catalase and coagulase positive. What organism was causing his infection?
2. Why were incision and drainage necessary to treat this infection? Why would antimicrobial agents alone not be effective in treatment of this infection?
3. Susceptibility results for this organism are seen in Fig. 36.4. How do you interpret these susceptibility results? Explain

the likely reasons for the results seen with drugs 2 and 4. How do results of the testing of drug 2 explain the progression of his infection despite a large intramuscular dose of ceftriaxone followed by oral cephalexin? Also explain the findings for drugs 7 and 8. How should the isolate infecting this patient be treated?

4. What test is being used to test vancomycin (drug 5)? Why is this test being used and what does it show?

5. What virulence factor is particularly associated with skin and soft tissue infections (SSTIs)? Explain its mechanism of action. This virulence factor and this type of antibiogram are associated with a particular strain of this organism. Briefly discuss the evolving epidemiology of this strain.

6 What infection control precautions would be necessary for this patient? What are some of the potential unintended consequences of hospitalized patients who are colonized with this organism?

7. Why are these organisms viewed as a global threat?



Figure 36.3

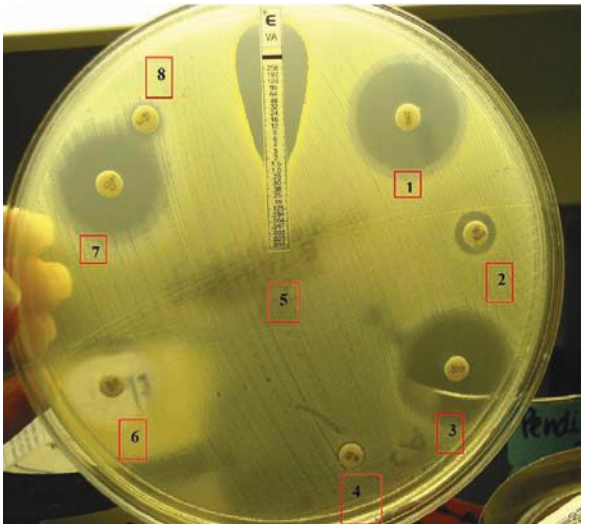


Figure 36.4 Disk diffusion susceptibility test of patient's isolate.

CASE DISCUSSION



1. The finding of Gram-positive cocci in clusters on Gram stain is consistent with staphylococci. The finding of a yellowish colony that is beta-hemolytic on 5% sheep blood agar is consistent with *Staphylococcus aureus*. The staphylococci are divided into two groups based on the biochemical test called the coagulase test; *S. aureus* is positive, while a group of >30 other staphylococcal species are negative. This group of organisms is referred to as the coagulase-negative staphylococci (CoNS). Three of the CoNS species are frequently encountered clinically. *Staphylococcus epidermidis* can infect implanted foreign bodies, such as pacemakers, cerebrospinal fluid shunts used to treat hydrocephalus, intravascular catheters, and artificial joints. *Staphylococcus lugdunensis* has been associated with skin and soft tissue infections (SSTIs) as well as native valve endocarditis. Although *S. lugdunensis* can cause SSTIs, it is less common than *S. aureus*. The other frequently encountered CoNS species is *Staphylococcus saprophyticus*, which causes urinary infections primarily in young, sexually active women. The isolate recovered from this patient was coagulase positive and was identified as *S. aureus*.

The patient's infection began as a folliculitis at the site of the ingrown hair, progressed to a cellulitis, and ultimately evolved into an abscess. Approximately 20% of adults are chronic nasal carriers of *S. aureus*, while an additional 60% may carry the organism intermittently. From the nose, the skin can become colonized. Studies have shown intermittent skin carriage rates as high as 40%, although most studies target the skin carriage rate at 10 to 15%. In all likelihood this individual's initial folliculitis was a result of the infecting *S. aureus* coming from skin colonization. Manipulation of the skin resulted in the spread of the organism to the dermis, leading to cellulitis and abscess formation.

2. The standard of care for an abscess is 2-fold: incision and drainage (Fig. 36.3) and antimicrobial therapy. The reason why antibiotics alone would not be sufficient is that abscess formation results in a loss of blood flow to the center of the infected area (the abscess). As a result, antibiotic levels in the center of the abscess would be low or, in a large abscess, completely absent, allowing the survival of the infecting organisms present there. Incision and drainage removes a large number of organisms and reduces the infected area, making penetration of much higher levels of antimicrobial agents to the infected tissue and killing of the infecting organism more likely.

3. The susceptibility test that was performed on this patient is a disk diffusion test for seven drugs and an E-test for one drug. The basis for disk diffusion susceptibility testing is described in the introductory chapter of this text, and the reader is referred there for further details. The antibiogram for this organism is as follows:

Drug 1: trimethoprim-sulfamethoxazole, to which the organism is susceptible
Drug 2: cefoxitin, to which the organism is resistant
Drug 3: doxycycline, to which the organism is susceptible
Drug 4: penicillin G, to which the organism is resistant
Drug 5: vancomycin with an MIC of 2 μg/ml by E-test (see answer 3 for more details)
Drug 6: gentamicin, to which the organism is susceptible
Drug 7: clindamycin, to which the organism is susceptible
Drug 8: erythromycin, to which the organism is resistant

This *S. aureus* strain is expressing two different resistance mechanisms against the β-lactam drugs. One is evidenced by its resistance to penicillin G. This resistance is due to the organism's ability to produce an enzyme, β-lactamase, that degrades the β-lactam ring of penicillin G, rendering this and the related widely used antimicrobials ampicillin, amoxicillin, and piperacillin inactive. Approximately 90 to 95% of *S. aureus* strains produce a β-lactamase that is encoded on the bacterial chromosome. Almost as soon as penicillin G was put into widespread therapeutic use, recognition of *S. aureus* strains resistant to penicillin G by virtue of β-lactamase production emerged. New agents including penicillinase-stable penicillins (oxacillin, nafcillin, and the oral agent dicloxacillin); first-, second-, and third-generation cephalosporins; and carbapenems were developed over the following decades. A characteristic all these drugs shared was that they were relatively stable in the presence of β-lactamase-producing *S. aureus*. However, a second resistance mechanism to β-lactam drugs soon emerged. The presence of this resistance is predicted by the cefoxitin result. Although cefoxitin is not a drug that is used to treat *S. aureus* infections, *S. aureus* strains expressing cefoxitin resistance predictably have alteration of a specific penicillin-binding protein, PBP2. The altered penicillin-binding protein, PBP2a, is encoded by *mecA*. All β-lactam antimicrobials have significantly reduced affinity for PBP2a relative to PBP2. This altered affinity is the basis for what we call methicillin resistance in *S. aureus*. This term is obviously a bit of a misnomer since this PBP alteration confers resistance to all β-lactam drugs, just not methicillin. The reason the term "methicillin-resistant *S. aureus*," or MRSA, became widespread is that methicillin was the drug used to treat serious *S. aureus* infections when this resistance was first encountered. It is critical to remember that no β-lactam antimicrobial has clinical efficacy against MRSA with the exception of a newly developed cephalosporin, ceftaroline, although some β-lactams may appear to have activity against *S. aureus in vitro*.

The only other antimicrobial to which this isolate is resistant is erythromycin. If the placements of the clindamycin (disk 7) and erythromycin (disk 8) disks are closely examined, it should be noted that they are closer together than the other disks in order to determine whether there is formation of a D-shaped zone of inhibition around the clin-

damycin. The D-zone occurs when erythromycin induces the production of an rRNA methylase encoded by the *erm* gene. Expression of this methylase can be either constitutive (always on) or inducible (on only in the presence of an inducer such as erythromycin). *erm*-specific methylation of the rRNA results in both erythromycin and clindamycin resistance. The D-zone test is assessing whether the inducible form of *erm* is present. The bacteria growing closest to the erythromycin disk are in the presence of an inducer, and therefore will be resistant to clindamycin; this resistance causes a "flattening" of the zone of inhibition in the area between the two disks, creating a characteristic D-shaped zone around the clindamycin disk (Fig. 36.3). If the constitutive form of *erm* was present, the organism would test as resistant to clindamycin independent of the presence of erythromycin. Clinical failures of clindamycin therapy for infections due to *S. aureus* strains with inducible *erm* genes are well documented in the literature. Mild SSTIs can be treated with oral antimicrobials. Because his isolate was resistant by virtue of altered PBPs to both of the drugs he was given initially, ceftriaxone and cephalexin (an oral cephalosporin), this patient next was given oral clindamycin. Some studies suggest that incision and drainage is all that is necessary to clear the infection, but the physician was being cautious.

4. Vancomycin is a key drug in treating MRSA infections, particularly severe ones as seen in this patient. Vancomycin (drug 5) is being tested using a special antimicrobial-impregnated strip called an E-test. The strip is designed to release a gradient of a specific antimicrobial agent into the agar. The point where the elliptical zone of bacterial growth inhibition (thus the name "E-test") meets the strip determines the MIC of the antimicrobial for the organism being tested. The vancomycin MIC is 2 μg/ml, which is at the upper level of susceptibility for this organism. Strains with vancomycin MICs of 4 or 8 μg/ml are referred to as vancomycin-intermediate *S. aureus*, or VISA, and are more likely to result in treatment failures. VISA strains are not reliably detected by disk diffusion techniques; thus the need for a MIC technique. The reduced susceptibility of VISA isolates is due to a thickening of the cell wall, resulting in "trapping" of vancomycin, a large, highly charged molecule. VISA strains should not be confused with vancomycin-resistant *S. aureus*, or VRSA. VRSA strains are still quite rare worldwide. Their resistance is due to the acquisition of the *vanA* gene from *Enterococcus faecium*. VRSA strains have high-level vancomycin resistance (MICs of 16 to ≥128 μg/ml).

5. Panton-Valentine leukocidin is a virulence factor that is specifically associated with SSTIs. It is a cytolytic pore-forming hexameric protein that can lyse a variety of cell types. It has particular affinity for polymorphonuclear cells and macrophages (thus the name "leukocidin"). With increasing frequency, *S. aureus* strains with a specific molecular signature have been documented to be responsible for significant SSTIs causing individuals to seek care in emergency departments. These strains are called community-associated MRSA, or CA-MRSA. CA-MRSA strains carry the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentin leukocidin and a small staphylococcal chromosomal cassette (SCC*mec* type IV) that harbors *mecA*. Among CA-MRSA isolates, a specific pulsed-field gel electrophoresis pattern

called USA300 predominates. Infections with this strain have attracted significant attention in the popular media because of outbreaks among a variety of athletic teams, day care centers, schools, and military units. More ominously, severe cases of pneumonia are being documented with increasing frequency. Cases of CA-MRSA necrotizing pneumonia have significant morbidity and mortality. One of the interesting things about CA-MRSA is that it remains susceptible to a variety of oral agents, in this case, clindamycin, doxycycline, and trimethoprim-sulfamethoxazole. This is in stark contrast to another group of MRSA strains that are classified as health care-associated MRSA, or HA-MRSA. Typically acquired in a health care setting, these strains are often resistant to oral agents and aminoglycosides, making vancomycin the primary therapeutic option, while three newer agents, daptomycin, linezolid, and ceftaroline, are important second-line drugs. For HA-MRSA, linezolid is often the only susceptible oral drug, and it is poorly tolerated and expensive. Thus, serious HA-MRSA infections are almost always treated with intravenous vancomycin. Because of its oto- and nephrotoxicity, vancomycin is complicated to give, since drug levels must be monitored to ensure that toxic levels are not accumulating.

6. MRSA, vancomycin-resistant enterococci, and *Clostridium difficile* are the most important bacterial causes of health care-associated infections. Patients who are colonized with MRSA are more likely to develop serious HA-MRSA infections, including postoperative wound infections, central venous catheter-related bacteremia, and ventilator-associated pneumonia. They may also spread MRSA to other patients directly or via common caregivers. A number of strategies have been advocated for preventing HA-MRSA infections, although evidence to support some of them is often contradictory. Strict adherence to hand washing is essential in preventing the spread of all health care-associated pathogens. Isolation and contact precautions for patients colonized or infected with MRSA is standard practice. Contact precautions include wearing gloves and gowns when entering the rooms of MRSA-colonized or -infected patients. Proper disposal of gloves and gowns coupled with hand hygiene is essential. One of the areas of controversy in the prevention of HA-MRSA infections is who should be screened for MRSA carriage and what laboratory method should be used for screening. This discussion is quite complex and is in a state of flux, so we will not attempt to cover it here. However, patients who are in isolation and on contact precautions often do not get the same level of care as patients who are not. This translates into fewer visits from health care providers, missed medicine doses, fewer assessments of vital signs, increased risks of falls, and not surprisingly, poorer satisfaction with health care. Additionally, patients who are admitted from long-term health care facilities may get "stuck" in the hospital if they are colonized with MRSA or vancomycin-resistant enterococci because a particular facility may not accept individuals with these multidrug-resistant infections.

Another issue of note is decolonization of MRSA-colonized individuals. Decolonization is done by applying a topical antimicrobial, mupirocin, to the nares to eliminate nasal carriage and bathing in either dilute solutions of chlorhexidine or bleach to decrease skin colonization, including inguinal sites.

In the community, CA-MRSA has been associated with a variety of sports activities. Most of the evidence to date suggests that this organism is spread from person to person either by direct contact (as in the case of football players, wrestlers, and fencers) or via fomites such as by sharing towels with colonized/infected individuals or by contact with training equipment that has been previously used by CA-MRSA carriers. Strict attention to personal hygiene, including good hand-washing practices, not sharing towels, and wiping down exercise equipment with disinfectant following use, could help reduce these infections.

7. CA-MRSA is now recognized as an important emerging human pathogen. A recent report in *JAMA* indicates that in the United States, MRSA is a more important cause of mortality than HIV. CA-MRSA has made a significant contribution to this mortality, and its importance as a human pathogen appears to be increasing. This strain's predilection to cause serious pulmonary infections made this organism of particular concern because it was feared that secondary bacterial superinfection due to CA-MRSA would greatly increase morbidity and mortality during any future influenza pandemic. CA-MRSA was found to be widespread in the United States by the early 2000s. Importantly, there is currently no evidence to suggest that increased numbers of secondary CA-MRSA pneumonia occurred during the influenza A/H1N1 pandemic of 2009 to 2011.

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This 65-year-old woman was bitten by her cat on the dorsal aspect of the right middle finger at 8:00 a.m. She rinsed the bite with water, and at 4:30 p.m. she noted pain and swelling in the finger and the dorsum of the right hand. She then noted pain in the axilla, red streaking up the forearm, and chills. On examination she had a temperature of 38°C and her right upper extremity was notable for swelling, erythema, warmth, and tenderness on the dorsum of the hand. Two small puncture wounds were seen on the proximal phalanx of the long finger, and erythema was visible over the extensor surface of the forearm. Axillary tenderness was also noted. Laboratory studies demonstrated an elevated white blood cell count of 12,000/μl with a left shift (the presence of immature neutrophils in the peripheral blood). Aspiration of an abscess on her finger was sent for culture, and the patient was taken to the operating room for incision and drainage of the abscess. A Gram stain of the organism causing this woman's infection is seen in Fig. 37.1, and Fig. 37.2 shows cultures on sheep blood and chocolate agars. The organism failed to grow on MacConkey agar, and spot tests from the blood agar plate were oxidase and spot indole positive.

1. Which organism was isolated on culture of the abscess? If this had been a human bite, what organisms might cause an infection?
2. What is the reservoir of this organism? How do humans most commonly become infected by this organism?
3. How can infection with this organism be prevented?
4. What other clinical syndromes can be caused by this organism?
5. If this patient had been scratched by a young cat rather than bitten and had subsequently developed regional lymphadenitis, what would be the likely organism?

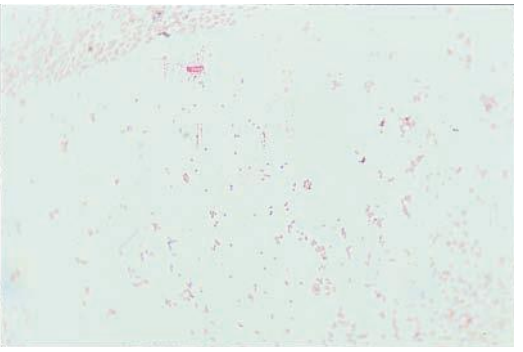


Figure 37.1

6. Domestic animals such as cats and dogs are vaccinated against what pathogen in order to protect humans? When should humans be vaccinated against this pathogen?



Figure 37.2



CASE DISCUSSION

1. Two organisms associated with domestic animal bites that are oxidase-positive, Gram-negative bacilli and fail to grow on MacConkey agar are *Pasteurella multocida* and *Capnocytophaga canimorsus*. The organism that was isolated from this patient's abscess was *P. multocida*. *P. multocida* can be differentiated from *C. canimorsus* on the basis of two characteristics. First, *P. multocida* is a Gram-negative coccobacillus, while *C. canimorsus* is a long, thin bacillus. Additionally, *P. multocida* is spot indole positive, while *C. canimorsus* is negative. A key feature of this case, which is typical of *P. multocida*, was the rapid onset of clinical signs of infection following the animal bite. One point worth emphasizing is that infections following cat and dog bites are commonly polymicrobial, often including both aerobic and anaerobic bacteria, with a median of five different bacterial isolates per culture when appropriate techniques are employed for the isolation of anaerobes.

Like those from cat and dog bites, human bite wound infections are typically due to a mixture of aerobic and anaerobic organisms that are part of the oral microbiota. Key organisms include facultative Gram-positive cocci in the *Streptococcus anginosus* group, the facultative Gram-negative bacillus *Eikenella corrodens*, and anaerobic Gram-negative bacilli within the genera *Prevotella* and *Fusobacterium*. Another important organism is *Staphylococcus aureus*, which likely arises from the skin microbiota of the injured individual. The emergence of community-associated methicillin-resistant *S. aureus* (MRSA) infections means that these infections must also be considered when choosing antimicrobials. Interestingly, facultative Gram-negative bacilli such as *Pasteurella* and *C. canimorsus* are not present in human bite wounds.

2. *P. multocida* is widely distributed throughout nature and is part of the normal flora in the nasopharynx of many mammals (both wild and domestic) and birds. Human infection is most likely to be associated with cat bites or scratches and less likely (though still quite commonly) to be caused by dog bites. Infections following bites by other members of the cat family, including lions, have been reported to cause *P. multocida* wound infections. In a minority of human infections, the patients have had no known animal exposure. Particular organisms are often associated with bites from specific animals. For example, *C. canimorsus* (*cani* = "dog"; *morsus* = "bite") infection may be transmitted by dog bites, and both *Streptobacillus moniliformis* and *Spirillum minus* are transmitted by rat bites. It is important to note that bites of domestic animals are responsible for hundreds of thousands of emergency department visits annually in the United States.

3. Infection can be prevented by limiting contact with cats and dogs. If a person is bitten or scratched by a cat or dog, the wound should be thoroughly cleaned as soon as possible. The animal should also be observed for sign of rabies, especially if rabies vaccination is not well documented.

4. In addition to soft tissue infection with rapid onset, other clinical syndromes seen with this organism following animal bites include osteomyelitis, tenosynovitis, abscess formation, and arthritis. Serious infections are more frequent after cat bites than after dog bites. It is speculated that the cat tooth, which is long and narrow, is more likely to cause puncture wounds that penetrate the tendon sheath (causing tenosynovitis) or periosteum (causing osteomyelitis). These infections are particularly problematic because they often occur on the hands and wrists. Because of the extraordinarily complex anatomy involved, infections of the hand and wrist, if neglected, can require complicated surgical debridement and loss of important motor function for the patient, either temporarily or permanently. Other uncommon complications include bacteremia with septic shock, meningitis, brain abscess, and peritonitis. Interestingly, there have been a fair number of reported cases of peritonitis due to *P. multocida* in which a cat bit into the tubing that was being used during peritoneal dialysis. Pneumonia due to cat exposure, rather than a bite, occurs as well.

5. Cat scratch disease is characterized by the development of a small lesion 1 to 2 weeks after a cat scratch, usually on the hand, wrist, or forearm. This lesion is followed 1 to 3 weeks later by regional lymphadenopathy, typically of a single or multiple nodes, most commonly in the axilla but sometimes in the cervical or epitrochlear region. Multiple sites are infrequently involved. The nodes may remain enlarged for several months and then resolve without treatment. The etiologic agent is a fastidious Gram-negative bacillus, *Bartonella henselae*. Although this organism can be grown from the blood of cats, it is rarely if ever recovered from the tissue of infected individuals. Diagnosis is likely to be sought in order to rule out other potential causes of lymphadenopathy such as malignancy. There are limited diagnostic tools clinically available to diagnose cat scratch disease. Although the antibodies that are tested for when using serology cross-react with similar organisms, the detection of a 4-fold rise in titer from acute- to convalescent-phase sera is diagnostic in the appropriate clinical setting. However, serology only provides a retrospective diagnosis. Multiple nucleic acid amplification tests have been described in the literature, but sensitive detection from human tissue often requires culture enrichment prior to molecular amplification, and this technique remains a research tool. Although the organism can be visualized in lymph node tissue with silver staining early in the disease course, this method is nonspecific and its sensitivity is unknown.

6. Both dogs and cats should be vaccinated against the neurotropic, single-stranded-RNA, enveloped virus rabies. Rabies is transmitted by the bite of a mammal, typically a dog. However, in the United States, cats are more likely to have rabies than dogs. This is probably because rabies vaccination is a requirement for dog licensure, and this licensure is required in most locales in the United States. On the other hand, only one state, Rhode Island, requires cat licensure, suggesting that cats are much less likely to receive rabies vaccination.

Rabies is endemic in many regions of the world, with travelers to Africa, South Asia, India, and certain regions of South and Central America at greatest risk of exposure. Rabies vaccination is recommended for individuals who are traveling to these regions and are likely to come in contact with dogs. More than 95% of cases that are imported into Europe and North America are due to dog bites. Of the small number of cases in the United States that are acquired in the absence of foreign travel, bats are often the source of the infection. In particular, parents are encouraged to have their children vaccinated if they may be exposed to dogs during their travels since they may be less careful about approaching these animals. Only 12% of travelers to regions where rabies is endemic are vaccinated. The reason for this low rate is thought to be the expense of the human rabies vaccine. The importance of this vaccine is illustrated by a case of rabies obtained by a U.S. soldier serving in Afghanistan who was bitten by a dog, was not offered postexposure prophylaxis, and developed rabies several months later and died. Unvaccinated people visiting countries where rabies is endemic should have a plan to get postexposure prophylaxis consisting of rabies immune globulin and vaccine if bitten by a dog, cat, monkey, bat, wolf, fox, or other mammal. This may include traveling to a place where such treatment is available. In the industrialized world, there is not as great a need to start postexposure prophylaxis immediately, since this risk is lower, especially if the animal responsible for the exposure can be observed or tested for the presence of rabies.

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An 18-month-old female presented to the emergency department with fever, a diffuse rash (onset 5 days before), and a swollen right hand. On examination she was irritable but alert. Her temperature was 39°C and her heart rate was increased at 180 beats/min. She had diffuse vesiculopustular lesions over her entire body (Fig. 38.1), with some areas showing older, crusted lesions. She had cellulitis of the right hand manifested by marked erythema, swelling, and tenderness. There were no mouth lesions, the lungs were clear, and the liver and spleen were not enlarged. Laboratory data were significant only for leukocytosis with a white blood cell count of 15,800/μl with 88% neutrophils. The chest radiograph was clear. A radiograph of the right hand showed only soft tissue swelling. The patient was treated with intravenous cefazolin. Improvement in the condition of her right hand was notable within 48 hours. This patient had a systemic viral infection with a complication of bacterial superinfection (cellulitis).

1. This patient had a characteristic rash (Fig. 38.1) at various stages of evolution. What was her underlying viral illness? What other causes of her skin rash should be considered in the differential diagnosis?
2. How is the diagnosis of infection with this pathogen made?
3. Describe the epidemiology of this viral infection and how it has changed since 1995.
4. What complications other than bacterial superinfection (as seen in this case) can occur as a result of this viral infection?
5. After acute primary infection with this virus, latent infection develops. What illness may occur years later as a result of viral reactivation? How do the clinical manifestations of this reactivation infection differ from those of primary infection?
6. What specific antiviral therapy has been shown to be efficacious? Are there any concerns about resistance?
7. What are the infection control issues related to this patient's illness?
8. Two different vaccines exist against this agent. How do they differ in terms of vaccine composition, target population, and efficacy?



Figure 38.1



CASE DISCUSSION

1. The patient's underlying viral illness was varicella (chicken pox). This illness is due to primary infection with varicella-zoster virus (VZV), which is a member of the herpesvirus family. These are enveloped, double-stranded DNA viruses. Varicella lesions develop in "crops" such that lesions can be seen in various stages of evolution, including vesicular, pustular, and crusted.

The differential diagnosis in this case includes impetigo (group A streptococcal infection), disseminated enteroviral infection, and disseminated herpes simplex virus infection in a child with underlying skin disease (e.g., eczema). This child had no history of a preexisting dermatologic disorder. Other viruses that cause "pox"-like lesions are in the *Poxviridae* family and include the orthopoxviruses and molluscum contagiosum virus. Molluscum contagiosum was unlikely in this case; in immunocompetent individuals, it remains localized and does not cause a sudden-onset systemic infection. However, the orthopoxviruses are important to consider, including monkeypox and smallpox. Although monkeypox is endemic in Central and West Africa and is rarely seen in the United States, there was an outbreak of monkeypox in the Midwest in 2003. This outbreak affected 72 individuals, all of whom had exposure to prairie dogs that had been housed at the same facility with imported, monkeypox-infected Gambian rats. Because of concerns about bioterrorism, the specter of smallpox must also be considered. Smallpox lesions, unlike those of chicken pox, are all at the same stage of development, whereas this patient's lesions simultaneously included vesicular, pustular, and crusted lesions. Smallpox lesions often occur on the palms and soles of the feet and are most concentrated on the face and extremities. This is in contrast to chicken pox lesions, which are rarely on the palms and soles and are more concentrated on the torso. If the patient had recently been vaccinated against smallpox, then disseminated vaccinia should also be in the differential. Noninfectious causes of skin rashes that may be confused with varicella include contact dermatitis, drug reactions, and insect bites.

2. In immunocompetent children, the diagnosis of chicken pox is often made on the basis of clinical findings alone. For adults and immunocompromised children, laboratory confirmation of VZV infection is frequently sought. A method that combines rapidity with sensitivity is direct fluorescent-antibody staining of scrapings taken from vesicular lesions. Culture techniques for detection of VZV include rapid centrifugation culture (i.e., shell vial) (Fig. 38.2) and standard tissue culture. Shell vial cultures, which take 2 to 4

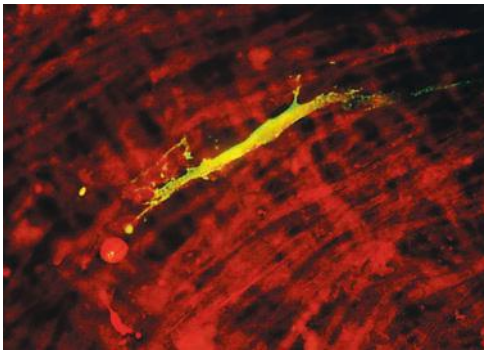


Figure 38.2

days, are both more rapid and more sensitive than standard tissue culture, which may take as long as 3 weeks to recover VZV. Nucleic acid amplification tests have also been developed, including quantitative assays. A recent study compared the sensitivity of direct fluorescent-antibody assay, shell vial culture, and two PCR assays and demonstrated 87.8, 46.3, and 97.6 and 100% sensitivity, respectively. Although highly sensitive and relatively rapid, nucleic acid amplification tests have not been approved by the FDA and therefore have limited availability.

3. VZV has a worldwide distribution. Disease is more common in temperate regions, with annual epidemics in the late winter and spring in areas with low vaccination rates. The virus is spread by the respiratory route and is highly infectious, with ~90% of nonimmune household contacts and 10 to 35% of nonimmune classroom contacts becoming infected. VZV can also be spread by direct contact with skin lesions and fomites. In 1995, a live attenuated vaccine was approved in the United States for prevention of primary varicella. In the prevaccine era, there were ~4 million cases of varicella annually in the United States, which translates to 15 to 16 cases per 1,000. In the first 5 years after introduction of the vaccine, the incidence dropped 76 to 87% in the United States.

4. In general, varicella causes much more severe illness in adults than in children. Immunocompromised children and nonimmune, pregnant women also are more prone to complications with this virus than is the general population. The severe illness seen with VZV in these patient populations is due in large part to the significant morbidity and mortality associated with varicella pneumonia. Other complications include hepatitis, arthritis, glomerulonephritis, myocarditis, pericarditis, pancreatitis, encephalitis, and cerebellar ataxia. Multiorgan involvement is associated with high mortality. Primary varicella during pregnancy can also cause intrauterine infection leading to fetal loss or an infant born with congenital varicella syndrome, which may include dermatomal scarring, limb hypoplasia, ocular defects, low birth weight, and mental retardation.

In addition, secondary bacterial infections of the skin lesions, as was seen in this case (cellulitis of the right hand), can also occur. These bacterial infections are most commonly caused by *Streptococcus pyogenes* and *Staphylococcus aureus*. VZV infections are associated with *S. pyogenes*-induced necrotizing fasciitis, as VZV skin lesions have been well recognized as an important portal of entry for *S. pyogenes*. Reye's syndrome, with encephalopathy, elevated transaminase levels, and elevated serum ammonia levels, can occur in children with varicella or influenza who take aspirin. It should be remembered that patients with VZV infection can have a prodrome characterized by fever, malaise, headache, and abdominal pain that is indistinguishable from many other viral illnesses. Therefore, infants and children with febrile illnesses should not be given aspirin.

5. Herpes zoster (shingles) is a reactivation of a latent VZV infection. The dorsal root ganglia are latently infected following primary infections. Cell-mediated immunity (CMI),

and not VZV antibody, is necessary to maintain latency. A loss in CMI, as is seen with increasing age, is associated with reactivation. Other risk factors for reactivation include CMI dysfunction (transplant, hematologic malignancies, HIV), diabetes, and even recent physiologic stress.

In herpes zoster, skin lesions appear in a single dermatomal distribution innervated by the specific dorsal root or extramedullary cranial ganglia where VZV had been latent. There are four groups of herpes zoster complications—cutaneous, visceral, neurological, and ocular. Perhaps the most debilitating complication is the persistent pain that can occur with the rash and persist even after the lesions heal. This persistent pain is called postherpetic neuralgia.

Rarely, skin lesions disseminate beyond the primary dermatome involved. In immunosuppressed patients, however, complicating viremia can occur, with dissemination to extradermatomal skin sites, lungs, liver, and the central nervous system. This condition, with extradermatomal sites of infection, is called disseminated herpes zoster. Patients with zoster are also infectious, although apparently not as infectious as patients with varicella.

6. Acyclovir is beneficial in treating VZV (both varicella and herpes zoster). In immunocompetent adults ≥50 years of age, treatment with both analgesics and antivirals is recommended, particularly in patients with ocular involvement. In immunocompetent patients <50 years old, antivirals are not necessary but can shorten the duration of illness. Because of its cost, acyclovir is often not used in uncomplicated cases. Thymidine kinase mutations in VZV conferring resistance to acyclovir have been described, though almost exclusively in immunocompromised patients. In one report, 27% of hematopoietic stem cell transplant recipients with persistent VZV infection had mutations possibly associated with resistance. Interestingly, in patients with disseminated disease, all infected sites may not harbor the resistant virus. Therefore, it is prudent to test multiple specimen types when screening for resistance mutations. Acyclovir-resistant VZV in immunocompetent patients appears to be rare, but has been reported.

7. Patients with varicella are very contagious. Secondary cases are frequently more severe. The increased severity is believed to be due to high viral inoculum. Hospitalized patients with varicella must be placed in respiratory isolation (airborne precautions), and strict infection control measures regarding skin contact (hand washing, use of gloves and gowns, etc.) must be implemented (contact precautions). Precautions must remain in place until lesions are dry and crusted. Only individuals who are nonimmune, including health care personnel, need to wear a mask. Ideally, nonimmune health care personnel should not care for a VZV-infected patient. Seronegative health care personnel who do come in contact with these infected patients should not have contact with other patients, especially immunocompromised ones, for a minimum of 2 weeks after exposure, the incubation period of this viral infection.

There are also infection control considerations when a nonimmune person has been exposed to VZV. Postexposure vaccine should be administered within 120 hours of exposure. For exposed individuals who cannot receive the live vaccine, a varicella-zoster immune globulin preparation (i.e., VariZIG) should be administered within 96 hours. This would apply to immunocompromised individuals, infants, and pregnant women.

8. There are two VZV vaccines—one to prevent varicella and one to prevent herpes zoster. Both are live, attenuated vaccines made from the same vaccine strain, but the difference is the titer of the virus in each vaccine. The herpes zoster vaccine has a much higher titer than that of the varicella vaccine (~14 times higher). A higher titer is needed to provide an immune booster to prevent herpes zoster reactivation or at least decrease the severity of disease. Because these are live virus vaccines, they should not be used in immunocompromised individuals, including those with a hematologic malignancy, congenital immunodeficiency, or symptomatic HIV infection. Persons receiving high-dose immunosuppressive drugs and pregnant women should also not receive these live vaccines.

The varicella vaccine is licensed for use in the United States for all children >12 months of age. Current recommendations call for the vaccine to be given in two doses—the first dose at 12 to 15 months of age and the second dose at 4 to 6 years of age. Adolescents and adults with no previous evidence of disease should receive two doses of the vaccine 4 to 8 weeks apart. The vaccine is very efficacious, vaccine failures are rare, and it has been shown to be particularly effective at preventing severe VZV disease. Postlicensure vaccine safety surveillance using the Vaccine Adverse Event Reporting System of the Centers for Disease Control and Prevention has shown the vaccine to be remarkably safe. Both vaccine-associated and natural infections have been noted postvaccination. Serious infections and deaths due to infection caused by the vaccine strain have been observed but are quite rare (1 death/1,000,000 doses of vaccine administered).

The herpes zoster vaccine is licensed for individuals ≥50 years of age and only requires one dose. The target population for vaccination is individuals ≥60 years of age due to the higher rate of herpes zoster and its complications in this population. The initial clinical trial data (≥60-year-olds) showed that the vaccine reduced the incidence of herpes zoster by 51% and the incidence of postherpetic neuralgia by 67%.

Two questions remain unanswered concerning the effect of these vaccines on the natural progression of disease. First, will individuals who receive the varicella vaccine be at risk for herpes zoster due to the vaccine strain later in life? Limited data suggest that they may, but that the rates and severity of herpes zoster are reduced compared with those in individuals who have natural disease. Second, will immunity wane in adults who received the varicella vaccine as a child? As natural disease declines, this could result in an at-risk population. Since adults are most vulnerable to severe varicella disease, this is a legitimate concern. Twenty-year follow-up data suggest that immunity persists, but these studies

were done in settings where natural disease continues to be common, offering the opportunity for immunized individuals to receive a "booster" effect from exposure to infected individuals.

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A 44-year-old female was transferred to the hospital by air ambulance after suffering a respiratory arrest in her doctor's office. She arrived intubated after being resuscitated. Her past medical history was significant for her having suffered a dislocated left thumb 3 days previously at work while assisting a patient into a wheelchair. When she got home from work, a family member reduced the dislocation. Over the next 2 days she had gradually increasing ascending pain and swelling in her left arm. She visited her local emergency department on each of those 2 days. Both times she was given analgesics and sent home. On her third day of illness, she was visiting her primary care physician, where she had a cardiopulmonary arrest.

On physical examination she had a temperature of 39.1°C, heart rate of 197 beats/min, and blood pressure of 95/45 mm Hg. Her white blood cell count was 4,700/μl, her hemoglobin was 11.7 g/dl, and she had a creatinine of 1.9 mg/dl, a blood urea nitrogen of 32 mg/dl, and a creatine kinase of 3,307 units/liter. Physical examination of her left arm was consistent with a nonperfused extremity including a cold, cyanotic hand, blisters with skin necrosis between the wrist and elbow, and arm warm to the touch at the elbow and above.

She was begun on clindamycin and penicillin G and taken to the operating room, where an incision was made over her left humerus, which revealed necrotic tissue and dishwater fluid between tissue planes at the fascial level. Her arm was amputated at the shoulder. She had additional chest wall debridement down to the pectoralis major. Tissue Gram stain showed 4+ Gram-positive cocci in pairs and short chains (Fig. 39.1). Postoperatively the patient became increasingly hemodynamically unstable, had a cardiac arrest, and could not be resuscitated. It was subsequently learned that the individual with whom the patient was working when she suffered the thumb dislocation had been admitted with septic shock to another hospital. The organism recovered from the patient is shown in Fig. 39.2.

1. What organism caused this patient's infection?
2. What syndrome did this patient have? How does it explain the physical finding of a cold, cyanotic extremity on admission? What virulence factors does this organism produce that played a role in her clinical disease course? What is the typical outcome of this infection? What might have been done to make her case less severe?
3. How did this patient become infected? How can this be proven?
4. Two other individuals in the community had a similar illness to the case patient in the same week. There was no direct epidemiologic link among the three. How do you explain this observation?

5. What is the key risk factor for this syndrome in children? Why is this risk factor unusual in adults? What is being done to try to eliminate this risk factor in children?
6. Why was clindamycin part of the therapy for this patient? What susceptibility test would you need to do to ensure that the clindamycin might be active in this patient?

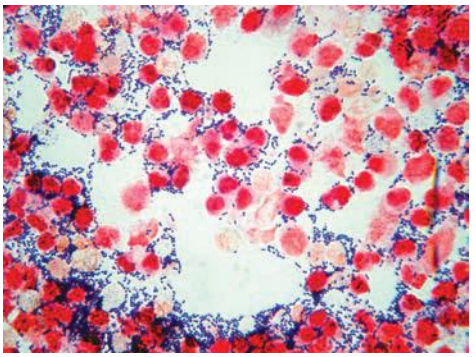


Figure 39.1 Direct Gram stain from tissue.

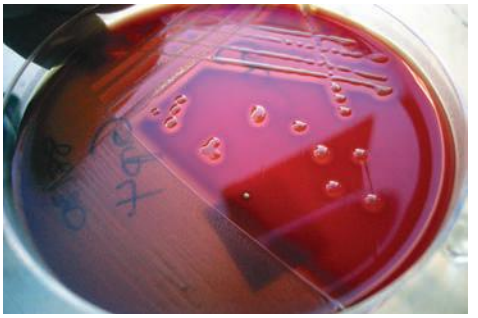


Figure 39.2 Isolate recovered from patient.

CASE DISCUSSION



1. The patient's infection was due to *Streptococcus pyogenes* (group A streptococcus or GAS).

2. This patient had streptococcal toxic shock syndrome (STSS) associated with necrotizing fasciitis. STSS is defined as recovery of GAS from a normally sterile site, shock as evidenced by decreased blood pressure (95/45 mm Hg in this patient despite the initiation of fluid resuscitation), and evidence of failure in two or more organ systems (this patient had evidence of renal, cardiac, and pulmonary failure based on laboratory findings). STSS is caused by the physiological response to the production of superantigens by GAS. Streptococcal pyogenic exotoxin A (SpeA) is the superantigen most commonly associated with STSS. This molecule binds nonspecifically to antigen-presenting cells and T lymphocytes, causing the T lymphocytes to produce massive amounts of proinflammatory cytokines, including tumor necrosis factor-α, interleukin-1β, interleukin-2, and gamma interferon. These high levels of cytokines activate the complement, coagulation, and fibrinolysis cascades, which are responsible for the abnormal physiological response that is characteristic of STSS.

In necrotizing fasciitis, highly virulent strains of GAS (see answer 4 for greater details) attach to the skin and penetrate through the dermis to the underlying soft tissue and muscle. The particular isolate infecting this patient was *emm* type 1 or M1. M1 organisms are more likely to cause invasive disease than most other *emm* types, with the possible exception of M3. M1 strains that are invasive have mutations in *covRS*, an operon that controls ~10% of the GAS genome, including a large number of virulence factor genes. When these mutations occur, there is downregulation in SpeB. SpeB is a cysteine protease that regulates the activity of several GAS virulence factors by degrading them. In addition, there is upregulation of a variety of virulence factors that allow it to evade the innate immune system locally as well as producing a variety of factors that either directly or indirectly destroy tissue. M protein, hyaluronic acid capsule, and extracellular streptodornase appear to be the key virulence factors in the organism's ability to evade phagocytosis. In Fig. 39.2, the colony of the organism recovered from this patient is highly mucoid as a result of high levels of hyaluronic acid production. This phenotype is a hallmark of GAS strains associated with invasive disease.

The manner in which streptodornase contributes to immune evasion has recently been delineated. Neutrophils secrete a substance known as NETs (neutrophil extracellular nets), which contain a complex of DNA, histones, proteases, and antimicrobial peptides. These NETs "trap" bacteria and are involved in neutrophil extracellular killing. Streptodornase is a DNase that degrades DNA, thus destroying the NET.

GAS also produces proteases, including C5a peptidase, that either directly or indirectly degrade complement components or cytokines. This reduces the migration of phagocytes to the site of infection.

GAS produces a variety of histotoxic molecules that allow the organism to spread along fascial tissue planes, causing massive tissue destruction. These include streptolysin S and O, pore-forming cytolysins that can lyse a wide variety of tissue types. Once cells are killed, the organism produces several different DNases and plasmin, a host-derived protease that can further degrade these tissues. The organism also produces at least two virulence factors that facilitate its spread through tissue. Hyaluronidase degrades hyaluronic acid found in ground substance, which acts as the "cement" in connective tissue, and streptokinase, which dissolves fibrin clots. In addition, streptokinase has high affinity for human plasminogen, degrading it to plasmin.

The patient's cold, cyanotic arm was characteristic of the massive tissue destruction, including thrombosis of the vascular bed, incurred during necrotizing fasciitis, resulting in nonviable tissue. STSS coupled with necrotizing fasciitis has a mortality ranging in some studies to as high as 60%, so the outcome in this case was, sadly, not unexpected.

One of the difficulties in diagnosing necrotizing fasciitis is that the pathology occurs in subcutaneous tissue. As a result, in the absence of an obvious injury, the skin may appear normal in as many as 50% of patients in the early stages of this illness. The clues in this case were her progressive arm swelling and continued arm pain, both of which were severe enough to warrant two trips to her local emergency department. Necrotizing fasciitis, because of its ability to spread rapidly along fascial planes, is a true surgical emergency. If surgical debridement and antimicrobial therapy are not implemented in the first 12 to 24 hours of symptomatic disease, the likelihood of a fatal outcome greatly increases. By the time she presented to her personal physician on the third day of illness, her disease had progressed beyond the point where she could survive. Perhaps the reason this disease has such a high mortality is that, as with this patient, it may not be recognized or considered until infection with this highly virulent organism has progressed beyond the point where the patient can be saved.

3. After the death of this patient (patient A), it was learned that she had dislocated her thumb while helping an individual (patient B) into a wheelchair. It was subsequently learned that patient B had impetigo-like lesions on her face at the time of patient A's injury and that patient B was admitted to a second hospital with septic shock the day after patient A died. GAS was isolated from the blood of patient B. The two organisms were serotyped, and both patients were infected with the M1 serotype. M1 is the either the first- or second-most-common serotype associated with invasive GAS and necrotizing fasciitis. Although this strain was serotyped, the current standard method for typing GAS is to do sequence analysis of the amino-terminal region of the M protein. Using this approach, >150 genotypes of GAS have been recognized. Pulsed-field gel electrophoresis confirmed these two isolates to be the same genotype. The conclusion was that patient A was infected by patient B, most likely at the time patient A dislocated her thumb helping patient B into a wheelchair.

Invasive GAS infections in health care workers obtained from infected patients have been described. Fortunately, these events are rare.

4. It is well recognized that community and regional outbreaks of invasive GAS disease primarily due to M types 1, 3, 12, and 28 occur. M typing of the isolates from these other two individuals revealed that one of the patients had M1 strain of the same pulsotype as the case patient while the other patient was infected with a different M type. During outbreaks of invasive disease, the level of GAS disease activity in the community or region increases but the great majority of patients have localized infections, typically pharyngitis and skin infections. It is believed that people who develop invasive GAS disease and especially those who develop STSS do not have antibodies to the specific M type that is circulating in the community nor do they have antibodies against SpeA, putting them at risk for this most severe manifestation of GAS disease. It may also be true that the individuals who become infected are unfortunate to have isolates with mutations in the *covRS* regulon that result in the upregulation of GAS virulence factors, while the vast majority of individuals who have localized skin and throat infections are not infected with these mutants.

5. Approximately 30% of children who develop necrotizing fasciitis have had a recent case of chicken pox (varicella-zoster virus), usually within the past 1 to 3 weeks. Children either with chicken pox or recovering from it have a 6-fold-increased risk of necrotizing fasciitis. Chicken pox lesions are "itchy," and children can inoculate GAS on their skin into their lesion by scratching these itchy lesions; in a small percentage of children, GAS necrotizing fasciitis can result. Unlike in adults, GAS necrotizing fasciitis in children has a low mortality: <10%. Most adults had chicken pox as children, so they are no longer prone to having this risk factor. We have seen one case of fatal necrotizing fasciitis secondary to chicken pox in an adult. The deceased was infected by his young child, who developed chicken pox during an outbreak in his day care center.

Beginning in 1995, a live, attenuated varicella-zoster vaccine has been used in children in the United States. This vaccine has proven to have an efficacy of 85% and has the likely added benefit of reducing the number of cases of necrotizing fasciitis secondary to chicken pox, although there are no organized studies to demonstrate this point.

6. The combination of penicillin and clindamycin is recommended for treatment of GAS necrotizing fasciitis. Clindamycin is included in the therapeutic regimen because in animal models it has been shown to reduce GAS toxin production, especially SpeA. Unlike penicillin, it is not prone to an inoculum effect nor does it lose activity against stationary-phase organisms. Both are problems with penicillin therapy. The number of organisms found in the tissue may be too large for penicillin to kill efficiently. In addition, because of the rapid growth of GAS in tissue, many of the organisms may be at "stationary" phase, or no longer growing. Penicillin only kills actively growing organisms. Oddly, there are no good clinical studies that demonstrate the efficacy of clindamycin in the treatment of STSS. Given the severity of this disease, its relative rarity, and positive animal model data, it is unlikely that such trials to demonstrate efficacy will be forthcoming.

Detection of clindamycin resistance in Gram-positive cocci including GAS is not straightforward. Strains of GAS and other Gram-positive organisms may have an inducible

form of clindamycin resistance due to the presence of an *erm* (erythromycin ribosomal methylase) gene. By standard susceptibility tests, *erm*-containing GAS isolates appear susceptible. However, when the inducer, erythromycin, is placed 12 mm from the clindamycin disk, the zone of inhibition will flatten on the side of the disk adjacent to the erythromycin disk. This zone looks like the letter "D" (Fig. 39.3); thus the term "D test." Isolates that have inducible resistance may undergo mutation to a constitutive form of the enzyme during clindamycin therapy, resulting in the organism becoming clindamycin resistant. Given the severity of STSS, it is of value to know if the organism has inducible clindamycin resistance. Recent studies suggest that this resistance mechanism is unusual, being found in 2% of GAS isolates.

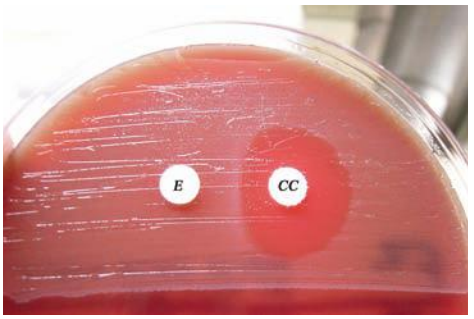


Figure 39.3 D test with erythromycin (E) and clindamycin (CC).

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The patient was a 50-year-old male who 7 months ago developed acute swelling, erythema, and synovitis of his right hand. He visited his personal physician, who obtained a radiograph of the hand, which was negative. He was started on prednisone, but his symptoms persisted. Three months into his disease course he went to see a rheumatologist, who diagnosed him with seronegative rheumatoid arthritis and added methotrexate. The condition of his hand worsened over the next 3 months. Over the 3 weeks prior to being seen at our institution, he developed ulcerative lesions on his hand draining bloody serous fluid. He stated that the hand had become much more erythematous. On physical examination he had large, weeping ulcerative lesions across the joints on all four fingers on his right hand. The skin over the joints was very red and mildly tender. The dorsum of the right hand was swollen, and there was lymphadenopathy tracking up his arm, but he did not have axillary lymphadenopathy. The left hand was normal and he had no other symptoms. His vital signs were normal. His erythrocyte sedimentation rate was 80 mm/hour. A biopsy was performed, which revealed granulomas. An acid-fast stain of the tissue is seen in Fig. 40.1. Approximately 3 weeks later the organism seen in Fig. 40.2 was growing on a Lowenstein-Jensen slant incubated at 30°C. When it was determined that the patient had granulomas in his hands, further history was elicited for him. His physicians learned that he had gone fishing on the Chesapeake Bay 3 weeks prior to his initial presentation.

1. What is seronegative rheumatoid arthritis? Did this patient meet the American College of Rheumatology criteria for rheumatoid arthritis? Besides rheumatoid arthritis, what other possible explanation might account for his symptoms?
2. Why was methotrexate added to his therapeutic regimen? Why do you think that his condition worsened, and why did it take him so long to seek further medical care?
3. Figure 40.1 indicates the group of organisms with which this patient was infected. In what genus is the organism infecting this patient most likely to be? Figure 40.2 shows the organisms before and after exposure to light. What difference do you see? To what group of organisms does this organism belong?

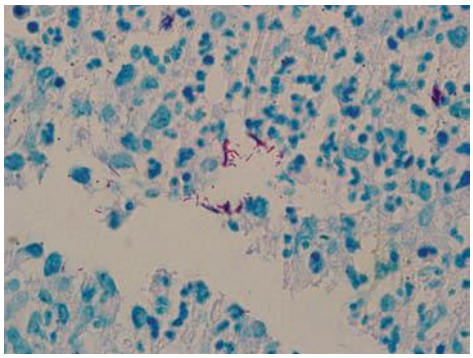


Figure 40.1 Kinyoun stain of biopsy of right hand (×1,000 magnification).

4. With what organism was he most likely infected? Why do you think the diagnosis was initially missed? How was eliciting further history helpful?
5. Most cultures done for organisms belonging to the genus infecting this patient are cultured at 35 to 37°C. However, when culturing for this genus, skin, soft tissue, and joint infection cultures are typically done at 30°C. Why?
6. Skin testing for one of the organisms in the genus infecting this patient is widely done. What is the skin test used, and for what organism in this genus is this skin testing done? What would be the likely result of skin testing in this patient? How might this affect management of this patient?

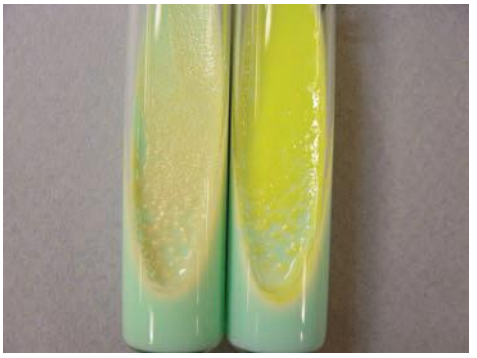


Figure 40.2 Organism prior to (left) and after (right) light exposure.

CASE DISCUSSION



1. Approximately 10 to 20% of patients with rheumatoid arthritis are seronegative, meaning they do not have rheumatoid factor, i.e., autoantibodies directed against the Fc portion of immunoglobulin G. Rheumatoid arthritis is an autoimmune disease that causes joint pain and in its most severe manifestations causes deformity and destruction of the joints, resulting in loss of function. By the time the patient saw the rheumatologist, he did meet the American College of Rheumatology's definition of rheumatoid arthritis because he had more than 10 small joints involved (all three small joints on the four fingers of his right hand) and had experienced symptoms for more than 6 weeks (see http://www.rheumatology.org/practice/clinical/classification/ra/ra_2010.asp). He did not have an erythrocyte sedimentation rate recorded at the time of his initial visit, and records from his rheumatology visit were not available.

Other possible explanations for his swollen joints include Lyme disease, for which he did not have an appropriate history; other chronic bacterial or fungal joint infections; rheumatic fever; and a wide variety of autoimmune and inflammatory diseases.

2. Anti-inflammatory therapies are a standard therapeutic approach for rheumatoid arthritis. Methotrexate is recommended as initial therapy for rheumatoid arthritis. Methotrexate is a folate antagonist that reduces purine and pyrimidine synthesis and thus cell proliferation, including that of T lymphocytes, which are believed to play a central role in the inflammatory process in this disease. One of the side effects of any anti-inflammatory agent is that it can increase the likelihood of infection by reducing the immune-mediated clearance of organisms. In this case, the prednisone/methotrexate combination masked the inflammation that was present as a result of his infection. The relatively slow progression that was seen was likely due to the slow growth rate of the organism infecting the patient (see answer 3 for details).

3. The biopsy from this patient's hand showed an acid-fast bacillus. The major group of organisms that are acid fast is the genus *Mycobacterium*. *Nocardia* is another higher-order bacterium that could cause the disease course observed in this patient. However, it is partially acid fast, so it would not appear like the organism in Fig. 40.1. The difference between an acid-fast and a partially acid-fast organism is that the decolorizing agent in an acid-fast stain contains both alcohol and dilute acid, while the decolorizing agent in a partially acid-fast stain contains only dilute acid. Mycobacteria are not decolorized by a solution containing alcohol and acid, while *Nocardia* species are. It should be noted that both organisms have a beaded, Gram-positive appearance on Gram stain. Other organisms that cause slowly progressive, indolent skin, soft tissue, joint, and bone infections on the hand include fungal agents such as *Sporothrix schenckii*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and a wide range of environmental dematiaceous fungi. Ulcerative

lesions can be seen with other environmental mycobacteria, the fungi listed above, and *Leishmania*. However, the patient did not have an appropriate travel history for the tropical disease leishmaniasis, so it was not considered further.

Figure 40.2 shows the organisms growing prior to and after exposure to light. Organisms that produce pigment independent of exposure to light are called scotochromogens. Organisms that make pigment only when they are exposed to light, as seen in Fig. 40.2, are called photochromogens. The pathogenic photochromogenic mycobacteria that are mostly likely to be encountered clinically are *Mycobacterium marinum* and *Mycobacterium kansasii*.

4. Given that the acid-fast bacillus in this case was a photochromogen, and considering the location of the infection and the slow rate of disease progression, the organism causing this infection is most likely *M. marinum*. When a photochromogen grew from the patient, we thought immediately that it was *M. marinum* because this organism most commonly causes skin, soft tissue, and joint infections on the extremities, with the vast majority on the hands. Sequencing of the 16S rRNA gene confirmed that the organism was *M. marinum*. It is a common environmental organism that is found in fresh-, brackish, or salt water. *M. marinum* is almost always associated with trauma involving water. It may take as long as 6 weeks to grow from a clinical specimen, although most isolates will be recovered by 2 to 3 weeks. Interestingly, direct acid-fast bacillus stains for clinical specimens are only positive in ~10% of *M. marinum* infections, so the finding in this case was unusual but may reflect how long the infection had to progress.

The most common form of *M. marinum* infection is called "fish tank granuloma," which occurs in people who have breaks in their skin while cleaning fish tanks contaminated with this organism. However, other sources of infection are well known. *M. marinum* is a hazard for commercial and recreational fishermen and -women, with trauma caused by fish fins or teeth or the shells of shellfish.

When this patient was originally seen by his personal physician, he had a negative radiograph and did not have any obvious trauma to the hand, could not remember any episodes of trauma, and did not own a fish tank or remember any contact with water beyond everyday bathing. As a result, his personal physician had no reason to suspect an infection, especially one due to an environmental *Mycobacterium* sp. However, once the identity of the organism was determined, the patient was requestioned and specifically asked about possible water exposures. He remembered that he had gone on a fishing trip to the Chesapeake Bay 3 weeks prior to developing symptoms, although he did not remember any traumatic event during the trip.

That it took more than 7 months to establish the diagnosis of this infection was not surprising. Several studies suggest that it takes as long as 12 months to diagnose *M. marinum* infection. This is likely due to the organism's indolent nature and slow growth rate and to physicians' inability to diagnose this infection because of a lack of experience with infections caused by this organism.

5. The most important human pathogen among the mycobacteria is *Mycobacterium tuberculosis*. Because it is adapted to growing in humans, it grows readily at 35 to 37°C. As a result, human specimens are cultured at this temperature to ensure the recovery of the most pathogenic of the mycobacteria. However, environmental mycobacteria (also referred to as nontuberculous mycobacteria) make up the bulk of the mycobacterial species to which humans are exposed. Many of these organisms, which include *M. marinum*, grow better at 30°C. In fact, *M. marinum* grows poorly, if at all, at 35 to 37°C. Since environmental mycobacteria are much more likely to cause skin, soft tissue, and joint infections, it is recommended that these specimens be cultured at both 30°C and 35 to 37°C.

6. A common strategy for screening patients to detect infection with *M. tuberculosis* is to do a skin test in which 5 tuberculin units are injected intradermally into the volar surface of the forearm. If the patient is infected with *M. tuberculosis* (tuberculosis [TB]), an area of induration will appear after 48 to 72 hours. This induration is due to a delayed-type hypersensitivity reaction to purified protein derivative present in the skin test material. Induration of 5, 10, or 15 mm is considered positive depending on the patient population tested, with 5 mm being used as a cutoff for immunocompromised patients, 10 mm for high-risk individuals such as those with documented exposures, and 15 mm for individuals with a low index of suspicion. It should be remembered that 90% of patients who are infected with *M. tuberculosis* are latently infected and will not have clinical symptoms.

Of patients with *M. marinum* infections, more than half will have a false-positive TB skin test. If these patients are tested, it may result in further testing such as a chest radiograph or even the initiation of *M. tuberculosis* therapy. *M. tuberculosis* therapy in patients with *M. marinum* infections is problematic because the mainstay of *M. tuberculosis* therapy is isoniazid, an agent with known liver toxicity and no activity against *M. marinum*. Given the high rate of false-positive TB skin tests in this clinical setting and the low pretest probability of this being *M. tuberculosis*, TB skin testing really has no role in the management of this patient.

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This 12-year-old girl was in her normal state of good health when she developed a fever of several days' duration. She had no localizing symptoms except for the development of a large rash on her back (Fig. 41.1). Her history was notable in that she lived in Connecticut near the New York State border and had recently been walking through tall grass where her sister was taking horseback riding lessons.

1. With what organism was she infected? What disease did she have?
2. What in her history is suggestive of this disease? How is this disease transmitted?
3. How, in the absence of a characteristic rash, is the diagnosis of this disease established?
4. She was appropriately treated with antibiotics and did well. What complications can occur in patients with this disease, particularly those in whom there is no treatment or inadequate therapy?
5. What efforts can be taken to prevent this illness?



Figure 41.1



CASE DISCUSSION

1. This patient was infected with the spirochete *Borrelia burgdorferi*, which is the etiologic agent of Lyme disease. Her symptom of a nonspecific fever is consistent with Lyme disease, but it is the presence of the characteristic erythematous annular rash, referred to as erythema migrans, demonstrated in Fig. 41.1, that is diagnostic. The rash typically has a target-like appearance with expanding borders. Some patients will have these lesions at multiple sites. This patient had one rather prominent lesion.

2. The patient lives in Connecticut, a state with a very high incidence of Lyme disease. In fact, the disease was initially described in (and named for) Old Lyme, a town in Connecticut. Other regions in which Lyme disease is endemic include other areas in the northeastern United States, Minnesota, Wisconsin, northern California, and much of Europe, particularly between 35° north latitude and 60° north latitude.

B. burgdorferi is spread to humans by ticks of the genus *Ixodes*. In the northeastern United States, the white-footed mouse appears to be the primary reservoir of *B. burgdorferi*, which is present in the mouse's bloodstream. This mouse is also the preferred host for the *Ixodes scapularis* tick, formerly known as *Ixodes dammini*, the major vector of this spirochete. In other geographic locales, other *Ixodes* species act as major vectors.

Given that the patient was walking through tall grass, the sort of environment where ticks are likely to be found, she may well have had an ixodid tick attach to her during this time. The tick is frequently found in woody areas, but also can be found in grassy areas. All three stages in the life cycle of the tick, i.e., larva, nymph, and adult, can feed on a human host, but only the nymph and adult stages can transmit the disease. Nymphs and adults that were infected after feeding on a *B. burgdorferi*-infected mouse (or other small mammal) pass the organism to humans during the blood meal, probably by regurgitating the spirochetes into the wound. Transfer of the spirochetes from the infected *I. scapularis* ticks to humans appears to require 36 to 48 hours of attachment. The events that occur in the tick during the attachment are complex, with the spirochetes migrating from the gut to the salivary glands. Additionally, the organism has to express a protein called outer surface protein C that enhances infectivity of the spirochete for the human host. It is believed that this migration and phenotypic change explain the need for prolonged attachment prior to transmission. Ticks removed before the 36 to 48 hours probably do not transmit the spirochete. However, the nymph stage of the tick is extremely small (described as the size of a pencil point), so the tick bite may go unnoticed. Less than half of patients with documented Lyme disease are able to recall a tick bite.

3. The case definition of Lyme disease that is used for surveillance purposes is the presence of an erythema migrans rash ≥5 cm in diameter or laboratory confirmation of infection with objective evidence of musculoskeletal, neurologic, or cardiovascular manifestations

of Lyme disease. Unfortunately, the organism itself is difficult to grow from clinical specimens and requires complex media that are not available in most clinical laboratories, making culture a low-yield procedure that is used almost exclusively in research settings. Because of this, isolation of this organism from clinical specimens is not routinely attempted. Although PCR is used in some settings, such as in cases of arthritis and central nervous system disease, its low sensitivity limits its usefulness. The current laboratory recommendation is a two-test approach for the serologic diagnosis of Lyme disease. The serum specimen should first be tested using either an enzyme immunoassay or an indirect immunofluorescent assay. Positive or equivocal specimens should then be tested with the more specific immunoglobulin G and immunoglobulin M Western blot (immunoblot). The sensitivity and specificity of the serologic tests vary in relation to the time in the course of the illness during which the specimen was obtained, with the tests being more accurate later in the disease course. The performance of different laboratories in Lyme serology testing varies greatly, with both false-positive and false-negative results occurring with increased frequency in certain laboratories.

4. Approximately 90% of patients with *B. burgderfori* present as this patient did with fever and skin rash and respond to antimicrobial therapy with no sequelae. Involvement of joints with clinical arthritis is seen in ~5% of patients. A smaller subset (<5%) may present with a clinical picture of headache and have cerebrospinal fluid (CSF) with a lymphocytic pleocytosis. Other neurologic symptoms and signs, such as cranial nerve VII palsy, peripheral neuropathy, meningoencephalitis, and subacute encephalopathy, may occur. Cardiac involvement with conduction defects and consequent arrhythmias also occurs in ~1% of patients. Coinfection with another tick-borne pathogen, *Babesia microti*, the major etiologic agent of babesiosis, has been reported to occur in patients with Lyme disease.

One of the most difficult challenges in infectious diseases is the management of patients who present with an entity that has been characterized as "chronic Lyme disease." Late Lyme neuroborreliosis, a rare complication of this illness, is characterized by increased CSF protein, CSF lymphocyte count, and the production of intrathecal antibody. These patients typically have changes on magnetic resonance imaging and peripheral neuropathy. Antimicrobial therapy may prove helpful in this group.

However, many more patients present with chronic Lyme disease without objective evidence of disease. Often, these patients have serologic evidence of prior infection and attribute nonspecific symptoms such as fatigue, headache, sleep disturbances, poor concentration, depression, irritability, and dizziness to chronic infection. Alternatively, they may have a specific diagnosis such as multiple sclerosis that they believe is in error. A final group is patients with a prior history of erythema migrans who continue to have nonspecific symptoms 1 year or more after the initial infection. Placebo-controlled studies of antimicrobials have demonstrated that antimicrobial therapy offered little or no benefit over placebo in this group of patients. Careful placebo-controlled trials have not been performed in the first two groups of patients.

Finally, recurrence of Lyme disease may occur when patients are infected with a *B. burgdorferi* strain that differs from that causing the patient's initial infection.

5. Prevention of Lyme disease is similar to the prevention of other tick-borne diseases. In areas of endemicity, the use of appropriate clothing, including long pants, long-sleeved shirts, and closed-toe shoes, is important when exposure to ticks may occur. The use of tick repellents, including the chemical *N,N*-diethyl-*m*-toluamide (DEET) on skin and clothing and permethrin on clothing, is an additional precaution. Finally, examination of the skin after walking in an environment in which tick exposure is a possibility allows for the removal of ticks before they are able to transmit *B. burgdorferi*.

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The patient was a 6-year-old female from North Carolina seen during the month of May. She was in her usual state of good health until 10 days prior to admission, when she had a tick removed from her scalp (Fig. 42.1). She developed a sore throat, malaise, and a low-grade fever 8 days after tick removal. She was seen by her pediatrician after developing a pink, macular rash, which started on her palms and lower extremities and spread to cover her entire body. The pediatrician's diagnosis was viral exanthem. One day prior to admission she developed purpura, emesis, diarrhea, myalgias, and increased fever. On the day of admission she was taken to her local hospital emergency department because of mental status changes and was admitted to the hospital. Her physical examination was notable for diffuse purpura; periorbital, hand, and foot edema; cool extremities with weak pulses; and hepatosplenomegaly. Her laboratory studies demonstrated an Na^+ level of 125 mmol/liter, platelet count of 26,000/μl, white blood cell count of 14,900/μl, hemoglobin level of 8.8 g/dl, and greatly prolonged coagulation times. Ampicillin and chloramphenicol therapy was begun, and she was intubated and transferred to our institution; she died soon after arrival.

1. Which infectious agents are spread by ticks? Was the observation that a tick had been removed from her scalp important in this case?
2. What is the etiologic agent of this infection? What physical and laboratory findings are consistent with this infection? In what patient population does the disease tend to be most severe? What explains this severity?
3. Briefly describe the pathogenesis of the organism causing this patient's illness.
4. Which condition(s) does her physical findings on admission suggest? List three organisms that can cause these types of physical findings.
5. Were her family members at increased risk for this infection? Explain.
6. What specific test(s) is available for diagnosis of this infection?



Figure 42.1



CASE DISCUSSION

1. Ticks are vectors for *Borrelia burgdorferi* (the agent of Lyme disease), other *Borrelia* species (which cause relapsing fever), *Francisella tularensis* (the agent of tularemia), *Babesia microti* (the agent of babesiosis), *Rickettsia rickettsii* (the etiologic agent of Rocky Mountain spotted fever [RMSF]), *Ehrlichia chaffeensis* (causing human monocytic ehrlichiosis), *Anaplasma phagocytophilum* (the agent of human granulocytic anaplasmosis), Colorado tick fever virus, Powassan virus, and other rickettsiae and viruses not found in the United States. *R. rickettsii* is endemic in the state of North Carolina, with Oklahoma or North Carolina reporting the largest number of RMSF cases on a yearly basis. Ninety percent of cases occur between the months of April and September, a period when adult ticks, the stage that spreads the organism, are most actively feeding. For *R. rickettsii* to be transmitted to humans, the infected tick must be attached for a minimum of 6 to 10 hours, and more than 24 hours may be required for transmission to occur. The incubation time after tick exposure ranges from 2 to 14 days, with a median of 7 days. The patient's development of symptoms 8 days after tick exposure is consistent with *R. rickettsii* infection. The tick removed from this child was *Dermacentor variabilis*, the common dog tick. This tick is known to transmit *R. rickettsii*, *F. tularensis*, *E. chaffeensis*, and *A. phagocytophilum*. The other tick that commonly acts as a vector for *R. rickettsii* in North America is *Dermacentor andersoni*, the Rocky Mountain wood tick. Although rare, other modes of acquisition of RMSF infections include needlesticks, blood transfusions, or lab accidents that occur when working with the organism.

2. The most likely etiology of the patient's infection is *R. rickettsii*, which causes RMSF. The physical finding of a skin rash, in the presence of fever, myalgias, vomiting, and diarrhea, with a history of a tick bite in a geographically compatible area, is highly suggestive of RMSF. Hyponatremia (low Na^+ level) is commonly seen in RMSF, as are low platelet counts and increased coagulation times, both of which can be manifestations of disseminated intravascular coagulation (DIC), an often fatal complication of this infection.

This case took place in North Carolina. The failure of her pediatrician to recognize that she had RMSF most certainly contributed to her demise. Patients with a skin rash (seen in 90% of patients with RMSF), fever, and tick exposure in North Carolina have RMSF until proven otherwise. Mortality with RMSF is ~1 to 3% with timely, appropriate antimicrobial therapy with doxycycline but may reach as high as 25% in untreated individuals.

In the United States, where RMSF epidemiology has been most closely scrutinized, African-Americans are the population in which the disease has the greatest severity. The reasons for this appear to be 2-fold. First, in dark-skinned individuals, the rash, which is the key diagnostic feature of this infection, may be difficult to appreciate. This may delay the diagnosis and appropriate treatment, which can have grave clinical consequences, most importantly, increased mortality. Second, African-Americans often have poorer

access to medical care than do many other groups of Americans, which may delay the diagnosis and thus treatment of this rapidly progressive disease.

3. *R. rickettsii* is an obligate intracellular pathogen that during the early stages of the 20th century was considered a virus. This is a highly evolved organism with a comparatively small genome due in part to its lack of genes for glucose metabolism and for lipid and nucleic acid synthesis. Thus, this organism is an energy parasite and must obtain ATP from the host, as well as lipids, purines, and pyrimidines. The organism has a tropism for endothelial cells and can infect this cell type throughout the body. This *R. rickettsii* tropism is due to the presence of a specific receptor on the endothelial cell surface called Ku70. Once bound, the organism is taken into the cell by endocytosis and then lyses the endosome, allowing it to replicate in the cytoplasm. There it attaches host cell actin filaments that allow the organism to move through the cytoplasm to the cell membrane, where it can be taken up by adjacent cells by an endocytosis-like process. Over time (hours), this infectious process results in disruption of endothelial tight junctions, resulting in vascular leakage. Additionally, infection of the endothelial cells results in endothelial damage, which leads to platelet activation and consumption; and clotting due to thrombin formation, which can lead to hemorrhage and vascular occlusion.

4. The finding of cool extremities with weak pulses is suggestive of shock. Edema in this case is the result of increased vascular permeability, suggesting damage to endothelial cells, a well-known mechanism of *R. rickettsii*-induced pathologic changes. Purpura in the setting of septic shock strongly suggested that this patient was suffering from DIC. Her platelet count and coagulation times were consistent with DIC. Almost any aerobic or facultative, Gram-negative organism can cause septic shock and DIC; these include *Neisseria meningitidis*, *Pseudomonas aeruginosa*, members of the family *Enterobacteriaceae*, *Pasteurella multocida*, *F. tularensis*, *Vibrio vulnificus*, and *Haemophilus influenzae*. *Streptococcus pneumoniae*, *R. rickettsii*, and many other systemic bacterial infections can also cause septic shock. In the southwestern United States, *Yersinia pestis* should also be considered in the differential diagnosis of patients with septic shock and DIC.

5. RMSF is a tick-borne disease. There is no evidence of person-to-person spread. However, family clusters of RMSF have been described. In one such cluster, five family members developed disease with two dying of fulminant infection. A hyperendemic focus of ticks was found on the wooded property of the family. None of the ticks were found to be infected with *R. rickettsii*, but another rickettsial species (*R. amblyommii*) thought to be nonpathogenic for humans was found. Since only a small percentage of ticks (0.5%) are typically infected with *R. rickettsii*, these findings are not surprising. Therefore, it would be unusual but possible for another family member to develop RMSF at about the time this child did.

The combination of increasing human intrusion into wooded habitats that are infested with large numbers of ticks along with improved diagnostic techniques for tick-borne

diseases such as RMSF, *Ehrlichia* spp., and *B. burgdorferi* has resulted in the recognition of tick-borne diseases as important emerging infectious diseases. In a recent study of patients with a history of tick bite and unexplained fever in North Carolina, 16 of 35 patients had evidence of either RMSF or ehrlichiosis.

6. Serologic tests are the most widely used diagnostic tests for detection of an RMSF infection. Of those commercially available in the United States, the most widely used test is an indirect fluorescent-antibody assay, which detects *R. rickettsii* antigens. Cross-reactions with other rickettsial species may occur. Enzyme-linked immunosorbent assays are also used but are neither as sensitive nor as specific as the indirect fluorescent-antibody assay. One of the major problems with the serologic tests is that early in the disease course, the individual may not have mounted a sufficiently strong immune response to result in a positive serologic test result. Follow-up serologic tests 1 to 4 weeks later may prove to be positive. In patients in whom RMSF is a distinct possibility, a negative serologic test result should not preclude the use of antimicrobial therapy since a fulminant, fatal disease course with this organism, as was seen in this case, is not unusual.

Other methods described in the literature include the direct detection of *R. rickettsii* in tissue biopsy specimens by using direct fluorescent-antibody assay or PCR. The direct fluorescent-antibody assay has the advantage of being very rapid and, in skilled hands, very specific. However, its sensitivity is dependent on the quality of the tissue biopsy. A negative test does not rule out this diagnosis. This test is available in only a very limited number of laboratories. Immunohistochemical techniques can also be used to detect this organism in fixed tissues.

PCR has also been applied to the detection of *R. rickettsii* but it is not yet widely available. PCR is more sensitive in biopsies of the skin rash than it is from samples of blood. This is not surprising since the organism is an obligate intracellular pathogen that specifically infects endothelial cells, a cell type likely to be found in biopsy material but not in blood. The organism can also be isolated from blood by inoculation of guinea pigs, embryonated eggs, or tissue culture using the shell vial technique. However, cultivation of this organism is extremely dangerous and is attempted only in a few highly specialized laboratories.

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The patient was a 58-year-old male with a 3-month history of a nonhealing ulcer on his right thigh. It began as a pimple and progressed. He had no constitutional symptoms and no response to oral or topical antibacterials (agents not specified in the history). He had no lesions elsewhere, nor did he have trauma at the site of inoculation. His prior travel history was significant for his having returned from the Amazon rain forest region of Peru 2 weeks prior to the onset of symptoms. In Peru, he stayed in an open-sided hut that did not have screens or windows. He had countless insect bites and had no pretravel immunizations, nor did he take malaria prophylaxis. In the United States, he lived in a rural community with three cats and one dog. Physical examination was essentially unremarkable, including normal vital signs, except for the lesion on his right thigh (Fig. 43.1). It was described as a superficial ulcer surrounded by a large area of erythematous indurated plaques. The lesion was nontender, nonpurulent, and without discharge. He had a normal complete blood count, electrolytes, and liver function tests. He was referred to the dermatology department, where a punch biopsy was performed (Fig. 43.2).

1. What was the organism causing his infection? Based on geography, you should be able to name the genus and species.



Figure 43.1 Initial lesion in Peru.



Figure 43.2 Lesion 1 month later at time of punch biopsy.

2. What stage of the organism is seen in Fig. 43.3? What stage is found in human infection? What are their roles in the disease cycle of this organism? Why is a punch biopsy from the raised edge of the lesion essential for diagnosis?

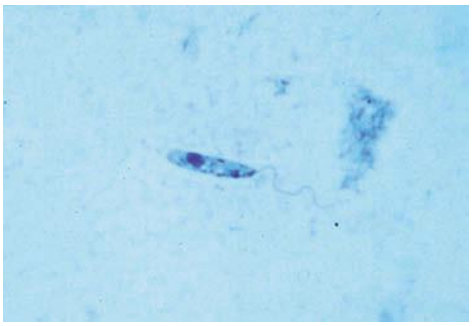


Figure 43.3 Microscopic examination from skin biopsy (magnification, ×1,000).

3. Three clinical syndromes are caused by this organism. What are they? Which one did this patient have? What was his risk for developing the other two? What organisms are associated with each one?

4. How is this disease diagnosed? Why is establishing the diagnosis useful?

5. How do you think this patient became infected? What steps could he have taken to prevent himself from getting infected?

6. This infection is generally seen in three distinct populations in the United States. How and where do they get it?



CASE DISCUSSION

1. This patient had an infection due to *Leishmania* (*Viannia*) *brasiliensis*. *Leishmania Viannia* is a subgroup of the genus *Leishmania*, with *L.* (*Viannia*) *brasiliensis* being the most common cause of cutaneous disease in patients who have been in the Amazon basin, including the region of Peru in which this patient had traveled (see answer 3 for further details). Leishmaniasis is one of the most common reasons that individuals who have traveled to South America and develop skin lesions consult a physician, especially if the lesion is slowly healing, as was the case here.

2. The stage of the organism seen in Fig. 43.3 is a promastigote. It is the infectious stage of the parasite. This flagellated protozoan stage develops in the gut of the sand fly. It migrates to the sand fly proboscis, where it is injected into the skin during the sand fly's blood meal. The organism is taken up by macrophages, where it converts to the round, nonflagellated amastigote form. The amastigote is taken up during a blood meal by the sand fly, converting back to the promastigote in the sand fly gut and completing the life cycle.

The amastigote form proliferates in the phagolysosome of the macrophage (Fig. 43.4). The result of this infection is an ulcerative papulonodular lesion with raised edges, although these edges are not readily apparent in Fig. 43.1 and 43.2. The lesion will have a central area of necrosis. In early stages of infection, the raised edges of the lesion contain macrophages filled with amastigotes, surrounded by lymphocytes. It is for this reason that the appropriate diagnostic specimen is a biopsy of the edge of the lesion. If a biopsy is performed in the central area of the lesion, where crusting is apparent (as can be seen in Fig. 43.2), few organisms will be observed. This lesion will continue to expand outward until cell-mediated immunity eliminates the parasite. Over time, granulomatous inflammation with lymphocyte predominance occurs and the macrophages filled with amastigotes are eliminated. Data suggest that the cytokines interleukin-2 and gamma interferon play a key role in the elimination of this parasite through macrophage activation. As healing continues, granulation tissue forms that contains both giant and epitheloid cells. In most cases, the lesion slowly heals and leaves a scar. This disease process occurs over many months. Because the lesions frequently occur on the face, disfiguring scars are not uncommon in areas where *Leishmania* is endemic.

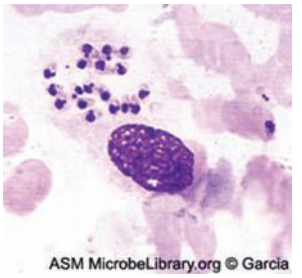


Figure 43.4 Organism grown on culture from the biopsy (magnification, ×400).

3. The three stages of leishmaniasis are cutaneous (as seen in this patient), mucocutaneous, and visceral. Cutaneous leishmaniasis can cause both a localized, typically self-limited disease (as seen in this case) and a diffuse, chronic one. In Europe, Africa, and Asia, cutaneous

leishmaniasis is usually caused by *Leishmanina tropica* and *Leishmania major*. In South and Central America, *L.* (*Viannia*) *brasiliensis* (Amazon basin) and *Leishmania mexicana* (Central America and Mexico) predominate. In the diffuse cutaneous form of leishmaniasis, the patient does not mount a cellular immune response to the parasite. Lesions characterized by the presence of amastigote-filled macrophages as well as the presence of free parasites in tissue are observed. Because of the failure of the immune response, there is no immune-mediated necrosis seen. These lesions can disseminate throughout the skin and be found throughout the body surface. These infections do not respond well to antiparasitic treatment. A third variant of cutaneous leishmaniasis is leishmaniasis recidivans. In this chronic disease, typically caused by *L. tropica*, there is recurrence at the site of the initial lesion after a period of dormancy as long as 15 years. Alternatively, some data suggest that the lesions represent reinfection rather than recurrence.

Mucocutaneous leishmaniasis is a rare manifestation and is seen primarily after cutaneous infections with *L.* (*Viannia*) *brasiliensis*, the most commonly recognized *Leishmania* species in South America. As the name of the disease implies, this is a disease found primarily in the nasal and oral mucous membranes. The organism spreads either hematogenously or from adjacent lesions. The lesions are the result of a severe inflammatory response to the parasite despite the fact that few parasites can be seen in the mucocutaneous lesions. This hyperimmune response causes necrosis of soft tissues including the nasal septum, soft palate, lips, and gums. Interestingly, it has been postulated that a double-stranded RNA virus found within *L.* (*Viannia*) *brasiliensis* may stimulate host Toll-like receptor 3, inducing the release of proinflammatory cytokines, which contributes to the severity of the disease process. Other species that cause cutaneous leishmaniasis are rarely associated with this form of this disease.

The third form of the disease is visceral leishmaniasis, also known by the names "kala-azar" and "black fever." This disease is seen primarily in the South Asian subcontinent. Most cases globally are due to *Leishmania donovani*. Other species that are associated with visceral disease are *Leishmania infantum* (in infants, as the name implies) and *Leishmania chagasi* (in the Americas). The disease is characterized by infection of the macrophages sequestered within the reticuloendothelial system, including the bone marrow, but most impressively causing massive hepatosplenomegaly resulting in protruding abdomen. Both the liver and spleen are filled with amastigote-infected mononuclear cells. This results in defects in immune clearance that put patients at increased risk for a wide variety of bacterial infections. Additionally, because of infection in the bone marrow, anemia, leukopenia, and thrombocytopenia are key features of advanced disease.

4. Cutaneous leishmaniasis is often diagnosed clinically by physicians who are experienced with this infection. However, this disease is not endemic in the United States, Canada, and Northern Europe, and thus few physicians there will have seen an actual case. Since cutaneous leishmaniasis can be confused with other conditions, including leprosy, tertiary syphilis, dimorphic fungal infections, and noninfectious causes, laboratory support

of this diagnosis is helpful in determining appropriate management and therapy. In this case, the amastigotes were observed in a Giemsa-stained biopsy of the lesion (Fig. 43.4). This indicates that the organism load was quite high. It is important to note that the yeast phase of *Histoplasma capsulatum* is similar in shape and size to the amastigotes. However, the internal structure of amastigotes is quite different from that of *Histoplasma*. A skilled pathologist should be able to easily differentiate the two organisms.

Later in the disease course, organism burden is likely to be less and microscopic examination insensitive. Culture is more sensitive than microscopic examination. The organism was grown on culture using triple N medium, an enriched medium designed for the recovery of *Leishmania*. When grown on artificial medium, the promastigote form is seen (Fig. 43.3). In patients with suspected *Leishmania* infection, this culture medium can be obtained in the United States from the Division of Parasitic Diseases and Malaria at the Centers for Disease Control and Prevention (CDC). They will also perform the culture. The most sensitive method for detection of *Leishmania* is nucleic acid amplification testing (NAAT). Although FDA-approved NAATs are not currently available, the same laboratory at the CDC does provide this service. NAAT is most useful in the late stages of cutaneous disease, when culture and direct microscopic examination are less sensitive because of low organism burden.

5. The patient was infected by the bite of the sand fly, the vector for this organism. Essentially all cases of cutaneous disease are obtained in this manner. Sand flies primarily feed at dawn and dusk, so it is during those periods that humans are most at risk. Leishmaniasis is primarily a zoonotic disease, with humans often being accidental hosts.

This patient had several reasons for developing this infection. First, he slept in an open-air shelter without screens, greatly increasing his risk of sand fly bites. In fact, in his history he admitted to having "countless" insect bites. He did not use any insect repellent, nor did he wear long-sleeved clothing. The use of screens, insect repellent, and long-sleeved clothes is a recommended measure for the prevention of *Leishmania*.

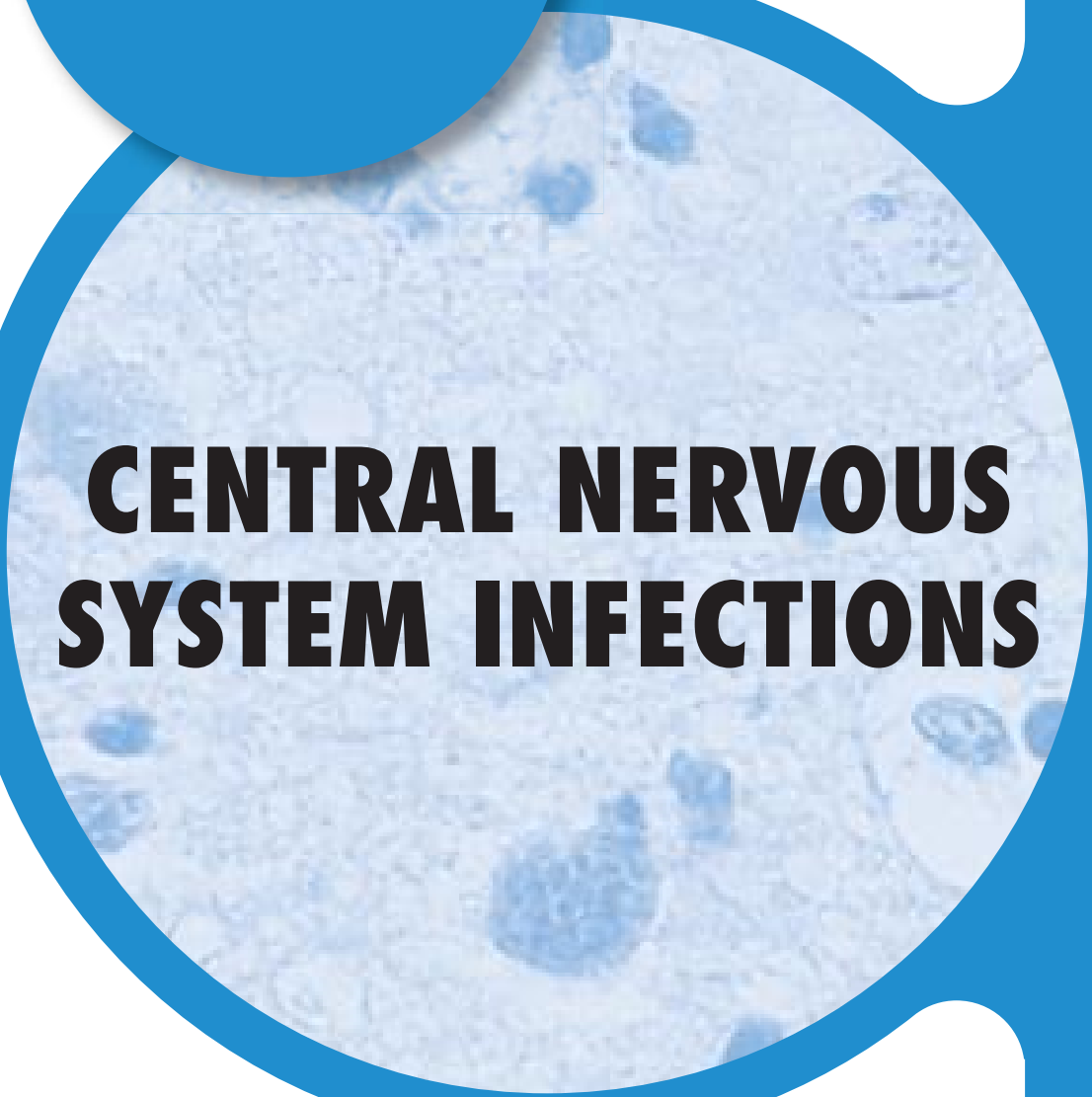
6. Three distinct populations in the United States and some other northern industrialized countries are at increased risk for development of cutaneous leishmaniasis. They are (i) travelers, especially those who do adventure tourism such as the patient described here; (ii) military or other personnel who are deployed to Iraq or Afghanistan; and (iii) emigrants who return to visit family and friends in their native countries where the disease is endemic. With the growing popularity of adventure tourism in the Amazon basin, cases of cutaneous leishmaniasis are on the rise in the industrialized North. With the return of the majority of troops from both Iraq and Afghanistan, the number of cases in those populations is declining.

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SECTION FIVE



CENTRAL NERVOUS SYSTEM INFECTIONS

INTRODUCTION TO SECTION V

An infection of the central nervous system (CNS) must be regarding as potentially life-threatening until a definitive diagnosis is determined. Such infections are infrequent compared with the others we have discussed thus far, but they are very important because of the high mortality rates and the serious sequelae associated with them, including learning disabilities, difficulties with speech, motor skill disorders, seizures, and both hearing and visual loss. The most frequent CNS infections are meningitis, encephalitis, and abscess. Intoxication caused by tetanus and botulinum toxins can affect the CNS, causing spastic or flaccid paralysis, but these diseases are quite rare in the developed world.

Pyogenic meningitis is typically caused by bacteria, often those that have an antiphagocytic capsule, and is acute in onset. The cerebrospinal fluid (CSF) is usually cloudy, with over 1,000 white blood cells per µl with neutrophils predominating; increased protein levels due to inflammation; and decreased glucose due in part to metabolism by white blood cells. Without appropriate antibiotic therapy—as defined by the selection of antibiotics that not only are active against the bacterial cause of the infection, but also achieve antibacterial levels in the CNS—the infection may quickly be fatal. In some cases, such as acute bacterial meningitis due to *Streptococcus pneumoniae*, rapidly treating the patient with high doses of corticosteroids prior to the initial intravenous infusion of systemic antibiotics may be helpful as well. Other causes of pyogenic meningitis to keep in mind include a number of potential bioterrorism agents such as *Yersinia pestis* and *Bacillus anthracis*.

Other causes of meningitis include viruses, fungi, and such slowly growing bacteria as *Mycobacterium tuberculosis*. In these cases, the CSF is more likely to be grossly "clear" due to a lower white blood cell count, typically in the range of 100 to 500/µl. Except for very early in the disease course, the predominant cell type is mononuclear, primarily lymphocytes. CSF glucose levels are frequently normal, but may be decreased in over half of the patients with fungal or mycobacterial infections. CSF protein levels are frequently normal except with *M. tuberculosis*, where they are typically quite elevated.

Bacterial meningitis is most common in the very young, the very old, and the immunocompromised. Group B streptococci are the most common cause of neonatal meningitis (newborns to 2 months). *Listeria monocytogenes* is another organism that causes neonatal disease. It also is an important agent of meningitis in those individuals who are immunosuppressed due to defects in cell-mediated immunity. Gram-negative enteric bacilli, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter* spp., may also cause neonatal meningitis. Congenital syphilis, which may manifest itself during the neonatal period, frequently will have a CNS component, neurosyphilis. Bacterial meningitis is most commonly seen in children 2 months to 5 years of age. Until recently, *Haemophilus influenzae* type b was the most common cause of bacterial meningitis in this age group in developed countries. The widespread use of conjugated *H. influenzae* type b vaccine has resulted in a dramatic decline in the incidence of this disease. *Streptococcus pneumoniae* and *Neisseria meningitidis* are now the leading causes of meningitis in this age group and in the elderly.

Recent trials of a conjugated 7-valent and now 13-valent *S. pneumoniae* immunization in children less than 2 years of age have shown that the immunization has an efficacy of greater than 95% in preventing invasive pneumococcal disease. The widespread use of this immunization has resulted in a significant drop in pneumococcal meningitis cases not just in children, but also in adults. Individuals with head trauma, including a breach in the integrity of the skull or the meninges following neurosurgery, are also at risk for developing bacterial meningitis. The organisms most frequently associated with this type of bacterial meningitis are coagulase-negative staphylococci, *Propionibacterium acnes* (especially in patients with CNS shunts or who have undergone neurosurgical procedures), *Staphylococcus aureus*, and *Pseudomonas aeruginosa.* In patients who have had meningitis more than once with *S. pneumoniae*, it is worth seeking a previously unrecognized anatomic defect in the skull due to what may have seemed to be minimal trauma, in some cases in the distant past. *M. tuberculosis* meningitis is seen primarily in children and the immunosuppressed.

Viral meningitis is typically caused by enteroviruses other than the polioviruses. Enteroviral meningitis is seen primarily during the summer months in infants and young children. In a small minority of cases, an infection by an enterovirus may invade motor neurons in the CNS and cause a case of flaccid paralysis that is clinically indistinguishable from the flaccid paralysis that can be the result of infection by one of the polioviruses. Flaccid paralysis is a very rare (approximately 1 in 3,000,000 doses) complication of the use of the live, attenuated poliovirus immunization. Notably, HIV can both cause viral meningitis and predispose to other causes of meningitis (see below in the paragraph on fungal meningitis) as a result of its suppression of cell-mediated immunity.

Herpes simplex virus can cause a (usually benign) meningitis associated with primary genital tract infections. This is not to be confused with herpes simplex encephalitis, which can occur in neonates, in adults with primary orolabial infection (most often due to herpes simplex virus type 1), or during reactivation of latent infection in adults. This form of herpes infection can produce necrotic lesions in the brain resulting in long-term sequelae or death.

Fungal meningitis is seen primarily, but not exclusively, in the immunocompromised. It is of particular importance in AIDS patients, in whom *Cryptococcus neoformans* is far and away the most important cause. Racial predisposition may also increase the risk of individuals to certain infectious complications. This is most well documented in meningitis as a result of disseminated disease by the thermally dimorphic organisms *Coccidioides immitis* and *Coccidioides posadasii*. Meningitis is far more likely to occur following inhalation of infectious arthroconidia by African-Americans and Asians, including Filipinos (for whom the risk of disseminated disease is approximately 80-fold greater than is the case for Caucasians), than among Caucasians, who are far more likely to have a self-limited flu-like illness without dissemination to the meninges. In addition to the above racial predisposition, defects in cell-mediated immunity also increase the risk of coccidioidal meningitis.

Encephalitis due to infectious agents is due primarily to viruses. Herpes simplex viruses are probably the most common cause of viral encephalitis encountered in the

developed world. Arthropod-borne viruses such as West Nile, Eastern equine, Western equine, St. Louis, and La Crosse encephalitis viruses are encountered in the United States. West Nile virus was introduced into the United States via infected birds or mosquitoes, most likely from the African continent. In many eastern states, an epidemic of rabies in animals is continuing. Few cases of human rabies are reported in the United States; most are acquired either from the bite of rabid dogs that occurred when the patient was in a developing country or, for those cases acquired in the United States, following contact with rabid bats. Recently, a human case of rabies was identified as originating from raccoons, animals that have, with rabies infecting them, migrated northward along the east coast of the United States over the course of years. Veterinary and human public health are inextricably linked. Nearly all cases of human rabies are fatal.

Parasites may also cause CNS infection. The most frequently encountered parasite causing CNS infections in the developed world is *Toxoplasma gondii*. Encephalitis due to this organism occurs primarily in AIDS patients and represents reactivation of latent infection. One of the most common causes of a clinical presentation of CNS infection in the developing world is cerebral malaria. Cysticercosis is a major cause of adult onset of seizures in certain areas of the developing world where pork is a source of protein in the diet. This disease occurs when eggs of the pork tapeworm *Taenia solium* are ingested. The parasite is unable to complete its life cycle in humans, and cyst-like lesions occur throughout the body, including the brain. An ameba, *Naegleria fowleri*, causes a rare and frequently fatal form of meningoencephalitis. It is found in individuals who swim in warm freshwater during the summer months. In the United States, a number of infants who have had contact with the feces of raccoons have acquired a parasitic infection due to the raccoon roundworm, *Baylisascaris procyonis*. While this infection is uncommon, the presence of eosinophils in the CSF is a clue to this diagnosis. Another parasitic infection associated with eosinophilic meningitis is *Angiostrongylus cantonensis*, which is obtained by the ingestion of uncooked snails or slugs that are infected with this organism or by the ingestion of raw vegetables, such as in salads, that have been contaminated by infected snails or slugs. In the writing of this and other sections in this book, the authors have to wrestle with the question of what is common enough to be clinically useful and whether less commonly seen entities are educationally of importance due to a particular aspect of the infection or to the host response, such as the characteristic presence of significant numbers of eosinophils in the CSF in a relatively small number of specific infections.

Brain abscesses occur through direct extension from a contiguous site such as an infected paranasal sinus, following head trauma, or by hematogenous spread from another infected site. Typically, patients with abscesses due to hematogenous spread have either endocarditis or a lung abscess. **Septic emboli**, which are small blood clots containing infectious agents, are released from the primary infection site and enter the bloodstream. The emboli lodge in capillaries in the brain, causing localized hemorrhage and producing sites for the initiation of infection which evolve into brain abscesses. The organisms most frequently causing brain abscess in immunocompetent individuals are *S. aureus* and organisms usually found in the oropharynx or in dental infections, including the viridans group

streptococci, *Actinomyces* spp., and anaerobic bacteria. In immunocompromised individuals, *Aspergillus* spp., *Mucor*, *Rhizopus*, and *Nocardia* spp. can cause brain abscesses. In trauma patients, *S. aureus* and Gram-negative rods are frequently seen. In diabetic patients (especially those with ketoacidosis), rhinocerebral zygomycosis due to *Mucor*, *Rhizopus*, and other fungi within the *Zygomycetes* can extend from the sinuses into the brain, causing extensive necrosis.

There are geographically defined CNS infections due to pathogens transmitted by ticks and other ectoparasites. For vector-borne infections, the geographic locations at which infected arthropod vectors occur will determine where these diseases are likely to be acquired. So, someone who has the CNS manifestations of Lyme disease is likely to have acquired the infection in regions of North America or Europe in which ticks infected with the spirochete *Borrelia burgdorferi* are most likely to be present. Similarly, Rocky Mountain spotted fever, which causes CNS manifestations and is transmitted by ticks infected by *Rickettsia rickettsiae*, is likely to be acquired in specific areas of endemicity in North America. Other infections with CNS manifestations that are found in specific geographic locations and are transmitted by vectors include diseases caused by *Plasmodium falciparum* (which may cause cerebral malaria), those viruses that cause a tick-borne encephalitis, *Yersinia pestis* (plague, which may cause bacterial meningitis), and a variety of rickettsial agents. It is worth noting that though patients may not have traveled to a region in which a particular pathogen is endemic, the pathogen, in effect, may have traveled to them. For example, there have been rare cases of malarial infections in people who were infected by mosquitoes that were imported into a malaria-free area from a malaria-endemic area, such as people who acquire malaria near an international airport.

While measles is efficiently transmitted from person to person by the respiratory route, the geographic range of endemicity of this disease does not include developed countries, as a result of the widespread use of the available live, attenuated vaccine. In regions of the world in which the populace has a low rate of immunization, measles in children may be common and have a mortality rate that ranges from 5 to 25%, most often due to secondary infections. With respect to CNS infections, approximately 1 in 10,000 measles-infected children will develop a progressive and uniformly fatal encephalitis (subacute sclerosing panencephalitis, or SSPE). This infectious complication is more common in boys than girls and is more likely to occur in children who were infected with measles when they were younger than 2 years of age. Notably, these children have a persistence of measles virus RNA in the neurons and the glial cells of the CNS. The refusal of parents to immunize their children against measles will increase disease prevalence and the likelihood of developing this rare but fatal neurologic complication.

Taking a very good clinical, exposure, and travel history is of key importance in rapidly identifying the possible causes of a patient's CNS infection so that appropriate therapy may be begun—whether broad or specific coverage with antibacterial, antiviral, antiparasitic, or antifungal agents—as well as to determine whether or not the patient is contagious.

TABLE V CENTRAL NERVOUS SYSTEM INFECTIONS

ORGANISM	GENERAL CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Bacteria			
Actinomyces spp.	Branching, Gram-positive bacilli, usually anaerobic	Individuals with aspiration pneumonia	Brain abscess
Bacillus anthracis	Spore-forming, Gram-positive bacillus	Individuals with severe anthrax infection; victims of bioterrorism	Meningitis
Citrobacter spp.	Enteric Gram-negative bacilli	Neonates	Meningoencephalitis with abscess
Clostridium botulinum	Toxin-producing, anaerobic, Gram-positive bacillus	Infants; adults who ingest botulinum toxin; wound botulism occurs but is not common; toxin is a potential bioterrorism agent	Botulism, flaccid paralysis
Coagulase-negative staphylococci	Catalase-positive, Gram-positive cocci	Individuals with foreign bodies, e.g., shunts or bolts	Meningitis
Escherichia coli	Lactose-fermenting, Gram-negative bacillus	Neonates	Meningitis
Group B streptococci (*Streptococcus agalactiae*)	Catalase-negative, Gram-positive cocci	Neonates, immunocompromised adults	Meningitis
Haemophilus influenzae type b	Gram-negative, pleomorphic bacillus	Unvaccinated children; more common in countries without availability of the vaccine	Meningitis
Listeria monocytogenes	Catalase-positive, Gram-positive coccobacillus	Neonates, adults with cell-mediated immunity defect	Meningitis, rhomboencephalitis
Mycobacterium tuberculosis	Acid-fast bacillus	Children; patients with AIDS; more common in foreign-born patients than in those born in the United States	Tuberculous meningitis, CNS[a] tuberculomas
Neisseria meningitidis	Oxidase-positive, Gram-negative diplococcus	All ages; outbreaks in college students and military; those in the "meningitis belt" of sub-Saharan Africa; visitors to the Hajj	Meningitis
Nocardia spp.	Aerobic, partially acid-fast branching bacilli	Individuals with pulmonary or cutaneous nocardiosis	Brain abscess

Oral streptococci (*Streptococcus sanguis*, *Streptococcus mutans*, etc.)	Alpha-hemolytic, Gram-positive cocci	Individuals with aspiration pneumonia, endocarditis	Brain abscess
Prevotella sp., *Porphyromonas* sp.	Anaerobic, Gram-negative bacilli	Individuals with aspiration pneumonia	Brain abscess
Pseudomonas aeruginosa	Oxidase-positive, Gram-negative bacillus	Individuals with head trauma or foreign bodies	Meningitis
Staphylococcus aureus	Catalase-positive, Gram-positive coccus	Individuals with head trauma or foreign bodies	Meningitis, brain abscess
Streptococcus pneumoniae	Catalase-negative, Gram-positive coccus	Primarily young children and elderly	Meningitis
Fungi			
Aspergillus spp.	Acute-angle, septate hyphae in tissue	Neutropenia with invasive aspergillosis	Brain abscess
Coccidioides immitis and *Coccidioides posadasii*	Dimorphic mold	Geographically limited to Lower Sonoran life zone; increased rate of disseminated disease in non-Caucasians and immunocompromised, especially AIDS	Meningitis
Cryptococcus neoformans	Encapsulated, round yeast	Cell-mediated immunity defect, especially AIDS	Meningitis, cryptococcoma
Mucor sp., *Rhizopus* sp.	Ribbon-like, aseptate hyphae in tissue	Diabetics, neutropenic individuals	Necrotizing encephalitis, rhinocerebral zygomycosis
Parasites			
Acanthamoeba sp.	Ameba	Immunocompromised or immunocompetent	Granulomatous amebic encephalitis or keratitis
Naegleria fowleri	Ameba	Individuals who dive into warm freshwater; the use of tap water in nasal rinsing	Typically fatal amebic meningoencephalitis
Plasmodium falciparum	Delicate, ring forms in red blood cells	Individuals who visit or reside in areas where malaria is endemic	Cerebral malaria

(continued next page)

TABLE V **CENTRAL NERVOUS SYSTEM INFECTIONS** *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Schistosoma spp. (not including avian schistosomes)	Trematodes (blood fluke)	Individuals who are in contact with free-living cercariae in freshwater	Inflammatory response involves granuloma formation following the deposition of eggs in brain or spinal cord with symptoms dependent upon egg location; brain involvement includes encephalopathy, visual defects, seizures, motor deficits; spinal involvement includes lower limb radicular pain, muscle weakness, bladder dysfunction, decreases in sensation
Taenia solium	Larval cyst	Individuals who ingest *T. solium* eggs	Seizures, calcified or noncalcified lesions in brain; calcified lesions in muscle
Toxoplasma gondii	Large cysts in tissue	Cell-mediated immunity defect, especially AIDS patients	Encephalitis, abscess
Viruses			
Arthropod-borne viruses (arboviruses), including Eastern equine encephalitis virus, St. Louis equine encephalitis virus, West Nile virus, and the tick-borne encephalitis viruses	Both enveloped and nonenveloped ssRNA[b] viruses	Children and adults bitten by an infected arthropod vector; tick-borne encephalitis virus has also been transmitted to humans from drinking animal milk	Encephalitis; fatality rate depends upon specific virus and age of individual
Cercopithecine herpesvirus type 1 (also known as herpesvirus simiae)	Enveloped dsDNA[c] virus	Acquired from contact, bites, or material from the rhesus macaque	Encephalitis and other CNS involvement that requires lifelong antiviral medication
Echovirus/ coxsackievirus	Nonenveloped ssRNA viruses	Children and adults during summer months	Aseptic meningitis
Herpes simplex virus	Enveloped dsDNA virus	Neonates, individuals with primary genital herpes, individuals with primary or recurrent herpes infection	Necrotizing encephalitis; benign, aseptic meningitis; necrotizing hemorrhagic encephalitis

Human herpesvirus 6	Enveloped dsDNA virus	Immunocompetent; hematopoietic stem cell transplants	Encephalitis in immunocompetent individuals; limbic encephalitis in hematopoietic stem cell transplant recipients
Human immunodeficiency virus (HIV)	Enveloped RNA retrovirus	AIDS patients	AIDS-associated dementia; predisposes to other CNS infections
JC virus	Nonenveloped dsDNA virus	AIDS patients; immunocompromised hosts following solid organ transplantation; following the use of selected biologics; infection with no symptoms in immunocompetent individuals	Progressive multifocal leukoencephalopathy
Lymphocytic choriomeningitis virus	Enveloped ssRNA virus that is relatively resistant to desiccation	Acquired from wild mice and pet and laboratory rodents (including hamsters, guinea pigs, mice); contact with or inhalation of an aerosol of urine, blood, saliva, droppings, or nesting materials of infected rodents; rodent bite; congenital infection; rarely by organ transplantation from infected donor	Aseptic meningitis; infection during the first or second trimester of pregnancy can cause developmental defects including hydrocephalus, psychomotor retardation, and blindness
Measles virus	Enveloped ssRNA virus	Nonvaccinated individuals; respiratory transmission; highly contagious	Subacute sclerosing panencephalitis (SSPE) following infection, especially in children infected prior to age 2, males > females
Polioviruses	Nonenveloped ssRNA viruses	Nonvaccinated individuals; live, attenuated vaccine recipients (approximately 1 in 3,000,000), especially in the immunocompromised	Flaccid paralysis; weakness; post-polio syndrome years after the infection
Rabies virus	Enveloped ssRNA virus	Individuals bitten or scratched by nonvaccinated, rabid dog, cat, or other mammal; bat contact or bite; rare cases from transplanted organs or corneas from people who died of undiagnosed rabies	Rabies; may be “furious” or “dumb” rabies

[a] CNS, central nervous system.

[b] ssRNA, single-stranded RNA.

[c] dsDNA, double-stranded DNA.



The patient was a 45-year-old male with a long-standing history of cirrhosis of the liver secondary to alcohol abuse, chronic hepatitis C infection, and diabetes. The patient presented to the emergency department with altered consciousness, confusion, and agitation. A family member noted that the patient had nausea and vomiting prior to arrival. No seizure activity or fevers were noted.

On physical examination his vital signs were normal but he was confused and agitated, making a neurologic examination not possible. Chest was clear to auscultation. He was without rashes. He did have abdominal distension with ascites. Laboratory tests were significant for a peripheral white blood cell (WBC) count of 27,800 cells/μl with 95% neutrophils. The patient was anemic and had a blood glucose level of 483 mg/dl. Because of his high WBC count and altered mental status, a lumbar puncture was performed, which revealed an opaque cerebrospinal fluid (CSF) containing 5,600 red blood cells (RBCs)/μl and 31,400 WBCs/μl with 95% neutrophils and 5% monocytes. The patient's CSF protein was 1,422 mg/dl and CSF glucose was 124 mg/dl. A Gram stain of the patient's CSF is shown in Fig. 44.1. The organism's susceptibility is shown in Fig. 44.2. He was begun on vancomycin and ceftriaxone. The patient's condition deteriorated rapidly, and despite intubation and aggressive cardiac resuscitation, he died of cardiopulmonary arrest in the emergency department.

1. Based on the patient's history and physical and laboratory findings, what condition did this patient likely have? What would be your differential diagnosis in this case? What did the CSF Gram stain tell you?

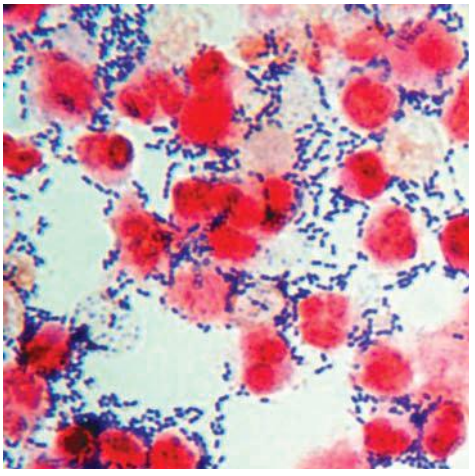


Figure 44.1 CSF Gram stain from patient.



Figure 44.2 E-test susceptibility test of the organism grown from the patient's CSF. PG, penicillin G; TX, ceftriaxone.

2. Briefly describe the pathogenesis of the infection caused by the organism that infected this patient. What risk factor did this patient have that predisposed him to this infection?
3. What is an E-test susceptibility test? What did you learn from this patient's susceptibility test results? Why were these two agents chosen for testing? Over the past 2 decades, how has antimicrobial resistance changed in this organism? What has been driving that change?
4. Disseminated infections with this organism are becoming infrequent in the United States, especially in the very young and very old. Why has this occurred? What impact does this observation have on patients such as the one seen in this case?
5. Invasive infections with this organism are very common in a selected subpopulation of African-Americans. What is this subpopulation, and why are they at increased risk for infection with this organism? In particular, what preventive measures are useful in this patient population?



CASE DISCUSSION

1. The patient's altered mental status and history of alcohol abuse could indicate alcohol withdrawal, especially since the patient's vital signs were normal. However, his history of cirrhosis of the liver, alcoholism, and diabetes put the patient at increased risk for infection. Given his presentation of altered mental status, confusion, and agitation, some type of central nervous system infection should be considered. His elevated peripheral WBC count further supports the idea of an infection. Given his presentation, a lumbar puncture was obtained and was grossly abnormal. In a patient with altered mental status, elevated WBC count, and abnormal CSF cell count, glucose, and protein, two types of infection are most likely, either encephalitis or meningitis. Encephalitis is most commonly caused by viruses, with herpes simplex virus and the arboviruses being the most common. The CSF findings in patients with viral encephalitis or meningitis would be a few hundred WBCs with a predominance of lymphocytes, a normal glucose (approximately two-thirds the level in peripheral blood), and a slightly elevated or normal protein. This is obviously quite different from what was found in this patient. Patients with bacterial meningitis have a WBC count of >1,000 cells/μl with a neutrophil predominance, a glucose that is considerably less than two-thirds of the peripheral blood glucose level, and an elevated protein. This patient had a highly inflammatory CSF with >30,000 WBCs, so many cells in fact that the patient's CSF was opaque.

How should the presence of RBCs be interpreted in this patient? Sometimes CSF specimens can be difficult to obtain, especially when the patient is not cooperative, as might be expected with a patient who is agitated. In such a situation, some amount of peripheral blood may contaminate the CSF during collection. How can we distinguish a "bloody" tap from a CSF showing inflammation? A simple rule is that peripheral blood has 500 to 1,000 RBCs for each WBC. In this case, the ratio was ~6 WBCs for each RBC, indicating that the CSF was inflammatory. Patients with inflamed meninges due to bacterial infection have elevated protein in the CSF. The normal CSF protein value is 15 to 45 mg/dl; this patient's CSF protein was ~1,400 mg/dl higher than that. This is not surprising, though, given the very large number of cells present in the CSF. The CSF glucose requires some analysis. Normal CSF glucose ranges from 50 to 75 mg/dl, and the patient had a CSF glucose level of 124; why was it elevated? The answer is, it was not! Remember that the normal value for CSF glucose is two-thirds that of the peripheral blood glucose. Since this patient's peripheral glucose was 483 mg/dl, a "normal" CSF glucose for him would be roughly 300 to 330 mg/dl. His actual CSF glucose was 124 mg/dl, or one-quarter of his peripheral level. He clearly had a reduced CSF glucose. Why is CSF glucose reduced in bacterial meningitis? Over the years, far too many medical students have told me it is because the "bacteria use it up." Not so. Remember, the bulk of the biomass in this patient's CSF was due to his neutrophils. How do they get energy to phagocytize the organisms present? They metabolize glucose in the CSF. The bacteria contribute to the low CSF glucose level, but the WBCs are primarily responsible.

With the CSF parameters indicating that the patient likely had bacterial meningitis, what organisms should be considered? In a 45-year-old patient with bacterial meningitis, the possible organisms are very limited. In patients in this age range, more than half of the cases will be due to *Streptococcus pneumoniae*. The Gram stain picture of Gram-positive diplococci is consistent with that organism. Without the Gram stain findings, though, *Neisseria meningitidis* would also need to be considered. *N. meningitidis* is problematic because certain serogroups, A and C, are associated with epidemic spread. Care providers who have close contact with patients with *N. meningitidis* meningitis—such as people involved in cardiac resuscitation, as was done in this case—may require prophylactic antimicrobials to ensure that they do not become infected.

The Gram stain findings were such that three other much less frequently encountered organisms would need to be considered and appropriate antimicrobial therapy given. *Listeria monocytogenes* is a Gram-positive coccobacillus and certainly might be appear like the organism on this Gram stain. It is highly unlikely that this patient had *L. monocytogenes* meningitis. Not only is *L. monocytogenes* meningitis rare, but more importantly, this intense inflammatory response would be highly unusual with this organism. Because *L. monocytogenes* is clinically resistant to ceftriaxone, vancomycin was added, in part to cover that organism. Antimicrobial therapy is discussed in greater detail in answer 3.

Two other organisms would also need to be considered based on the Gram stain. Group B streptococcus (*Streptococcus agalactiae*) is a much more common cause of meningitis in neonates. Nevertheless, diabetic patients have an increased incidence of *S. agalactiae* infection—primarily skin and soft tissue infections but also meningitis. Group A streptococcus (*Streptococcus pyogenes*) may also cause meningitis but is even less common. Finally, for completeness, *Streptococococcus suis* has been associated with adults with meningitis. This patient did not appear to be at risk for infection with this organism as it is seen primarily in people who are in close contact with hogs.

Based on the clinical findings of bacterial meningitis, the Gram stain (Fig. 44.1), and the alpha-hemolytic organism seen in Fig. 44.2, this patient had *S. pneumoniae* meningitis. The organism's identity was confirmed by inhibition of growth around an optochin disk, a key phenotypic characteristic of *S. pneumoniae* (Fig. 44.3).

The large number of Gram-positive organisms and WBCs was a poor prognostic sign. The mortality of *S. pneumoniae* meningitis has been reported in different case series to be between 15 and 40%.

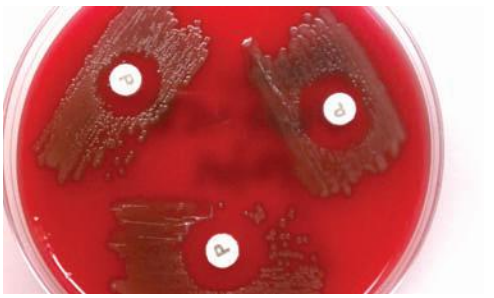


Figure 44.3 Optochin disks showing zone of growth inhibition characteristic of *Streptococcus pneumoniae*.

2. *S. pneumoniae* is part of the microbiota of the nasopharynx. It is aspirated into lung and finds its way to the alveolar space. To get to the alveolar space, it must evade the innate immune response of the bronchial tree and

alveolar space. Two factors produced by *S. pneumoniae* play an important role in this evasion. All virulent strains of *S. pneumoniae* produce capsular polysaccharide. There are >90 different capsular types, with 13 of those types being responsible for approximately 80 to 95% of invasive infection. Capsules act in two ways to evade innate and adaptive immunity. First, they are antiphagocytic. Second, the surface charge of these encapsulated bacteria is such that they are not entrapped in mucus and thus are not cleared efficiently by ciliary action.

A second important virulence factor is pneumolysin. Pneumolysin is released from the bacteria during lysis. It has at least three different roles in pathogenesis. It can bind to the Fc portion of IgG and activate complement, depleting it from the bloodstream; it inhibits ciliary beating, further impairing ciliary clearance; and it binds and damages type 2 pneumocytes, facilitating entry of the organism into the bloodstream in the alveolar space.

If the organism is not cleared by the spleen and liver, key organs in the clearance of bacteria from the bloodstream, it can be carried to the central nervous system, where it can adhere to endothelial cells and be translocated across the blood-brain barrier. Once in the central nervous system, the organisms release a variety of bacterial products, such as cell wall lipoteichoic acid and peptidoglycan, which elicit a strong cytokine-mediated inflammatory response that is responsible of much of the tissue damage characteristic of this infection.

In normal individuals, this is a rare event, because innate and adaptive immunity will kill the organism long before it reaches the meninges. Three factors are important in allowing this to occur and put this patient at increased risk for invasive disease with pneumococci: alcohol abuse, diabetes, and cirrhosis of the liver. All three adversely affect innate immunity. The strongest association of these three risk factors with invasive pneumococcal infection is alcohol abuse. There are at least four factors that explain this association. First, alcohol causes a decreased cough reflex, which allows this common member of the upper airway microbiota access to the bronchial and alveolar spaces. *S. pneumoniae* is found as a component of the nasopharyngeal microbiota of ~20 to 40% of adults and is probably the most virulent of the commonly found members of that bacterial community. Alcohol also adversely affects ciliary clearance by decreasing beat frequency. When organisms get to the alveolar space, they are more likely to survive because there is impairment in neutrophil recruitment and function. Additionally, the antimicrobial proteins lysozyme and lactoferrin have been shown to have impaired killing of pneumococcus in bronchoalveolar lavage fluid of alcoholic patients.

The problem of alcohol abuse was further magnified by the presence of diabetes in this patient. In high-glucose environments, neutrophil function is impaired due to a suppression of ATP generation. This results in impairment of neutrophil adherence, chemotaxis, phagocytosis, and bacterial killing.

Finally, patients with cirrhosis of the liver have decreased complement levels because hepatic cells are a key source of complement in the body. Because complement plays a key proinflammatory role in response to infection, the lowering of complement levels results in poorer opsonophagocytic response and clearance of this organism.

3. The E-test is a modification of the disk diffusion susceptibility test. A lawn of bacteria containing a specific starting bacterial population of the patient's pneumococcal isolate is applied to the surface of a Mueller-Hinton agar plate supplemented with 5% sheep blood. After the lawn dries (it takes a few minutes), the two test strips in this case, penicillin G (PG) and ceftriaxone (TX), are applied to the plate. These two drugs were tested because they are the two most commonly used and effective agents for treatment of pneumococcal meningitis. A gradient of increasing concentration of antimicrobial is bound to the strips and when it comes in contact with the agar surface is released and "diffuses" into the agar. The strip is marked with different drug concentrations. Where the organism's growth meets the strip determines the MIC of the organism. The zone of growth inhibition observed is elliptical; thus the name "E-test." For this patient's organism, the penicillin G MIC was 0.064 μg/ml and the ceftriaxone MIC was 0.032 μg/ml. Because the organism was causing a central nervous system infection, the criteria for determining whether the organism is resistant to these antimicrobials are different than if it was causing pneumonia. Antimicrobials cross the blood-brain barrier at only a fraction of the level at which they will be found in the bloodstream. As a result, the MIC indicating resistance is much less when testing *S. pneumoniae* isolates from the CSF; for penicillin G a MIC of ≥0.12 μg/ml and for ceftriaxone a MIC of ≥2 μg/ml is considered resistant. As can be seen from the results, the patient's isolate was susceptible to both. His poor outcome was a result not of him receiving an antimicrobial agent for which his organism was resistant but rather the progression of disease prior to the patient's presentation.

For the first 3 decades of the antimicrobial era, *S. pneumoniae* was uniformly and exquisitely susceptible to penicillin G. Starting in the late 1970s reports of penicillin G-resistant strains started to emerge. By the 1990s, the era of multidrug-resistant *S. pneumoniae* infections was a reality in much of the industrialized world. Molecular studies demonstrated the global spread of highly resistant strains. What led to this unfortunate occurrence?

Two factors are important, one due to human activity and the other to the evolutionary power of microbial populations. By the mid-1980s, there was recognition that large amounts of antimicrobial agents were being used inappropriately to treat viral infections. What was not recognized was that this inappropriate use of antimicrobials was resulting in the emergence of commensal organisms in the microbiota that were resistant not only to penicillin G but also to many other oral antimicrobials that were also often misused, including erythromycin and trimethoprim-sulfamethoxazole. When penicillin-resistant strains of *S. pneumoniae* emerged, elegant molecular genetics studies determined that the resistant genes for penicillin originally emerged in the oral commensal streptococci and were spread to *S. pneumoniae* via transformation and recombination. Pneumococcal strains that are resistant to all commonly used oral antimicrobials are frequently seen in children, especially in those who attend group child care, a setting in which spread and high carriage rates of drug-resistant pneumococci are common.

4. The recognition that drug-resistant pneumococci were becoming a major problem was a major impetus for the development of improved pneumococcal vaccines. For several decades, a 23-valent pneumococcal capsular vaccine has been available and recommended for use in the elderly and in immunocompromised adults. What is meant by a "23-valent" vaccine? It contains capsular polysaccharide from the 23 most common serotypes of *S. pneumoniae* found to cause invasive disease. This vaccine is not recommended for children because it lacked efficacy in children <2 years of age, the other major target group of invasive pneumococcal disease. The 23-valent polysaccharide vaccine's efficacy was modest, ~60%, but it was still recommended. The success of conjugated polysaccharide *Haemophilus influenzae* type b vaccine in the late 1980s to early 1990s laid the groundwork for the development of a conjugated *S. pneumoniae* vaccine. Because of technical concerns, the initial conjugated pneumococcal vaccine was only 7-valent but the serotypes chosen caused at least 80 to 90% of invasive disease and also contained the serotypes of *S. pneumoniae* most likely to be drug resistant. The vaccine has been a huge success, resulting in a 75% reduction in pneumococcal meningitis in children <5 years of age. An important additional benefit is that herd immunity against *S. pneumoniae* has been enhanced. Pneumococcal meningitis has declined by a third in adults >65 years of age despite the fact that they did not receive vaccine. It is known that the 7-valent vaccine reduces carriage rates of pneumococci in children. It is believed that this reduction in carriage has reduced the spread of pneumococci in the community.

One of the drawbacks of the 7-valent vaccines is that other pneumococcal strains increased in frequency and became much more common causes of invasive disease. Two serotypes in particular, 19A and 22F, were responsible for a significant number of cases.

With the recognition that the 7-valent vaccine did not cover emerging serotypes, a 13-valent vaccine was developed and approved for use in children in 2010 and in adults >50 years of age in 2011. The additional serotypes include 19A but not 22F. Further reduction in invasive pneumococcal disease can be anticipated.

Current vaccination recommendations for immunocompromised patients such as the one described in this case with chronic liver disease would be to use the 23-valent pneumococcal polysaccharide vaccine. The efficacy data in this population are limited. Recommendations for the use of conjugated pneumococcal vaccines in this patient population are under discussion. Nevertheless, immunocompromised patients are likely to benefit from the herd immunity that the 13-valent vaccine induces.

5. Patients with sickle cell anemia are at greatly increased risk for invasive pneumococcal disease due to functional asplenia. The spleen is the major filtering organ that eliminates *S. pneumoniae* from the bloodstream. Sickle red cells cause vaso-occlusive infarction in the spleen, which over time results in splenic atrophy and a decreased ability to clear encapsulated bacteria, especially pneumococci. Patients <3 years of age with sickle cell anemia are given prophylactic antimicrobials, typically penicillin, as well as the 13-valent conjugated vaccine. After the initial conjugate vaccine, two doses of 23-valent polysaccha-

ride vaccine are recommended, one at 2 years and another at 3 to 5 years of age. The 7-valent vaccine strategy has been shown to reduce invasive pneumococcal disease in this patient population. As can be seen, vaccine strategies in special populations can be complex compared with the general population.

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The patient was a 21-year-old Hispanic migrant farmworker who was 27 weeks pregnant. She presented with complaints of fever, headache, chills, frequency and urgency of urination, decreased appetite, and a 1-day history of diarrhea and decreased fetal movement. On physical examination she had a temperature of 38.3°C, abdominal tenderness, and tachycardia. Her chest was clear on auscultation, and no cervical discharge or tenderness was seen on pelvic examination. Her laboratory studies were significant for a white blood cell count of 21,300/μl. A cervical specimen assayed for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using PCR was negative. Two blood cultures were drawn, and the patient was begun on ampicillin-sulbactam and gentamicin.

The next morning the patient complained of right costovertebral tenderness and abdominal pain. On ultrasound there was no fetal movement, and intrauterine fetal demise was suspected. Labor was induced, and a stillborn infant was delivered vaginally. Cultures of blood, placenta, and umbilical cord all grew the organism seen in Fig. 45.1 and 45.2.

1. What organisms do you think were likely infecting this patient? If you learned that the organism was catalase positive, how would that help you decide what organism was infecting this patient?
2. What is the significance of headache in this patient's history? What is the natural history of this disease in pregnancy?
3. What other patient populations are at risk for infection with this organism?
4. How is this organism spread? Was the woman's ethnicity important in this case? What special systems are in place in the United States to try to reduce the number of cases caused by this organism? What special characteristic of this organism may be important in its spread?
5. This organism is classified as a facultative intracellular organism. Briefly describe how this organism is able to evade the host's immune system and survive intracellularly.

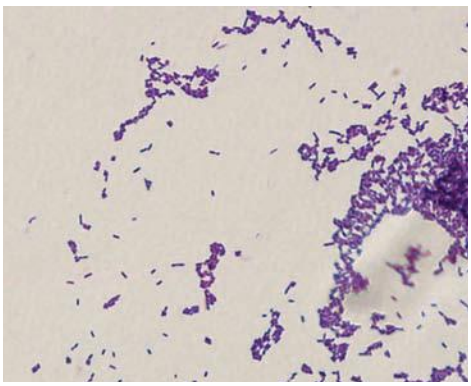


Figure 45.1 Gram stain of the organism isolated from the placental culture.

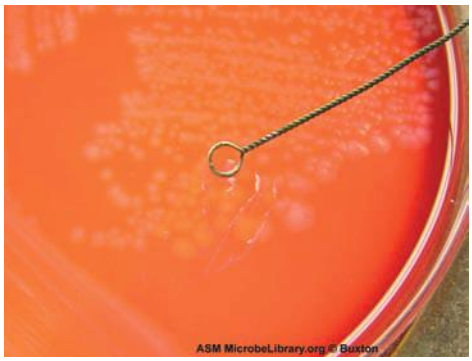


Figure 45.2 Organism growing on 5% sheep blood agar. Note that the organism is weakly beta-hemolytic.



CASE DISCUSSION

1. The Gram stain and colonial morphology of the organism infecting the mother and her child are consistent with two organisms that are frequently associated with fetal demise, group B streptococci (GBS) and *Listeria monocytogenes*. On Gram stain, the organisms, for all practical purposes, are indistinguishable even though textbooks indicate that they are morphologically distinct, GBS appearing as cocci and *L. monocytogenes* as bacilli. In clinical specimens, GBS frequently appear as Gram-positive diplococci while *L. monocytogenes* organisms appear as Gram-positive, diphtheroid-like bacilli. These organisms can appear so much alike on Gram stain that even a skilled microscopist may not be able to distinguish them. The colonial morphology of these organisms on a 5% sheep blood agar plate is identical, with both organisms producing grayish white colonies surrounded by a narrow zone of beta-hemolysis (see Fig. 45.2).

Although the organisms have many similar characteristics, they can be easily distinguished in the laboratory based on the catalase test: *L. monocytogenes* is catalase positive while GBS are negative. It is important to identify these organisms accurately for both therapeutic and epidemiological reasons. (See case 46 for further discussion.) The disease course is similar for these two organisms, with the highest morbidity and mortality seen in low-birth-weight children who have poorly developed lungs. The incidence of neonatal infection with GBS is much higher than that for *L. monocytogenes*. Because early-onset disease is quite rare with *L. monocytogenes*, there is no organized attempt to screen women for this organism prenatally at 35 to 37 weeks of gestation as there is for GBS. There also are no available clinical trial data that show that intrapartum antibiotic therapy prevents early-onset disease with *L. monocytogenes*.

2. A prominent feature of the clinical spectrum of *L. monocytogenes* disease is meningitis. Headache is an important clinical symptom of meningitis, as it is of many clinical syndromes. Meningitis due to *Listeria* is almost always seen in neonates or severely immunocompromised patients. The headache seen in the mother is part of the flu-like illness that is commonly seen in pregnant women who are bacteremic with *L. monocytogenes*. The disease is usually self-limiting in pregnant women but can have devastating consequences for their unborn children.

The organism, which can be vertically transmitted, causes chorioamnionitis, resulting in septic abortion, stillbirth (as was seen in this case), premature birth, and serious infections in the early neonatal period (0 to 3 days), including pneumonia, bacteremia, and meningitis.

Like GBS, *L. monocytogenes* can also cause late-onset disease in infants. Late-onset neonatal disease occurs after the first week of life up to the third month. These children tend to have been born at term, and meningitis is more common in late-onset disease.

3. Besides pregnant women and their unborn children, serious *Listeria* infections are usually limited to immunocompromised individuals. In particular, organ transplant recipients, patients with AIDS or malignancy (especially lymphoma and chronic lymphocytic leukemia), and those receiving corticosteroids are at high risk for developing serious infections with this organism. Meningitis is a prominent feature of the disease spectrum in these patients. Mortality rates with systemic listeriosis are estimated to be 20%, with most fatalities in newborns and the immunocompromised.

4. Listeriosis is a food-borne infection. Dairy products (particularly soft cheeses), undercooked chicken, and prepared meats such as hot dogs and cold cuts have all been implicated as vehicles for the transmission of *Listeria*. Interestingly, the largest outbreak of listeriosis in this decade was due to the consumption of cantaloupe raised on a single farm in Colorado, resulting in the death of 33 of the 147 infected individuals (22%).

This patient's ethnicity may be important because two large outbreaks have been associated with consumption of Mexican cheeses, a food product more likely to be consumed by this ethnic group. One outbreak occurred in the Latino community in Los Angeles in 1985. In this outbreak, it was noted that there was a high rate of fetal and neonatal infections and death due to *Listeria* infection in Latinos that was not occurring in other populations in the city. Through careful epidemiologic study, it was learned that a soft cheese referred to as "Mexican-style cheese," which was sold and consumed primarily in the Latino community, was contaminated with *L. monocytogenes*. Further studies showed that this cheese was frequently tainted with unpasteurized milk, and this unpasteurized milk was the ultimate source of the *Listeria*. A second, smaller outbreak in North Carolina resulted in 5 stillbirths and 3 premature births among 11 infected pregnant Hispanic women. In this outbreak, the food source was homemade soft cheese made from contaminated milk that was traced to a single dairy. It was sold door-to-door in the Hispanic community.

Outbreaks of *Listeria* infection due to contaminated foods may not be readily recognized because small numbers of cases (<50) may be spread throughout the United States. In addition, the median incubation period of invasive disease (bacteremia, meningitis) with this organism is 3 weeks. By the time cases are recognized, the potential food source may no longer be available for culture. In addition, implicated foods such as hot dogs or cold cuts may be distributed from a central location under many different brand names to locations throughout the country, making the connection between a particular food and a specific supplier more difficult. Surveillance programs that monitor food-borne diseases, such as the Centers for Disease Control and Prevention-sponsored FoodNet and PulseNet, play an important role in recognizing these outbreaks and in limiting their spread by effecting producer recalls of tainted products. The number of *Listeria* cases has remained stable in the United States over the past decade, giving credibility to these control efforts.

The reason why *L. monocytogenes* can be transmitted by foods that are almost always refrigerated before consumption is due to the fact that this organism can grow at 4°C. This is an unusual characteristic for an organism that causes human disease. Most human pathogens grow in a temperature range between 20° and 37°C. Another human pathogen, *Yersinia enterocolitica*, can also grow at 4°C. Not surprisingly, the vehicle of transmission for outbreaks of disease due to *Y. enterocolitica* has also been dairy products and processed meats.

In addition, *Listeria* can grow in high-salt and acidic conditions, further explaining its ability to survive in processed foods. This organism's ability to produce certain "stress" proteins allows it to survive when exposed to such hostile environments. The production of stress proteins has been essential for the evolutionary survival of an organism that has soil as it natural environment.

L. monocytogenes is one of the leading causes of serious food-borne illnesses. It is estimated that there are as many as 200 deaths annually in the United States due to this organism. Recently, *L. monocytogenes* food-borne outbreaks of febrile gastroenteritis in immunocompetent individuals have been described. This disease entity appears to be different from invasive disease seen in immunosuppressed and pregnant women. The incubation period is short (12 to 26 hours), and gastroenteritis symptoms are prominent.

5. *L. monocytogenes* produces a cell surface virulence factor called internalins that allows it to invade epithelial cells. After invasion, the organism is found in a vacuole within the host cell. The organism produces a second virulence factor, listeriolysin O. Listeriolysin O lyses the vacuole, allowing the organism to enter the cytoplasm of the host cell, where it can multiply. There, a third virulence factor, the cell surface protein ActA, mediates the binding of host cell actin to one end of the bacterium. This actin "tail" propels the bacterium to the periphery of the cell, where the bacterium induces the formation of protrusions called filopodia. These filopodia are taken up by adjacent cells, where this infective process can begin anew. Because the bacterial cells are never exposed to the extracellular environment, humoral immunity plays little if any role in the immune response to this organism. Rather, cell-mediated immunity is central to control of infection with this organism, explaining why individuals with defects in cell-mediated immunity, such as transplant recipients and individuals with hematologic malignancy, have increased risk of invasive disease with this organism.

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The patient was a 3½-week-old male who was born at term by cesarean section. At birth he had a left diaphragmatic hernia that was repaired soon thereafter. He required intubation at that time and continued to require respiratory support. Over a 24-hour period, the infant developed bulging anterior fontanelles, increased respiratory and heart rates, wide fluctuations in blood pressure, and difficulties maintaining adequate tissue perfusion, and his peripheral white blood cell (WBC) count increased from 6,300 to 13,700/µl. The child began to have focal seizures as well. A cerebrospinal fluid (CSF) examination showed 3,900 WBCs/µl with 92% neutrophils, a glucose level of 2 mg/dl, and a protein level of 350 mg/dl. A Gram stain of the child's CSF is shown in Fig. 46.1. The organism isolated from the CSF is shown in Fig. 46.2.

1. What is your diagnosis for this patient? Is it consistent with his physical and laboratory findings? Explain.
2. What organism was most likely causing his infection? What other organism has similar Gram stain and colonial morphology? What simple, rapid test would you use to distinguish these two organisms?

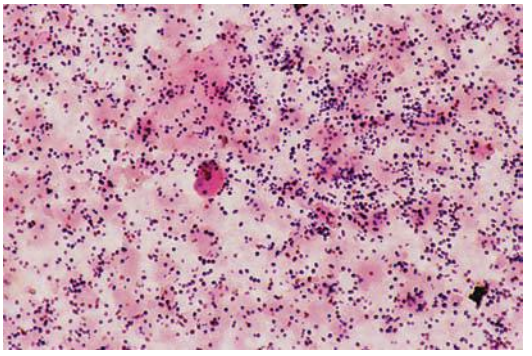


Figure 46.1

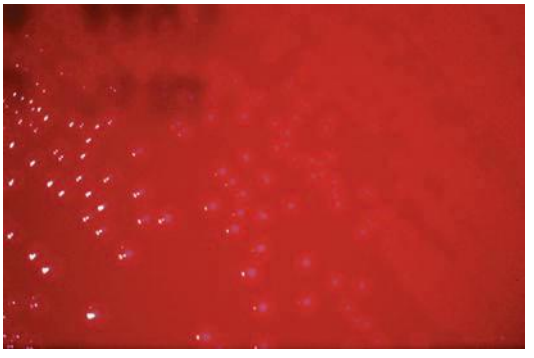


Figure 46.2

3. There are two forms of this infection in neonates. Compare and contrast these two forms. Which form did this patient have?
4. Beside infections in neonates, what other populations are at risk for invasive infection with this organism?
5. Describe the key virulence factor produced by the infecting organism and discuss its role in pathogenesis of infection.
6. Vaccines are currently under development for this organism. Describe the components that you would include in this vaccine. Who should receive this vaccine? Why would they receive it?
7. Since vaccines against the organism are not currently available, discuss strategies for prevention of neonatal infections with this organism. How effective have they been in preventing early-onset disease? How effective have they been in preventing late-onset disease?



CASE DISCUSSION

1. This patient had bacterial meningitis. The physical finding of bulging anterior fontanelles is due to inflammation and swelling of the meninges caused by infection. His increased respiratory and heart rates, fluctuations in blood pressure, and difficulties in maintaining adequate tissue perfusion are all signs of sepsis and are often seen in individuals with bacterial meningitis. Seizures are common in patients with meningitis. His CSF findings of 3,900 WBCs/μl with a neutrophil predominance, low glucose, and high protein are all consistent with bacterial meningitis. The finding of Gram-positive cocci (Fig. 46.1) in the microscopic examination of CSF is conclusive evidence of bacterial meningitis.

2. The bacteria that most frequently cause neonatal meningitis are the group B streptococci (GBS, or *Streptococcus agalactiae*). The Gram stain and colonial morphology seen in Fig. 46.1 and 46.2 are consistent with GBS. However, *Listeria monocytogenes*, a much less frequent cause of neonatal infection, can be confused with GBS on Gram stain, even though *L. monocytogenes* is classified as a Gram-positive coccobacillus. On sheep blood agar both organisms have very similar colonial morphology and both are weakly beta-hemolytic. The clinical disease these organisms cause is indistinguishable. Accurate identification is important when therapeutic choices are being made because cephalosporin therapy is not effective against *L. monocytogenes*. Accurate identification is also important in understanding the epidemiology of disease caused by these two organisms. Accurately distinguishing these organisms can be easily accomplished by the catalase test. Catalase is an enzyme that catalyzes the following reaction: $H_2O_2 \rightarrow 2H_2O + O_2$ (gas). Catalase activity can be detected by smearing the test organism on a glass slide and placing a drop of hydrogen peroxide (H_2O_2) on the smear. A bubbling reaction due to the release of O_2 occurs immediately if the organism produces catalase. *L. monocytogenes* is catalase positive; GBS is catalase negative. The patient's isolate was catalase negative; further testing including serogrouping confirmed it to be GBS.

3. Both GBS and *L. monocytogenes* can cause both early- and late-onset infections in neonates. In early-onset disease, the organism is spread vertically from mother to infant, as evidenced by the mother and child having both the same serotype and the same genotype. In the mother, the GBS or *L. monocytogenes* infection may manifest itself as a mild febrile illness, with the mother having flu-like symptoms, or she may be an asymptomatic carrier. The neonate is generally infected *in utero* or during passage through a colonized birth canal. Neonates with early-onset disease often are ill at birth or become symptomatic in the first week of life, generally within the first 3 days. Risk factors for early-onset GBS disease include maternal colonization with GBS, gestation <37 weeks, prolonged rupture of membranes, young maternal age, and black race. The major focus of infection is the lungs, which are poorly developed and poorly functioning in low-birth-weight babies.

Infected neonates often present with pneumonia and sepsis and less commonly with meningitis. Mortality in early-onset disease is 4 to 6%, which is down significantly from the 50% mortality seen in early-onset GBS disease in the 1970s. Approximately 75% of GBS neonatal infections are early onset.

Late-onset disease usually occurs between 10 and 14 days of age but can be seen up to 3 months after birth. Late-onset disease is typically seen in full-term infants. The epidemiologic link between mother and child is more tenuous in late-onset disease, with only 50% of the children having the same serotype as that colonizing their mothers. A major focus of infection in late-onset disease is the central nervous system, with meningitis being more common than it is in early-onset disease. Unlike early-onset disease, late-onset disease incidence has remained stable. Mortality is not as high in late-onset disease, but it is still significant. Surviving infants may have neurologic sequelae, including blindness, hearing loss, and developmental and educational delay.

4. Given the great emphasis on preventing GBS in neonates, it is not surprising that 70% of invasive GBS infections occur outside of the neonatal period. Of those, about 90% occur in nonpregnant adults, with 6% in pregnant women and 3% in children from 3 months to 14 years of age. Diabetes mellitus is the most common risk factor for invasive GBS infections. Invasive GBS infections are 14 times more likely to occur in diabetics than in persons of the same age without diabetes. GBS bacteremia in diabetics is frequently secondary to cellulitis or foot ulcers. These ulcers are frequently seen as a result of peripheral vascular disease, which is common in diabetics. Other underlying conditions associated with invasive GBS infections are cardiovascular disease, solid cancers, alcoholism, and cirrhosis. The disease is much more common in those 65 years of age or older, and the mortality in that population is 15%, which is three times as high as the 5% seen in neonates. Along with early-onset disease, GBS infections during pregnancy can be responsible for septic abortion and stillbirth, as well as bacteremia, chorioamnionitis, endometritis, and urinary tract infections.

5. The key virulence factor of GBS is the capsule. There are 10 described serotypes (Ia, Ib, and II through IX), with Ia, III, and V being the most common in neonatal disease. Serotype III accounts for the majority of late-onset GBS disease and about one-third of cases of early-onset disease. However, there is significant variability in serotype prevalence globally. Type V is an important cause of invasive disease in adults and is responsible for approximately one-third of those cases; it has also been increasingly associated with neonatal disease. Like *Haemophilus influenzae* type b capsule, GBS capsule inhibits complement-mediated phagocytosis, allowing the organism to evade the immune system. Capsule-specific antibodies reverse this inhibition. There is an inverse correlation between the amount of maternal anti-capsular polysaccharide IgG antibodies found in infants and their risk of developing either early- or late-onset GBS disease.

6. Currently the GBS vaccine is experimental. The most important component of any vaccine against GBS would be capsular polysaccharide. Because cross-immunity is not conferred among GBS serotypes, a vaccine should contain capsular polysaccharide from the common GBS serotypes (see answer to question 5). However, this approach is complicated as the prevalence of serotypes varies with geography. Polysaccharides are T-cell-independent antigens. Primary and even secondary immune response may be poor in some individuals. Coupling the polysaccharides to a protein carrier molecule produces an antigen that elicits a T-cell-dependent immune response, resulting in a more predictable and protective immune response to the polysaccharide antigen. Tetanus toxoid coupled with different capsular polysaccharide serotypes has been shown to elicit a protective response in animals and in humans. To address the challenges associated with capsular type specificity, recent developments focusing on conserved surface antigens (such as cell surface and pili proteins) have shown promise for potential candidates for global vaccines.

The target population in which disease must be prevented is the fetus/neonate. This population cannot be effectively vaccinated. However, the mother, who can pass protective IgG transplacentally, can be. Animal studies have shown protection in neonates challenged with GBS whose mothers have been previously vaccinated. However, vaccinating pregnant women and designing clinical trials to do so can be problematic, largely due to the fear of the risk of birth defects. Therefore, the target group will likely be adolescents or women prior to pregnancy. A GBS vaccine might also be useful in diabetics, who are at a much greater risk for infections than the general population.

7. Until GBS vaccines are proven safe and efficacious, prevention of GBS infection in the neonatal period is dependent on the use of prophylactic antibiotics. Currently there is agreement that intrapartum intravenous administration of penicillins, or clindamycin or erythromycin in penicillin-allergic mothers, is a successful prophylactic strategy for GBS neonatal infection.

Initially published in 1997, guidelines for the prevention of perinatal GBS disease were revised in 2010 by the Centers for Disease Control and Prevention along with a number of medical professional societies. Since only 15 to 35% of pregnant women are colonized with GBS in the genitourinary tract, it is not necessary to give intrapartum antibiotics to all women during labor. It is recommended that women be screened by culture at 35 to 37 weeks of gestation for the presence of GBS in their vagina and rectum. If positive, then they should be offered intrapartum antibiotics. Women who have had a previous child with GBS neonatal infection or have had GBS bacteriuria during pregnancy should be given intrapartum antimicrobial agents, as these both indicate a high level of GBS colonization. In addition, women without prenatal care who deliver at <37 weeks of gestation, have intrapartum fever of ≥38°C, or have rupture of membranes for >18 hours should receive intrapartum antibiotics.

The combination of GBS screening and intrapartum prophylaxis has succeeded in reducing the incidence of early-onset disease from 1.7 cases per 1,000 births in the 1990s

to 0.35 cases per 1,000 births currently (an ~80% decline). This result has been seen despite the fact that there is not yet universal compliance with these guidelines as well as 25 to 32% resistance to erythromycin and 13 to 20% resistance to clindamycin among GBS isolates. GBS remains universally susceptible to penicillin. Intrapartum antibiotic therapy has not had any impact on the incidence of late-onset GBS disease.

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A 10-year-old female presented in August to the emergency department of a North Carolina hospital with a 3-day history of worsening headache and petechial rash. She began feeling ill approximately 1 week prior to presentation, with increasing fatigue, intermittent chills, and lower back pain. She had 1 day of nausea, vomiting, and diarrhea, along with a yellowish nasal discharge. Her headache, which began 3 days previously, was exacerbated by light and rapid movement. Over-the-counter medications had no effect on her headache. The patient stated that her "head hurts everywhere" and her neck felt stiff. In addition to the headache and general malaise, she had a small pinpoint rash over her legs, trunk, and upper extremities. The only known sick contacts were two younger cousins with fever and rash. She had a history of a tick bite earlier in the summer.

The patient's physical exam was within normal limits with the exception of slight pain with neck flexion. She had negative Brudzinski's and Kernig's signs. Her laboratory tests, including a complete blood count, sedimentation rate, and urinalysis, were within normal limits. Cerebrospinal fluid (CSF) obtained by lumbar puncture showed <1 red blood cell/μl and 32 white blood cells/μl with 31% neutrophils, 36% lymphocytes, and 33% monocytes. CSF protein and glucose were both normal at 43 mg/dl and 54 mg/dl, respectively. CSF Gram stain showed no neutrophils and no organisms. Bacterial cultures of blood and CSF were obtained, and a viral PCR was ordered on the CSF. While remaining laboratory results were pending, the patient was started on ceftriaxone and doxycycline. However, once the PCR test was reported as positive, all antibiotics were discontinued and the patient was discharged home.

1. Did this patient have meningitis? Explain your answer.
2. What were the most likely causes of her infection?
3. Describe the epidemiology of the virus that caused her infection, including other clinical syndromes associated with this group of viruses.
4. How are these viral infections prevented and treated?
5. One of the members of this group of viruses is near global eradication. Describe the efforts that have led to this success and what concerns remain.



CASE DISCUSSION

1. The finding of >3 white blood cells per μl in the CSF indicates that the patient may have had meningitis. Brudzinski's and Kernig's signs, if positive, indicate meningeal irritation. These signs have a diagnostic sensitivity as low as 5%, but specificity for meningitis is high. Therefore, the fact that these signs were negative in this patient does not rule out meningitis. The patient reported that her neck was stiff, which was also appreciated on physical exam. The normal levels of protein and glucose measured in her CSF indicate that this was not likely bacterial meningitis, but could still be mycobacterial, fungal, or viral meningitis. The CSF cell count showed approximately equal numbers of neutrophils and lymphocytes. Although viral meningitis is typically associated with a lymphocytic predominance in the CSF, there is often a neutrophilic predominance in the first 48 hours of disease. Because fungal and mycobacterial meningitis in children are rare in the United States, the most likely source of her meningitis is viral. A PCR test confirmed the diagnosis.

2. The most common cause of pediatric viral meningitis, particularly during the summer and fall months, is enterovirus. However, herpes simplex virus and arboviruses cannot be excluded without additional laboratory testing. Given that the patient presented with headache and rash in North Carolina during the summer, Rocky Mountain spotted fever (RMSF) must also be in the differential. The concern for RMSF is why she received doxycycline. Since the most likely cause of her illness was enterovirus, an enterovirus PCR was ordered on her CSF, which was positive. This result was reported while the patient was still in the emergency department; therefore, the patient was able to be discharged home with her empiric antibiotic coverage discontinued.

3. Enteroviruses include the coxsackieviruses (A and B), echoviruses, polioviruses, and the numbered enteroviruses (e.g., enterovirus 71). Recently, parechoviruses (formerly echoviruses 22 and 23) have been shown to be genetically distinct from the enteroviruses. Although parechoviruses are also associated with meningitis, it is important to note that most enteroviral PCR tests will not detect the parechoviruses. Enteroviruses belong to the family *Picornaviridae*, along with rhinovirus and hepatitis A virus. The primary mode of enteroviral transmission is the fecal-oral route, but it can also spread via the respiratory route and by fomites. Approximately 85% of viral meningitis cases in the United States are due to enteroviruses, although very few enteroviral infections result in meningitis. In fact, most enteroviral infections are subclinical to mild. Similar to other childhood exanthems, enteroviral infections in children may present with only fever and rash, as was presumably the case for this patient's cousins. Enteroviral infections typically peak in the spring to fall months in temperate regions.

The nonpolio enteroviruses are ubiquitous and cause a wide range of diseases. Hand-foot-and-mouth disease (HFMD) is characterized by fever, mouth blisters/ulcers, and rash

(often on the hands and feet) and is most commonly caused by coxsackievirus A serotype 16 (A16). In 2011 to 2012, there was a large outbreak of HFMD in the United States caused by coxsackievirus A6. This outbreak was widespread and included more severe manifestations of HFMD, including some hospitalizations. Coxsackievirus A and enterovirus 70 have caused outbreaks of conjunctivitis. Coxsackievirus B is the most common cause of viral meningitis in children <3 months of age (~60%). Typically, the mother has a subclinical or mild enteroviral illness that is passed to the infant shortly after birth. In these young infants, enteroviral infection can be particularly severe, including meningitis and pericarditis. Enteroviruses 70 and 71 are particularly neurotropic enteroviruses and often cause more severe disease, including meningoencephalitis, acute flaccid paralysis, and Guillain-Barré syndrome. Enterovirus 71 has caused HFMD outbreaks worldwide, including cases of brain stem encephalitis. Echoviruses make up the majority of the remaining enteroviral meningitis cases, including several viral meningitis outbreaks in the United States.

The polioviruses are unique among the enteroviruses in that they can cause severe paralysis, including permanent disability and death, and in the fact that infections due to polioviruses are vaccine preventable. During the 1940s and 1950s, there were ~35,000 cases of poliomyelitis annually in the United States. However, after the development of two vaccines in the 1950s, poliovirus was eradicated from the United States in 1979. Active poliovirus transmission still occurs in other areas of the world (see the answer to question 5 for more information on worldwide polio eradication). As with other enteroviruses, the majority of individuals who are infected with polioviruses are asymptomatic. Fewer than 1% of those infected become paralyzed, and of these, 5 to 10% die due to paralysis of the respiratory muscles.

4. Because enteroviruses are spread primarily by the fecal-oral route, good hand-washing technique aids in preventing the spread of infection. Frequent hand washing and surface disinfection helps interrupt the transmission cycle, particularly in day care or school settings, where enteroviral infections often spread. Further, since enteroviruses can also spread by the respiratory route and by fomites (in particular HFMD), it is recommended to avoid all close contact, including kissing, hugging, and sharing eating utensils and cups. There is no vaccine available for the nonpoliovirus enteroviruses. To prevent poliovirus infections in the United States, vaccination with an enhanced form of the inactivated polio vaccine is currently recommended at the following ages: 2 months, 4 months, 6 to 18 months, and 4 to 6 years. The vaccine contains all three poliovirus serotypes. The live, attenuated oral polio vaccine is no longer recommended in the United States due to the rare complication of vaccine-associated paralytic poliomyelitis (1 in 2.4 million doses, or 5 to 10 cases annually). Since 1979, the only indigenous cases of polio reported in the United States have been associated with the live oral vaccine. Previously, the oral vaccine was preferred because it conferred both humoral and mucosal immunity, and the live, attenuated nature of the vaccine provided herd immunity by fecal-oral spread to

unvaccinated contacts. The oral poliovirus vaccine is very effective (>95%) and continues to be used worldwide.

There is no enterovirus-specific treatment, and most patients fully recover from their infections without intervention. Treatment of enteroviral infections is supportive. For example, hydration and pain relief for mouth sores might be indicated for those with HFMD. Patients with enteroviral meningitis occasionally require hospitalization to provide the appropriate level of supportive care. However, most patients are initially admitted to determine the diagnosis, some of which (i.e., bacterial meningitis, herpes simplex virus meningitis, and RMSF) have specific therapeutic interventions and higher morbidity and mortality. The use of PCR to provide a rapid, accurate laboratory diagnosis of enteroviral meningitis has been shown to result in hospital cost savings by reducing the length of hospitalization and unnecessary antimicrobial use in both pediatric and adult patients.

5. Two poliovirus vaccines became available in the mid-20th century: the Salk inactivated vaccine in 1955 and the Sabin live, attenuated oral vaccine in 1962. The two vaccines successfully eradicated poliovirus transmission in most industrialized countries, with the last indigenous U.S. case seen in 1979. The strategy to make the world polio free is based on increasing worldwide vaccination coverage, particularly in children, as well as enhancing surveillance for acute flaccid paralysis cases. Once there are no (or very few) humans susceptible to poliovirus infection, fecal shedding of polioviruses into the environment will cease, and the transmission cycle will be interrupted. Global vaccination efforts have resulted in a decrease in worldwide poliomyelitis incidence from 350,000 cases in more than 125 countries in 1988, to 1,652 cases in 4 countries in 2007, to only 223 cases reported worldwide in 2012. The Western Hemisphere has been polio free since 1991. Poliovirus type 2 appears to have been eradicated worldwide since 1999, but types 1 and 3 are still detected in clinical disease and environmental surveillance. Polioviruses have remained endemic in Afghanistan, Nigeria, and Pakistan, and in 2013 they reappeared after initial eradication in Kenya, Somalia, and Ethiopia.

As the goal of worldwide eradication of polioviruses approaches, it will be important to define what strains, if any, should be stored in laboratories. This has been a contentious issue for smallpox virus, which is a potential agent of bioterrorism and biowarfare. Other issues include the cessation of routine oral poliovirus vaccination. This World Health Organization recommendation is based on the concern for circulating vaccine-derived poliovirus, which is the attenuated vaccine virus that accumulates genetic changes that render it virulent and therefore a source of outbreaks. To discontinue the oral vaccine, the inactivated vaccine will need to become less expensive so as to be broadly available in the developing world. Lastly, continued vigilance in the surveillance program for acute flaccid paralysis will be necessary until complete eradication is confirmed. Funding for the surveillance program in the posteradication era is a concern.

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The patient was a 38-year-old HIV-positive male with a CD4 count of 80/μl. The patient had a 1-week history of progressively worsening headache, photophobia, lethargy, and fevers to 38.5°C. On the morning of his admission, he became confused, disoriented, and ataxic, having fallen three times. On physical examination he was lethargic and could only answer a few questions before falling asleep. His vital signs were all within normal limits. Chest examination and radiograph were normal. He had a head computed tomography scan that was also normal. Because of his declining mental status and history of headache and photophobia, a lumbar puncture was done. The cerebrospinal fluid (CSF) revealed 32 white blood cells per μl with 89% lymphocytes and 6% monocytes, a glucose level of 22 mg/dl, and a protein level of 89 mg/dl. A Gram stain of his CSF is shown in Fig. 48.1. Serum and CSF tests for the presence of a specific antigen were positive. The organism that was recovered from his CSF and blood is shown growing on a sheep blood agar plate in Fig. 48.2.

1. What organism was most likely was causing his illness? Are his CSF parameters (cell count and chemistries) consistent with infection with this organism? What other organisms are frequently seen causing central nervous system infections in this patient population?
2. What virulence factor does this organism produce, and what is its role in the pathogenesis of this disease?
3. What other patient populations are at increased risk of this infection? A new species closely related to the organism infecting this patient has recently been found to be causing an ongoing outbreak in the northwestern portion of North America. What is this organism and how does it differ from the organism infecting this patient?

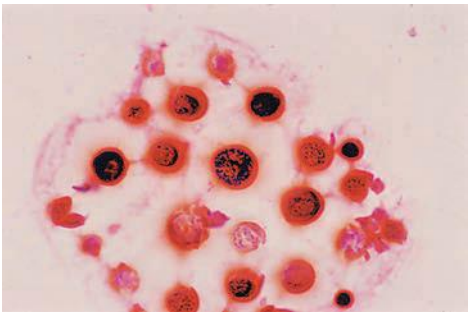


Figure 48.1

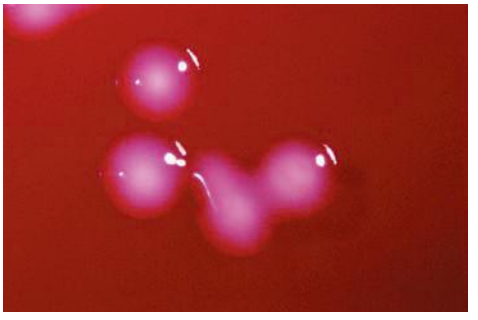


Figure 48.2

4. What is the specific antigen that was found in his serum and CSF? Explain two different ways this antigen test is used in managing HIV patients. What other organism will give a positive reaction in this test?
5. How did this patient become infected? Beginning in the mid-1990s, what changes have occurred in the epidemiology of infection with this organism? Why have these changes occurred? What is an important consequence of these changes?
6. Three months later, the patient again presented with symptoms consistent with his initial illness. A CSF Gram stain obtained at this time is seen in Fig. 48.3. His CSF antigen titer on his first admission had been 1:100,000. It had dropped to 1:200 after therapy and on his latest admission was 1:1,600. How do you interpret his CSF antigen titers?
7. How should this patient be managed to prevent future infections with this organism?

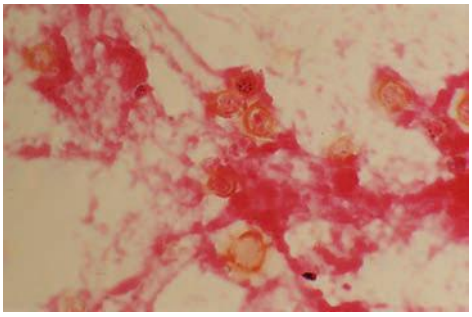


Figure 48.3



CASE DISCUSSION

1. The Gram stain reveals round yeast cells that are Gram variable. The most common such agent causing central nervous system infection in HIV-infected individuals with CD4 counts of <200/μl is *Cryptococcus neoformans*. Both the Gram stain and the colonial morphology seen in Fig. 48.1 and 48.2 are consistent with this organism. Further biochemical characterization, including a rapid urease test (Fig. 48.4) and the production of melanin on birdseed agar, confirmed this organism as *C. neoformans*.



Figure 48.4 Patient isolate on right; negative control on left.

CSF cell counts of <100/μl are commonly seen in HIV-infected patients with cryptococcal meningitis. CSF glucose levels are frequently normal but may be low as was seen here. CSF protein levels are also frequently normal but may be elevated as was seen in this patient.

Toxoplasma gondii and HIV both are common causes of central nervous system infection in HIV-infected patients. Both cause an encephalopathic picture. A variety of other agents must be considered when *C. neoformans* and *T. gondii* have been ruled out in these patients. Some of the more important of these include *Mycobacterium tuberculosis*, *Nocardia* spp., *Treponema pallidum* (neurosyphilis), the herpes viruses (cytomegalovirus and herpes simplex virus), dimorphic fungi including *Histoplasma capsulatum* and *Coccidioides immitis*, and encapsulated bacteria.

2. On Gram stain, it is often possible for the skilled observer to detect a capsule surrounding the yeast. The capsular material often stains Gram negative while the yeast cell stains Gram positive. This explains the Gram-variable appearance of this organism. Alternatively, the capsule can be nicely demonstrated using India ink, a negative staining technique. With this method, the yeast cell is seen in a dark background surrounded by a clear "halo." This halo is due to the inability of the ink to penetrate the capsule surrounding the cell.

The cryptococcal capsule is antiphagocytic. In animal studies, the capsular polysaccharide has been shown to inhibit cell-mediated immunity as well. Finally, soluble cryptococcal capsular polysaccharide has been shown to activate the alternative complement pathway. In patients with a high concentration of circulating capsular polysaccharide, this may result in depletion of complement, causing inefficient opsonization and reduced phagocytosis of this organism.

3. Although individuals with normal immune systems may acquire infection with *C. neoformans*, the patients at elevated risk of infection are those who are immunocompromised, with deficiencies in cell-mediated immunity. This includes solid organ transplant

recipients who receive antirejection agents, individuals who receive corticosteroid therapy for other conditions, and patients with lymphoma. Recently, there have been cases documented in patients who received tumor necrosis factor-α antagonists that have been used to treat inflammatory conditions such as rheumatoid arthritis.

A second pathogenic cryptococcal species, *Cryptococcus gattii*, has caused clinical disease in Vancouver Island, British Columbia, Canada, and the northwestern United States, primarily in Washington and Oregon. This organism differs from *C. neoformans*, the organism infecting this patient, in several ways.

First, *C. gattii* appears to be more likely to infect immunocompetent hosts, although as would be expected, it infects immunocompromised patients as well. Second, this organism is more likely to cause clinical pulmonary disease, and animal model data support this observation. Third, this organism is more likely to produce cerebral cryptococcomas. Finally, mortality rates as high as 35% have been reported for *C. gattii* infections in North American patients. Until the North American outbreak, *C. gattii* was thought to be limited to tropical and subtropical locales, with *Eucalyptus* trees being an important habitat. In Australia, where this organism is endemic, Aboriginal Australians are 10 times more likely to be infected, suggesting a genetic predilection. The extent of *C. gattii* infection globally is unknown since most laboratories do not try to distinguish the two species and both are positive in the cryptococcal antigen test, a widely used diagnostic tool.

4. The specific antigen that was found in this patient's CSF and serum was the capsular polysaccharide of *C. neoformans*. Capsular polysaccharide is detected using latex particles coated ("sensitized") with antibodies specific for this antigen. Alternatively, enzyme-linked immunosorbent assays have been developed for the quantitative detection of this antigen. Most laboratories use latex agglutination to detect cryptococcal antigen.

These cryptococcal antigen detection methods can be used in two ways. One is diagnostically. An HIV-infected patient who presents with central nervous system symptoms such as headache and lethargy and has cryptococcal antigen detectable in his or her serum is at high risk for having cryptococcal meningitis. In HIV-infected patients with culture-proven cryptococcal meningitis, >99% have a positive cryptococcal antigen test. The second way in which the antigen test can be used is to follow response to therapy. Cryptococcal antigen can be quantitated in both serum and CSF by serially diluting these body fluids and determining the highest dilution (most dilute) that produces a positive agglutination reaction. This test can be used to follow the patient, with titers falling with successful therapy. Increases in titer may herald relapse (see answer to question 5 for further details). Both *C. neoformans* and *C. gattii* are positive in this test. Patients with sepsis due to *Trichosporon asahii* and *Capnocytophaga canimorsus* can have a false-positive cryptococcal antigen test.

5. Cryptococcal meningitis usually begins as an asymptomatic pulmonary infection. The patient is infected with *Cryptococcus* by inhaling it from the environment. The organism's

natural habitat is soil; it grows particularly well in pigeon droppings and other bird guano. Areas with large pigeon populations, such as urban parks, are places where exposure to this organism may be increased. Asymptomatic pulmonary infection may progress to fungemia and meningitis in the individual with defects in cell-mediated immunity, especially HIV-positive patients, the population in whom this infection is most frequently seen.

The widespread use of highly active antiretroviral therapy (HAART) began in the United States in 1995. HAART is a combination of antiretroviral drugs that usually includes a protease inhibitor. The effect of this therapy is to preserve immune function in some HIV-infected patients and to reconstitute it in others. The end result has been a reduction of opportunistic infections and a significant decrease in morbidity and mortality in HIV-infected patients. There was a marked decline in the number of cases of cryptococcal meningitis in our patient population at the University of North Carolina Hospitals beginning in the last quarter of 1994, a time period when many of our patients had begun to receive HAART in clinical trials. Most HIV patients who develop cryptococcal meningitis today are patients who have undiagnosed HIV infections. They typically have CD4 counts of <200/μl and often have CD4 counts of <100/μl.

However, the widespread use of HAART in patients such as the one presented here has led to the recognition of a new clinical syndrome, immune reconstitution inflammatory syndrome, or IRIS. IRIS can occur secondary to a variety of AIDS-related opportunistic infections. Many patients with documented cryptococcal meningitis have a "relapse" of meningitis usually between 1 to 2 months after initiating HAART therapy. The patients have clinical signs and symptoms of cryptococcal meningitis with fever, headache, and altered mental status; CSF white blood cell counts of 25 to 100/μl with a predominance of lymphocytes, and increased opening pressure during lumbar puncture. These patients generally, but not always, have negative cultures and declining CSF cryptococcal antigen levels. The disease process is believed to be due to a heightened inflammatory response to circulating antigens. Intensive antifungal therapy with the use of a therapeutic lumbar puncture to control intracranial pressure is currently recommended. Use of immune modulators in cryptococcal IRIS is controversial but is recommended in severe disease.

6. In an HIV-infected patient who has been treated for cryptococcosis with a corresponding drop in CSF or serum antigen titers, a subsequent rising CSF cryptococcal antigen level coupled with clinical symptoms may herald a relapse of his cryptococcal infection. In addition to being used to determine cryptococcal antigen levels, the CSF specimen should be cultured. Microscopic examination of CSF may be misleading in diagnosing active infection since nonviable yeast cells can remain visible in the CSF for weeks to months. Recent studies of cryptococcal meningitis relapse have shown that in most patients, the same genotype causes the initial infection and the relapse. In some patients, the genotype causing relapse is different from the genotype causing the initial infection. This second observation can be explained in two ways. One, the patient did not relapse but has developed a "new" infection. Since most patients do not make antibodies

to cryptococcal antigens, reinfection with a different genotype of *C. neoformans* would not be surprising. Alternatively, it has been shown that patients may be infected with multiple genotypes. In the initial infection one genotype could be predominant, while a second genotype could be predominant in the relapse.

7. Guidelines have been developed for the prevention of relapse of cryptococcal meningitis. The strategy is 2-fold. First, HAART should be used in HIV-infected patients. Because of concerns about cryptococcal IRIS, there has been some concern about starting HAART therapy concurrently with antifungal therapy. Further studies are needed to determine the timing of HAART in a patient being treated for cryptococcal meningitis. HIV-infected patients who develop cryptococcal meningitis have, as a rule, CD4 counts of <200/μl, and the risk of developing initial infection or relapse increases as the CD4 count declines below 200/μl. HAART has been shown to successfully reconstitute the immune response in many individuals, as evidenced by CD4 counts rising above 200/μl. In addition to attempts to reconstitute the immune system or prevent its decline, HIV-infected patients who develop cryptococcal meningitis receive antifungal prophylaxis until the CD4 count stabilizes above 200/μl. Oral fluconazole is the drug of choice for this purpose. There are two major problems with lifelong prophylactic antimicrobial therapy. First, compliance is an issue, especially when the patients must take very complex drug regimens including multiple antiretroviral agents. Most relapses of cryptococcal meningitis are due to failure to comply with either HAART or fluconazole prophylaxis or both. Second, development of drug resistance must also be considered as a potential problem. Fluconazole-resistant strains have been recovered from HIV patients who received fluconazole prophylactically, although these are currently rare.

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The patient was a 13-year-old male who had awakened 4 days previously with a headache. After 1 day of headache he visited the emergency department, where a head computed tomography scan was performed, which was negative. The patient subsequently presented with 3 days of vomiting, fevers to 38.5°C, and continued headache. His headaches had become increasingly severe throughout the 4 days and were accompanied by agitation and confusion. He had difficulty answering questions at nighttime. Two weeks previously he had visited the North Carolina mountains, where he spent approximately 8 hours at a swimming hole.

On physical examination his vital signs were normal, his neck was supple, and he did not have lymphadenopathy. A lumbar puncture revealed 1,354 red blood cells/μl and 198 white blood cells/μl with 86% neutrophils and 9% mononuclear cells. The cerebrospinal fluid (CSF) protein was 55 mg/dl and glucose was 71 mg/dl. Herpes simplex virus (HSV) PCR was negative, as was Rocky Mountain spotted fever and Lyme disease serologic testing. The bacterial Gram stain and culture were also negative. He was empirically started on vancomycin, ceftriaxone, acyclovir, and doxycyline, but when his bacterial culture and HSV PCR results were known, all antimicrobials except doxycycline were discontinued.

During hospitalization his mental status deteriorated, his heart rate dropped to 30 to 40 beats/min, and he was intubated in the pediatric intensive care unit. Head computed tomography and magnetic resonance imaging scans showed no evidence of increased intracranial pressure. He gradually improved and was discharged home after 7 days of hospitalization when his mental status returned to baseline. A serologic test revealed the etiologic agent of disease.

1. What was the clinical diagnosis of this patient? How do the case history and laboratory tests help to narrow this diagnosis?
2. The diagnosis of this infection is made by serologic testing, but the case presentation gives adequate clues that an educated guess can be made about the etiology of the infectious agent. What is the agent that caused his infection? To what larger group of organisms does this pathogen belong?
3. Describe the epidemiology of the agent that caused his infection. How did this patient likely get infected?
4. What are the advantages and disadvantages of using serologic testing to diagnose this infection? Why was a NAAT not the diagnostic test of choice?
5. Was this patient's outcome typical of this infection? Explain.



CASE DISCUSSION

1. This patient likely had encephalitis, or inflammation of the brain. Meningitis, on the other hand, is inflammation of the membranes and fluid surrounding the spinal cord. Although many symptoms of meningitis and encephalitis overlap (such as fever, headache, stiff neck, photophobia, fatigue, nausea, and vomiting), symptoms of encephalitis depend on the area of the brain affected and may also include cognitive dysfunction (confusion and impaired judgment), altered speech or gait, and even seizures. Often meningitis and encephalitis present concomitantly as meningoencephalitis, which can be clinically indistinguishable from encephalitis.

A variety of agents can cause meningitis or encephalitis, including bacteria, fungi, parasites, and viruses. The negative bacterial culture of the CSF rules out the most common bacterial pathogens causing meningitis in adolescents, including *Neisseria meningitidis* and *Streptococcus pneumoniae*. It is important that the lumbar puncture be performed prior to the administration of any antimicrobials so that the negative predictive value of the bacterial culture can be properly assessed. In addition, the number of white blood cells observed in the CSF is much lower than that typically seen with bacterial meningitis. Note, however, that there is a neutrophilic predominance in the CSF cell count. This can be confusing in the early stages of central nervous system disease, as many viruses cause an initial neutrophilic predominance followed by lymphocytic predominance. Lastly, the CSF chemistries are not consistent with bacterial meningitis, as the CSF protein is only slightly elevated and the CSF glucose is within normal limits. Although fungi can cause meningitis and encephalitis, this is more common in immunocompromised persons or individuals receiving steroidal injections for chronic back pain. Similarly, parasitic causes of meningitis and encephalitis are unusual. Although the patient had been in a swimming hole, potentially predisposing him to *Naegleria* infection and primary amebic meningoencephalitis, the water in the mountains was likely not warm enough to support the growth of the trophozoite stage of *Naegleria*. This is the infectious, pathogenic phase of the organism and likely to be found only in waters warmer than 25°C. In addition, his disease would likely have been more aggressive and potentially fatal. Parasitic encephalitis due to *Taenia solium* or *Toxoplasma gondii* occurs primarily in HIV-positive patients and those with other immunocompromising conditions. Viruses are the most commonly diagnosed cause of meningitis and encephalitis. The most common causes of viral meningitis are HSV and enteroviruses, while the most common causes of viral encephalitis are HSV and the arboviruses. Viral meningitis or encephalitis presents with CSF cell counts and chemistries consistent with this patient's. The serologic result is needed for a definitive diagnosis, but all available data support a diagnosis of viral encephalitis.

2. The incubation period, signs and symptoms, age of patient, and travel history are all consistent with La Crosse virus encephalitis. The incubation period is typically 5 to 15 days. Most infected patients are asymptomatic or have a nonspecific febrile illness, but

those with more severe disease present with fever (5%), meningitis (17%), meningoencephalitis (56%), or encephalitis (21%). Children under the age of 16 have a higher risk of developing acute encephalitis following infection, approximately half of whom will have seizures. Since La Crosse virus is transmitted by mosquitoes, it is a member of the larger group of viruses called the arboviruses (for arthropod-borne viruses). The arboviruses include many different viral families, but all are transmitted via blood feeding of a variety of vectors, including mosquitoes, biting midges, flies, and ticks.

3. La Crosse virus was first described in the 1960s after it was isolated from the brain of a fatal pediatric encephalitis case in La Crosse, WI. The virus has since been shown to cause infections not only in the upper Midwest but also in the mid-Atlantic region of the United States, with the highest incidence in West Virginia, North Carolina, Tennessee, and Ohio. The virus is transmitted primarily by the day-feeding Eastern treehole mosquito, *Ochlerotatus* (*Aedes*) *triseriatus*, with chipmunks and squirrels being the primary vertebrate hosts (or amplifying hosts). Humans are incidental hosts. As the name suggests, this mosquito is found in or near woods containing hardwood trees. The mosquitoes lay their eggs in treeholes and other places where water accumulates, such as discarded tires. La Crosse virus can be passed transovarially in the mosquito and can survive the winter, resulting in large-scale amplification of infected mosquitoes in spring and summer months. The virus can also be transmitted by a secondary day-feeding mosquito vector, the Asian tiger mosquito (*Aedes albopictus*), which expands the potential for human infections throughout the southeastern United States. Since the patient had visited a swimming hole in a hardwood forest 2 weeks prior to presentation, he likely got the infection during his visit to the North Carolina mountains.

4. The laboratory diagnosis of most arboviral diseases is best achieved using serum and CSF for serologic testing. There is an FDA-cleared indirect immunofluorescence assay for the detection of IgM and IgG to the California serogroup viruses from serum. As a California serogroup bunyavirus, La Crosse virus cross-reacts with other California serogroup viruses (California encephalitis, Jamestown Canyon, Keystone, snowshoe hare, and Trivittatus), but since it is the most common in the United States, a positive serologic result is a presumptive positive for La Crosse virus. Confirmatory testing should be performed by a public health laboratory using a plaque-reduction neutralization test and/or a capture enzyme-linked immunosorbent assay. Positive serologic results that indicate a confirmed case of La Crosse virus infection are a 4-fold or greater rise in antibody titer in acute- and convalescent-phase sera obtained 3 to 4 weeks apart, a positive IgM test from serum with virus-specific neutralizing antibodies demonstrated in the same or later specimen, or detection of virus-specific IgM antibodies in CSF. IgM antibodies are usually detectable 3 to 8 days after onset of illness and persist for 1 to 3 months, although IgM has been detected in some patients months to years after acute infection. Since most arboviral serologic testing is done through public health labs or large reference laboratories,

the results are often not obtained while the patient is still hospitalized. A confirmed diagnosis often requires both acute- and convalescent-phase sera and/or confirmatory testing, which grossly delays the diagnosis. Single-specimen testing has 62% sensitivity for IgM and 71% for IgG, with high specificity compared with paired serologic testing. Thus, a presumptive diagnosis may be possible with just one specimen in the appropriate clinical setting. Nucleic acid amplification tests (NAATs) for viral detection often provide rapid results with high sensitivity, but the arboviruses are an exception. In most arboviral diseases there is a short-lived viremia that ends shortly after disease presentation; thus, the sensitivity of these tests with blood or CSF is not high. However, the positive predictive value of a positive NAAT in this setting is nearly 100%. NAAT for arboviruses is the most sensitive from brain tissue and therefore is generally reserved for fatal cases where a combination of NAAT, histopathology with immunohistochemistry, and viral culture can be performed. NAATs can also be used to screen field-collected mosquito pools.

5. As mentioned above (see the answer to question 2), most people infected with La Crosse virus do not present with acute encephalitis. This patient had severe neuroinvasive disease, of which there are only ~100 cases reported annually in the United States. He had a significant hospital course requiring intubation and intensive care, but his mental status returned to baseline. In 2012, 97% of patients with neuroinvasive La Crosse virus infections were hospitalized; only 1 died. Children with La Crosse virus encephalitis infrequently have neurologic sequelae such as epilepsy, learning disabilities, or cognitive defects (2 to 15%). Death following infection is even more uncommon, with a reported case-fatality rate of 0.3 to 1.9%. Although there is no specific treatment for La Crosse encephalitis, it is generally considered to be a relatively benign disease with low morbidity and mortality.

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A 29-year-old man, who was originally from the Cape Verde Islands, was in his usual state of good health until the day of admission, when his family noted that he could not speak and could not move the right side of his body. He was taken to the hospital emergency department. Neurologic examination was notable for aphasia. On ophthalmologic exam, the optic nerves appeared normal. The patient also complained of hearing voices. His recent medical history was notable for negative HIV antibody test results. A head computed tomogram (CT) scan was performed; it was remarkable for the presence of a 1-cm ring-enhancing lesion with focal edema in the left temporal lobe (Fig. 50.1) and multiple small, thin-walled lesions less than 5 mm in diameter.

A lumbar puncture was performed. The opening pressure was within normal limits and the cerebrospinal fluid (CSF) demonstrated no cells, a normal glucose level, and a normal protein level.

1. What is the differential diagnosis of this patient's intracranial process? Serum and CSF were sent for serologic studies to the Centers for Disease Control and Prevention (CDC), where the clinical diagnosis was confirmed serologically. What is the likely diagnosis?
2. Are there any additional tests that might help to establish this diagnosis while the serologic studies are pending?
3. Which parasite causes this infection?
4. How do people become infected with this parasite? What is the outcome of the different types of infection that this organism causes? What in this patient's history indicates that he is at increased risk for this infection?

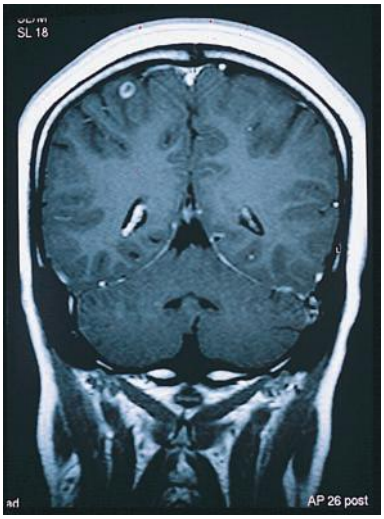


Figure 50.1



CASE DISCUSSION

1. This patient's signs and symptoms are consistent with a seizure and postictal neurologic abnormalities. Hearing voices can be explained by the temporal lobe location of the lesion. The presence of multiple intracranial lesions is consistent with a noninfectious process, such as cancer with metastases to the brain, as well as with several infectious processes. Multiple brain lesions would be consistent with central nervous system toxoplasmosis in an immunocompromised host. This patient had a negative serology for HIV and had no other clinical history suggesting immunosuppression, so toxoplasmosis was less likely. Central nervous system tuberculosis occasionally presents with tuberculomas in the brain and cannot be ruled out on the basis of the information provided in the history. A negative purified protein derivative (PPD) skin test or interferon gamma release assay (IGRA) would be helpful in decreasing the probability of the diagnosis of tuberculosis. Multiple brain abscesses are another possibility, but none of these possibilities would adequately explain the calcifications seen on radiologic examination (see the answer to question 2). As part of this patient's evaluation, serologic studies were performed by the CDC, which confirmed the diagnosis as cysticercosis, a parasitic infection due to the larval form of the tapeworm *Taenia solium*. Both serum and CSF should be sent for serologic studies in the setting of suspected cerebral cysticercosis.

2. An X-ray study of the soft tissues will frequently demonstrate "rice grain" calcifications (Fig. 50.2) in skeletal muscle that are essentially diagnostic of cysticercosis. An additional test that is of low yield but suggestive of cysticercosis is the presence of eosinophils in the CSF, a finding that can also occur in a number of other conditions. This patient, however, did not have any white blood cells in his CSF, so there would not be any CSF eosinophils.



Figure 50.2

3. Infection with the larval forms of the parasite *T. solium*, the pork tapeworm, causes cysticercosis. Infection with the beef tapeworm, *Taenia saginata*, does not cause cysticercosis in humans.

4. The form of infection that a patient develops due to *T. solium* is based on how she or he becomes infected with this parasite. Humans can be infected in three ways: by ingesting larvae in contaminated food, or by ingesting eggs from contaminated food or water or from unclean hands. Patients who develop adult *T. solium* tapeworm are infected by eating raw or undercooked pork containing cysticercus larvae. Following their ingestion, cystic-

ercus larvae are liberated in the stomach. The larval form migrates and attaches to the small intestine. Over a period of months it develops into the adult tapeworm. The worms reproduce there, and eggs are excreted in the feces. In this form of the disease, humans are the definite host. The worm is highly adapted to its definitive host, and may cause little clinical disease. As a result, most individuals with *T. solium* tapeworms will be asymptomatic.

However, humans can become intermediate hosts in the same manner that pigs do, by ingesting eggs excreted by humans. This can occur either by ingestion of food or water contaminated with human feces containing *T. solium* eggs, or by autoinfection, where eggs are ingested after defecation and failure to properly clean hands. When *T. solium* eggs are ingested, either by autoinfection from the hands of infected individuals or in fecally contaminated food or water, gastric acid and pancreatic enzymes cause the release of oncospheres (motile larvae) that penetrate the intestinal wall and are disseminated in the blood, from which they can encyst in a variety of tissues including the brain, eyes, and skeletal muscle. The formation of tissue cysts only occurs following ingestion of the eggs and does not follow ingestion of cysts. In this form of the infection, humans are intermediate hosts; the parasite's life cycle cannot be completed, and the larval form never develops into the adult worm.

In neurocysticerosis, the most common form of human clinical disease, the most prominent features are seizure activity, which occurs in approximately 80% of patients, and headache, which occurs in 40%. The lesions that lead to clinical symptoms are most commonly found in the brain parenchyma. The cysticerci lodge in small vessels in the parenchyma and form cysts. When these larval forms eventually die, calcification occurs around the degenerating larval form. In most infected patients, the natural history of disease is believed to be the development of parenchymal cyst, death of the larva, and resolution of the cysts without clinical symptoms. In a subset of patients, probably <10%, seizures may occur. It is thought that these seizures are triggered by an inflammatory reaction that occurs in response to the release of antigens from the dying larvae. Seizures are more common in patients who have multiple cysts. They may also occur in patients who have larval-associated calcifications, although the exact reason for these seizures is not known. This form of the disease is treated with antihelminth agents such as albendazole or praziquantel, corticosteroids, and antiepileptic drugs. There are no proper double-blind clinical trials that support the use of any of these therapies, although symptomatic treatment with antiepileptic and anti-inflammatory drugs is intuitive. The value of antihelminth therapy has not been established but it nevertheless continues to be used.

A second, much less common form of disease is extraparenchymal neurocysticercosis. In this form of the disease, cysts are most frequently found in the brain ventricles and may cause mechanical obstruction of cerebrospinal fluid flow, resulting in hydrocephalus and inflammation with the potential for brain herniation. Patients with this form of the disease typically do not have seizures but rather symptoms associated with hydrocephalus such as headache, nausea, dizziness, and vomiting. Surgical intervention is needed to relieve the

hydrocephalus and surgically remove any cysts, as this form of the disease can be fatal. Many of the deaths due to neurocysticercosis that occur globally are likely due to this form of disease.

Most of the world's estimated 50 million cysticercosis cases occur in countries with poor sanitation. Most of the cases identified in the United States are immigrants from areas of Mexico and other Latin American countries with poor sanitation. It is also a common disease in sub-Saharan Africa (where the Cape Verde Islands are located), India, China, Southeast Asia, and Indonesia. Cysticercosis is the major cause of adult-onset seizure disorders in Latin America, where it is responsible for neurologic symptoms in ~400,000 people. In the United States, it is responsible for approximately 10% of adults presenting with seizures in Los Angeles. This patient's geographic location and his adult-onset seizure disorder are significant factors to consider in the differential diagnosis.

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The patient was a 36-year-old HIV-positive male with a CD4 count of 60/μl. He previously had *Pneumocystis jirovecii* pneumonia for which he continued to receive prophylaxis with aerosolized pentamidine. At presentation he also had intermittent diarrhea due to *Cryptosporidium*. He was on a study protocol evaluating the efficacy of a highly active antiretroviral therapy (HAART). He had had compliance issues with his antiretroviral therapy in the past, and at this presentation it was unclear whether he had been taking his antiretrovirals regularly. It was known that he had missed some of his aerosolized pentamidine treatments.

He presented with complaints of headaches, weakness, and difficulty maintaining his balance while walking. A friend who brought the patient to the emergency room related that the patient had seemed quite agitated over the previous few days, his speech was slurred, and he made several inappropriate comments. The friend brought the patient to the emergency room when the patient failed to recognize him.

On physical examination the patient was afebrile with normal vital signs. He was not oriented to time or place, his speech was slurred and inappropriate, and he was unable to count backward from 100 by 7. A lumbar puncture was performed, which revealed 68 white blood cells per μl with 78% lymphocytes and 20% monocytes, a protein level of 67 mg/dl, and a glucose level of 55 mg/dl. A cryptococcal antigen test of the cerebrospinal fluid (CSF) was negative. Imaging of the brain revealed multiple ring-enhancing lesions (Fig. 51.1).

1. What clinical entity did this patient have? Did he have meningitis, encephalitis, a brain abscess, a brain tumor, or something else? Do you think an infectious agent caused this patient's symptoms? How would you confirm this diagnosis?

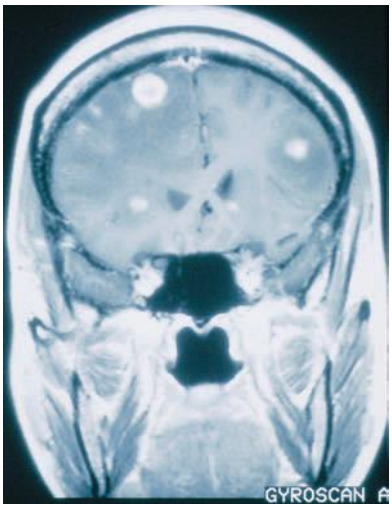


Figure 51.1

2. Do you believe this represents an acute infection or a reactivation of a latent one? Explain your answer.
3. Name two ways individuals can become infected with this organism. What stage of the parasite is found in each transmission mode? How could each mode of transmission be prevented?
4. What other populations are at increased risk for debilitating or life-threatening infections with this organism?

CASE DISCUSSION



1. The differential diagnosis of ring-enhancing lesions seen on head computed tomography scan includes both infectious and noninfectious diseases. The presence of this type of lesion indicates that there has been some breakdown in the normally intact blood-brain barrier.

Of noninfectious causes, malignancy is the most common process. The presence of multiple ring-enhancing lesions would suggest the possibility of metastatic cancer (for example, squamous cell carcinoma of the lung) with spread to multiple sites within the brain. However, in a patient with AIDS, a far more likely malignancy would be central nervous system (CNS) lymphoma. This must be considered in this patient's differential diagnosis.

Of infectious causes, a process that can spread hematogenously to multiple sites within the brain is a possibility. Such processes would include septic emboli from a vegetation on a heart valve, as would be seen in infective endocarditis; from other endovascular infections; or from lung abscesses. These processes can result in multiple brain abscesses with resulting loss of the blood-brain barrier, which would be seen as ring-enhancing lesions on head computed tomography. It would be important to know if this patient was actively injecting illicit drugs or if he had other infections that could result in infective endocarditis.

CNS infection with *Mycobacterium tuberculosis* may result in CNS tuberculomas, and similarly, infection with *Cryptococcus neoformans* may result in the presence of cryptococcomas. Although both infections occur with an increased frequency in patients with AIDS, these are not extremely common causes of ring-enhancing CNS lesions.

The most common cause of ring-enhancing CNS lesions in patients with AIDS is toxoplasmosis, caused by *Toxoplasma gondii*. The definitive diagnosis of cerebral toxoplasmosis requires the visualization of tachyzoites (actively replicating form of *Toxoplasma*) during histologic examination of a brain biopsy. (Figure 51.2 shows a positive biopsy from another patient with toxoplasmosis.) A presumptive diagnosis can be made using either molecular or serologic tests. Although a positive *Toxoplasma* PCR from a brain biopsy or CSF is diagnostic, the clinical sensitivity of PCR varies greatly depending on the target being amplified and can be well below 50%, particularly for CSF; the sensitivity of PCR on blood can be even lower. Further, there are no commercially available assays for *Toxoplasma* PCR, making such testing difficult for many laboratories. On the other hand, serologic testing for *Toxoplasma* is widely available. Although a positive IgG indicates *Toxoplasma* exposure, it does not distinguish reactivation disease from latent infection. It should be noted that *Toxoplasma* IgM antibodies can persist for years

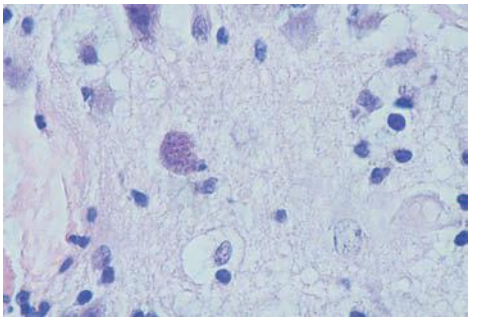


Figure 51.2

after primary infection, but are rarely seen with cerebral toxoplasmosis. A negative *Toxoplasma* serology, particularly in immunocompromised patients, does not exclude the diagnosis of cerebral toxoplasmosis. However, a positive result in the appropriate clinical setting can provide a presumptive diagnosis leading to a trial of anti-*Toxoplasma* therapy. A presumptive diagnosis is confirmed when the patient clinically responds to appropriate therapy within 10 to 14 days. *T. gondii* CNS infections are seen less commonly in developed countries than they used to as a result of the use of HAART. In this patient, his lack of compliance with HAART therapy likely resulted in his continued immunosuppression, which permitted the progression of a dormant *T. gondii* infection.

2. *Toxoplasma* infection in AIDS patients is typically due to reactivation of a latent parasitic infection rather than an acute infection. The initial infection, which is often not noticed, may have occurred years prior to the patient's infection with HIV and subsequent suppression of cell-mediated immunity.

3. Infection with *T. gondii* most commonly occurs by ingestion of infective oocysts. An infected cat, the definitive host for *T. gondii*, sheds unsporulated oocysts that require several days before they convert to infective oocysts. Ingestion of infective oocysts in the environment (from cat feces) or of trophozoites present in cysts in undercooked or raw meat is the means by which primary infections typically occur. Alternatively, transplacental infection, in which a fetus acquires *T. gondii* during the parasitemia that occurs during the mother's primary toxoplasma infection, can occur as well. These infections are potentially fatal, especially in the first trimester of pregnancy. Transmission of *T. gondii* has also been documented to occur as a result of organ transplantation and blood transfusions.

Prevention of toxoplasmosis includes eating meat that has been properly cooked (to kill any viable cysts) and, particularly for pregnant women, only changing the litter box within 24 hours after the cat has defecated. This does not give the oocysts time to become infectious. The use of gloves and hand washing when handling cat litter is important to prevent fecal-oral spread of the oocysts. If the pregnant woman is a gardener, wearing gloves while working with soil is another important preventive measure, because cats may defecate there as well. In some countries, such as Austria and France, prenatal screening and treatment for toxoplasmosis are performed, though the risk-benefit ratio of such a program is unclear.

4. In addition to AIDS patients, infants who acquire congenital toxoplasmosis may have a poor outcome. Clinically, the infected infant may have cerebral calcifications, seizure disorder, hydrocephalus, or ocular involvement. The disease may be fatal, or it may result in learning disabilities. Often congenital toxoplasmosis can be diagnosed *in utero* using PCR on amniotic fluid.

Another group of patients at high risk for toxoplasmosis is organ transplant recipients, particularly heart and heart-lung transplant recipients. In addition to reactivation, which may occur due to immunosuppression that is used to prevent organ rejection, an explana-

tion for the increased risk in this patient population is that a solid organ, such as the transplanted donor heart, may contain viable trophozoites within cysts. Needless to say, in transplanting the heart, a large amount of muscle mass that may be latently infected with viable trophozoites will be transferred to the organ recipient. This risk in cardiac transplant patients is high enough that many centers routinely give such patients prophylactic anti-*Toxoplasma* therapy beginning at the time of transplant.

Other patients at increased risk for reactivation resulting in toxoplasmosis are those with lymphoma and leukemia who are immunosuppressed as a result of their underlying disease as well as cytotoxic drug therapy.

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The patient was a 6-month-old male who presented with a 3-day history of increasing hypotonia and a 1-day history of dehydration. Three days prior to admission the mother had noticed decreased suck while breast-feeding. The next day it took him 1 hour to take his normal amount of breast milk. He normally took only 15 minutes. During the evening 2 days prior to admission, the parents noticed that the infant had generalized weakness with decreased movement and difficulty sitting up. That evening the child would not breast-feed. The morning prior to admission the parents noted that the infant was increasingly floppy and took only 1 oz of breast milk (versus his usual 4½ oz) and 1 oz of juice. He had trouble with gurgling in the back of his throat, very poor head control, and increased floppiness. They took him to a local physician, who noted severe hypotonia and dehydration. The parents were told to take their son to the local emergency room. On review of systems, it was noted that the child began to be constipated 4 weeks previously and had had only two small stools over the last 6 days. His diet consisted of breast milk, occasional sweet potatoes, and rice cereal. Abnormal findings on physical examination consisted of generalized hypotonia with head lag. Cerebrospinal fluid findings were within normal limits. The patient was admitted to the pediatric intensive care unit. Because of increasing respiratory difficulty, he was intubated. He remained on the ventilator for 6 days, was extubated, and was discharged home 11 days after admission. A stool specimen obtained at admission was positive in a mouse lethality test.

1. What condition did this child have? What clinical clues are present that help you make this diagnosis?
2. What organism caused this condition? Briefly describe the epidemiology and pathogenesis of this disease.
3. What is the mouse lethality test? Why can't some other test like enzyme immunoassay, culture, or PCR be done to diagnosis this illness?
4. How is this form of the disease typically treated?
5. What other forms of disease can be seen with this organism? Describe the similarities and differences in these different forms of the disease.
6. Why is there increased concern about this organism among governmental agencies such as the Department of Defense, the Centers for Disease Control and Prevention, and the Federal Bureau of Investigation?
7. The agent causing this child's illness has been used therapeutically. How is it used, and for what conditions is it used? What risks have been associated with its therapeutic use?



CASE DISCUSSION

1. This patient had infant botulism. This diagnosis must be considered in any infant who has the constellation of symptoms seen in this child. The disease is characterized by descending paralysis. The initial signs of the disease include constipation; poor suck (which is typically noticed by breast-feeding mothers); and increasing hypotonia, frequently first characterized by the child's inability to hold up its head and by ptosis (drooping of the upper eyelids). Because infant botulism is rare, the clinical manifestations seen are frequently attributed to sepsis, central nervous system infections, or other, more esoteric diagnoses such as Guillain-Barré syndrome or inborn errors of amino acid metabolism. The misdiagnosis of sepsis may further complicate the disease course of these children. (See answer to question 4 for further details.)

2. The patient is infected with *Clostridium botulinum*. *C. botulinum* produces a neurotoxin called botulinum toxin, which, on a weight basis, is the most potent biologic toxin known. There are seven types of botulinum toxin, designated A to G, with types A, B, and E being responsible for most human disease. Two other *Clostridium* species, *C. baratii* and *C. butyricum*, have been shown to cause human disease via the production of botulinum toxin.

Infant botulism is the most commonly seen form of botulism in the United States. The highest incidences of disease are in California, due primarily to type A botulinum toxin, and in the Delaware Valley area of Pennsylvania and New Jersey, involving primarily type B botulinum toxin. Toxin A-producing strains are the predominant type in soil in California, while toxin B-producing strains predominate east of the Mississippi River. This disease occurs sporadically; no outbreaks of infant botulism have been reported. In this disease, spores of the organisms are ingested either in foodstuffs (e.g., honey) or from dust. The peak incidence of infant botulism is at 2 to 4 months of age, with formula-fed babies developing disease earlier than breast-fed babies, perhaps reflecting differences in the intestinal microbiome of those two populations. It is important to note that most cases of infant botulism occur around the time of weaning. At this time, the infant's intestinal microbiota undergoes significant change and it is this change that is believed to provide an ecological niche for the growth of and toxin production by this organism. An adult form of "infant botulism" has also been postulated since the source of some cases of adult botulism cannot be identified. These individuals typically have had gastrointestinal surgery, have chronic gastrointestinal disease such as inflammatory bowel disease, or have had recent antibiotic treatment. A toxin-producing organism can be recovered from the gastrointestinal tract of these patients.

C. botulinum produces toxin in the gastrointestinal tract that is absorbed into the bloodstream and binds irreversibly to the presynaptic nerve endings. The toxin, like many exotoxins, consists of two chains or subunits designated A (enzymatically active) and B (binding). For the toxin to be biologically active, it must be cleaved by a protease. Once

cleaved, the B subunit binds to neurons and forms a pore in the neuronal membrane through which the A subunit can enter the cell. There it blocks the release of the neurotransmitter acetylcholine by preventing its exocytosis. Acetylcholine release is necessary for the excitation of the muscle fibers. This blockage of neurotransmitter release results in the flaccid paralysis seen in this disease.

3. The mouse lethality assay is the standard test used to detect botulinum toxin in serum, foodstuffs, and stool specimens. Mouse lethality studies are used for research and epidemiologic purposes to study other microbial toxins including those responsible for paralytic shellfish poisoning (algal saxitoxins) but are clinically used almost exclusively for botulinum toxin detection. In this test, serum, food, or stool filtrates are injected intraperitoneally into mice and the animals are observed over a 96-hour period for the development of paralysis. Typically the paralysis begins in the hind legs and eventually results in the death of the animal. To ensure that this paralysis is specifically due to botulinum toxin, control mice are injected with an aliquot of the serum or stool specimen that has been preincubated with polyvalent antiserum raised against the different botulinum toxin types. Animals receiving this mixture will not develop paralysis if the paralysis is due to botulinum toxin. This assay is very sensitive and specific, but it is also laborious and slow, so alternative methods would be beneficial.

The diagnosis of botulism is dependent on the demonstration of toxin in feces, serum, or in the case of food-borne botulism, implicated food (see answer to question 5 for more details on this form of botulism). Enzyme immunoassays for botulinum toxin detection are generally not as sensitive as the mouse lethality assay and as such have not replaced this assay.

Culture has been found to be useful for epidemiologic purposes but has limited diagnostic utility because it is less sensitive than the mouse lethality test for the detection of *C. botulinum* toxin in feces.

Molecular detection of *C. botulinum* toxin genes directly in feces by amplification techniques such as PCR would seem like an ideal approach for detection of this organism. Although a number of reports have described PCR techniques for detection of *C. botulinum*, few have been used with feces. Thus far, PCR has not proven to be more sensitive than the mouse lethality test for detection of *C. botulinum* in feces. Enrichment cultures may enhance the sensitivity of PCR, but clinical studies of this approach are limited.

4. Death from botulism is typically due to respiratory arrest from paralysis of the diaphragmatic muscles involved in breathing. As a result, mechanical ventilatory support is central to therapy for all forms of botulism. Typically, ventilatory support is required for 2 to 8 weeks while the affected nerve endings regenerate.

Human botulism immune globulin (BIG-IV) has been developed to treat infant botulism. This antitoxin is made from pooled human plasma from volunteers immunized with a pentavalent botulinum toxoid who made high titers of neutralizing antibodies to botulinum

toxin A and B. The plasma is treated in such a manner as to inactivate a variety of viruses (HIV and hepatitis B and C, to name a few) that can infect humans. Treatment with this antitoxin reduced the length of hospital stay, time on a ventilator, and time in an intensive care unit. These data make it clear BIG-IV must be considered an important component of therapy for infant botulism. However, BIG-IV cannot reverse the pathology that has already occurred since the toxin was internalized.

Many patients with infant botulism are initially believed to have sepsis. As a result, they may receive empiric antimicrobial therapy, which includes an aminoglycoside. Aminoglycosides are contraindicated in patients with botulism because they have been shown to potentiate the activity of the toxin. Therefore, in patients with ptosis or hypotonia, the use of aminoglycosides should be avoided until the diagnosis of botulism can be ruled out.

5. There are two other forms of naturally occurring botulism, food-borne and wound botulism. In food-borne botulism, the toxin is preformed in food, typically canned goods or smoked fish (especially type E toxin) or meat, and ingested with the food. This disease is an intoxication rather than an infection, as is seen in both infant and wound botulism where toxin is produced in the host. Food-borne botulism tends to cause outbreaks of disease. These outbreaks are most commonly associated with improperly home-canned vegetables and usually are small, affecting only family members. In these outbreaks, spores of *C. botulinum* are not killed by the canning or other food-processing techniques. An anaerobic environment is produced, the spores vegetate, and the organism grows, producing toxin. When the food is then consumed, it is either not heated at all (smoked meats or fish) or not heated to temperatures sufficient to inactivate the toxin (canned vegetables and soups). Outbreaks in which large numbers of individuals become ill are almost always associated with commercially prepared foods. Alaska has the highest rate of food-borne botulism in the United States, due to the consumption of native Inuit foods such as fermented fish and fermented marine mammals, in which anaerobic conditions permit the growth of *C. botulinum* and the elaboration of its toxin.

In wound botulism, the organism is introduced into a wound with devitalized tissue. This dead tissue provides an anaerobic environment where the organism can grow and produce toxin that can enter the bloodstream. Wound botulism has been seen with increasing frequency in users of black tar heroin who inject the drug by "skin popping," i.e., intradermal injection of the drug. In wound botulism, the organism can be detected in the wound by culture in addition to detection of the toxin in serum. These patients should be treated with both antitoxin and antibiotics. Penicillin G is the antibiotic of choice. These patients may also require ventilatory assistance. Interestingly, two patients have been described in the literature who have had two episodes of wound botulism associated with intradermal drug use, and another individual has been reported to have had two episodes of food-borne botulism. This suggests that the initial toxin dose was not sufficient for them to mount a protective immune response that could prevent subsequent episodes.

6. Botulinum toxin has been "weaponized" by several countries. During the Gulf War, missiles with warheads containing botulinum toxin were reported to have been produced by Iraq, although there was never any evidence that these bioweapons were used. Botulinum toxin is recognized as a potential weapon of bioterrorists. The toxin in crude form is easily produced. Animal studies have shown that the toxin can enter the bloodstream following inhalation, making it theoretically possible to deliver this agent by aerosol. Contamination of various foods is another possible scenario by which this toxin could be used to attack a population. Because the medical management of botulism often requires ventilatory support, and the number of ventilators and the skilled individuals to support their use is limited, a successful bioterrorism attack on a large population with this toxin is of great concern to governmental agencies.

7. Botulinum toxin has been developed to treat disorders associated with hyperactivity of cholinergic nerve endings. A small dose, between 30 and 300 units, is injected into the target muscle. There is a long list of spastic disorders that have been treated successfully with botulinum toxin, but FDA-approved indications are limited and include neck dystonia, strabismus, blepharospasm, and certain cosmetic uses (wrinkles around the eyes and the forehead). Interestingly, there are experimental data that suggest botulinum toxin may be useful in the treatment of migraine headaches. The toxin is active for weeks, so it is given infrequently and in minute quantities to minimize the likelihood that individuals chronically treated with botulinum toxin will develop neutralizing antibodies that render it biologically inactive. When administered as recommended, this agent is remarkably safe. However, a recent event occurred where four individuals including the treating physician received botulinum toxin for cosmetic reasons. The toxin used was an unlicensed preparation designed for research rather than human use. The physician miscalculated the dose and it was estimated that each individual received the equivalent of from 20 to 40 human lethal doses. All four required long-term hospitalization (at least 6 weeks), including a minimum of 5 weeks of ventilator support. Fortunately, none of the four died. The treating physician was sentenced to 3 years in prison for using this unlicensed toxin preparation.

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SECTION SIX



SYSTEMIC INFECTIONS

INTRODUCTION TO SECTION VI

Systemic infections can be caused by many different infectious agents: bacterial, fungal, viral, and parasitic. One common finding for all systemic infections is the need for a **portal of entry**. The portal of entry can be via the skin (as in mosquito-borne diseases such as malaria), via the oral route (as in typhoid fever), via sexual contact (as in HIV infection), as a blood-borne pathogen (as in hepatitis B virus infection), via the respiratory tract (as in measles), and by vertical transmission via transplacental infection (as in congenital cytomegalovirus infection). In many cases of systemic infection, **colonization** occurs prior to the dissemination of the infectious agent throughout the body. In some diseases (e.g., tetanus and diphtheria), the infection itself is caused by a noninvasive organism and the systemic symptoms are caused by the dissemination of a **toxin** that is responsible for the disease. In most cases, however, the etiologic agent is disseminated via the hematogenous route.

If the portal of entry is clinically determined by physical examination or on the basis of radiologic studies, it can help to predict the etiology of the systemic infection. Thus, a patient who has bacteremia and on examination has physical findings consistent with pneumonia may well have *Streptococcus pneumoniae* in the blood. Similarly, an individual with flank pain and the presence of costovertebral angle tenderness compatible with pyelonephritis may have bacteremia with *Escherichia coli*.

When a patient has bacteremia or fungemia, crucial questions that should be answered quickly include:

1. What is the organism(s) causing the infection?
2. What is the portal of entry and, potentially, the infected site (lung, kidney, biliary tree, intra-abdominal or pelvic infection, skin, etc.)?
3. During the time that the organism has been in the blood, has it seeded other sites?

Certain principles are worth recognizing in assessing where an organism may have seeded during the time of bacteremia or fungemia. First of all, anatomically abnormal sites are more likely to become infected than are anatomically normal sites. Thus, during the bacteremia that may occur from dental work, an abnormal or prosthetic heart valve is more likely to become infected with oral streptococci than is a normal valve. Similarly, during staphylococcal bacteremia, an abnormal joint, such as may occur in a patient with rheumatoid arthritis, or a prosthetic joint is more likely to become infected than is a normal joint. As a final example, elderly patients with atherosclerotic arteries are more likely to have infection of these blood vessels during *Salmonella* bacteremia (especially *S. enterica* serovar Choleraesuis) than are younger individuals without atherosclerotic arteries.

Second, specific organisms are more likely to seed certain locations than are other organisms. For example, the yeast *Candida albicans* is more likely than are many other organisms to cause the serious eye infection endophthalmitis if it is present in the blood. This is of particular importance in the intensive care unit setting, in which many of the

cases of *Candida* bloodstream infections occur and where the patients are very often unable to sense or communicate any change in vision. Knowledge about what *C. albicans* may "seed" during a bloodstream infection should prompt regular eye examinations in an unconscious patient with candidemia. This may prevent irreversible damage, including blindness, in an eye.

Patients may have certain risk factors or defects in host defenses that predispose them to specific types of infections. Examples of defects in host defenses that predispose to certain specific types of infections include breaches in the integrity of the skin (patients with burns, patients with invasive medical devices), defects in cell-mediated immunity (AIDS, corticosteroid use), defects in humoral immunity (hypogammaglobulinemia), decreased splenic function (splenectomy, sickle cell disease), quantitative defects in neutrophils (neutropenia following chemotherapy), qualitative defects in neutrophils (chronic granulomatous disease, Chediak-Higashi syndrome), and deficiencies in the complement system. It is important to be able to recognize these risk factors when they are present and to understand the defect that predisposes the patient. Conversely, it is important to be able to suspect a specific defect in host defenses when a patient presents with a systemic infection. It may be that the defect is only recognized as the result of a specific infection, such as the presence of deficiencies in the complement system when *Neisseria meningitidis* bacteremia is diagnosed. Likewise, colon carcinoma may be first suspected as a result of the identification of a bacteremic infection as due to *Streptococcus gallolyticus* subsp. *gallolyticus* (formerly *Streptococcus bovis* biotype I) or *Clostridium septicum*.

Protection of the host from a systemic infection can occur as a result of acquired immunity due to a prior infection or due to a vaccination against that agent. Unfortunately, efficacious vaccines are not available for the majority of infectious agents, and in many diseases, infection does not lead to protective immunity.

Important agents of systemic infection are listed in Table VI. Please note that virtually all bacteria can potentially be isolated from the blood under circumstances of specific host defects, such as the presence of an intravenous catheter. Many of the etiologic agents listed have a particular organ tropism (such as the liver for hepatitis viruses) but may also cause systemic illness.

TABLE VI SELECTED SYSTEMIC PATHOGENS

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Bacteria			
Acinetobacter spp.	Glucose-nonfermenting, Gram-negative bacilli	Exogenous	Health care-associated UTI,[a] health care-associated pneumonia, health care-associated and line-related bacteremia
Bacillus anthracis	Aerobic, Gram-positive bacillus	Exogenous; zoonosis; possible agent of bioterrorism	Cutaneous, pulmonary (with hemorrhagic mediastinitis), gastrointestinal, bacteremia, meningitis
Bartonella henselae	Fastidious, Gram-negative bacillus	Exogenous; cats appear to be primary host	Cat scratch disease; bacillary angiomatosis (in immunocompromised hosts)
Borrelia burgdorferi	Spirochete	Exogenous; tick to human	Lyme disease; rash, arthritis, nervous system and cardiac manifestations
Brucella spp.	Oxidase-positive, fastidious, Gram-negative bacilli	Exogenous; zoonosis; possible agent of bioterrorism	Lymphadenopathy, hepatosplenomegaly, genitourinary, bone, and CNS[b] infection
Clostridium botulinum	Anaerobic, Gram-positive bacillus	Exogenous; improperly canned food; possible agent of bioterrorism	Botulism; flaccid paralysis with prominent cranial nerve symptoms
Clostridium perfringens	Anaerobic, Gram-positive bacillus	Exogenous	Wound infection, gas gangrene, bacteremia, gangrenous cholecystitis, food poisoning
Clostridium tetani	Anaerobic, Gram-positive bacillus	Exogenous	Tetanus
Coagulase-negative staphylococci	Catalase-positive, coagulase-negative, Gram-positive cocci	Endogenous	Health care-associated and line-related bacteremia, prosthetic valve endocarditis
Corynebacterium diphtheriae	Aerobic, Gram-positive bacillus	Exogenous	Diphtheria
Enterobacter spp.	Lactose-fermenting, Gram-negative bacilli	Endogenous	Community-associated and health care-associated UTI, bacteremia, endocarditis
Enterococcus spp.	Catalase-negative, Gram-positive cocci	Endogenous	Wound infections, health care-associated UTI, bacteremia, intra-abdominal infections
Escherichia coli	Lactose-fermenting, Gram-negative bacillus	Endogenous	Community-associated and health care-associated UTI, bacteremia, intra-abdominal infections, health care-associated pneumonia

Francisella tularensis	Fastidious, Gram-negative bacillus	Exogenous; zoonosis; tick to human; direct contact with animal; inhalation; ingestion of contaminated food or water; possible agent of bioterrorism	Skin ulcer, lymphadenopathy, ocular involvement, bacteremia, pneumonia
Group A streptococci (*Streptococcus pyogenes*)	Catalase-negative, Gram-positive cocci	Exogenous	Pharyngitis, cellulitis, bacteremia, scarlet fever, necrotizing fasciitis, pneumonia, poststreptococcal glomerulonephritis and rheumatic fever
Group B streptococci (*Streptococcus agalactiae*)	Catalase-negative, Gram-positive cocci	Endogenous	Sepsis, meningitis, cellulitis, chronic wounds and UTIs (diabetic)
Klebsiella pneumoniae	Lactose-fermenting, Gram-negative bacillus	Endogenous	Community-associated and health care-associated UTI, bacteremia, intra-abdominal infections
Mycobacterium avium complex	Acid-fast bacilli	Exogenous	Disseminated disease
Mycobacterium tuberculosis	Acid-fast bacillus	Respiratory; may be exogenous (primary) or endogenous (reactivation)	Chronic pneumonia with or without cavitation, adenopathy, gastrointestinal involvement, pleural involvement, peritonitis, meningitis, bone infection, genitourinary infection, miliary tuberculosis
Neisseria gonorrhoeae	Oxidase-positive, Gram-negative diplococcus	Exogenous; direct sexual contact; vertical, mother to child	Urethritis, cervicitis, pharyngitis, pelvic inflammatory disease, proctitis, bacteremia, septic arthritis, conjunctivitis
Neisseria meningitidis	Oxidase-positive, Gram-negative diplococcus	Exogenous	Meningitis, bacteremia, pneumonia
Pasteurella multocida	Oxidase-positive, Gram-negative bacillus	Exogenous; zoonosis (often animal bite or scratch)	Cellulitis, bacteremia, osteomyelitis, meningitis, pneumonia
Proteus mirabilis	Lactose-nonfermenting, Gram-negative bacillus	Endogenous	Community-associated and health care-associated UTI, bacteremia
Pseudomonas aeruginosa	Glucose-nonfermenting, oxidase-positive, Gram-negative bacillus	Exogenous	Community-associated and health care-associated UTI, health care-associated pneumonia, health care-associated bacteremia, chronic pulmonary infections in patients with cystic fibrosis, chronic wounds and osteomyelitis
Rickettsia rickettsii	Rickettsial organism	Exogenous; tick to human	Rocky Mountain spotted fever
Salmonella enterica serovar Typhi	Lactose-nonfermenting, Gram-negative bacillus	Exogenous; human to human via contaminated food or water	Typhoid fever, bacteremia, intestinal disease, perforation of colon

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TABLE VI SELECTED SYSTEMIC PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Staphylococcus aureus	Catalase-positive, coagulase-positive, Gram-positive coccus	Endogenous, exogenous	Skin infections, bacteremia, endocarditis, osteomyelitis, septic arthritis, pneumonia, food poisoning
Streptococcus pneumoniae	Catalase-negative, Gram-positive coccus	Endogenous	Community-acquired pneumonia, sinusitis, otitis media, bacteremia, meningitis, endocarditis, septic arthritis, peritonitis
Treponema pallidum	Spirochete (does not Gram stain)	Exogenous; direct sexual contact; vertical, mother to child	Primary, secondary, latent, and late syphilis; can affect any organ
Viridans group streptococci	Catalase-negative, Gram-positive cocci	Endogenous	Dental caries, endocarditis, bacteremia, abscesses
Yersinia pestis	Gram-negative bacillus	Zoonosis; flea to human; person to person in pneumonic form; possible agent of bioterrorism	Localized lymphadenopathy (bubonic), high-grade bacteremia, pneumonia, meningitis
Fungi			
Aspergillus spp.	Molds with septate hyphae	Exogenous	Pneumonia, sinusitis, external otitis, allergic processes, disseminated infection
Blastomyces dermatitidis	Dimorphic mold	Exogenous	Pneumonia, meningitis, bone and prostate infections
Candida albicans	Yeast, often germ tube positive	Endogenous	Thrush, vaginal yeast infection, diaper rash, esophagitis, health care-associated bloodstream infection
Candida spp., non-*albicans*	Yeasts, germ tube negative	Endogenous	Thrush, vaginal yeast infection, health care-associated bloodstream infection
Coccidioides immitis	Dimorphic mold	Exogenous	Pneumonia, meningitis, bone infection
Cryptococcus neoformans	Encapsulated yeast	Exogenous	Meningitis, pneumonia, bloodstream infection
Histoplasma capsulatum	Dimorphic mold	Exogenous	Pneumonia, disseminated infection
Paracoccidioides brasiliensis	Dimorphic mold	Exogenous	Ulcerative mucosal lesions in the mouth, nose, larynx and oropharynx, lung, skin, other organs
Penicillium marneffei	Dimorphic mold	Exogenous	Disseminated disease in immunocompromised; skin lesions, lung involvement
Zygomycetes	Molds with aseptate hyphae	Exogenous	Pneumonia, sinusitis, invasive infection

Parasites			
Babesia microti	Can be seen on peripheral blood smear	Exogenous; tick to human	Babesiosis
Leishmania donovani	Amastigotes in tissue touch preparation	Exogenous (*Phlebotomus* sand flies)	Kala-azar
Plasmodium spp.	Can be seen on peripheral blood smear	Exogenous (*Anopheles* mosquitoes)	Malaria
Strongyloides stercoralis	Nematode	Exogenous; endogenous (autoinfection and hyperinfection)	Gastrointestinal, pulmonary (pneumonia, wheezing); disseminated in hyperinfection
Taenia solium	Tapeworm	Exogenous (consumption of pig meat; fecal-oral from humans passing eggs)	Gastrointestinal infection, cysticercosis (brain, muscles, other organs)
Toxoplasma gondii	Protozoan	Exogenous; endogenous (reactivation)	CNS, ocular, hepatic, pulmonary
Viruses			
Coxsackieviruses	Nonenveloped, ssRNA[c] viruses	Children and adults during summer months	Aseptic meningitis, myocarditis, hand-foot-and-mouth disease, pleuritis
Cytomegalovirus	Enveloped, dsDNA[d] virus	Immunocompromised; newborns	Pneumonia, hepatitis, gastrointestinal ulcers, congenital infection
Dengue viruses	Enveloped, ssRNA viruses	Exogenous (*Aedes* mosquitoes)	"Breakbone fever," headache, fever, rash, myalgia, sometimes hemorrhagic fever/shock
Epstein-Barr virus	Enveloped, dsDNA virus	Often present in saliva; exogenous	Mononucleosis, lymphoproliferative disorders
Filoviruses (Ebola virus, Marburg virus)	Enveloped, ssRNA viruses	Reservoir in nature is unknown; nonhuman primates and humans have been sources; possible agents of bioterrorism	Hemorrhagic fever with high mortality
Hantaviruses	Enveloped, ssRNA viruses	Rodent excreta	Pneumonia, hemorrhagic fever with renal dysfunction
Hepatitis A virus	Nonenveloped RNA virus	Fecal-oral	Hepatitis
Hepatitis B virus	Enveloped DNA virus	Blood-borne; sexual contact; vertical	Hepatitis, chronic carriers, cirrhosis, hepatocellular carcinoma
Hepatitis C virus	Enveloped, ssRNA virus	Blood-borne	Hepatitis, chronic carriers, cirrhosis, hepatocellular carcinoma

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TABLE VI SELECTED SYSTEMIC PATHOGENS *(continued)*

ORGANISM	GENERAL CHARACTERISTICS	SOURCE OF INFECTION	DISEASE MANIFESTATION
Hepatitis D virus	Enveloped, ssRNA virus	Blood-borne; requires hepatitis B coinfection	Fulminant hepatitis; requires coinfection with hepatitis B virus
Hepatitis E virus	Nonenveloped, ssRNA virus	Fecal-oral	Hepatitis, increased severity in pregnant women
Herpes simplex viruses	Enveloped, dsDNA viruses	Person to person, including via sexual contact; reactivation of latent infection; during passage through the birth canal	Genital, oral, ocular, encephalitis, neonatal infection, esophagitis (immunocompromised)
Human herpesvirus type 6	Enveloped, dsDNA virus	Person to person	Exanthema subitum (roseola)
Human herpesvirus type 8 (Kaposi's sarcoma-associated herpesvirus)	Enveloped, dsDNA virus	Person to person, including sexual transmission	Kaposi's sarcoma in HIV-infected individuals
Human immunodeficiency viruses (HIV-1 and -2)	Enveloped RNA retroviruses	Blood-borne; sexual contact; vertical	AIDS
Human T-cell lymphotropic virus type 1	Enveloped RNA retrovirus	Blood-borne	Tropical spastic paraparesis, T-cell leukemia
Mumps virus	Enveloped, ssRNA virus	Respiratory spread	Mumps, parotitis, pancreatitis, orchitis, meningitis
Parvovirus B19	Nonenveloped, ssDNA virus	Person to person, including vertical transmission	Erythema infectiosum, arthritis, transient aplastic crisis, hydrops fetalis
Rubella virus (German measles)	Enveloped, ssRNA virus	Person to person, including vertical transmission	Inapparent or subclinical infection in adults; birth defects in infants
Rubeola virus (measles)	Enveloped, ssRNA virus	Respiratory spread	Measles, pneumonia, encephalomyelitis, subacute sclerosing panencephalitis
Smallpox	Enveloped, dsDNA virus	Must be assumed to be due to bioterrorism or biological warfare; respiratory spread; direct contact	Prominent vesicular rash; up to 30% mortality
Varicella-zoster virus	Enveloped, dsDNA virus	Respiratory spread; direct contact; reactivation of latent infection	Chicken pox; zoster (may disseminate)
Yellow fever virus	Enveloped, ssRNA virus	Exogenous (*Aedes* mosquitoes)	Severe hepatitis; significant mortality rate

[a]UTI, urinary tract infection. [b]CNS, central nervous system. [c]ssRNA, single-stranded RNA. [d]dsDNA, double-stranded DNA.



This 53-year-old man with a past medical history of non-insulin-dependent diabetes mellitus and hypertension was in his usual state of health until 4 days prior to admission, when he developed fatigue, fever, chills, and a cough occasionally productive of green sputum. Over the 2 days prior to admission, he had drenching sweats, increasing dyspnea, and left-sided pleuritic chest pain. The patient had smoked 2 packs of cigarettes a day for 40 years.

His physical examination was notable for an increased respiratory rate of 22 per minute, and crackles were heard over the right middle, left middle, and left lower lung fields. A chest radiograph demonstrated right lower lobe, left lingular, and left lower lobe infiltrates.

A Gram stain of the patient's sputum contained >25 polymorphonuclear leukocytes per low-power field and 4+ (many) Gram-positive diplococci. Culture of the sputum grew 4+ (many) *Streptococcus pneumoniae* as well as normal respiratory flora. One set (both bottles) of two sets of blood cultures drawn prior to the administration of antibiotics grew the organism shown on Gram stain in Fig. 53.1. The organism growing from a subculture of the blood is shown in Fig. 53.2. Further biochemical testing revealed the organism to be catalase positive and coagulase negative.

1. The organism described in this case belongs to a group of organisms. What is that group? With what types of infections are these organisms specifically associated?
2. What is the significance of this patient's blood culture isolate?
3. Name three key factors necessary to ensure the detection of bacteremia in a patient such as the one described in this case.

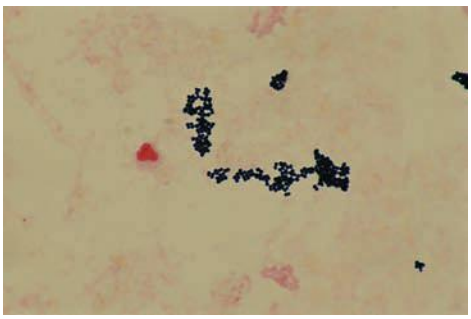


Figure 53.1



Figure 53.2

4. What is the clinical impact of the type of blood culture isolate recovered from this patient in terms of length of stay, antibiotic administration, and additional testing?
5. What can be done to prevent this type of blood culture isolate in a health care facility?

CASE DISCUSSION



1. Gram-positive cocci in clusters that are catalase positive are most likely staphylococci. The coagulase test helps to differentiate *Staphylococcus aureus*, which is coagulase positive, from the other staphylococci, which are often grouped together as "coagulase-negative staphylococci." Coagulase-negative staphylococci are a heterogeneous group of several different species. These organisms are most likely part of the human skin microbiota. They are commonly found in small numbers in cultures of skin and soft tissues and in this setting are frequently viewed as not contributing to the disease process (i.e., contaminants). However, coagulase-negative staphylococci can readily grow as biofilms on solid surfaces. As a result, they are now recognized as important causes of infections of a wide variety of catheters and prosthetic devices including intravascular catheters, prosthetic joints, penile pumps, ventriculoperitoneal catheters used in the treatment of hydrocephalus, drive lines for cardiac assistance devices, pacemakers, peritoneal dialysis and hemodialysis catheters, and central venous pressure lines, to name a few. Because these organisms grow as biofilms on these lines and devices, the only manner in which these infections can usually be successfully eradicated is by their removal. Needless to say, this can only be done at considerable risk and expense to the patient. These line and prosthetic device infections can be due to several of the >30 species of coagulase-negative staphylococci that have been described. The species most commonly associated with these infections is *Staphylococcus epidermidis*, which is well recognized to grow as biofilms on solid surfaces.

Two species of coagulase-negative staphylococci are of special significance. *Staphylococcus lugdunensis* (Fig. 53.3) can be confused with *S. aureus* on sheep blood agar in part because the organism can be beta-hemolytic and appear slightly yellow. In addition to causing line and prosthetic device infections, this is the only species of coagulase-negative staphylococci that is recognized to cause skin and soft tissue infections including boils and abscesses. Perhaps of even more importance clinically is the observation that this organism can cause native valve endocarditis, something not associated with other coagulase-negative staphylococci. Importantly, *S. lugdunensis* is infrequently resistant to oxacillin, unlike the other coagulase-negative staphylococcal species.



Figure 53.3 *S. lugdunensis* on sheep blood agar.

Staphylococcus saprophyticus is a second species of coagulase-negative staphylococci that is typically considered a pathogen. It is recognized as a cause of cystitis primarily in sexually active young women. Interestingly, this organism has not been associated with pyelonephritis. Susceptibility testing is not required for this

organism, as it remains fully susceptible to drugs commonly used to treat cystitis, including sulfa-containing drugs, fluoroquinolones, and nitrofurantoin.

This patient's blood isolate was not identified to the species level by the clinical microbiology laboratory but was identified as a coagulase-negative staphylococcus. In most cases, this information is sufficient for the clinician.

2. This patient's blood culture isolate is considered a contaminant. When coagulase-negative staphylococci are causing bacteremia, it is typically from an endovascular source such as an intravascular catheter. In this clinical setting, the patient has what is classified as a "continuous bacteremia" because organisms are being continuously released from the biofilm on the catheter. As a result, if the patient had a catheter-related infection, both blood cultures should have had the same organism. Since this patient did not have an intravascular source and had not received antimicrobials, the best explanation for his finding is that the organism was picked up from the skin during venipuncture and contaminated the blood culture.

This interpretation of his blood culture results is supported by his clinical picture, which is most consistent with community-acquired pneumonia due to *S. pneumoniae*. Approximately two-thirds of patients with pneumococcal pneumonia do not have positive blood cultures for the pneumococcus. The recovery of coagulase-negative staphylococci from the patient's blood is not at all consistent with his clinical picture and should not lead his doctor to alter antibiotic therapy.

In addition to coagulase-negative staphylococci, other organisms that are often contaminants include skin microbiota such as *Propionibacterium acnes*, *Micrococcus* spp., diphtheroids, *Bacillus* spp., and viridans group streptococci.

3. The three essential factors to ensure that bacteremia, if present, will be detected are (i) obtaining the blood culture prior to the initiation of antimicrobial therapy, (ii) obtaining adequate volumes of blood, and (iii) obtaining multiple blood culture sets.

The first of these three is relatively self-explanatory. Certain organisms such as *S. pneumoniae* and *Neisseria meningitidis* are rapidly killed by appropriate antimicrobials. Thus, drawing blood cultures after antimicrobial administration may result in false-negative cultures.

It has been found that one of the key factors in detecting bacteremia is the amount of blood drawn. For adults, an adequate volume of blood is 10 to 20 ml. The reason for the need to draw such a large volume of blood to detect bacteremia is that the number of organisms per milliliter of blood in adults during bacteremia is often <1.

Finally, multiple blood cultures are needed so that contaminated blood cultures can be differentiated from true bacteremia. In most academic medical centers, the most common organisms recovered from blood cultures are the organisms that were seen in this case, coagulase-negative staphylococci. Approximately 80% of the time coagulase-negative staphylococci are contaminants, but 20% of the time they are causing bacteremia.

For example, consider a patient who has a central venous line who develops fevers, erythema, and tenderness at the catheter site. Multiple sets of blood cultures are positive for coagulase-negative staphylococci, and the patient's central venous catheter line tip, when removed, grows >15 colonies of the same organism on culture. This patient is far more likely to have a real infection with this organism than is the patient described in this case. The laboratory staff are often asked by clinicians, "Is this organism a contaminant or is it a real pathogen?" This question is not one that the laboratorian can answer. It is up to the patient's physician to weigh his or her clinical observations with the laboratory data to judge the significance of the patient's blood culture isolate.

When more than one set of blood cultures are positive for coagulase-negative staphylococci, it is more likely that the positive blood culture represents a true pathogen. With modern technology such as automated phenotypic identification systems and matrix-assisted laser desorption ionization–time of flight mass spectroscopy, it is now fairly easy to determine the species of coagulase-negative staphylococci. The finding of the same species from two or more blood cultures indicates that the patient has a true bacteremia. What about the situation when there are different species of coagulase-negative staphylococci in multiple blood cultures? This situation has not yet been resolved.

If multiple blood cultures need to be collected, what is a sufficient number? Depending on the laboratory, the number could range from two to four. Blood cultures are typically collected in pairs, and collecting two pairs for a total of four cultures per septic episode is a common practice. Collecting additional blood cultures beyond four will only rarely improve pathogen recovery and is not recommended.

Certain organisms are almost always considered true pathogens. These include *S. aureus*, *S. pneumoniae*, *Listeria monocytogenes*, *N. meningitidis*, *Candida* spp., *Cryptococcus neoformans*, dimorphic fungi, group A and B streptococci, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*. Recovery of any of these even in a single blood culture would be considered clinically significant.

4. Contaminated blood cultures are expensive. It is estimated that each contaminated blood culture costs between $5,000 and $10,000 per episode. For example, if a hospital has a contamination rate of 2.5% (the national average) and does 20,000 blood cultures (a number that would be done in a typical 500- to 700-bed hospital), then the excess cost would be $2.5 million using the $5,000/contaminated blood culture cost estimate. Why is the cost so high? Patients with contaminated blood cultures, on average, stay in the hospital an additional day. They have additional diagnostic procedures performed, but perhaps most importantly, they receive unnecessary antimicrobials. Patients with contaminated blood cultures receive antimicrobials at the same rate as those with true bacteremia. Risks associated with unnecessary antimicrobials include allergic reactions; inability to detect other infectious agents; and modification in the patient's microbiota, which will increase the risk for colonization with multidrug-resistant organisms such as vancomycin-resistant

enterococci or methicillin-resistant *S. aureus*. It also increases the risk for the development of *Clostridium difficile* infection.

5. Strict attention to the method by which cultures are obtained is important. In particular, the use of tincture of iodine as the skin antiseptic has been shown to be superior to the use of an iodophor. In addition, some hospitals have phlebotomists who are specifically trained in proper techniques for obtaining blood for culture. Studies have shown that blood culture contamination rates are lower in these hospitals. Having specially trained staff to draw blood cultures should be cost-effective given the high costs associated with the management of patients who have contaminated blood cultures.

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This 39-year-old injection drug user (actively using cocaine on the date of admission) was admitted with cellulitis of the right arm after experiencing fevers for several weeks. He had been treated with outpatient antibiotics without relief of either associated chills or dizziness. Two sets of blood cultures were obtained on admission. A transthoracic echocardiogram demonstrated a 1-cm vegetation on the ventral surface of the aortic valve. The patient left the hospital against medical advice but was readmitted 2 days later for antimicrobial therapy.

Past medical history was notable for multiple hospital admissions for both cellulitis and abscesses primarily involving the patient's arm. He had had multiple drug rehabilitation treatment attempts without success.

Physical examination demonstrated a thin, unkempt man in no acute distress with multiple "needle track" marks on both his upper and lower extremities. No splinter hemorrhages or signs of embolic phenomena were noted on the extremities. Cardiac exam was notable for a grade II/VI systolic murmur best heard at the left sternal border. The spleen tip was palpable. The right arm had a 10-by-6-cm excoriated area with surrounding induration.

Gram stain of an organism detected in both sets of the blood cultures obtained at admission is shown in Fig. 54.1. Growth of the organism on a blood agar plate is shown in Fig. 54.2. The organism grew in broth containing 6.5% NaCl, hydrolyzed esculin in the presence of bile (i.e., was bile esculin positive), and was catalase negative.

1. What type of infection did this patient have?
2. What organisms frequently cause this type of infection in injection drug users? What organism was causing his infection? What new laboratory technique has resulted in a better understanding of the etiologies of the type of infection this patient had?
3. How does injection drug use predispose the patient to this type of infection? Briefly describe the pathogenesis of this infection. Describe what other organs may be secondarily infected and the mechanism by which secondary infections occur.

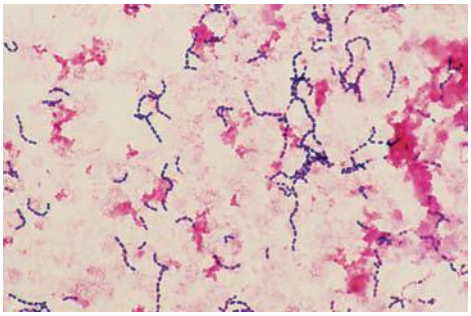


Figure 54.1



Figure 54.2

4. For what other infectious agents is this individual at increased risk?
5. When considering antimicrobial therapy for this infection, what general strategy should be employed?
6. What major antimicrobial resistance problems are associated with this organism? What strategies have been employed to reduce the spread of these organisms?

CASE DISCUSSION



1. This patient had bacterial endocarditis. The keys to making this diagnosis are the detection by echocardiogram of a vegetation on his aortic heart valve and the presence of continuous bacteremia in his bloodstream as detected by his positive blood cultures. The use of criteria for the diagnosis of infective endocarditis has been advocated, and this patient would meet the Duke clinical criteria for endocarditis on the basis of the community-acquired enterococcal bacteremia and the presence of the vegetation. Physical findings consistent with endocarditis include enlarged spleen and the presence of a heart murmur. When looking for signs of bacterial endocarditis, the presence of splinter hemorrhages in the nail beds should be sought. The use of transillumination of the nail beds in a darkened room may help to show splinter hemorrhages that would otherwise not be seen on physical examination. Twenty to 40% of patients with endocarditis have these cutaneous findings due to embolic events secondary to endocarditis. This patient did not have these lesions.

2. The most common agents of bacterial endocarditis in injection drug users are *Staphylococcus aureus*, viridans group streptococci, *Candida albicans*, *Enterococcus* spp., and Gram-negative bacilli including *Pseudomonas aeruginosa*. The organism description indicates that this patient was infected with an organism belonging to the genus *Enterococcus*. Further phenotypic characteristics would be required to determine to which species this organism belonged.

The application of broad-range 16S and 18S rRNA PCR and sequencing directly on heart valve tissue has greatly enhanced our understanding of the etiologies of "culture-negative" endocarditis. Culture-negative endocarditis is exactly as it sounds: the organism that is causing the endocarditis cannot be recovered on routine blood cultures. Using this molecular technique, we better appreciate the importance of two zoonotic pathogens, *Coxiella burnetii* and *Bartonella* spp., as causes of bacterial endocarditis. Another advantage of this technique is that it can detect bacteria or fungi in the heart valve tissue of patients who are blood culture negative and on appropriate antimicrobial therapy since molecular detection requires only the presence of DNA, not living organisms. The disadvantage of this technique is that it requires that heart valves be removed, as this methodology is not particularly sensitive on blood samples.

3. The pathogenesis of bacterial endocarditis is dependent on damage to heart valves, which typically produces turbulent blood flow. Turbulence in blood flow may result in the deposition of platelets and fibrin, the initial stage in formation of vegetations. Injection drug users do not use "sterile technique" when they inject drugs. Therefore, transient bacteremia with either skin flora (*S. aureus*, viridans group streptococci, enterococci, or *Candida* spp.) or environmental organisms (*P. aeruginosa*) may occur following drug injection. These organisms have been shown to adhere readily to thrombotic lesions on the

heart valve. The adherent bacteria begin to grow, and platelet and fibrin deposition continues, resulting in an enlarging vegetation. As this vegetation continues to grow, small pieces containing fibrin, platelets, and bacteria may "break off," causing septic emboli. Septic emboli enter the bloodstream and can become lodged in the vascular bed, resulting in localized hemorrhage and infection. Common secondary infections due to septic emboli include brain, kidney, spleen, lung, and liver abscesses. Anatomically, injection drug users are more likely to have "right-sided" endocarditis affecting the tricuspid valve than are other people with infective endocarditis.

4. Injection drug users often share needles or reuse needles used by others, exposing themselves to blood from other individuals. As a result, they are at increased risk for many blood-borne infectious agents. The most common and important agents acquired by this behavior are HIV; hepatitis B, C, and D viruses; and in geographically appropriate areas, human T-cell lymphotropic virus type 1. Uncommon infections can also be acquired from injectable drugs. As a result, tetanus due to infection with *Clostridium tetani* and other life-threatening soft tissue clostridial infections are well documented in injection drug users. The practice of licking needles may result in bacteremia with oral flora.

5. Enterococci are susceptible to very few antimicrobial agents. Most strains are susceptible only to vancomycin and ampicillin/penicillin G. Enterococci are often tolerant to these agents, meaning that the organisms are inhibited but not killed by the specific antimicrobial agent. This is problematic when treating patients with endocarditis because phagocytic cells provide little help in clearing the infection on the heart valve so that killing of organisms within vegetations is very much dependent on antimicrobial activity. Studies *in vitro* and in animals have shown that aminoglycosides, although inactive alone at concentrations achievable in the bloodstream, greatly enhance the killing power of ampicillin/penicillin G or vancomycin when one of these cell wall-active agents is given in combination with the aminoglycoside. This enhancement of antimicrobial killing power when drugs are given in combination is known as synergy. Typically, either ampicillin or vancomycin is given in combination with gentamicin (or, if there is high-level resistance to gentamicin, streptomycin) to treat enterococcal endocarditis. Major resistance problems have developed, however (see answer to question 6).

The other important problem when treating a patient with endocarditis is the poor penetration of antimicrobial agents into the infected vegetation. As a result, therapy for bacterial endocarditis is typically long-term, lasting 4 to 6 weeks.

6. Drug resistance has become a major problem in *Enterococcus* spp. There are three major problems with acquired drug resistance in organisms belonging to this genus, in addition to the organism's well-known intrinsic resistance to cephalosporins, monobactams (aztreonam), clindamycin, and trimethoprim-sulfamethoxazole. Isolates have obtained

genes that encode for resistance to ampicillin/penicillin G, aminoglycosides, and perhaps most disturbingly, vancomycin. Resistance to ampicillin/penicillin G in enterococcal isolates is due primarily to modification of penicillin-binding proteins.

All strains of enterococci are resistant to aminoglycosides at concentrations achievable in serum, e.g., gentamicin MIC of 16 to 64 μg/ml. This is known as low-level resistance. Fortunately, as discussed in the answer to question 5, aminoglycosides can be used synergistically with cell wall-active agents. Unfortunately, strains of enterococci have been recognized that have high-level resistance to gentamicin (MIC ≥500 μg/ml). This high-level resistance is due either to modification of the aminoglycoside binding site on the ribosome or, more commonly, to the production of enzymes that modify and thus inactivate the aminoglycosides. Isolates that have high-level resistance to gentamicin generally possess high-level resistance to tobramycin and amikacin but not to streptomycin; however, high-level resistance to streptomycin has also been reported. When enterococci demonstrate high-level resistance to aminoglycosides, the synergy between cell wall-active agents and the aminoglycoside is lost.

Perhaps the most disturbing trend in enterococcal drug resistance is the development of resistance to vancomycin. Vancomycin has generally been thought of as the "drug of last resort" for multidrug-resistant Gram-positive organisms. The emergence of vancomycin resistance has challenged that dogma. Several genes have been described that confer resistance to vancomycin in enterococci, with *vanA* and *vanB* being the most common and clinically important. These resistance genes can be transferred among strains of enterococci on plasmids. Vancomycin resistance is due to the production of enzymes that modify the vancomycin target, significantly reducing the ability of vancomycin to block cell wall synthesis. Vancomycin-resistant enterococci (VRE) are frequently resistant to ampicillin and high levels of aminoglycosides as well. When these isolates are detected in serious infections such as endocarditis, there are few options. Additional antimicrobials that have good activity against enterococci include the streptogramin combination of quinupristin and dalfopristin (which is only active against *Enterococcus faecium*), daptomycin, and the oxazolidinone linezolid. The last two are active against both *E. faecium* and *Enterococcus faecalis* and are most frequently used to treat VRE infections. Resistance in enterococci has already been reported for all three of these agents.

It is well recognized that VRE are more common in patients who have previously received vancomycin and have prolonged hospital stays. Judicious use of vancomycin is important; because of concerns about VRE, many institutions control the use of this antimicrobial. Because enterococci are part of the normal gut microbiota, the gastrointestinal tract is a reservoir for VRE. Studies have shown that patients who carry drug-resistant organisms in their gastrointestinal tract frequently contaminate their environment. Therefore, strict infection control measures, including patient isolation and barrier nursing precautions (the wearing of masks, gloves, and gowns and the strict enforcement of hand washing), may prevent health care-associated spread of this organism.

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The patient was a 46-year-old male who presented with complaints of fever. Ten days previously the patient had returned from a holiday trip during which he visited his family in Guatemala. He was both in urban areas and at the Caribbean coast. He received many mosquito bites and did not take malaria prophylaxis. He had one episode of diarrhea during his visit, but that resolved within 1 day.

One week prior to presentation he began to develop malaise, headache, and fevers with some sweating and chills. Over the 3 days prior to presentation he had fevers throughout the day to 39.0°C with drenching sweats. He also complained of intermittent headaches. He did not complain of stiff neck, diarrhea, or abdominal pain. His urine appeared somewhat darker than normal to him. He had normal oral intake.

On physical examination his temperature was 38.5°C, his blood pressure was 133/85 mm Hg, and his pulse was 145 beats/min. He had no nail bed hemorrhages and no petechial lesions. The remainder of his physical examination was within normal limits. He had a white blood count of 13,500 cells/μl with 10,000 neutrophils/μl. A peripheral blood smear is shown in Fig. 55.1. Figure 55.2 shows a triple sugar iron (TSI) slant of the organism that was recovered from the patient's blood.

1. Given his travel history, name three organisms that are likely to have been responsible for his fever. How do his peripheral blood smear and the TSI slant in Fig. 55.2 help to narrow the list? What about his physical examination rules out one of the pathogens?
2. How did this patient likely become infected with this organism?
3. This infection typically occurs in individuals who return from foreign countries or are immigrants from those countries. What countries are the most common source for this infection for patients in the United States?

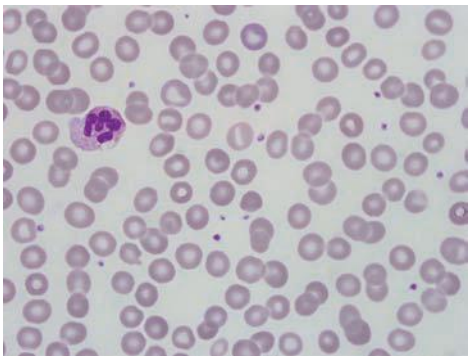


Figure 55.1 Patient's peripheral blood smear.



Figure 55.2 TSI slant of isolate recovered from blood culture.

4. Briefly describe the pathogenesis of infection with this organism.
5. Explain why this organism infects only humans but other closely related organisms can infect a range of zoonotic hosts.
6. If this patient worked in the food industry, what would need to be documented before he could return to work and why?
7. What antimicrobial resistance problem has recently been described for this organism? What is likely driving the emergence of this resistance?
8. How might this individual have avoided becoming infected with this organism?

CASE DISCUSSION



1. The major agents of febrile illness in a traveler returning from Central America, especially one who visited rural areas, would be malaria, dengue fever, and typhoid fever, which is caused by *Salmonella enterica* serovar Typhi. The patient's failure to take malaria prophylaxis means that his physician should be aggressive in ruling out this diagnosis because of the significant morbidity and mortality associated with this disease. The early onset of fever soon after returning from an area in which malaria is endemic is more typical of *Plasmodium falciparum* than *Plasmodium vivax*, which tends to have a more extended incubation period. Paradoxically, *P. vivax* infection is much more common in returning travelers from Guatemala, with *P. falciparum* being only rarely seen. When malaria is suspected, as was the case here, a peripheral blood smear is examined for the various stages of the *Plasmodium* protozoan. The negative peripheral blood smear supports the notion that the patient did not have malaria. However, false-negative smears can occur especially with *P. vivax*, so multiple smears collected near fever spikes would be needed to confidently rule out malaria. Multiple smears were done and were all negative.

A second infection that would need to be considered is dengue fever (also called "breakbone fever"). However, during physical examination this patient had neither the skin rash nor the extremely painful joints that are both hallmarks of this disease, making this diagnosis less likely.

The finding of a small ring of H_2S on TSI agar slants at the top of the butt (Fig. 55.2) is characteristic of *S.* Typhi. The vast majority of other *Salmonella* isolates will produce H_2S throughout the butt of the slant. The less intense H_2S reaction, along with serotyping performed by a public health laboratory, confirmed that the patient had *S.* Typhi infection.

2. *S.* Typhi only infects humans (see answer to question 4 for greater details). It is transmitted from humans to humans primarily by ingestion of fecally contaminated food or water. As the historical case of Typhoid Mary taught us, *S.* Typhi excreters who fail to wash their hands after defecating and then prepare food are one common source of this organism. The other common source is water that is drawn from sources that have been contaminated by feces from individuals excreting the organism and is then ingested. Most cases of *S.* Typhi in the United States are obtained either during travel abroad or from individuals who recently immigrated. Excellent sanitation has essentially eliminated endemic cases of *S.* Typhi in the United States.

3. Acquisition of *S.* Typhi is most common in individuals who have traveled to the Indian subcontinent. Other geographic locales where acquisition of *S.* Typhi is high are Vietnam, Mexico, Central America, the Philippines, and Haiti. Interestingly, acquisition of *S.* Typhi in travelers to sub-Saharan Africa is less common than the acquisition of

rickettsial infections. Geographical information is important when considering empiric antimicrobial therapy in the returning traveler with a febrile illness.

4. A high inoculum (10^6 CFU/ml) is needed because of this organism's susceptibility to stomach acid. After the organisms have survived transit through the stomach, they multiply in the small intestine. They invade the intestinal mucosa of the ileum via M cells. M cells are specialized epithelial cells that play a role in gut mucosal immunity. Normally antigens from the lumen of the gut are taken up by these cells and are then processed by antigen-presenting cells in the Peyer's patches. The organism has a series of genes encoding proteins involved in invasion of epithelial and epithelial-like cells. This series of genes is located in a region of the bacterial chromosome called a pathogenicity island. The typhoid bacilli subvert the function of the M cells to invade the Peyer's patches. Within the Peyer's patches, they are phagocytized by macrophages, where they survive and multiply. From the Peyer's patches they can be carried to the bloodstream via the lymphatics.

5. Sequencing of the entire genome of two strains of *S.* Typhi has provided insight into the narrow host range of this organism. Large numbers of mutations have occurred in the genome, resulting in the inactivation of as many as 5% of the genes of this organism. At least some of these inactivated genes have been recognized in other *Salmonella* serovars to play a role in adhesion and persistence of the organism in the intestinal tract. If the repertoire of adhesion proteins and proteins involved in intestinal persistence is reduced, it is logical that the host range of the organism may also be reduced.

6. Approximately 1 to 3% of patients who have typhoid fever will become chronic carriers of *S.* Typhi. They excrete large numbers (>10^6 CFU/ml) of *S.* Typhi organisms in their feces and can continue to do so for many years. To illustrate this point, we saw an 80-year-old patient who had his gallbladder removed. He gave his surgeon a history of having had typhoid fever when he was 20 years old but had had excellent health since then. The surgeon sent a swab of the patient's gallbladder for culture, and it grew *S.* Typhi 60 years after his original infection!

Food workers who are carriers and do not practice good hygiene could contaminate the food they handle, spreading the organisms to large numbers of individuals. As a result, workers in the food industry who have had an *S.* Typhi infection should have three negative stool cultures before being allowed to return to work. Three stools are necessary because the organism is excreted intermittently from the biliary tree. These cultures should be done over a period of at least 5 to 7 days to prevent sampling error.

7. Approximately 30% of *S.* Typhi isolates in the United States are resistant to multiple drugs, including the three drugs that have long been considered front-line therapy for typhoid fever: chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. Not surprisingly, multidrug-resistant organisms are widespread in countries where sanitation is

poor, such as countries on the Indian subcontinent and in Southeast Asia. These organisms have been imported to the industrialized world, including the United States.

Perhaps even more ominous has been the rapid evolution of fluoroquinolone resistance in *S.* Typhi. With the emergence of multidrug-resistant *S.* Typhi on the Indian subcontinent and in Southeast Asia, fluoroquinolones, specifically ciprofloxacin and ofloxacin, became the agents of choice to treat not just enteric fever but also diarrheal diseases. In those countries, antimicrobial agents are freely available over the counter, leading to increased antimicrobial pressure and selection of resistant strains. By 1993, resistance to nalixidic acid, the quinolone precursor of the fluoroquinolones, was being recognized in 4% of *S.* Typhi isolates in Vietnam. By 2005, 97% of *S.* Typhi isolates were nalixidic acid resistant. Importantly, multidrug-resistant strains, including those that are nalixidic acid resistant, have spread to the Indian subcontinent from Vietnam but interestingly not to Laos, Indonesia, or China. Why is this observation important? First, we need to remember that more than half of those individuals who develop *S.* Typhi infection after travel obtain it on the Indian subcontinent, which will influence the selection of empiric antimicrobial therapy. Second, resistance to nalixidic acid is typically a result of a mutation in the quinolone resistance determining region of the DNA gyrase, while two mutations are required for fluoroquinolone resistance. However, because of numerous reports in the literature of fluoroquinolone treatment failures in systemic *Salmonella* infections in patients with only a single mutation, screening for nalidixic acid resistance is now performed for all clinical *Salmonella* isolates in the United States. It is recommended that physicians be alerted when *S.* Typhi strains resistant to nalidixic acid are recovered so a consultation with an infectious disease practitioner can be initiated. Strains with two mutations that test as resistant to fluoroquinolones are still rare in Asia. However, if the experience with the emergence of nalidixic acid is any guide, it is likely that this resistance will also emerge, eliminating the last class of oral agent as a therapeutic choice for treatment of this infection.

8. Because of the increasing problem of antimicrobial resistance in *S.* Typhi, prevention of infection has become of even greater importance. This is particularly true for travelers to the Indian subcontinent and Vietnam, where rates of multidrug-resistant *S.* Typhi are high. The simplest way to avoid becoming infected by *S.* Typhi is to avoid consuming fecally contaminated food and water. However, this is simply not practical in many parts of the world, where fecal contamination of food and water is the norm. Travelers to areas where sanitary conditions are poor should consider vaccination against *S.* Typhi. This seems to be particularly true for individuals such as the patient in this case who visit friends or relatives in the developing world. Although they represent a small fraction of individuals who travel from the industrialized to the developing world, it is estimated that 80% of typhoid fever cases occur in travelers who visit friends or relatives. It is thought that these individuals have less control over the food and water that they consume, and because many originally lived in the countries that they visit,

they may be more willing to eat food from street vendors, a well-recognized source of enteric infections in travelers.

Two vaccines are currently available and have been found to be protective. One is derived from the Vi polysaccharide antigen found on the surface of the typhoid bacilli. A single injection of this vaccine is given, with boosters recommended every 2 years. Vaccine efficacy is between 65 and 70%. This vaccine is not recommended for use in children <2 years of age.

The other is a live, attenuated oral vaccine. It is given as four oral doses over a 1-week period. It elicits primarily a cell-mediated response, and immunity with this vaccine lasts for ~7 years. Estimates of vaccine efficacy range from 60 to 85%. Booster doses after the initial series of four oral doses are currently recommended after 5 years. A disadvantage of the oral vaccine is that it must be kept refrigerated, which may be problematic for vaccine campaigns in tropical areas. Currently this vaccine is not recommended for children <6 years of age, HIV-positive individuals, or individuals with other immunocompromising conditions such as steroid use, radiation, or chemotherapy. Because this is a live, attenuated bacterial vaccine, it should not be administered to individuals receiving antimicrobials until at least 24 hours after the completion of this therapy.

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A 62-year-old man with a past medical history of heart disease, diabetes, unexplained gastrointestinal bleeding, and diverticulosis presented to the hospital with a chief complaint of increasing abdominal distention and abdominal pain. His physical examination was notable for fever, a blood pressure of 97/40 mm Hg, and abdominal distention and tenderness. He had an elevated white blood cell count and was found to have a rising serum creatinine, consistent with acute renal failure. A paracentesis (obtaining fluid from within the abdomen aseptically via needle) demonstrated 18,000 white blood cells/μl, 80% of which were neutrophils. A computed tomography scan demonstrated possible extraluminal air in the sigmoid colon.

The patient was seen by the surgical service, which felt that he should be medically stabilized, as surgical intervention would have a high mortality rate due to his cardiac and renal status and the presence of hypotension. The patient was given broad-spectrum antibiotics (vancomycin for aerobic Gram-positive cocci, cefepime for aerobic Gram-negative rods, and metronidazole for anaerobes) and managed medically.

He received a central venous pressure line and fluids and was monitored in the intensive care unit. After receiving a prolonged course of broad-spectrum antibiotics, he developed a fever. Cultures of blood were positive for an organism that was not effectively treated by any of his antibiotics. A Gram stain of the broth from the positive blood culture is shown in Fig. 56.1. It is ovoid and reproduces by budding. The organism is seen growing on a chocolate agar plate in Fig. 56.2. When this Gram stain result was known, peptide nucleic acid fluorescent *in situ* hybridization (PNA FISH) was performed on the isolate. That result is seen in Fig. 56.3.

1. Based on the Gram stain of the organism in blood cultures, what organisms should be considered as the potential pathogen in this patient?
2. What is PNA FISH? What does the PNA FISH result tell you about this organism? Why is PNA FISH done with this genus of organisms?
3. Is this organism part of the indigenous microbiota of humans? What unintentional consequence can antibacterial therapy have on this microbiota?

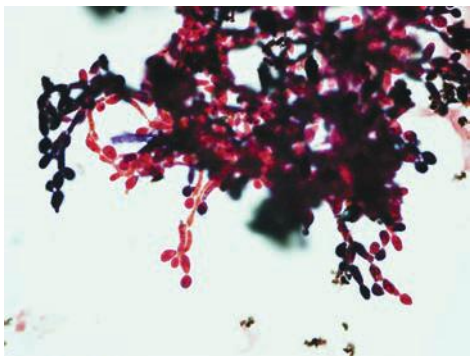


Figure 56.1 Gram stain from blood culture bottle.

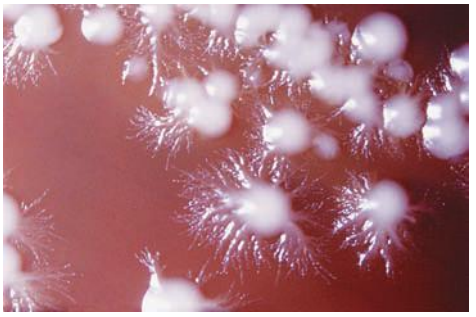


Figure 56.2 Organism growing on chocolate agar.

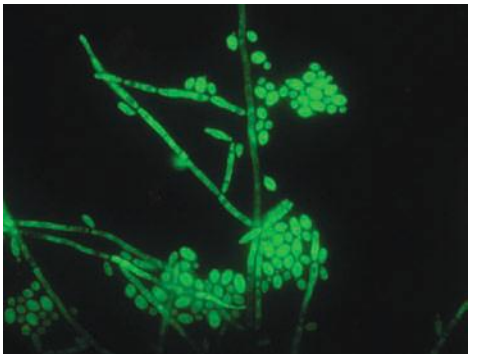


Figure 56.3 PNA FISH of blood culture.

4. What risk factors did this patient have for the development of the infection with this organism?
5. The epidemiology of invasive infection with organisms of this genus is changing. Describe the patient populations in whom these invasive infections will most likely occur and why understanding this epidemiology is important.

CASE DISCUSSION



1. The presence of ovoid yeast cells that reproduce by budding is consistent with a *Candida* species. Please note that on Gram stain these organisms, typically considered to be Gram positive, may also appear Gram negative (Fig. 56.1). *Candida* species are resistant to all antibacterial agents, including the ones this patient was receiving. The five most common *Candida* spp. recovered from bloodstream infections in order of frequency are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. These five species are responsible for >95% of episodes of fungemia caused by this organism. *Histoplasma capsulatum* is also often ovoid and may demonstrate budding, but is not confused with *Candida* species because of its smaller size. In addition, it grows more slowly than do *Candida* species. Other yeasts that are being seen with increasing frequency and may appear as ovoid yeasts on Gram stain include *Trichosporon* spp. and *Malassezia* spp. *Trichosporon* is the most common genus of yeast after *Candida*, causing fungemia in patients with hematologic malignancies, a patient population that has a high mortality from fungemia. *Malassezia* is a cause of fungemia mainly among neonates and pediatric patients receiving parenteral nutrition. The catheter through which the parenteral nutrition is supplied becomes colonized with this yeast, which is part of the skin microbiota. This contaminated catheter then seeds the bloodstream with this yeast. Immunocompromised adults may also develop *Malassezia* in the same manner as infants and children, but this occurs much less frequently. Although isolates of *Cryptococcus neoformans* typically demonstrate round yeast cells, this species should also be considered when yeast is isolated from a blood or cerebrospinal fluid sample.

2. Rapid identification of yeast is an important strategy in the management of fungemia. PNA FISH is a 20- to 60-min test that detects specific nucleic acid sequences directly from positive blood cultures. The PNA FISH test used to identify *Candida* species can differentiate the *Candida* species into four different groups based on the presence of a specific hybridization reaction or a negative reaction. It detects the five most common *Candida* species causing fungemia, differentiating fluconazole-susceptible (*C. albicans* and *C. parapsilosis*), fluconazole-nonsusceptible (*C. glabrata* and *C. krusei*), and fluconazole-susceptibility-uncertain (*C. tropicalis*) spp. Yeasts that fail to react in this assay are either other *Candida* species or some other genus of yeast likely to be found in blood cultures such as *Trichosporon*, *Malassezia*, or *Cryptococcus*.

Why is rapid identification of *Candida* causing bloodstream infection important? Ninety-day survival following *Candida* fungemia is only 50 to 70% depending upon the infecting species. There are three factors that are important in explaining this poor prognosis. First and most importantly, the patients have extensive comorbidities (see answer 4 for more details), which in the face of an invasive fungal infection may result in increased mortality. Second, invasive disease due to *Candida* is difficult to diagnose and may be missed if organisms are not detected in blood cultures. The diagnosis of a significant number of

unrecognized cases of invasive candidal disease is made at autopsy. The third problem is that the patient's antifungal therapy may be delayed, not given at all, or inappropriate due to resistance. All *C. glabrata* strains are considered nonsusceptible to fluconazole, a widely utilized antifungal agent, as are isolates of *C. krusei* (see answer 5 for further discussion). However, the other *Candida* species that cause invasive disease are likely to be susceptible to fluconazole. When *Candida* is observed in a blood culture by Gram stain (Fig. 56.1), clinicians would like to know what antifungal to use as soon as possible for the reasons just discussed. However, economics must also be considered. Two classes of antifungal agents, the azoles (e.g., fluconazole) and the echinocandins (e.g., micafungin or caspofungin), are used to treat candidemia. Echinocandins are significantly more expensive than fluconazole. Institutions that have implemented this strategy have found cost savings of anywhere from $400 to $1,700 per case without negatively affecting outcomes.

3. *Candida* species are a part of the indigenous microbiota of the skin, gastrointestinal tract, oropharynx, and vagina. When the microbiota is altered due to antibacterial agents, the resident bacterial microbiota, which competes with *Candida* species for nutrients or produces metabolites that inhibit *Candida* species growth, is reduced or eliminated. As a result, C*andida* is no longer inhibited, and it may become a predominant member of the indigenous microbiota.

Candida can cause both superficial and invasive disease. In the immunocompetent host, these infections are found primarily along mucous membranes, with candidal vaginitis a common complication of bacterial urinary tract infections treated with antibacterial agents. Thrush, a superficial candidal infection of the oropharyngeal mucosa, can occur in young children on antimicrobials but in adults may also be a harbinger of HIV infections and the development of AIDS. In the immunocompromised host, especially those with neutropenia, fungemia can result. For further discussion of invasive candidal disease, see answer 4.

4. This patient had a number of risk factors for the development of *Candida* fungemia. As previously described, treatment with broad-spectrum antimicrobials likely increased the number of *Candida* organisms present in the microbiota of the skin and mucous membranes. Because *Candida* species do not normally invade the bloodstream, a breakdown of host defenses must occur to allow a blood-borne infection (e.g., candidemia). The two most important defenses in this regard are (i) the presence of an intact barrier (skin) between the blood vessels and the environment and (ii) the presence of an adequate number of functioning neutrophils. In this patient, it is likely that the breach in skin integrity at the site of the central line insertion resulted in seeding of the bloodstream with *Candida* from the skin microbiota. Another possibility in this case is that the patient developed candidemia from another source, possibly as a consequence of the breach in colon integrity that caused his peritonitis.

Other factors associated with candidemia that were present in this patient included diabetes (which may have affected neutrophil function) and being cared for in an intensive care setting. Factors that are associated with candidemia but were not present in this patient include corticosteroid use; antifungal prophylaxis secondary to organ or stem cell transplant; neutropenia during stem cell transplantation or malignancies; and graft-versus-host disease, especially when there is a breach in a mucosal membrane.

5. The incidence of invasive *Candida* infections has increased over the past decade. Although the *Candida* species associated with infection have not changed, the species of *Candida* that predominate in specific populations have. For example, in neonates who develop line-related candidemia, fluconazole-susceptible *C. albicans* will predominate, making fluconazole the choice for empiric therapy. On the other hand, neutropenic human stem cell transplant recipients and solid-organ transplant recipients are more likely to have *C. glabrata* fungemia, in part because they may receive prophylaxis with fluconazole, to which *C. glabrata* strains are not susceptible. In that setting, empiric treatment with echinocandins may be prudent. Patients with hematologic malignancy are likely to have a more equal distribution of *Candida* species causing candidemia. In this setting, the more active and expensive echinocandins are the most prudent empiric choice, making detection and rapid identification especially useful in this patient population.

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The patient was a 34-year-old male who presented with a 6-week history of acute, intractable lower back and right leg pain. A magnetic resonance imaging (MRI) study was done and showed a large extradural defect at the L4-5 space in the lumbar spine. The MRI study was consistent with a possible herniated disk, and he was admitted for surgery. A frozen section done during the operative procedure showed acute inflammation. A biopsy from the lumbar spine was sent for pathologic testing and culture. The tissue showed acute and chronic inflammation as well as scattered giant cells consistent with granulomatous inflammation. On the basis of the operative findings, purified protein derivative (PPD) and control skin tests were placed immediately postoperatively. The patient was anergic. Laboratory studies were within normal limits except for an elevated erythrocyte sedimentation rate. Cultures of biopsy material and blood cultures obtained postoperatively grew the organism shown in Fig. 57.1 (Gram stain) and Fig. 57.2 (growth on sheep blood agar). This organism was rapidly urease positive.

When the identity of the organism infecting this patient was known, a more extensive social and travel history was elicited. It was learned that 11 months earlier he had visited his family in Mexico. During his visit, both his mother and a brother had a febrile illness. He also admitted to consuming goat milk and cheese obtained from his father-in-law, who raised goats.

1. What organism do you think was causing his infection? You should be able to give the species name based on a clue in the case. What is the clue?
2. Why was a PPD test done on this patient? What does anergic mean? What skin test antigens are used to test for anergy?
3. In what organs are lesions usually seen with infection due to this organism? Explain the probable steps in the pathogenesis of this patient's infection.
4. What factors concerning the pathogenicity of this organism should be taken into account when deciding on antimicrobial therapy to manage the infection?
5. If this patient had no identifiable risk factor for the organism that was infecting him, what possibility must be considered?

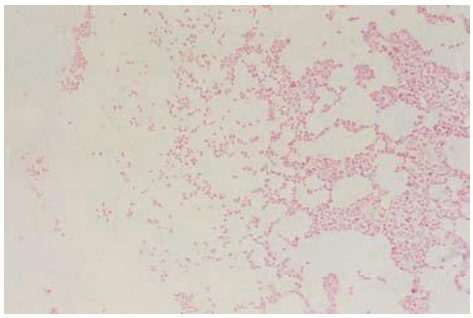


Figure 57.1

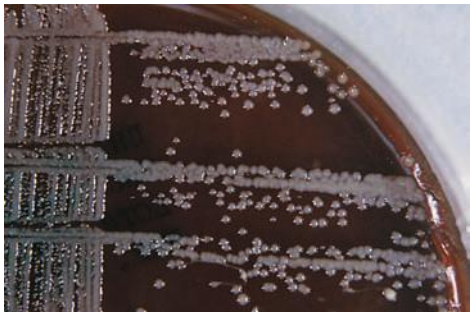


Figure 57.2



CASE DISCUSSION

1. This patient had a paravertebral abscess due to *Brucella melitensis*. Paravertebral abscesses are a common complication of brucellosis, occurring in ~20% of patients. *Brucella* spp. are tiny Gram-negative rods that will grow only on enriched laboratory medium. *B. melitensis* produces a highly active urease enzyme, so the positive urease test is consistent with this organism. Although the diagnosis of brucellosis was established in this patient on the basis of a positive culture, in most cases the diagnosis is established on the basis of serologic testing. There are two widely used serologic tests, an enzyme immunoassay for *Brucella* specific antigen and tube agglutination tests. Recent reports suggest that tube agglutination is the more accurate of the two approaches, and the Centers for Disease Control and Prevention recommends that tube agglutination be used to establish this diagnosis. In areas where *Brucella* is not endemic, tube agglutination is reasonably accurate for establishing the diagnosis of brucellosis, although cross-reactions can occur with a wide variety of Gram-negative rods, including but not limited to *Francisella*, *Salmonella*, *Yersinia*¸ and specific strains of *Escherichia coli*. Although a single titer of ≥1:160 in the tube agglutination test is sensitive, it is not necessarily specific, especially in areas in which *Brucella* is endemic. To definitely establish the diagnosis of brucellosis by serology, a 4-fold change in tube agglutination is required.

Although PCR has been used to establish the diagnosis of brucellosis, it is not generally available. One of the problems with using PCR for the diagnosis of brucellosis is that its DNA can persist for as long as 3 years after the resolution of clinical disease, so false-positive PCR results are of concern in patients who live in areas in which *Brucella* is endemic.

Brucellosis is a zoonotic infection and is usually obtained in one of three ways: (i) ingestion of unpasteurized dairy products, primarily from cows or goats; (ii) direct inoculation through cuts or scratches when caring for or attending the birth of (veterinarians, farm workers/herders) or rendering (abattoir workers) cattle (*Brucella abortus*), sheep (*B. melitensis*), pigs (*Brucella suis*), goats (*B. melitensis*), or (rarely) dogs (*Brucella canis*); or (iii) via an exposure in a microbiology laboratory. The key clue in identifying the organism infecting this patient was his history of consuming dairy products from goats when visiting his family in Mexico. It is estimated that between 15 and 40% of goats in Mexico are infected with *B. melitensis*, and this organism is present in the milk of infected animals. *Brucella* attack rates are much higher in Mexico than the United States, in part because of the consumption of unpasteurized goat dairy products; brucellae are killed by pasteurization. It has been suggested that *B. melitensis* can survive stomach acidity better than other *Brucella* species. Other countries with high rates of human infections are in the Middle East, with Iran, Turkey, and Syria having the highest number of reported cases.

2. *Brucella* and *Mycobaterium tuberculosis* have similar clinical (fever, night sweats, malaise, and weight loss) and pathologic (granuloma formation) features. Both can cause

osteoarticular disease such as vertebral spondylitis. Because of this, the diagnosis of *M. tuberculosis* infection was considered in this patient. Vertebral spondylitis due to *M. tuberculosis* is called Pott's disease. Because this patient had a pathologic process consistent with extrapulmonary *M. tuberculosis* infection, he had a skin test to see if he would react to the skin test antigen of *M. tuberculosis* PPD. A positive skin test in this clinical setting would be strong evidence that the patient was infected with *M. tuberculosis*. The patient was found to be anergic, which means that he did not give a positive skin test response to additional control antigens that were given concurrently with the PPD. The other antigens were ones most patients would have been exposed to in the past, and thus they should have positive skin test reactions to them. Failure to respond to ubiquitous antigens is evidence of anergy and is frequently seen in immunosuppressed patients, especially HIV-infected patients with low CD4 cell counts. The skin test for *M. tuberculosis* is uninterpretable in anergic individuals. Antigens used for anergy testing include diphtheria and tetanus toxoids, streptokinase-streptodornase (enzymes produced by group A streptococci), and antigens derived from either *Candida* sp. (yeast commonly found on skin and mucous membranes) or mumps virus.

3. *Brucella* is typically found in organs of the reticuloendothelial system, including liver, lungs, spleen, lymph nodes, kidneys, and bone marrow. The localization of brucellae in the reticuloendothelial system is due to the ability of this organism to survive and multiply within phagocytes. Survival within phagocytes leads to granuloma formation in infected organs. The steps in the pathogenesis of this patient's infection include ingestion of contaminated milk or cheese; increased organism survival in the acidic conditions found in the stomach; translocation across the gut wall; phagocytosis with intracellular survival and multiplication of the bacteria, followed by lysis of the parasitized phagocytes; dissemination of bacteria to bone marrow in the spine; phagocytosis with intracellular survival, multiplication, and granuloma formulation; and eventually abscess formation in the paravertebral area. The extradural process detected by the MRI study was a granuloma/abscess caused by the localization to the spine of his *Brucella* infection.

4. The ability of brucellae to survive within phagocytes makes antimicrobial therapy difficult. Many antimicrobial agents penetrate poorly if at all into phagocytes. As a result, relapse following antimicrobial therapy is common with brucellosis. Two treatment strategies are important for optimal management. First, therapy should be long-term, with antimicrobial regimens of 6 weeks frequently being recommended. Long-term therapy is necessary because relapse is frequently reported even with this prolonged regimen. Second, the use of antimicrobial agents that are active against brucellae and can penetrate into the phagocyte are desirable attributes. Because of its lipid solubility, rifampin can penetrate into white cells. It is active against brucellae as well. Because of the rapid rate at which most bacteria, including *Brucella* spp., develop resistance to rifampin if it is used as monotherapy, this agent should only be used in combination with other drugs active

against brucellae. The combination of rifampin with doxycycline has proved to be effective clinically, although the combination of doxycycline and streptomycin may be less likely to result in failure.

5. If no identifiable risk factors are present, the possibility that the patient is the victim of bioterrorism must be considered. *Brucella* is an important cause of laboratory-acquired infections, suggesting that it can be obtained via aerosols. Individuals may become ill after exposure directly to the organism or to the actual bioterror target, herd animals such as cattle or sheep that have fallen ill. Unusual numbers of cases of brucellosis in farmers, herders, feedlot workers, and veterinarians would be a cause for concern because infection with *Brucella* species is rare in the United States. This organism is on the list of critical biological agents along with smallpox virus, *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, and others. In the absence of an identifiable risk factor for acquiring brucellosis, contact should be made with both public health and law enforcement authorities.

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A 37-year-old Panamanian man with AIDS and a history of *Pneumocystis jirovecii* (previously named *P. carinii*) pneumonia, treated syphilis, and a reactive purified protein derivative skin test for which he received prophylactic isoniazid for 1 year was admitted to an outside hospital for persistent fever, weight loss, and pancytopenia. On admission to the outside hospital, the patient had a white blood cell count of 1,700/μl, a hemoglobin level of 8.7 g/dl, and a platelet count of 39,000/μl. A bone marrow examination demonstrated pancytopenia, granulomas, and the presence of yeast. The patient was begun on intravenous amphotericin B. Desiring to be closer to his family, he left the outside hospital and came to our institution for evaluation.

On examination, he was a cachectic, weak-appearing man in no acute distress. He had oral thrush and 3+ pitting lower extremity edema to his knees. The culture of his bone marrow from the outside hospital was positive for a dimorphic fungus that was subsequently identified both morphologically and by nucleic acid probe. The bone marrow aspirate showing the infecting organism is shown in Fig. 58.1. Growth of the organism incubated at 30°C is shown in Fig. 58.2. Microscopic morphology of the organism growing at 30°C is shown in Fig. 58.3.

1. What are the dimorphic fungi? With which of these agents was the patient infected? Which of these have an increased rate of dissemination in patients with AIDS?
2. Does the patient's country of origin, Panama, give any additional clues? Are there particular environments in which this organism is more likely to be found?
3. How does this organism appear at body temperature? At room temperature?
4. Clinically, how does disseminated disease with this organism occur in patients who have not recently been exposed to this organism?

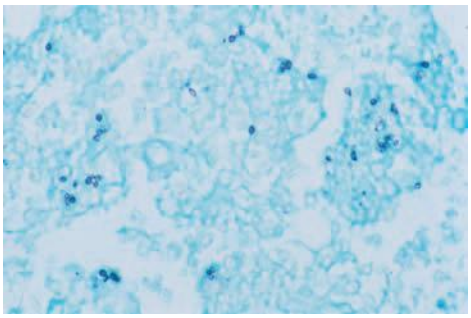


Figure 58.1



Figure 58.2

5. What other infections can invade the bone marrow, leading to fever, weight loss, and pancytopenia in patients with AIDS?
6. Culture is a rather slow method (on the order of several weeks) of diagnosing a disseminated infection with this organism. What other methodology is clinically useful in diagnosing patients suspected of having disseminated disease with this organism?

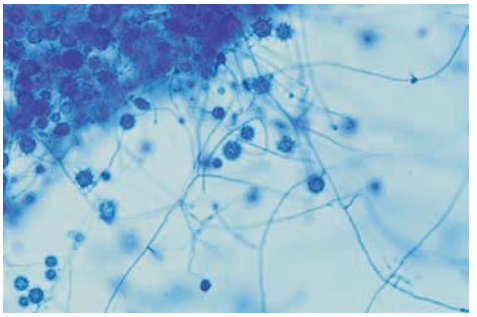


Figure 58.3

CASE DISCUSSION



1. Dimorphic fungi exist in the mold form at 25 to 30°C and as a yeast or a yeast-like form at body temperature. The dimorphic fungi include *Blastomyces dermatitidis*, *Coccidioides immitis/posadasii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffei*, and *Sporothrix schenckii*. Of these fungi, *C. immitis/posadasii*, *H. capsulatum*, and *P. marneffei* all have an increased rate of dissemination in patients with AIDS, and the mortality of infection with *P. brasiliensis* is high. This patient was infected with *H. capsulatum*, the etiologic agent of histoplasmosis. Identification is based on the mold form of the fungus. In Fig. 58.3, the characteristic tuberculate macroconidia of *H. capsulatum* can be seen.

2. Histoplasmosis was originally described in Panama. Regions where *H. capsulatum* is endemic include many areas in the midwestern and south central United States, especially in the Ohio, Mississippi, and Missouri River valleys. What is not well appreciated, however, is that much of the Caribbean basin is also a region in which this disease is endemic. In fact, of patients admitted to Boston Medical Center Hospital over the past several years who were subsequently found to have histoplasmosis, all were originally from the Caribbean basin, with Puerto Rico the most common site of origin. This, of course, reflects the patient population at this institution, but it also serves to illustrate that the geographic distribution of this disease is not limited to certain regions within the United States.

In nature, although *H. capsulatum* does not infect birds, soil that is rich in nitrogen, primarily from bird or bat droppings (near chicken coops, pigeon roosts, or starling roosts or in caves, etc.), is an environmental source for this organism.

3. At body temperature, *H. capsulatum* is found in the yeast form (Fig. 58.1). Within the body, it is typically present as small, oval cells with thin walls and is often found intracellularly. At room temperature, conversion to a mold occurs (Fig. 58.2). The tuberculate macroconidia of the mold form of *H. capsulatum* are shown in Fig. 58.3.

4. Although most cases of disseminated histoplasmosis complicating AIDS are likely due to exogenous infection, the disease can also occur as a result of reactivation of a latent infection. Like infections with *Mycobacterium tuberculosis*, an acute infection with *H. capsulatum* is usually contained by the body's cell-mediated immunity. An exposure to *H. capsulatum* in an immunocompetent host typically results in an immune defense to wall off the organism. If the host's cell-mediated immunity is compromised, as it can be, for example, by infection with HIV, the balance between the host and the pathogen will be tilted in favor of the pathogen. The host's cell-mediated immunity may no longer be able to keep the *H. capsulatum* in check, and in some cases disseminated disease occurs. In patients with HIV who have disseminated histoplasmosis, the standard recommendation for therapy is

to treat with liposomal amphotericin for 4 to 6 weeks followed by 1 year of oral itraconazole, which is more active against *H. capsulatum* than the more widely used fluconazole. Because of drug interaction caused by itraconazole, therapy may be shortened if adequate immune reconstitution using highly active antiretroviral therapy (HAART) can be achieved.

5. The other common infectious agents that cause pancytopenia in AIDS patients and invade the bone marrow are members of the *Mycobacterium avium* complex, *M. tuberculosis*, and those *Leishmania* species that cause visceral leishmaniasis. Disseminated *M. avium* complex infections typically occur when an HIV-infected individual's cell-mediated immunity is markedly suppressed, as can be demonstrated by a low number of CD4-positive cells. Both of these mycobacterial infections can be diagnosed by appropriate mycobacterial culture techniques. Although visceral leishmaniasis has not been reported from Panama, sand fly vectors do exist in parts of the country that could potentially transmit the infection if it was introduced into Panama.

6. An enzyme immunoassay that detects *H. capsulatum* polysaccharide antigen in urine, blood, and bronchoalveolar lavage (BAL) fluid has been developed that has good sensitivity and specificity in AIDS patients with disseminated histoplasmosis. The time to the detection of this polysaccharide antigen is much more rapid than is the incubation time required for a positive culture. In addition, the level of antigen falls in response to successful antifungal therapy and can be used to monitor cases for relapse. The drawback of this test is that it is generally not performed in hospitals and must be sent to a reference laboratory, extending the time until a result is available. Cross-reactions with other dimorphic fungi, most notably *Blastomyces* and *Coccidioides* spp., occur. Recent studies have demonstrated that this enzyme immunoassay is very sensitive when BAL fluid is tested. However, the procedures that are used to perform a BAL and to process BAL specimens vary from institution to institution, which may affect the test performance characteristics of the enzyme immunoassay for BAL specimens. In addition, the majority of patients who have been studied were infected with HIV, and the test performance characteristics are not yet well established for patients who are not infected with HIV. It is worth noting that in some cases of disseminated histoplasmosis, the organisms can be seen in smears of peripheral blood or of the buffy coat. When this is seen, the diagnosis of disseminated histoplasmosis can be made quite rapidly.

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The patient was a 58-year-old man who was seen in the HIV clinic for the evaluation of a new rash. He was taking antiretroviral therapy and had a recent undetectable HIV viral load and a normal CD4 count of 631 cells/ml. Two months prior to his evaluation, he had had anonymous unprotected sex with another man. At presentation he complained of 2 weeks of mild temperature elevation, generalized achiness, and a diffuse rash. On examination, he was afebrile and was covered in innumerable papular, red lesions on his extremities, torso, palms, and soles (Fig. 59.1 and 59.2). There were no oral lesions. He had mild alopecia.

A rapid plasma reagin (RPR) test done at this clinic visit gave a positive titer of 1:256. A *Treponema pallidum* particle agglutination (TPPA) assay was also performed and was positive.

1. How do you interpret the results of his RPR and TPPA tests?
2. Why were two tests necessary to establish the diagnosis of this infection? Why are serologic tests the standard method that is used to diagnose this bacterial infection?
3. What new testing strategy for this organism has recently been advocated by the Centers for Disease Control and Prevention (CDC)? What are the strengths and weaknesses of this approach?



Figure 59.1 Patient's rash on the back and upper arm.



Figure 59.2 Patient's rash on palms.

4. Why are patients, especially females, frequently unaware that they are infected with this organism?
5. Describe the natural history of this disease in patients who go untreated. In what stage of the illness was this patient? How does the disease process differ in HIV patients?
6. For what other infections was this patient at increased risk?
7. What is the current status of antibiotic resistance in the organism that caused this patient's infection?



CASE DISCUSSION

1. The positive RPR test, when confirmed by a positive TPPA assay, is consistent with the diagnosis of syphilis.

2. *Treponema pallidum* is the etiologic agent of syphilis. Unlike other common agents of sexually transmitted infections (STIs), this organism cannot be cultured on an artificial medium or in tissue culture cells. The reason for this is that *T. pallidum* lacks a number of genes that encode specific metabolic and biosynthetic processes that must be functioning in order for the organism to grow *in vitro*, which requires it to scavenge amino acids, nucleotides, and lipids from the host. As a result, laboratory detection has long relied on either direct microscopic examination using dark-field microscopy or serologic testing. Dark-field examination is comparatively insensitive and should not be used on oral lesions because the mouth harbors other spirochetes as a part of its normal microbiota. Because syphilis is rare outside the setting of an STI clinic, most laboratorians lack the necessary experience to reliably perform dark-field microscopy.

Syphilis serologic testing has traditionally been performed using a two-step algorithm. In the initial step, a screening test is performed, followed by a confirmatory test if the screening test is positive. The desired characteristics of a screening test are that it be highly sensitive (detect all people with the disease state in question), rapid, easy to do and interpret, and inexpensive. While the RPR test has a high sensitivity in secondary syphilis, it misses approximately one-third of cases in primary syphilis and a similarly notable fraction of people with latent and tertiary syphilis, such as in many cases of neurosyphilis. Screening tests often are not highly specific (they may detect patients who do not have the disease state in question), and so a confirmatory test is needed to confirm the results of the screening test. The confirmatory test is frequently more complex and more expensive, but it is much more specific than the screening test, which in practice means that a positive result on the confirmatory test indicates the patient has the disease state for which she or he has been tested.

In this case, the RPR test was performed as the screening test. RPR is a nontreponemal test in that it detects the presence of an antibody (reagin) that reacts with a purified beef heart extract (cardiolipin-lecithin and cholesterol particles) and not with a treponemal antigen. An RPR test may be positive either due to a treponemal infection or due to one of many other causes, as it is not specific for syphilis. As a result, there are many different infectious (e.g., leprosy, malaria, and infectious mononucleosis) and noninfectious (e.g., systemic lupus erythematosus) causes of a positive RPR result. Other treponemal diseases can cause a positive RPR test as well. It is uncommon for a patient from the United States to have a cause other than syphilis result in an antibody titer as high as 1:256.

The use of a confirmatory treponemal test, which may include either the TPPA assay or the fluorescent treponemal antibody absorption (FTA-ABS) test, can confirm the clin-

ical diagnosis of syphilis in a patient with a positive nontreponemal screening test such as the RPR. These tests look for antibodies to treponemes. Though they are more specific than the RPR test, they are in some cases technically difficult to perform and would therefore be poor choices for screening tests.

Once an individual has syphilis, the treponemal antibody tests remain positive even if the patient has been appropriately treated and the disease has been cured. By contrast, in most cases of treated syphilis, the RPR titer progressively declines and ultimately becomes undetectable. As a result, a new positive RPR result in a patient who was treated for syphilis and had an undetectable (nonreactive) RPR following therapy is assumed to indicate a reinfection. Of note, in some patients, particularly those with syphilis of long duration, the RPR titer remains positive at a low titer ("serofast") following therapy. As a result, a patient who has an increase from the low, positive titer should be evaluated under the assumption that the patient has been reinfected and, once again, has syphilis. Infection with *T. pallidum* and appropriate treatment for syphilis does not prevent another syphilis infection in the future.

3. The CDC, the Association of Public Health Laboratories, the United Kingdom Health Protection Agency, and the International Union against Sexually Transmitted Diseases all currently advocate a new testing algorithm for the detection of *T. pallidum* in which a *T. pallidum*-specific immunoassay is performed and, for positive specimens, an RPR test with titer is done as the confirmatory test (Fig. 59.3). This testing approach is called a reverse algorithm because the treponeme-specific test is initially performed as a screening test and the less specific nontreponemal test is performed as a confirmatory test. As with the standard syphilis serology algorithm, this approach is less sensitive in the primary stage of syphilis but has excellent performance in untreated secondary, latent, and tertiary syphilis. The reason these groups advocate this approach is that the treponemal

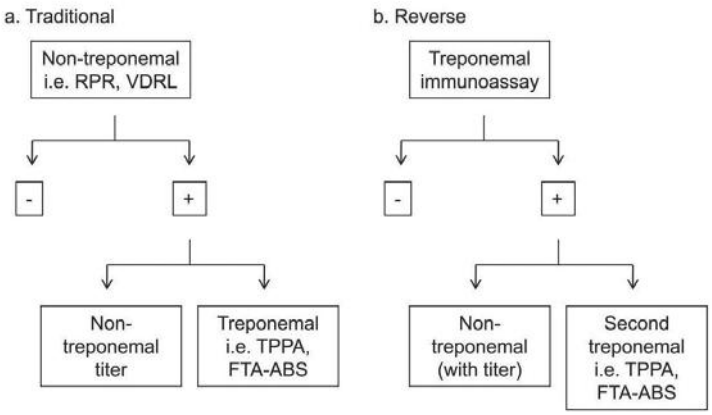


Figure 59.3 Syphilis testing algorithms.

screening test can be automated, making it simple to screen large numbers of specimens accurately. Additionally, it may have greater sensitivity in the primary phase of syphilis than do the nontreponemal screening tests. The major drawback of the reverse syphilis algorithm is that patients who previously were infected with syphilis and were successfully treated will be positive in the screening or treponeme-specific part of this algorithm and negative in the confirmatory or nontreponeme part of the algorithm. When this occurs, a third test such as TPPA must be done. This too can be positive in a previously infected and treated patient, making management of such a patient complex.

4. Infection by *T. pallidum* results in a painless ulcer (or multiple ulcers) called a chancre. The chancre, which is the hallmark of primary syphilis, occurs from 9 to 90 days (average of 21 days) following infection. In addition to being painless, the chancre will disappear even if the patient is untreated. This combination of a lack of pain and the fact that the chancre disappears is why many cases of syphilis are undetected early in the course of disease. In addition, the painless chancre may be unseen in women as it may not be anatomically apparent in a cursory examination of the external genitalia. An examination of material from the chancre under dark-field microscopy may demonstrate the presence of spirochetes (*T. pallidum*).

Given that women can be infected with *T. pallidum* and be unaware of this, there is one more problem that can occur. An additional means by which humans can become infected is transplacentally. Congenital syphilis was a tremendous problem early in the 20th century, and screening for syphilis in all pregnant women is currently recommended.

5. Following infection, primary syphilis, as evidenced by the presence of a chancre (see answer to question 4), occurs following an incubation period of 9 to 90 days. Dissemination of treponemes throughout the body can subsequently result in a diffuse symmetrical rash, often including the palms and soles, as well as generalized lymphadenopathy and abnormal liver function tests, and may result in "moth-eaten" alopecia. Fever may occur, as may ocular involvement. This is secondary syphilis, which occurs 6 weeks to 6 months after exposure. The rash may be macular, papular, pustular, papulosquamous, or follicular during secondary syphilis but will ultimately disappear. Lesions on mucosal surfaces (mouth, throat, and cervix) may occur. The rash contains many treponemes and the patient can infect others. There may be one or more relapses of secondary syphilis including the generalized rash. Once several years have passed, the patient progresses to latent syphilis. At this time there are no signs or symptoms of syphilis. A fraction of patients do not subsequently progress to late syphilis, but many do. During late syphilis, there may be vascular destruction, typically obliterative endarteritis of the terminal arterioles and the small arteries, as well as destructive lesions (gummas). Neurosyphilis and cardiovascular syphilis may occur in late syphilis. Nontreponemal tests such as the RPR test are often negative in late syphilis.

This patient had a generalized rash that was consistent with secondary syphilis. Alopecia, chronic fevers, and arthralgia and myalgia may also be seen in secondary syphi-

lis, but are not as specific findings as was the skin rash that the patient presented, which included involvement of the palms and soles. Men who have sex with men have an increased risk for both HIV and syphilis. It is estimated that 25% of all syphilis cases in the U.S. occur in individuals coinfected with HIV and that HIV-infected patients are over 50 times more likely to contract syphilis than the general population. The clinical presentation of syphilis in HIV-infected patients is similar to that in non-HIV patients, although HIV-infected patients with low CD4 counts may have a more severe, rapidly progressive disease course. Additionally, HIV-infected patients with CD4 counts of <350/μl are much more likely to have neurosyphilis. As a result, antimicrobial treatment regimens are prolonged in AIDS patients. Given his low viral load and fairly high CD4 count, this patient would likely not require prolonged antimicrobial therapy.

6. Given that this patient had syphilis, an STI, he was at risk for other STIs. In fact, the presence of an infection that causes genital ulcers, as does syphilis, has been established as a risk factor for HIV infection. Other infections that should be sought include gonorrhea, *Trichomonas*, and chlamydial infection.

7. *T. pallidum* continues to remain susceptible to penicillin G therapy, the treatment of choice for this organism. Unfortunately, resistance to an important second-line drug used in penicillin-allergic individuals, azithromycin, is emerging, especially in China, where this drug is used with some degree of frequency.

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The patient was an 18-year-old female who presented to the ear, nose, and throat clinic complaining of hoarseness and difficulty in swallowing. She had a 1-week history of sore throat, fever, fatigue, and myalgia. Her examination was significant for enlarged tonsils touching at the midline with exudate present. Bilateral tender anterior and posterior cervical lymphadenopathy, as well as splenomegaly, was present. Her complete blood count showed a hematocrit of 44% and a white blood cell count of 7,000/μl with 40% neutrophils, 28% lymphocytes, 12% atypical lymphocytes, and 20% monocytes. Liver function tests showed an aspartate transaminase (AST) level of 155 U/liter, an alanine aminotransferase (ALT) level of 208 U/liter, and an alkaline phosphatase level of 189 U/liter. Electrolytes were normal. Lateral neck radiographs showed a clear airway; the chest radiograph was negative. She was admitted to the hospital. A throat culture was sent to rule out gonococcal infection and beta-hemolytic streptococci. Viral serologic tests were ordered. She was treated with intravenous hydration, clindamycin, and steroids. On hospital day 2, a Monospot test result was positive. The clindamycin therapy was stopped, and oral prednisone was given. Her condition showed some improvement, with decreased tonsillar size evident on examination by hospital day 5.

1. What was the differential diagnosis? What viral serologic tests should have been ordered given her physical examination and initial laboratory findings?
2. What was the etiology of her illness? How was her diagnosis confirmed?
3. Briefly describe the epidemiology of infections with this etiologic agent.
4. Why was this patient given corticosteroids?
5. What complications can the agent causing her infection produce in immunosuppressed hosts? What is our current understanding of how this occurs? How are these complications detected in the laboratory?



CASE DISCUSSION

1. Her clinical presentation of fever, pharyngitis, cervical lymphadenopathy, and splenomegaly is consistent with infectious mononucleosis. Hepatitis, as was seen in this patient, is seen in ~80% of cases. Infectious mononucleosis is typically due to either Epstein-Barr virus (EBV) or cytomegalovirus (CMV). Acute HIV infection can also present as an infectious mononucleosis-like syndrome (see case 65). Given her significant pharyngitis, her physician also considered group A streptococcal infection and, because she was sexually active, gonococcal pharyngitis. Her enlarged tonsils raised the possibility of retropharyngeal abscess, often a surgical emergency. Given her significant lymphadenopathy, acute toxoplasmosis would also need to be included in the differential diagnosis. Her fever and elevated liver enzymes might suggest she had an acute hepatic infection.

The serologic tests that should be done would be a test for heterophile antibodies such as the Monospot test (see answer to question 2 for further details), CMV IgM antibodies to diagnose acute CMV-associated mononucleosis, and a fourth-generation HIV antibody/antigen combination enzyme immunoassay. IgM antibodies for hepatitis A, surface antigen and antibodies to core antigen of hepatitis B virus, and hepatitis C virus antibodies might also be sought, although their diagnostic value in this particular clinical setting likely would be minimal.

2. This patient had infectious mononucleosis due to EBV. This diagnosis was based on her clinical presentation, her physical findings, a white blood cell count and differential count consistent with infectious mononucleosis, and a positive Monospot test result. Her diagnosis was confirmed by specific EBV serologic tests. The positive Monospot test result indicated that the patient had heterophile antibodies. Heterophile antibodies, detected by an agglutination reaction to antigens of sheep or horse erythrocytes coated on latex beads, represent a nonspecific activation of B cells. These antibodies are present in 90% of EBV-infected patients at some time during acute illness. The heterophile antibody test is helpful when positive. The test is highly sensitive in adolescents and adults, although less so in children younger than 4 years. This patient's diagnosis was confirmed by EBV-specific serologic tests. There are a variety of serologic patterns that could be consistent with acute infection, including viral capsid antigen (VCA) IgM, VCA IgG, and early antigen-diffuse (EA-D) IgG positivity. However, Epstein-Barr nuclear antigen-1 (EBNA-1) IgG should be negative in primary infection. Detection of VCA IgM antibodies is the most accurate serologic test for diagnosis of acute EBV infection. EBV DNA viral load assays have been shown to be helpful in cases of indeterminate serologic results.

In patients with a negative Monospot, EBV-associated mononucleosis cannot be completely ruled out until results of EBV-specific serologies are known. CMV can also cause a heterophile-negative acute mononucleosis syndrome, so CMV IgM and IgG serologic tests should also be done in heterophile-negative individuals.

False-positive heterophile tests are unusual and are typically seen in patients with lymphoma or hepatitis.

3. EBV is often referred to as the "kissing disease" because it is typically acquired by intimate oral contact such as kissing. EBV replicates in oropharyngeal epithelial cells and B cells. The virus is shed continuously in saliva for months after acute infection and is shed intermittently thereafter. Following acute infection, B cells become latently infected and the viral genome forms a circular episome in the nucleus. The virus persists primarily in B cells and possibly also epithelial cells.

In patients <10 years of age, EBV infections are typically either asymptomatic or very mild and do not attract clinical attention. In the small subset of individuals who are not infected early in life, EBV infections can be more severe. Disease incidence is highest in adolescents and young adults (15 to 24 years), such as the patient discussed in this case. These individuals may have significant morbidity, with a spontaneously resolving illness lasting 2 to 3 weeks being the norm. Acute EBV infection is a frequent reason for hospitalizations among college students and an important cause of lost training days in military recruits. Deaths from acute EBV infections are rare and are typically due to airway obstruction caused by swelling of lymphoidal tissue, splenic rupture, or encephalitis.

EBV has been associated with an unusual form of lymphoma found primarily in equatorial Africa, Burkitt's lymphoma. It has also been associated with nasopharyngeal carcinoma, particularly in Southeast Asia. In both of these malignancies, EBV DNA can be found in the tumor cells. EBV has been associated with malignancy in HIV-infected patients and a lymphoproliferative disorder (LPD) in transplant recipients. These latter two disorders are discussed in the answer to question 5.

4. The use of steroids in uncomplicated cases of mononucleosis is controversial. However, in patients with significant tonsillar enlargement where concern exists that airway obstruction may occur, patients should be hospitalized and corticosteroid use is indicated, as it has been shown to quickly reduce this enlargement. Steroid use is also indicated to treat EBV-induced hemolytic anemia and thrombocytopenia. Several antiviral agents, including acyclovir and ganciclovir, have *in vitro* activity against EBV, but there is a paucity of clinical data regarding their *in vivo* efficacy, particularly in the setting of acute infectious mononucleosis.

5. EBV infection (either primary or reactivation) can result in more severe outcomes in immunosuppressed patient populations with defects in cell-mediated immunity, especially in HIV-infected patients with low CD4 counts and in patients receiving a solid-organ transplant or allogeneic hematopoietic stem cell transplant. In both individuals with AIDS and transplant recipients, an LPD can occur. The spectrum of disease associated with EBV infections in immunosuppressed individuals ranges from asymptomatic viremia to a mononucleosis-type illness to LPD.

In individuals with AIDS, LPD can result in an oral hairy leukoplakia that causes a white corrugated oral lesion typically seen on the side of the tongue. This is a nonmalignant condition where replicating virus can be detected. EBV-associated non-Hodgkin's lymphoma is a common malignancy found in HIV-infected individuals. HIV-infected persons are 60 to 200 times more likely to develop non-Hodgkin's lymphoma than HIV-uninfected individuals. Other EBV-associated lymphomas seen in HIV-positive persons include Burkitt's lymphoma, Hodgkin's lymphoma (~15 times increased risk), primary effusion lymphoma (nearly exclusive to HIV), primary central nervous system lymphoma (~1,000 times increased risk), and diffuse large B-cell lymphoma. HIV-associated lymphomas are a result of diminished immune surveillance against viruses and virally infected tumor cells. Therefore, an important aspect of therapy is improvement in immune status in HIV-infected individuals.

The LPD seen in transplant recipients specifically is called posttransplant lymphoproliferative disorder (PTLD). PTLD generally occurs during the first year posttransplant, with a median onset of 2 months after hematopoietic stem cell transplant and 6 months after solid-organ transplant. EBV-seronegative transplant recipients are at much greater risk for PTLD than are seropositive individuals. PTLD presents with nonspecific symptoms such as fever, malaise, and anorexia along with a rapidly developing mass typically in the engrafted organ or nearby. Transplants of lymphoid-rich organs, such as lung or intestine, result in a higher risk of PTLD, presumably due to an increase in EBV-positive donor B cells. Transplant recipients of these organs are also more intensely immunosuppressed, which may also contribute to the increased risk. Although PTLD only occurs in 3 to 10% of adult solid-organ transplant recipients, its mortality is 40 to 60%. Since children are more likely to be EBV seronegative, the rate of PTLD is higher (~50%) in pediatric solid-organ transplant recipients. PTLD can result from either reactivation or primary infection, which can be from the transplanted organ or from exposure in the community. PTLD will often resolve with reduction of immunosuppressive therapy, but this may not always be possible. Other therapies used for established PTLD include rituximab (anti-CD20 monoclonal antibody) and traditional chemotherapy.

EBV latently infects B cells. A transmembrane protein called latent membrane protein 1 (LMP1) can be expressed by latently infected cells. LMP1 is found on the surface of lymphoma cells from patients with PTLD and HIV-associated lymphomas. LMP1 has been shown to bind a cytoplasmic signal-transducing molecule called TRAF. TRAF activates another protein, NF-κB transcription factor, which enters the nucleus and causes B cells to proliferate. For a more detailed discussion of the mechanisms of EBV-associated diseases, see reference 6.

Although serologic tests can be used to determine pretransplant serostatus, they are generally not helpful in diagnosing LPD in immunosuppressed patients; serologic results in the setting of immunosuppression may not be accurate. Monitoring EBV DNA viral load from either whole blood or plasma is the preferred method for detecting EBV-associated infections in immunocompromised individuals. A positive viral load is not necessarily indicative of LPD but can be an early indicator for additional testing, such as

imaging and histopathology. Patients with PTLD nearly always have a high EBV viral load. Detection of a high EBV viral load prior to the onset of symptoms may indicate impending PTLD and signal preemptive therapy (i.e., reduction in immunosuppression). The definition of a "high" viral load is controversial, and each lab must establish its own clinical thresholds for predicting LPD. Further, EBV viral loads correlate with tumor burden and can, therefore, be used to monitor therapeutic efficacy.

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The patient was a 19-year-old female who presented to the emergency department (ED) in mid-August with fever and altered mental status. Four days previously she had been seen in an outside clinic with complaints of fever, headache, nausea, diarrhea, dizziness, myalgia, and fatigue. She was told she had a viral syndrome and was sent home with supportive therapy. Two hours prior to her admission, the patient called a friend and asked to stay with the friend because she was not feeling well. When the patient arrived at the friend's home, the patient could not open the door. When the friend saw the patient, the friend immediately brought her to the ED. The friend related the following history in the ED. The patient had returned from Togo 3 weeks previously. While there she took doxycycline prophylaxis, but her level of compliance was unknown.

On physical examination she had a temperature of 38.7°C, heart rate of 127 beats/min, respiratory rate of 30 per minute, and blood pressure of 100/61 mm Hg. She was confused but arousable. The remainder of her physical examination was significant only for some scleral icterus. Laboratory data were significant for a creatinine level of 2.5 mg/dl, hemoglobin of 8.7 mg/dl, and platelet count of 66,000/μl. A peripheral blood smear was taken at the time of admission (Fig. 61.1).

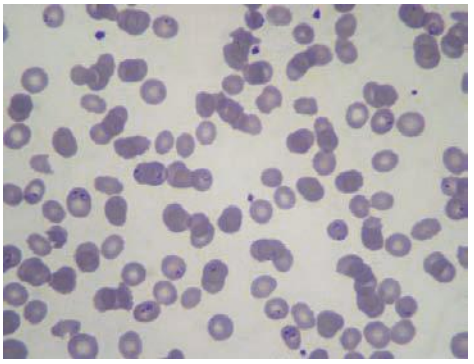


Figure 61.1

1. What is the organism (genus and species) present in her peripheral smear? Why is it important to be able to determine the species of this organism?
2. Where is this organism endemic?
3. The organism infecting this patient is not endemic in the United States, Canada, United Kingdom, Northern Europe, or Japan, and almost all individuals infected in these regions acquire it by travel to regions of endemicity. Give two explanations for how patients can obtain this infection in regions where it is not endemic.
4. What does the patient's smear tell you about the severity of her illness? Briefly explain the pathogenesis of her illness and how this pathogenesis contributes to her disease state. What did this individual fail to do that likely contributed to the severity of her infection?
5. What population in Togo is likely to have a disease state that mimics hers? Why is her disease course similar to that seen in this population in Africa?

6. The patient was begun on appropriate antimicrobials and given an exchange transfusion soon after admission. What do we mean by an exchange transfusion? Her parasitemia immediately posttransfusion was 15% but increased to 20% after 1 hour. This points out one of the shortcomings of exchange transfusion; what is it? She had a second exchange transfusion 24 hours later. Her parasitemia at the time of that procedure was 3%. Posttransfusion it was <1%. How do you explain the differences seen between the first and second transfusions?

7. The organism infecting this patient causes hundreds of millions of cases of disease worldwide and in excess of 500,000 deaths annually. However, there is evidence that cases of this disease are declining by 5% annually. Name two possible explanations for this decrease. A new approach currently undergoing clinical trials shows great promise to reduce infections with this organism in young children. What is this approach?



CASE DISCUSSION

1. Figure 61.1 demonstrates the delicate ring forms that are consistent with trophozoites of *Plasmodium falciparum*. Note the red blood cells (RBCs) infected with multiple ring forms. The presence of the trophozoites along the margin of the RBC is called appliqué. These findings are most frequently observed in, but are not limited to, *P. falciparum*, which is also the species of human malaria most frequently associated with mortality. Other species of malaria include *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium knowlesi*, and *Plasmodium malariae*.

Species identification of *Plasmodium* is important in selection of antimalarial agents. Because resistance in *P. falciparum* is constantly evolving, the interested reader is directed to the website of the Centers for Disease Control and Prevention for the most recent recommendations for treatment: http://www.cdc.gov/malaria.

Drug resistance is an important consideration in selection of antimalarial therapy. *P. falciparum* obtained in Africa must be considered to be chloroquine and sulfadoxine-pyrimethamine resistant. In addition, resistance to a newly developed antimalarial drug, artemisinin, is being recognized in Cambodia but is not widespread in Africa as of this writing. Because of this resistance, artemisinin-based drug combinations are being developed and thus far are efficacious. These combinations are infrequently used in the United States but are widely used in regions where malaria is endemic.

Therapeutic approaches must also take into consideration the life cycle of the specific *Plasmodium* species causing the infection. Globally *P. falciparum* is the most frequently encountered malaria species, but both *P. ovale* and *P. vivax* cause a significant number of malaria cases. Both *P. ovale* and *P. vivax* have a hypnozoite phase in the liver, while other species do not. The hypnozoite phase of the parasite allows *P. ovale* and *P. vivax* to cause a latent infection in which the parasite can persist in the liver for months to years before causing infection. Evolutionarily this makes sense because both *P. ovale* and *P. vivax* are found in more temperate climates where for at least part of the year the vector of the parasite, *Anopheles* mosquitoes, may be dormant because of cold temperatures. Antimalarials such as mefloquine and atovaquone-proguanil, widely used in the industrialized world, are not effective against the hypnozoite phase. Primaquine has activity against this phase and is used in combination with other antimalarials to treat *P. vivax*- and *P. ovale*-infected patients. Because *P. falciparum* lacks a hypnozoite phase, primaquine is not needed to treat it.

2. *P. falciparum* is the most common cause of malaria in sub-Saharan Africa, including Togo. *P. falciparum* is found in tropical areas primarily along the equator, with most cases of this disease found in equatorial West Africa. The parasite has global distribution in tropical areas, with infections occurring in the Amazon, jungle regions in Southeast Asia (Cambodia, Vietnam, and Thailand), the Philippines, and New Guinea. Occasional cases have been reported in both Jamaica and Hispaniola, the island that is shared by Haiti and

the Dominican Republic. These areas of the world provide an ideal habitat for the vector of this parasite, the *Anopheles* mosquito.

In the United States, malaria was found along much of the mid-Atlantic seaboard and the Gulf of Mexico until the 1940s, suggesting that environmental conditions are in place that would allow malaria transmission in these regions. The vector for malaria is widespread throughout the United States, so there is, in theory at least, the potential for endemic malaria to return to the United States. Given these factors, the importance of maintaining a public health infrastructure to prevent the return of endemic malaria must be emphasized.

3. Only a small number of malaria-infected patients (<1%) obtain the infection in the United States each year. Most are due to either vertical transmission from mother to child (the mother is typically an immigrant from a region where malaria is endemic) or blood transfusion. A very small number of cases are actually acquired in the United States from infected *Anopheles* mosquitoes. Three different scenarios have been suggested. One is that the individual acquires malaria via a mosquito that has taken a blood meal from an infected individual who had recently entered the United States from a region where malaria is endemic. An example of this mode of transmission has been reported from Long Island in New York State. This type of transmission only occurs if environmental conditions, i.e., temperature, humidity, and breeding grounds for the *Anopheles* mosquito, are favorable for the malaria developmental cycle in the mosquito. The second scenario is that infected mosquitoes are carried by jet planes from tropical regions. This is called "airport malaria." Studies have shown that viable female *Anopheles* mosquitoes can be found on jets that have flown from tropical regions to the industrialized North. Individuals living near international airports in both the United Kingdom and the United States have developed malaria without having traveled to regions of endemicity. Finally, injection drug users with malaria who come to the United States and share needles with individuals there have also transmitted malaria, although this is a highly unusual occurrence.

4. The patient's parasitemia (the percentage of RBCs infected) was calculated to be 30%, a finding consistent with severe malaria. Any definition of severe malaria would include a parasitemia of ≥10%, although some have stated that >2% parasitemia is associated with severe disease. A parasitemia of >10% is found in ~40% of patients with severe malaria, and these individuals have the highest mortality. There are several laboratory and clinical findings associated with severe malaria. This patient had evidence of renal (increased creatinine), central nervous system (mental status changes), and respiratory distress (respiratory rate of 30 per minute, and she required intubation) along with anemia and thrombocytopenia.

The pathogenesis of *P. falciparum* begins with the bite of an infected female *Anopheles* mosquito. The sporozoite stage of the parasite is present in the salivary gland of the mosquito

and enters the bite wound via the mosquito's saliva. From there, the sporozoites are carried by the bloodstream to infect hepatocytes. The parasites multiply in the hepatocytes and are released into the bloodstream as the merozoite phase. The merozoite phase then infects erythrocytes. As the *P. falciparum* parasites mature in RBCs, knobs develop on the surface of these cells. A malaria-derived protein found on the surface of the infected RBCs (*P. falciparum* erythrocyte membrane protein-1 [PfEMP1]) is believed to play a central role in the pathogenesis of malaria. PfEMP1 mediates the adherence of infected RBCs to endothelial cells in the microvasculature of the brain, kidney, lung, and other organs. In addition, it is thought to be responsible for the rosetting of uninfected RBCs to infected ones. The end result of infected RBCs binding to the endothelial cells and the rosetting of uninfected RBCs is a process known as sequestration. Sequestration is believed to cause obstruction of the microvasculature in the central nervous system, lungs, and kidneys. This obstruction, along with the production of increasing levels of cytokines induced by the infection, is thought to play a central role in cerebral malaria and acute respiratory distress syndrome, two potentially fatal manifestations of *P. falciparum* infection. Consequences of this blockage include decreases in glucose and oxygen levels in the brain tissue. Similar microvascular blockage in the kidney, coupled with high levels of circulating hemoglobin, is thought to cause the renal failure seen in *P. falciparum*-infected patients.

Failure to take malaria prophylaxis is the major reason why individuals from regions where malaria is not endemic develop malaria when visiting a region of endemicity. More than 95% of adults from regions where the disease is not endemic who develop severe malaria have not taken appropriate antimalarial prophylaxis. This patient reported taking antimalaria prophylaxis only sporadically during her visit to Togo. In addition, it is likely that she failed to notify her clinician on her initial medical visit that she had recently been in a region of endemicity, although the physician's apparent failure to elicit this history is also of concern.

5. Severe malaria is seen in two distinct patient populations: travelers from regions where malaria is not endemic who travel to regions where it is endemic, and young children who live in regions of malaria endemicity. The reason these two patient populations have similar disease courses and are most prone to severe disease is that both groups tend to be "immunologic virgins" when infected with *Plasmodium*. Adults in regions of malaria endemicity who survive childhood have some level of immunity and when they do get infected generally have quite mild disease. The exception would be adults with AIDS, in whom the severity of disease is influenced by CD4 counts.

6. Exchange transfusion is a controversial strategy for treating severe malaria. It is typically only performed in patients whose parasitemia is >10%. In this technique, the patient's blood is removed and replaced with transfused blood. A meta-analysis has shown that it has no benefit over chemotherapy, but an appropriate case-controlled study has not been performed. It is likely that such a study will never be done because the resources to

perform it in a region of the world such as equatorial West Africa, where there are sufficient numbers of severe malaria cases, are better used for other approaches such as prevention (see answer to question 7 for more details).

The finding of increased parasitemia after the initial transfusion is not surprising for two reasons. First, infected RBCs are sequestered on the surface of endothelial cells. These are not removed during exchange transfusion and provide a ready reservoir to infect the new RBCs that are present as a result of the transfusion. However, the patient in this case was on antimalarials, so why weren't the parasites killed? Clearance of malaria by chemotherapy takes a minimum of 18 hours and as long as 72 hours. Viable parasites were still present at the time of initial exchange transfusion, which occurred soon after admission to the hospital. The second exchange transfusion occurred 24 hours later; by that time, the antimalarial agents with which she was being treated, quinine and clindamycin, had begun to kill the parasite, as was reflected in the significantly reduced parasitemia at the time of the second exchange transfusion. The very low parasitemia 1 hour after the second exchange transfusion suggests that the sequestered *Plasmodium* had lost significant viability.

7. The decline in malaria cases and deaths has been attributed to improved prevention measures and diagnosis and treatment.

The *Anopheles* mosquito feeds primarily at night, making evening and nighttime precautions particularly important. Measures to prevent exposure to this mosquito include staying in well-screened areas in the evening and night; using mosquito netting, preferably impregnated with insecticides, when sleeping; wearing clothing that covers the trunk and extremities; using insect repellent containing *N*,*N*-diethyl-*m*-toluamide (DEET); and spraying sleeping areas with insecticides.

In addition, improved bedside diagnostics for detection of malaria are being used with increasing frequency in areas where malaria is endemic. The tests that are being used detect specific antigens of the different *Plasmodium* species with a specific focus on antigens of *P. falciparum*. The tests require a drop of blood applied to a nitrocellulose strip similar to a pregnancy test. The blood is lysed, and specific *Plasmodium* antigens migrate on the strip until they encounter impregnated antibodies that bind these specific antigens. The antibody-antigen complex can be detected by an enzymatic reaction. This testing approach is rapid, taking no more than 15 minutes. It is much easier and generally more accurate than the traditional thin and thick malaria blood smears, which are not available in most settings where malaria diagnosis is most needed.

Finally, attempts are ongoing to develop a malaria vaccine. Progress in developing such a vaccine has been slow, in part because of the exceedingly complex life cycle of the parasite. A number of different vaccine strategies have been proposed. One promising approach is a recombinant vaccine derived from the major surface protein of the infective stage of the parasite, the sporozoite. The target antigen is a polypeptide of circumsporozoite protein that has been fused with hepatitis B virus surface antigen. This vaccine has

been referred to as the RTS,S/AS01 vaccine. Recent reports of an ongoing clinical trial of this vaccine in children <17 months of age shows that it has an efficacy of 50% against *P. falciparum*. Although not as impressive as hoped, it is an important first step in developing a vaccine with high efficacy.

One of the challenges in developing this vaccine is the need to induce immunity such that the parasite is eliminated before it enters the hepatocyte. Once the sporozoite enters the hepatocyte and develops into merozoites, it undergoes significant antigen variation, making it unlikely that the immune response generated by this vaccine will be effective at the next stage of the disease.

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A 5-year-old African-American male with a known history of sickle cell disease presented to an outside hospital with 3 days of intermittent fevers and headache. The mother reported that he had had one episode of vomiting, decreased oral intake, and fatigue. The patient also complained of occasional abdominal and foot pain. At the outside hospital, his complete blood count showed numerous nucleated red blood cells (RBCs), a corrected white blood cell (WBC) count of 75,000/μl (normal, 5,000 to 14,500), a hemoglobin level of 3.1 g/dl (normal, 11.5 to 13.5), and a platelet count of 962,000/μl (normal, 150,000 to 440,000). The patient's baseline hemoglobin was 6 to 8 g/dl. After blood cultures were obtained, he was given ceftriaxone before transfer to our institution. He had had three prior hospitalizations, all for fever and upper respiratory viral syndromes. The patient had been on penicillin since early infancy and was up to date on vaccinations, including pneumococcus and *Haemophilus influenzae* type b vaccines.

Upon admission at our institution, the patient continued to be anemic, with a hemoglobin level of 2.5 g/dl and a hematocrit of 7.4% (normal, 34 to 40%). He subsequently received two transfusions of packed RBCs, which brought his hemoglobin up to 5.3 g/dl. Although blood cultures were negative to date, he was continued on ceftriaxone and vancomycin was added. A positive PCR result was obtained, which confirmed this patient's infection.

1. What syndrome did this patient have? What is the etiology of his infection? Why were his WBC and platelet counts so high?
2. How does infection with this organism normally present in childhood? Are there any differences in presentation when primary infection occurs in an adult?
3. This patient had sickle cell disease, which predisposed him to this infection as well as other infections. What infections are patients with sickle cell disease more susceptible to, and why? Why had he received life-long penicillin G therapy?
4. What other patient populations are at risk for severe infections due to this organism?
5. What methods are available to diagnose this infection? Include a discussion of their strengths and weaknesses.



CASE DISCUSSION

1. This patient had transient aplastic crisis due to parvovirus B19 infection. Parvovirus B19 (also called erythrovirus) is a nonenveloped, single-stranded DNA virus that replicates in and destroys erythroid progenitor cells. The host cellular receptor is the blood group P antigen. Thus, individuals who lack P antigen do not get infected with parvovirus. When parvovirus infects the bone marrow, RBC production is grossly diminished, if not halted, for several days. In most patients RBCs live for ~120 days, but in patients with sickle cell disease RBCs only live ~10 to 20 days. Therefore, in patients with sickle cell disease, parvovirus infection causes severe anemia, as evidenced by very low hemoglobin and hematocrit values. Transient aplastic crisis can occur in the general population, but it is generally only recognized in patients with chronic anemias, such as sickle cell disease, thalassemia, iron deficiency anemia, hereditary spherocytosis, or any disorder of hemoglobin gene expression. With the possible exception of necessitating a blood transfusion, transient aplastic crisis is generally self-limiting. However, a sudden drop in hemoglobin could lead to congestive heart failure, a cerebrovascular accident, or acute splenic sequestration, all of which were carefully monitored in this patient during his hospital stay. Interestingly, this patient had a pronounced leukocytosis (of all cell types) and thrombocytosis, which is not classically associated with parvovirus infection. More often, due to bone marrow suppression, there is a mild leukopenia and thrombocytopenia. However, because all WBC types were elevated in this patient, including immature WBCs, and numerous nucleated RBCs and increased platelets were seen, this indicates a general increase in erythropoiesis, which can be seen in sickle cell disease without parvovirus infection (i.e., sickle cell crisis). The child's complaint of abdominal and foot pain could also support that he was having a sickle cell crisis. Nonetheless, the patient had a decreased total RBC count and low hemoglobin and hematocrit, which is consistent with parvovirus infection.

2. Primary parvovirus infection in childhood normally presents as either a nonspecific viral illness (e.g., fever, coryza, myalgia, and headache) or erythema infectiosum (i.e., fifth disease of the childhood exanthems). Erythema infectiosum is preceded by a nonspecific viral prodrome, but several days later a characteristic "slapped cheek" rash appears that may be followed by a lacy erythematous rash on the extremities or torso (Fig. 62.1). The rash is more common and pronounced in children than adults. This patient presented acutely during the viral prodrome phase, so a rash would not yet be apparent. However, not all children develop a rash, and ~20% of children may be asymptomatic. Although relatively uncommon, severe complications such as myocarditis and encephalopathy can occur. Initial symptoms in adults may also be nonspecific, but adults have a higher incidence of arthralgia in knees, feet, and/or hands. Fewer than 10% of children have arthalgia, while up to 60 to 80% of adolescent and adult patients have joint pain. Some adults, particularly women, may have arthralgia as the only symptom of parvovirus infection (i.e.,

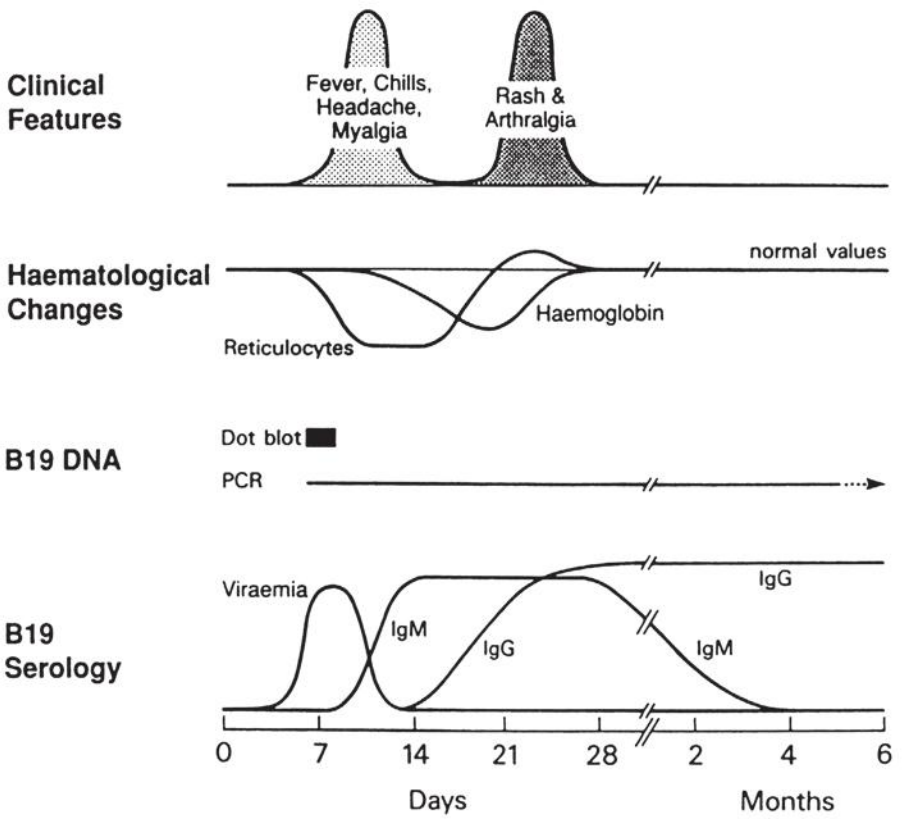


Figure 62.1 Parvovirus B19 infection in a healthy individual. (From Brown KE, Young NS, *Blood Rev* **9**:176–182, 1995, with permission.)

polyarthropathy syndrome); joint pain may last from 1 week to a few months. By the time the rash and/or arthralgia appears, the patient is no longer infectious or viremic (Fig. 62.1). The laboratory diagnosis of parvovirus infection in adults with arthralgia can be complicated by false-positive rheumatoid factor and anti-nuclear antibody tests, which can be seen in patients with parvovirus infection. Young adults may also present with papular, purpuric lesions on the hands and feet called "gloves and socks syndrome."

3. Patients with sickle cell disease are susceptible to infections caused by encapsulated bacteria, nontyphoid *Salmonella*, and *Plasmodium falciparum* (in countries where it is endemic). Encapsulated bacteria of particular significance are *Streptococcus pneumoniae* and *H. influenzae* type b. This is why a vaccination history was elicited for this patient. Historically, children <5 years old with sickle cell disease were at greatest risk for meningitis and septicemia due to *S. pneumoniae* and *H. influenzae*, but vaccination has reduced

the incidence of infections due to these organisms in developed countries. *S. pneumoniae* is responsible for ~75% of septicemia and meningitis cases in children with sickle cell disease. Because not all serotypes are included in the vaccine, the patient received prophylactic penicillin to prevent infections due to other *S. pneumoniae* strains. Additionally, during the course of this patient's acute illness, he received ceftriaxone to treat a potential *S. pneumoniae* infection, and vancomycin was added to cover for potentially resistant strains. Both antimicrobials were discontinued when blood cultures were negative at 5 days and an alternative diagnosis was available. Other organisms that cause infections less frequently are *Neisseria meningitidis* and *Escherichia coli*. The accumulative effects of red cell sickling and hemolysis on the spleen lead to functional asplenia between 6 months and 3 years of age. This predisposes patients to infections with encapsulated organisms. Opsonized bacteria (coated with antibodies or complement components such as C3b) can be removed directly by splenic or hepatic macrophages, but encapsulated organisms are poorly opsonized and require removal by the spleen. Further, hyposplenic and asplenic patients have also been shown to lack IgM memory B cells, which also hinders the immunologic response to encapsulated organisms. These predisposing factors ultimately result in an increased rate of localized infections becoming systemic. Studies have also suggested that defects in the alternative pathway of complement activation may contribute, but these findings have not been confirmed in large-scale studies.

Salmonella osteomyelitis is an infection of concern in all age groups with sickle cell disease and represents ~70% of hematogenous osteomyelitis cases. It is thought that devitalization of the gastrointestinal tract and bone due to repetitive sickle cell crises, saturation of the reticuloendothelial system with RBC breakdown products from chronic hemolysis, and underlying splenic and hepatic dysfunction contribute to the predisposition to *Salmonella* infections.

Although sickle cell trait has been shown to provide some protection from *P. falciparum* infection in countries where it is endemic, malarial infection is more severe in those with sickle cell disease due to increased sickling, hemolysis, and subsequent vessel occlusion. Malaria is the most common cause of sickle cell crisis in tropical Africa.

4. Parvovirus infection can cause complications in pregnant women and immunocompromised patients, including solid-organ transplant recipients, hematopoietic stem cell transplant (HSCT) recipients, and HIV-positive individuals. Approximately 25 to 45% of women of childbearing age have never been infected with parvovirus, as demonstrated by serologic studies. The incidence of parvovirus infection in pregnant women is generally ~1% but rises with epidemics. In part due to the high-level viremia associated with parvovirus infection, viral transmission across the placenta is high, leading to 25 to 50% of fetuses becoming infected. This is particularly true during the second trimester, when there is rapid expansion of fetal erythroid precursor cells. The risk of an adverse fetal outcome is ~10% and is highest when infection occurs during the first 20 to 22 weeks of gestation. Fetal infection can resolve without sequelae or can lead to severe complications,

including nonimmune hydrops fetalis due to severe fetal anemia, myocarditis, and spontaneous abortion. Once maternal infection is confirmed (see answer to question 5), the fetus should be monitored weekly by ultrasound for hydrops. Monitoring should continue for 12 to 20 weeks postexposure. If the fetus develops hydrops due to anemia, an intrauterine transfusion can reduce the risk of morbidity and mortality.

Parvovirus infection in immunocompromised patients (e.g., transplant recipients and HIV-positive patients with advanced immunodeficiency) can lead to chronic pure red cell aplasia. Patients present with a very low or absent reticulocyte count and chronic anemia that does not respond to erythropoietin. Other symptoms are less common, including fever. Rash and arthralgia are rare since these are mediated by antigen-antibody complexes in immunocompetent individuals. Posttransplant parvovirus infection appears to be more common in solid-organ transplant recipients than in HSCT recipients, possibly due to the intravenous immunoglobulin that is commonly used after HSCT. Primary parvovirus infection posttransplant is associated with very high viral loads and clinical disease. Interestingly, a recent study has shown that low-level viral loads persist in ~5% of solid-organ transplant recipients in the absence of associated anemia; most of these patients were seropositive. Further investigation is needed regarding the clinical relevance of persistently low viral loads in seropositive immunocompromised patients. In HIV-positive patients, early recognition of parvovirus infection could prevent unnecessary transfusions, as successful control of the infection has been demonstrated with antiretroviral therapy. Additionally, immunocompromised patients are at risk for transfusion-associated parvovirus infection. Although it is uncommon, packed RBCs are not screened for parvovirus and have been a source of parvovirus infection in susceptible patients.

5. The two main methods used for the laboratory diagnosis of parvovirus infection are serology and nucleic acid amplification tests (NAATs). Detection of IgM and IgG antibodies to viral capsid antigen is the preferred method to determine immune status. A positive IgM test generally indicates that infection has occurred in the preceding 2 to 4 months and is reportedly 89% sensitive and 99% specific. A positive IgG test (in the absence of IgM) indicates past infection. However, serologic testing is not reliable for determining the immune status of immunosuppressed patients, fetuses and neonates, or patients receiving intravenous immunoglobulin. Additionally, in acute primary disease (such as in this patient), serologic tests are likely to be negative. In these circumstances, NAAT should be used. Unfortunately, no FDA-cleared parvovirus NAATs are available, which limits the availability of this test. However, a number of laboratory-developed tests have been described, including some using commercially available analyte-specific reagents. The challenge in using analyte-specific reagents or laboratory-developed tests is the extensive validation work that must be performed by the laboratory prior to implementing clinical testing. Nonetheless, NAAT is the preferred method for diagnosing aplastic crisis in patients with sickle cell disease, pure red cell aplasia in immunocompromised patients, and

parvovirus-associated hydrops fetalis. Whole blood is the specimen of choice for aplastic crisis and pure red cell aplasia, while amniotic fluid is the specimen of choice to diagnose fetal infection with parvovirus. As mentioned above, acute infections nearly always have very high viral loads. This is a challenge for NAATs, as high-titer specimens represent a significant source for laboratory contamination. Therefore, it is prudent to have laboratory procedures in place that control for and detect possible parvovirus contamination in NAATs. Another challenge associated with NAAT is that the test detects DNA and not necessarily live virus. Parvovirus DNA has been detected for 6 months or longer from whole blood and over a year from bone marrow after initial infection. Therefore, it is critical to analyze NAAT results in clinical context, as a positive NAAT does not necessarily represent acute infection. Quantitative reporting of NAAT results may aid in the interpretation of positive results.

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SECTION SEVEN



ADVANCED CASES

INTRODUCTION TO SECTION VII

Our understanding of infectious diseases has continued to evolve in the first decade and a half of the 21st century. A number of issues have arisen that have challenged our ability to control and prevent infectious diseases. We are seeing new patient populations who are at risk for a variety of infectious diseases that either have newly emerged or were rarely encountered in the previous century.

Expanding, rapid travel, including adventure and medical tourism and military interventions, has created the need to understand global infectious disease threats that perhaps would not have been considered at the end of the last century. For example, an individual can visit a live poultry market in China one day and on the next day fly to airline hubs in any of numerous cities around the world, which has the potential for the global transfer of a novel influenza virus. Patients from America and Europe may choose to have surgical procedures performed, for a fraction of the cost of their usual care, in sophisticated medical centers in the developing world, where they may be exposed to organisms that cause clinical disease with which their physicians in the industrialized world may not be familiar.

Advances in health care have resulted in an increased lifespan and quality of life. These advances have led to new infectious disease challenges. Manufactured materials such as artificial joints, intravascular catheters, ventricular assist devices, and pacemakers may become infected by organisms once dismissed as "contaminants." Management of these infections may require the removal of these materials.

The use of immune modulators, including monoclonal antibodies, has improved the likelihood of successful solid organ and hematopoietic stem cell transplants and the treatment of graft-versus-host disease. Immune-modulating monoclonal antibodies have also improved the quality of life for a variety of patients including those with multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease. Unfortunately, these immune modulators place individuals at risk for a variety of infections due to such organisms as *Mycobacterium tuberculosis*, dimorphic fungi (e.g., *Histoplasma capsulatum*), *Pneumocystis jirovecii*, *Neisseria meningitidis*, JC and BK viruses, and organisms that arise from the patient's environment. These so-called "environmental organisms" include glucose-nonfermenting Gram-negative bacilli such as *Acinetobacter* species and *Pseudomonas aeruginosa*; higher-order bacteria such as *Nocardia*; environmental mycobacteria such as *Mycobacterium abscessus* and *Mycobacterium avium* complex; and fungi that are frequently found within the hospital environment including in the air-handling and water systems. These organisms do not typically cause disease in individuals with normally functioning immune systems, but can cause devastating infections in patients who are immunosuppressed either by their disease process or by the immunosuppressive drugs they are receiving. Further, the survival of these organisms in nature requires that they have developed mechanisms to survive in environments rich in antimicrobial substances such as naturally occurring antimicrobials. As a result, these organisms tend to be much more resistant to antimicrobial agents than are those organisms that have adapted over the centuries to humans, such as group A streptococci and *Treponema pallidum*.

One of the great challenges now facing medicine is the problem of multidrug-resistant (MDR) organisms. Three factors are important:

1. The emerging importance of environmental microorganisms that are adapted to surviving in antimicrobial-rich environments, such as *Acinetobacter* and *P. aeruginosa*
2. The indiscriminate and inappropriate used of antimicrobials. Two key examples are (i) the all-too-common practice of using antibacterial agents to treat viral respiratory illness in children and (ii) the use of antimicrobials in the feed of animals raised in industrialized farm settings, rather than preventing epidemics by vaccination and responsible husbandry.
3. The failure to develop agents with novel mechanisms of action to which organisms have difficulty developing resistance. This problem is particularly acute for Gram-negative bacteria for which antimicrobial agents with novel mechanisms have not been developed in this century.

There are multiple examples of MDR bacteria and their global spread, but we will discuss just three.

In the early 1990s, *Staphylococcus aureus* strains resistant to all antimicrobial agents with the exception of vancomycin became a common cause of health care-associated infection. These organisms, called methicillin-resistant *S. aureus* (MRSA), along with another important health care-associated pathogen, *Clostridium difficile*, were treated with vancomycin. The resulting antimicrobial pressure is believed to have made a major contribution to the emergence of another health care-associated pathogen, vancomycin-resistant enterococci (VRE). Bacteremia due to these organisms has a high mortality rate. In 1997, strains of *S. aureus* with reduced susceptibility to vancomycin were also described. These organisms, called VISA (vancomycin-intermediate *S. aureus*), are being seen with increasing frequency and are often clinically refractory to vancomycin treatment. Fortunately, new antimicrobial agents to cope with MDR Gram-positive organisms—linezolid, daptomycin, and ceftaroline—have been developed to treat infections due to MRSA, VISA, and VRE. Unfortunately, drug resistance to these new antimicrobials appears to be emerging. Vancomycin-resistant *S. aureus* strains are still rare, however, even though they were first recognized over a decade ago.

Carbapenem-resistant *Enterobacteriaceae* (CRE) and carbapenem-resistant *Acinetobacter* are a major global infectious disease threat. These organisms possess multiple mechanisms of resistance encoded on plasmids and other transposable genetic elements. As a result, the genetic machinery responsible for drug resistance to multiple antimicrobial agents can be easily spread among a variety of Gram-negative bacilli. Colistin, an antimicrobial with significant renal and ototoxicity, may be the only antimicrobial available to treat invasive disease with CRE and carbapenem-resistant *Acinetobacter*.

A final MDR bacterium of global importance is MDR *Mycobacterium tuberculosis* (MDR-TB). MDR-TB is typically resistant to the first-line antituberculosis medications isoniazid and rifampin and is frequently resistant to other first-line agents as well. The combination of MDR-TB and HIV has been proven to be practically deadly, especially in

the developing world where access to care is at best rudimentary. One of the greatest concerns about MDR-TB has been the emergence of extensively drug-resistant *M. tuberculosis*, or XDR-TB. XDR-TB is MDR-TB strains that are also resistant to oral fluoroquinolones such as moxifloxacin and one of the second-line injectable agents such as amikacin. MDR-TB is global in distribution and is most prevalent in the Russian Federation, Kazakhstan, China, India, the Philippines, Eastern Europe, South Africa, and Peru, with a moderate prevalence in Southeast Asia, Brazil, and the Middle East. There is little evidence of significant person-to-person spread of MDR-TB in the United States, but importation of cases is well recognized. XDR-TB is much less common globally, and few cases have been seen in the U.S.

Antimicrobial resistance is not just a problem in treating bacterial infections. One of the important strategies used in the management of hematopoietic stem cell transplantation is the use of prophylactic antifungal therapy during the period when patients are neutropenic. The reason that this is important is this patient population is at risk for invasive infections with both the endogenous fungi—including *Candida*, a component of the microbiota of the skin and mucous membranes—and exogenous fungi, such as *Aspergillus*. Either an azole or echinocandin may be used prophylactically to prevent invasive disease. Unfortunately, two fungi with reduced susceptibility to these agents, *Fusarium* spp. and *Trichosporon asahii*, are being seen with increasing frequency as causes of fungemia with significant morbidity and mortality.

Changes in lifestyle in the developed world and pressures due to population growth in the developing world may be bringing humans into ever-increasing contact with several newly recognized pathogens. The initial outbreak of the hantavirus pulmonary syndrome occurred in the Four Corners Region of New Mexico, Colorado, Utah, and Arizona and is believed to have been due to increasing contact between humans and the deer mouse, the reservoir of the Sin Nombre virus. A recent outbreak due to this viral agent has been seen in Yosemite Valley in California, a popular global vacation destination. Ehrlichiosis, babesiosis, and Lyme disease, which are all spread by ticks, are being seen with increasing frequency because humans are more frequently entering environments (either to live or for recreational purposes) where their exposure to infected ticks is increased. Increases in yellow fever and dengue fever in tropical regions may be due in part to clearing of forest for farmland as well as a failure of mosquito control. The impact of global climate change on mosquito-borne infections is an active area of research.

Recognition of West Nile virus in the metropolitan New York area in 1999 reminded us that the combination of worldwide jet travel and the presence of appropriate insect vectors can result in the establishment of seemingly exotic tropical infectious agents in new environmental niches in the industrialized world, including the United States, Canada, and northern Europe. In just 3 years this viral agent spread throughout the continental United States despite strong public health efforts to eradicate it through vector control measures.

Another virus, Chikungunya, has recently emerged in the Caribbean basin. This virus causes a disease similar to dengue virus and has the same mosquito vector. Chikungunya virus was thought to be a cause of disease primarily in Southeast Asia and the Indian Ocean region. This is another example of the potential for rapid global spread of a viral agent.

New variants of the influenza A virus may have emerged through reassortment events between avian and human viral strains. In 2009, a triple reassortant H1N1 influenza A virus began a global pandemic that has continued into 2014. Two avian influenza A variants, H5N1 and H7N9, have been associated with high mortality in humans, primarily in China. H5N1 has been spread by birds from China to other parts of Asia and to Africa and has resulted in a small number of deaths there. Prompt destruction of a large number of fowl at live poultry markets has thus far limited the spread of these viruses, but the specter of mutation or reassortment leading to efficient person-to-person viral spread is of grave concern.

The early 1980s were a time of great optimism for the control of infectious diseases. This optimism has been replaced by the realization that infectious diseases will not disappear any time soon. However, recent advances in the war on AIDS sparked by the development of several increasingly potent antiviral agents, coupled with the recognition that treatment in discordant couples can prevent the transmission of HIV, have given new hope that HIV infection might be controlled. *Haemophilus influenzae* type b and *Streptococcus pneumoniae* invasive disease have been brought under control in the developed world by the widespread use of conjugated vaccines. Strategies are urgently needed to make access to both of these vaccines global. Cases of many viral illnesses are in decline in the developed world, again because of the development of new vaccines. Perhaps we have finally reached the point where we fully recognize that we must be more judicious in our use of antimicrobials or lose their value. New infectious diseases will continue to be recognized, but so too will new strategies designed to prevent or diagnose and cure them. We must also hope that humans will not pervert the great advances in understanding of molecular biology of microorganisms to create chimeric "superbugs" which are both highly virulent and highly drug-resistant and can be used as weapons against fellow humans.

TABLE VII **AGENTS ASSOCIATED WITH COMPLEX CLINICAL DISEASE**

ORGANISM	IMPORTANT CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Bacteria			
Acinetobacter baumanii	Environmental Gram-negative bacillus	Exogenous, hospitalized patients	Respiratory, urinary, wound, and bloodstream infections
Anaplasma phagocytophilum	Morulae in neutrophils; organism is not visualized by Gram stain and does not grow on artificial media	Exogenous, ticks; predominantly males, especially hunters	Similar disease syndrome to ehrlichosis; mild to severe systemic disease
Bartonella henselae	Fastidious, Gram-negative bacillus	AIDS; individuals scratched or bitten by a cat, most often a kitten	Cat scratch disease; bacillary angiomatosis, hepatic peliosis, bacteremia
Borrelia burgdorferi	Spirochete	Exogenous, tick to human	Lyme disease; rash; arthritis; eye, cardiac, and nervous system manifestations
Brucella spp.	Gram-negative coccobacilli that are most often urease-positive; commonly diagnosed serologically	Individuals from developing countries with exposure to large herbivores (sheep, goats, cattle, camels, etc.); those ingesting cheese from Mexico or contaminated milk; slaughterhouse workers; laboratory accident in microbiology laboratory researchers and workers; possible agent of bioterrorism	Fever; infection of nearly any part of the body including liver, bone marrow, eye, skin, intra-abdominal, meningitic, GU[a], etc.
Burkholderia pseudomallei	Multidrug-resistant, nonfermenting Gram-negative bacillus	Patients exposed to rice paddies in Southeast Asia; possible agent of bioterrorism	Pneumonia, septic shock; reactivation disease may occur years later (similar to tuberculosis)
Clostridium septicum	Anaerobic, spore-forming, Gram-positive bacillus	Patients with gastrointestinal malignancies, other disorders which disrupt intestinal epithelium	Atraumatic gas gangrene, sepsis
Corynebacterium jeikeium	Gram-positive bacillus, often susceptible only to vancomycin	Hospitalized patients, presence of a central line	Line-related sepsis
Ehrlichia spp.	Morulae seen in cytoplasm of monocytes; organism is not visualized by Gram stain and does not grow on artificial media	Individuals bitten by infected tick	Monocytic ehrlichiosis, i.e., "Rocky Mountain spotless fever"

Enterococcus faecium, vancomycin resistant	Frequently resistant to first-line antimicrobials	Hospitalized patients	Urinary tract, wound, and bloodstream infections
Shiga toxin-producing *Escherichia coli* (STEC) including *E. coli* O157:H7 and other strains	Produces Shiga toxins I and II	Individuals who eat raw or inadequately cooked hamburgers, leafy vegetables that were in contact with animal excreta	Bloody diarrhea, hemolytic uremic syndrome
Francisella tularensis	Fastidious, Gram-negative bacillus	Exogenous; zoonosis; contact with wild rabbits or hares (hunting, skinning, etc.); tick to human; mosquito-borne in northern Europe; direct contact with animal; inhalation; ingestion of contaminated food or water; laboratory accident in microbiology laboratory researchers and workers; possible agent of bioterrorism	Skin ulcer, lymphadenopathy, ocular involvement, bacteremia, pneumonia
Gram-negative bacilli, multidrug resistant	Extended-spectrum β-lactamase production; AmpC; other mechanisms of resistance, carbapenemases	Hospitalized patients; residents of long-term care facilities; individuals returning from India	Urinary tract, wound, pulmonary, and bloodstream infections
Group A streptococci (*Streptococcus pyogenes*)	Gram-positive cocci in chains; superantigen producing, M1 and M3 serotypes	Children and adults, especially with varicella or immunocompromised	Necrotizing fasciitis, toxic shock syndrome
Helicobacter pylori	Helical, curved, Gram-negative bacillus	Adults	Gastric and duodenal ulcers
Mycobacterium abscessus	Acid-fast bacillus, environmental	Surgical patients, immunocompromised patients, cystic fibrosis patients	Post-surgical wounds, line-related sepsis, chronic bronchial infections
Mycobacterium tuberculosis, multidrug resistant	Acid-fast bacillus, resistant to isoniazid and rifampin	AIDS patients and their caregivers; homeless, prison inmates, immigrants	Tuberculosis; rapid, fatal disease course in AIDS/HIV-infected individuals
Neisseria meningitidis	Oxidase-positive, Gram-negative diplococcus	Exogenous	Meningitis, bacteremia, pneumonia
Nocardia spp.	Partially acid-fast bacilli/ beaded Gram-positive bacilli	Defects in cell-mediated immunity; exogenous	Respiratory infections; cutaneous infections; brain abscess

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TABLE VII AGENTS ASSOCIATED WITH COMPLEX CLINICAL DISEASE *(continued)*

ORGANISM	IMPORTANT CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Pseudomonas aeruginosa	Glucose-nonfermenting, oxidase-positive, Gram-negative bacillus	Exogenous, cystic fibrosis and chronic obstructive pulmonary disease patients; health care associated	Community and health care-associated UTI[b], health care-associated pneumonia and bacteremia, chronic pulmonary infections in patients with cystic fibrosis
Rhodococcus equi	Partially acid-fast, club-shaped bacillus	Exogenous (soil), AIDS	Bacteremia, pneumonia
Staphylococcus aureus, glycopeptide-intermediate and resistant	Rare strains of true resistance identified	Health care-associated; patients with chronic wounds	Wound, pulmonary, and bloodstream infections
Yersinia pestis	Gram-negative bacillus	Zoonosis; flea to human; person to person in pneumonic form; present in many parts of western United States, especially New Mexico; possible agent of bioterrorism	Localized lymphadenopathy (bubonic), high-grade bacteremia with septic shock, pneumonia, meningitis; natural multidrug resistance documented in Madagascar
Fungi			
Dematiaceous fungi: *Curvularia* spp., *Cladophialophora bantiana*, *Exophiala jeanselmei*, *Bipolaris* spp.	Molds with brown- or black-walled septate hyphae due to the presence of melanin	Superficial infection in immunocompetent patients; invasive infections in immunocompromised patients or following trauma	Skin infections; ocular infections, osteomyelitis secondary to trauma, sinusitis, CNS[c] infections in immunocompromised patients
Fusarium spp.	Hyaline, septate mold with banana-shaped macroconidia	Neutropenic patients receiving antifungal prophylaxis	Sinusitis, common cause of fungal keratitis (corneal infection) following trauma, disseminated disease with fungemia in immunocompromised hosts
Trichosporon spp.	Yeast	Neutropenic patients receiving antifungal prophylaxis	Superficial skin infection in immunocompetent host; disseminated disease with fungemia in immunocompromised hosts
Parasites			
Angiostrongylus cantonensis	Rat lungworm	Ingestion of snails or snail-contaminated vegetables	Eosinophilic meningitis, ocular involvement

Babesia microti	Can be seen on peripheral blood smear with "classic" but not always present "Maltese Cross"; may only have ring forms and need to be differentiated from *Plasmodium* spp.	Exogenous, tick to human; blood transfusions from infected donors	Babesiosis
Cyclospora spp.	8–10-μm cysts	Travelers to countries with poor hygiene; individuals eating imported food	Diarrhea
Echinococcus granulosus	Dog tapeworm	Ingestion of parasitic eggs due to exposure to dogs in areas in which dogs are fed raw livestock offal, especially of sheep	Human cystic echinococcosis; liver and lung cysts; GU tract, brain cysts, and retinal involvement are uncommon
Echinococcus multilocularis	Fox tapeworm (fox is definitive host, small mammals such as wild rodents are intermediate hosts)	Fecal-oral route by ingestion of parasitic eggs defecated in soil by foxes, coyotes, and dogs in the Northern hemisphere, including parts of Europe, former Soviet Republics in Asia, China, Alaska, Canada, and north-central parts of the United States	Human alveolar echinococcosis grows over years, mimics liver cancer, may involve contiguous strctures and lung, brain; mortality rate, 50 to 75%
Viruses			
BK virus	Nonenveloped, dsDNA[d] virus, polyomavirus	Renal transplants	Hemorrhagic cystitis
Chikungunya virus	Enveloped, ssRNA[e] virus	Spread by bite of the *Aedes* mosquito; recently emerged in the Carribean basin, Africa, Southeast Asia, Indian Ocean	Viral syndrome with fever, rash, and joint pain, similar to dengue but without hemorrhagic component
Dengue viruses	Enveloped, ssRNA viruses; four different serotypes	Travelers bitten by infected *Aedes* mosquitoes, especially in Caribbean basin; infection with one serotype is not protective against infection with another serotype	"Breakbone" fever, severe joint and muscle pain with rash; hemorrhagic fever; shock can occur; after infection with one serotype, an infection with a second serotype may predispose to hemorrhagic fever syndrome
Ebola virus	Enveloped, ssRNA virus	Epidemics in Africa; health care workers exposed to blood of infected patients	Hemorrhagic fever with very high mortality rate

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TABLE VII **AGENTS ASSOCIATED WITH COMPLEX CLINICAL DISEASE** *(continued)*

ORGANISM	IMPORTANT CHARACTERISTICS	PATIENT POPULATION	DISEASE MANIFESTATION
Hantaviruses	Enveloped, circular, ssRNA viruses	Children and adults in Argentina, southwestern U.S., Korea, parts of Europe	Acute respiratory distress syndrome, pneumonia, hemorrhagic fever
Human immunodeficiency virus-1 (HIV-1)	Enveloped, ssRNA retrovirus	Infants of HIV-infected mothers, sexually active adolescents and adults, injection drug users	Acute phase: mononucleosis-like infection; late phase: profound immunosuppression, multiple opportunistic infections
Influenza A H5N1	Enveloped, segmented, ssRNA virus	Human cases with high mortality rate	Severe respiratory disease associated with exposure to fowl in Asia
JC virus	Nonenveloped, dsDNA polyomavirus	AIDS patients and patients receiving immune modulators for autoimmune conditions such as Crohn's disease or multiple sclerosis	Progressive multifocal leukoencephalopathy
Marburg virus	Enveloped, ssRNA virus	Epidemics in Africa; health care workers exposed to blood of infected patients	Hemorrhagic fever with very high mortality rate
Nipah virus	Enveloped, ssRNA virus	In Malaysia and Singapore, associated with pigs; in Bangladesh, associated with the ingestion of raw date palm sap that has been contaminated by fruit bats	Epidemic of encephalitis with 40% mortality rate
West Nile virus	Enveloped, ssRNA virus	Introduction of mosquito-borne virus into North America; found in parts of Europe, Asia, Middle East, Africa	Ranges from asymptomatic infection to fatal encephalitis (usually in elderly)
Prions			
Variant Creutzfeldt-Jakob disease	Prion	Seen in United Kingdom and associated with outbreak of bovine spongiform encephalopathy, primarily in young adults	Fatal spongiform encephalopathy

[a]GU, genitourinary.

[b]UTI, urinary tract infection.

[c]CNS, central nervous system.

[d]dsDNA, double-stranded DNA.

[e]ssRNA, single-stranded RNA.

CASE 63

A 30-year-old male with Crohn's disease called his physician complaining of lack of mental focus, especially when trying to understand even remotely complex issues. He also had heaviness and pain behind his right eye, making it hard to visually focus. He stated he saw residual grainy images superimposed when his vision changed from one object to another. He denied blurry vision or double vision. He also noted that his equilibrium seemed off, stating that when looking down a hallway the floor did not look level. This has caused him to misstep at times, as he felt he needed to step down while walking. His depth perception also seemed to be affected, as evidenced by nearly hitting himself with a door when opening it. In addition, he occasionally could not find the right words (could not think of "stove" and kept calling the TV a "VCR"). These changes had subtly started during the previous week, but seemed to be progressing.

The physician sent the patient for magnetic resonance imaging (MRI), which showed a single area of increased FLAIR (fluid-attenuated inversion recovery) sequences and T2 signal in the left temporal-parietal region, predominantly adjacent to the occipital horn of the left lateral ventricle (Fig. 63.1). These lesions did not show post-contrast enhancement or a mass effect, which the radiologist determined to be consistent with a demyelinating lesion in the white matter. The patient was subsequently admitted for further evaluation.

The patient's complete blood count was within normal limits. A lumbar puncture was performed; the cerebrospinal fluid (CSF) had 1 total nucleated cell/μl with 91% lymphocytes and 9% monocytes. CSF protein was 50 mg/dl (normal, 15–45) and glucose was 49 mg/dl (normal, 50–75). CSF was submitted for routine bacterial culture and herpes simplex virus, cytomegalovirus, and JC virus PCRs. The Gram stain showed no leukocytes and no organisms. One of the viral PCRs was positive, confirming the diagnosis. Notably, the patient had been getting monthly infusions of a monoclonal antibody (natalizumab) for the past 3 years for his Crohn's disease.

1. What is the clinical diagnosis of this patient? What agent is responsible for his disease? Are his clinical presentation, radiographic findings, and laboratory results consistent with this diagnosis?
2. Describe what is known about the epidemiology and natural history of infection with this agent.
3. Briefly describe the pathogenesis of disease progression.

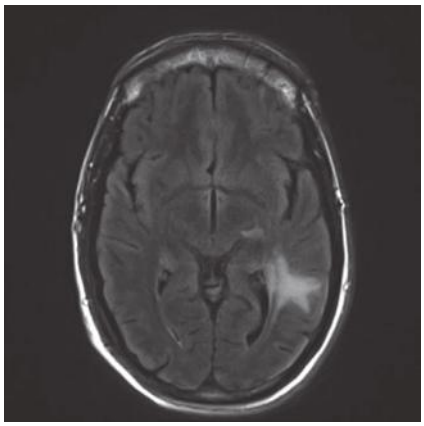


Figure 63.1

4. What is the mechanism of action for the monoclonal antibody this patient was receiving? In which patient populations is this therapeutic used? What other patient populations are at risk for the same disease?
5. What laboratory methods are helpful in determining patients at risk? What methods are available for the laboratory diagnosis of this disease?
6. How is this infection treated? What particular complication is associated with implementing therapy that is of great concern?

CASE DISCUSSION



1. This patient has progressive multifocal encephalopathy (PML), which is a demyelinating disease caused by JC virus (JCV). JCV is a nonenveloped double-stranded DNA polyomavirus that is intimately associated with PML. This patient was at risk for developing PML because of his extended natalizumab therapy (see answer 4 for more detail). Monoclonal antibody-associated PML presents with cognitive and behavioral disorders (54%), motor weakness (45%), visual deficits (41%), and language disturbances (24%). Interestingly, these patients typically have monofocal disease as opposed to the multifocal disease seen in other patient populations. PML affects the white matter of the brain, with the most commonly affected areas being the parietal and occipital lobes. By MRI, the lesions characteristically show fluid-attenuated inversion recovery (FLAIR) sequences, hyperintensity on T2-weighted imaging, and hypointensity on T1-weighted imaging. Only ~10% of lesions show contrast enhancement, and mass effect is usually absent. CSF findings are generally within normal limits. Approximately 15% of cases have a mild leukocytosis (<25 cells/ml) and 20 to 30% of cases may have mildly elevated CSF protein. Based on the information above, this patient's clinical presentation, radiographic findings, and laboratory results are all consistent with PML. The diagnosis was confirmed when the JCV PCR performed on his CSF was determined to be positive.

2. JCV is ubiquitous worldwide, infecting 55 to 85% of adults. Infection appears primarily to occur before late childhood, as seropositivity for JCV at 10 years of age is ~50%. The mode of transmission is largely unknown. Respiratory transmission has been postulated since JCV has been found in tonsils. Likewise, since JCV can be detected in the urine of ~20% of seropositive individuals, uro-oral transmission has been proposed. JCV has also been detected in raw sewage and shellfish, indicating a possible role for water- or food-borne transmission.

Primary infection with JCV does not appear to cause clinical disease, though there are a few reports of nonspecific fever and respiratory symptoms associated with seroconversion. It is likely that infected tonsillar lymphocytes carry JCV to the bone marrow and kidneys, where it remains latent. JCV DNA has been detected in brains of healthy individuals, indicating a possible role for latency in glial cells. With severe immunosuppression, viral reactivation may occur, leading to PML in certain individuals (see answer 3).

3. In an immunocompetent host with latent JCV, reactivation is prevented by a robust host immune system. However, with immunosuppression, JCV reactivation can occur. Current data suggest that, upon reactivation, JCV reaches the brain via infected B cells passing through the blood-brain barrier. Although not seen in this patient, the multifocal nature of PML supports the hematogenous spread of JCV. Once in the brain, JCV can infect glial cells. However, for this to happen, genetic rearrangement by mutations,

insertions, and deletions in the noncoding transcription regulatory region of the JCV genome must occur. These highly variable genetic changes increase the replication fitness of JCV and the number of binding sites for brain-specific transcription factors, which results in increased pathogenicity (i.e., PML). The necessity for JCV genomic changes for glial cell infection likely explains the low incidence of PML, given the large proportion of immunosuppressed patients who are JCV-seropositive. Another model exists in which the brain is the site of latency, but similarly to the hematogenous model, PML would only occur after genetic changes in the noncoding region and immunosuppresssion.

Once in the brain, JCV infects oligodendrocytes and causes them to lyse. Since oligodendrocytes create the myelin sheath protecting axons, the lysis of these cells leads to focal destruction of myelin and the spread of JCV to neighboring oligodendrocytes. Demyelinated axons are prone to injury, which can lead to permanent loss of the neuronal cell body and neurologic sequelae.

4. The patient was receiving natalizumab, a recombinant, humanized monoclonal antibody that binds to the α4 subunit of α4β1 and α4β7 integrins. By disrupting the action of these integrins, natalizumab inhibits leukocyte-endothelium adhesion processes necessary for efficient migration of leukocytes across the gastrointestinal endothelium and the blood-brain barrier. This results in reduced inflammation in the gastrointestinal tract and the central nervous system, respectively. Natalizumab is approved for use in patients with moderate to severe Crohn's disease and patients with multiple sclerosis. The risk of developing PML during natalizumab therapy increases with duration of therapy. The incidence is estimated to be 3.85 per 1,000 patients who have received more than 24 infusions, as was the case with this patient.

Patients receiving other immunomodulatory therapies are also at risk for developing PML, particularly if they result in $CD4^+$ and $CD8^+$ lymphopenia. Rituximab is a humanized monoclonal antibody against CD20 on B cells used in the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, Wegner's granulomatosis, and microscopic polyangiitis. Both natalizumab and rituximab have Food and Drug Administration (FDA) "black box" warnings about the risk of PML during treatment. Efalizumab, an anti-CD11a monoclonal antibody, was used in the treatment of psoriasis until its increased risk for PML (1 in 500) resulted in its withdrawal from the market. Post-transplant recipients receiving certain antirejection therapies, such as mycophenolate mofetil, are also at increased risk for developing PML.

HIV-positive patients with CD4 counts <100/μl have increased risk for PML, which is an AIDS-defining illness. The incidence of PML was 1 to 8% prior to the use of highly active antiretroviral therapy (HAART), but rates have since declined. PML still causes 3 to 5% of deaths in this patient population.

5. JCV antibody testing can help determine patients who are at risk for PML. However, initial serologic testing should be performed prior to immunosuppression, as false

negatives may occur otherwise. Although a positive antibody test indicates JCV latency, a negative test does not rule it out, as a 3 to 35% false-negative rate has been reported. PML risk stratification using JCV antibody testing is recommended prior to natalizumab therapy. Patients who are negative for anti-JCV should be retested every 6 months and followed closely for signs and symptoms of PML. False-positive serologic results also occur due to low-affinity cross-reacting antibodies against related polyomaviruses such as BK virus. Therefore, a two-step testing strategy has been recommended that includes an ELISA screening test followed by confirmation affinity testing.

Once a patient presents with a clinical syndrome compatible with PML, serologic tests should not be used diagnostically. Instead, PCR on the CSF for JCV DNA should be performed. Since there is not an FDA-cleared test for JCV nucleic acid detection, many laboratories have developed their own tests, leading to great variability in performance. The sensitivity of JCV PCR on CSF for diagnosing PML is 60 to 80%, while the specificity is 92 to 100%. Data from HIV-positive patients with PML indicate there may be value in performing quantitative PCR for JCV in the CSF. In patients not receiving HAART, increased JCV DNA levels (>3.64 $\log_{10}$ copies/ml) were associated with shorter survival time. Not surprisingly, high JCV DNA levels also correlated with low $CD4^+$ cell counts. In longitudinal studies of HIV-positive patients with PML, patients who initially had a negative JCV CSF PCR often had positive results on subsequent CSF specimens, indicating a possible need to retest patients who are PCR negative on an initial CSF. HIV-positive patients who received HAART and stabilized their PML had a reduction in JCV DNA levels, suggesting that quantitative PCR can be used to assess response to therapy. For patients who cannot be diagnosed using the combination of radiologic, clinical, and CSF testing, histological examination of a brain biopsy may be performed.

This patient's JCV quantitative PCR on CSF was positive, with over 2 million copies/ml present. The patient survived, but cannot take natalizumab to control his Crohn's disease and has neurologic sequelae including seizures.

6. The primary mode of treatment is to reduce the patient's immunosuppression. For HIV-positive patients, this is accomplished by initiating HAART. Monoclonal antibodies have a relatively long half-life, so in addition to discontinuing the immunomodulatory therapy, plasma exchange is often used to remove circulating monoclonal antibodies. A few drugs have shown possible benefit in treating PML, but none have been proven using randomized controlled trials. Of these drugs, mefloquine (antimalaria drug), cytarabine (chemotherapeutic for leukemias), and cidofovir (antiviral agent) likely have little, if any, effect on long-term survival with PML and are not currently recommended. However, mirtazapine (α_2-adrenergic, $5HT_{2A}$- and $5HT_3$-receptor antagonist used to treat depression) shows promise, as the receptor it blocks has been demonstrated to play a role in JCV infection in glial cells. This patient received mirtazapine in addition to five plasma exchange sessions.

A major concern associated with the reduction of immunosuppression is the development of immune reconstitution inflammatory syndrome (IRIS). In patients with PML, when the immune system is reconstituted by reducing immunosuppression, inflammatory cells migrate to the virally infected sites in the brain. This inflammatory process can cause damage to the brain, resulting in a rapidly progressing clinical course with worsening of neurologic deficits caused by PML. This is termed inflammatory PML or PML-IRIS and can be life-threatening if corticosteroids are not started immediately. PML-IRIS can occur in the weeks to months following plasma exchange or HAART therapy.

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A 35-year-old male presented to the emergency department complaining of nausea, vomiting, abdominal pain, and diarrhea of 6 hours' duration. He also complained of fever, chills, shakes, and severe leg pain ("like I've done a thousand squats"). Since the morning of hospitalization he also reported dizziness upon standing up, along with a faint headache, but no neck stiffness. His only medication on admission was eculizumab, taken every other week since he was diagnosed 1 year ago with paroxysmal nocturnal hemoglobinuria (PNH). His last dose was 4 days ago.

His PNH was well controlled with eculizumab. He drank three to seven alcoholic beverages a night, along with smoking occasional tobacco and marijuana. He was single, worked as a bartender, lived alone in town, and had no pets. He was unaware of any sick contacts at work and denied any travel, insect bites, or sexual contact in the past 3 months.

On exam he had a respiratory rate of 18 per minute, O_2 saturation of 94% while breathing room air, temperature of 37.8°C, seated blood pressure of 115/56 mm Hg, and heart rate of 111 beats/min. His blood pressure on standing was 99/56 mm Hg, with a heart rate increasing to 124 beats/min, and he felt dizzy. Overall, he was quite ill appearing but not in distress. He was alert and oriented, and his head, eye, ear, nose, and throat exam was unremarkable. His neck was supple, his lungs were clear to auscultation, and he was breathing normally. He was tachycardic but his heart sounds were otherwise normal. His abdomen was soft but diffusely tender without rebound or guarding, and his extremities were without edema. He had a faint, nonblanching rash on his chest.

His lab results were significant for a white blood cell count of 1,200/μl, with an absolute neutrophil count of 1,000/μl, an absolute lymphocyte count of 100/μl, and a platelet count of 68,000/μl. His hemoglobin was 13.3 g/dl, and he had a lactate dehydrogenase level of 497 units/liter. A chest radiograph was normal.

After his initial evaluation he was started on intravenous fluids for suspected dehydration secondary to his vomiting and diarrhea. Two hours later the patient developed fever to 39.4°C but otherwise was unchanged. Blood cultures were drawn, and he was begun on broad-spectrum antibiotics (vancomycin and cefepime). Two hours later his oxygen saturation dropped to 85% on room air, his heart rate increased to 130 beats/min, and his respiratory rate was 37 per minute. He was given oxygen by nasal cannula, and 1 hour later he was cyanotic and his oxygen saturation had declined to 80% on 100% O_2 via facemask. The patient was sedated and intubated but progressively worsened despite critical care management. One hour later he had a pulseless electrical activity cardiac arrest. Chest compressions were started and epinephrine, amiodarone, sodium bicarbonate, and calcium chloride were given. Attempts at resuscitation continued for 60 minutes

without recovery. At that point all measures were suspended, and the patient was subsequently pronounced dead.

Twelve hours later the microbiology lab called the emergency department with results from the patient's blood cultures (Fig. 64.1) and confirmed the likely cause of death in this patient.

1. Name the clinical condition that resulted in the death of this patient. What is the most likely cause of this condition based on the Gram stain seen in Fig. 64.1? For what other infection is this organism an important causative agent?
2. How is this organism typically spread? In what region of the world is infection with this organism most common?
3. Name two important virulence factors involved in the pathogenesis of disease caused by this organism. How do they contribute to the disease process?
4. What is PNH? What is eculizumab? Why are patients with PNH treated with eculizumab?
5. Eculizumab has a "black box warning" for infection by the organism that caused this patient's demise. What is a black box warning? As a result of the eculizumab black box warning, what is typically done for these patients? Why might that strategy be unsuccessful?
6. What antimicrobial resistance problems exist for this organism? Is it likely that this organism was resistant to vancomycin or to cefipime?
7. Eculizumab has been used to treat to treat a severe manifestation of an epidemic infectious disease. What is the infectious disease and what is the manifestation that is treated? Why was this treatment tried?

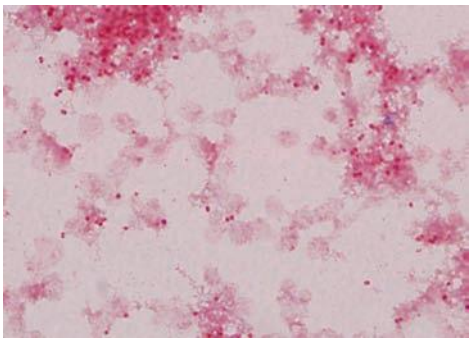


Figure 64.1 Gram stain of positive blood culture.



CASE DISCUSSION

1. The initial presentation in this patient was consistent with gastroenteritis with dehydration. He was begun on intravenous fluids for rehydration and initially was stable. However, he developed a high fever 2 hours after the initiation of rehydration therapy and was begun on broad-spectrum antimicrobials, including vancomycin to treat Gram-positive bacteria and cefepime to treat Gram-negative ones. The decreased systolic blood pressure, increased heart rate, and dizziness seen on his initial presentation were likely early signs of septic shock due to vasodilation and loss of fluid from the capillary bed (see answer to question 2 for greater detail). Given the fact that he was receiving eculizumab for his PNH, septic shock due to *Neisseria meningitidis* should have been considered on his initial presentation, especially since his vital signs were consistent with early septic shock (see answers to questions 5 and 6 for further details). The Gram stain of the organism from the patient's blood culture, a Gram-negative diplococcus, is consistent with *N. meningitidis*, and this was the organism recovered from his blood culture. *N. meningitidis* is well recognized as a cause of fulminant septic shock where death can occur within hours of initial presentation, as was seen in this case. Even in the era of broad-spectrum antimicrobials and sophisticated advanced life support technologies, mortality due to fulminant meningococcemia continues to be 10 to 15%.

N. meningitidis, as its name suggests, is, along with *Streptococcus pneumoniae*, a leading cause of bacterial meningitis in the developed world, particularly in children and young adults.

2. *N. meningitidis* is found only in humans, so it can be spread only from person to person. Approximately 10% of the population carries this organism asymptomatically in the nasopharynx. It is maintained in the population by person-to-person droplet transmission. Transmission is not particularly efficient as it requires prolonged close contact. Any activity where there is close personal contact, such as sleeping in the same bed, sharing saliva by kissing, drinking from the same container, or using the same eating utensils, is associated with an increased risk of transmission.

Disseminated meningococcal disease is most frequently seen in the meningitis belt of sub-Saharan Africa (Fig. 64.2). The reason for the increased numbers of cases seen there is not clearly understood, but it is important that individuals from the developed world who visit this region take appropriate precautions. See the answer to question 5 for further details on vaccination to prevent invasive meningococcal disease.

3. *N. meningitidis* has two important virulence factors: lipooligosaccharide, or endotoxin, embedded in the outer membrane of this Gram-negative organism; and capsular polysaccharide surrounding the outer membrane.

There are five common capsular serogroups of *N. meningitidis*: A, B, C, Y, and W135. These capsular polysaccharides protect the organism from phagocytosis, allowing the organism to evade immune clearance.



Figure 64.2 *N. meningitidis* meningitis belt in central sub-Saharan Africa (from reference 4).

During septic episodes, large quantities of bacterial lipooligosaccharide bind to Toll-like receptor 4, causing a "cytokine storm" that includes tumor necrosis factor-α, interleukin-1β, interleukin-6, and NF-χB. These cytokines cause vasodilation and a loss of fluid from the capillary network. Simultaneously, capillary damage from large numbers of activated polymorphonuclear leukocytes and macrophages along with the loss of blood pressure (hypovolemic shock) also decrease the oxygenation of major organs.

This action depletes the blood of essential clotting factors, leading to a multitude of small hemorrhages responsible for the petechiae that are often seen in patients with meningococcemia. When these lesions coalesce, they are called purpura. At the same time it inappropriately triggers the coagulation system in an attempt to stem the loss of blood from capillaries. This process causes the widespread depletion of clotting factors in what is known as disseminated intravascular coagulation. Petechiae and purpura (Fig. 64.3) can be seen in 60% of adults with disseminated meningococcal disease. It was unclear whether this patient actually had a skin rash consistent with meningococcal disease.



Figure 64.3 Large lesions are called purpura; small pinpoint lesions are called petechiae.

4. PNH is a cause of hemolytic anemia in which the alternate complement pathway mediates lysis of red blood cells (RBCs). This occurs as a result of mutation in a gene found in hematopoietic stem cells, *PIGA*, that encodes a glycosyltransferase. This enzyme is essential for the synthesis of a specific glycosylphosphatidylinositol moiety that plays a central role in anchoring specific proteins to the surface of hematopoietic cells that down-regulate the activity of the alternate complement pathway against RBCs. When these proteins are absent from the surface of RBCs, RBCs can be attacked by the alternate complement pathway and lysed.

Eculizumab is a humanized monoclonal antibody that is specific for the C5 component of complement. When it binds to C5, it blocks the formation of the membrane cytolytic attack complex (MAC) by the alternate complement pathway, thus preventing RBC lysis.

5. Biologic immune modulators including humanized monoclonal antibodies are finding increasing numbers of clinical applications. Because eculizumab blocks the formation of MAC, it has been recognized that patients receiving this biologic are at increased risk for invasive disease with *N. meningitidis*. Thus, patients receiving this monoclonal antibody are warned of an increased risk of invasive *N. meningitidis* infections and the need to receive meningococcal conjugate vaccine against serogroups A, C, Y, and W135 at least 2 weeks before the initial dose of eculizumab is given. A black box warning is exactly as it sounds: it is a box bordered by bold black lines in the package insert of any drug with potential life-threatening consequences that warns of that risk.

This increased risk is hardly surprising since clinical observations have shown that patients with defects in the terminal components of complement (C5 to C9) are at increased risk for invasive disease with this organism. The explanation for this observation is that patients whose serum is bactericidal for *N. meningitidis* are much less likely to develop disseminated infections because they can readily kill the organism *in vivo*, while those without bactericidal antibodies are at increased risk of disseminated infections. Curiously, patients receiving eculizumab will not have bactericidal serum since they cannot form MAC.

One of the problems with the meningococcal vaccine is that it does not induce immunity against *N. meningitidis* serogroup B, which is responsible for ~30% of all invasive meningococcal infections. As a result, patients receiving eculizumab who present with fever should be treated immediately with antimicrobials active against meningococci. Not surprisingly, this patient was infected with *N. meningitidis* serogroup B.

6. Because the patient was seriously ill, he was given broad-spectrum antimicrobial therapy including vancomycin to treat Gram-positive cocci such as *Staphylococcus aureus* and cefepime to treat Gram-negative bacilli such as members of *Enterobacteriaceae* and *N. meningitidis*. Because vancomycin is primarily active against Gram-positive organisms, it is not effective in the treatment of meningococcal infections. Cefepime, on the other

hand, is highly active against most Gram-negative organisms including *N. meningitidis*. The drawback of using cefepime in a clinical situation where there is a high index of suspicion for infection with *N. meningitidis* that is there are no good clinical trials supporting its use for the treatment of this specific organism.

Drug resistance in *N. meninigitidis* (meningococcus) seems to be following a similar path to that seen with *S. pneumoniae*. Thirty years ago *S. pneumoniae* was highly susceptible to all antimicrobials normally used to treat infections caused by this organism. Over the ensuing period, mutation and recombination events have resulted in strains of *S. pneumoniae* that are multidrug resistant. Three drug resistance problems are emerging in the meningococci. First, reduced susceptibility to penicillin is appearing with greater frequency. As with *S. pneumoniae*, this resistance is due to modification in penicillin-binding proteins. Recombination with commensal *Neisseria* species, which carry genes for these modified proteins, appears to be playing an important role in the emergence of this resistance. Since the advent of the penicillin therapeutic era in the middle of the 20th century, penicillin has been the drug of choice for treatment of serious meningococcal infections. As resistance emerges, empiric penicillin therapy for the treatment of meningococcal infections will need to be reconsidered, with third-generation cephalosporins such as ceftriaxone emerging as the new empiric therapy much as this class of drugs is now used to treat penicillin-resistant pneumococcal infections.

Because epidemic person-to-person spread of meningococcus can occur, one of the key strategies in controlling epidemics is the use of antimicrobial prophylaxis to prevent transmission. Two easily administered oral drugs have been widely used for this purpose, rifampin and ciprofloxacin. However, drug resistance to both drugs is emerging, although for now it is uncommon. Rifampin resistance is due to a specific mutation in the *rpoB* gene, which encodes RNA polymerase. Interestingly, rifampin-resistant meningococcus in animal models is less virulent than are sensitive strains, suggesting a significant fitness cost and calling into question whether rifampin-resistant meningococcus would cause serious disease.

The third problem, ciprofloxacin resistance, has also been recently recognized. As with rifampin, ciprofloxacin is used prophylactically. Resistance is due to mutations in the quinolone resistance determining region. As with penicillin resistance, there is some evidence that ciprofloxacin resistance was obtained in meningococcus via a recombination event with commensal *Neisseria* spp.

For now, both rifampin resistance and ciprofloxacin resistance are rare in the developed world, but it would not be surprising if they emerge in much the same manner that multidrug-resistant pneumococci have emerged and become a major clinical issue.

7. In the summer of 2011, the largest recorded outbreak of enterohemorrhagic *Escherichia coli* occurred in northern Germany. This outbreak had three unusual features: it was caused by an uncommon Shiga toxin-producing *E. coli* serotype, O104:H4; it caused a very high rate of hemolytic-uremic syndrome (HUS), affecting >20% of patients; and there

were a large number of adults with HUS. A brief case report described the success of eculizumab in treating three pediatric HUS patients. This treatment was tried since there had been other case reports that suggested that eculizumab could be used to treat HUS. After the initial report was met by much excitement in the medical community, a larger study done with patients during the same epidemic did not support the initial findings that eculizumab was beneficial in the treatment of HUS. However, this larger study was flawed because of its retrospective nature and lack of a well-matched control group. This experience emphasizes the importance of performing well-designed clinical trials before using an unproven agent in the treatment of disease. The problem, of course, is that patients during this outbreak had a life-threatening, rare disease for which there was no well-established therapy. The physicians took a chance by using this novel therapy. It did not appear to cause harm, but it was also not clear that it helped. Since doses of this biologic cost thousands of dollars, it is important to establish its clinical value, especially in light of its clear risks.

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A 21-year-old male presented to the emergency department with a 4-day history of headache, fever, myalgia, sore throat, nausea, and diarrhea. He described his headache as severe (pain level, 10/10) and worsened by bright light, noise, and movement. The patient had a history of migraine headaches, but he stated that this headache was different. He denied head injury, ear pain, chest pain, and dyspnea. He did not have any abdominal pain, dysuria, or skin rash. He denied alcohol and illicit drug use and had no recent travel, tick exposure, or known sick contacts.

His physical exam revealed right-sided tonsillar exudates and swelling but was otherwise unremarkable. Although he complained of neck pain with the headache, his neck was supple upon examination. A lumbar puncture was performed, which showed four nucleated cells and 88% lymphocytes. Cerebrospinal fluid (CSF) glucose and protein were within normal limits. His complete blood count showed a normal white cell count but a low absolute lymphocyte count of 700 cells/μl (normal, 1,500 to 5,000/μl). His head computed tomogram and chest radiograph were normal. CSF was sent for herpes simplex virus (HSV) PCR and bacterial culture. The CSF Gram stain showed no neutrophils and no organisms. Urine was obtained for bacterial culture and chlamydia and gonorrhea testing. Blood was drawn for routine bacterial cultures and a rapid Monospot test. The patient was admitted for further evaluation.

1. What was this patient's differential diagnosis given his clinical presentation? With what syndrome seen in young adults are these findings consistent? What are the viral etiologies of this condition?
2. What additional history should have been taken during his emergency department visit?
3. The patient's last HIV test was 4 months ago and was negative. Based on the history elicited upon admission (question 2), an HIV antigen/antibody combination test was ordered, which was positive, but confirmatory testing by the Multispot test was negative. Explain these results. What was this patient's specific diagnosis? What additional testing should be done to confirm his diagnosis?
4. What populations are at increased risk for infection with this agent?
5. Describe the pathogenesis of his infection, which resulted in the mononucleosis-like illness. What is the natural history of this infection?
6. How should this patient's infection be managed?
7. Discuss approaches to controlling the spread of this infection.



CASE DISCUSSION

1. The patient had an infectious mononucleosis-like syndrome. Epstein-Barr virus (EBV), cytomegalovirus (CMV), and HIV can cause a mononucleosis-like illness. Based on his severe headache, neck pain, and photophobia, meningitis was also a possibility, but tonsillar exudate and swelling would be unusual with meningitis. The low nucleated white cell count and normal glucose and protein in the CSF, along with a negative Gram stain, make bacterial meningitis unlikely. The predominance of lymphocytes in the CSF suggests possible viral meningitis, which is why the HSV PCR was ordered on the CSF. Another common cause of viral meningitis is enterovirus, which was not tested for in this patient. Notably, HIV can also cause aseptic meningitis.

2. Although a social history was taken regarding alcohol and drug use, a sexual history was not obtained when he first presented to the emergency department, even though sexually transmitted infections were considered (chlamydia and gonorrhea testing). Upon further questioning at the time of hospital admission, the patient revealed that he was sexually active as a man who has sex with men with unreliable condom use. His last sexual encounter had occurred 2 to 3 weeks previously with a now estranged partner.

3. His diagnosis was acute, primary HIV infection. During this early stage, there is a massive burst of viral replication. Plasma viral RNA and antigen (specifically, p24) are detectable before the host's antibody response becomes positive. The antibody response can take weeks to occur following initial infection, and how soon HIV antibodies are detected depends on the test. For example, "third-generation" enzyme immunoassay tests typically will detect HIV antibodies 3 to 4 weeks postinfection, whereas "second-generation" tests (e.g., Multispot and other rapid tests) may not be positive for another week or longer. The reason for this is based on the type of antibodies detected by the immunologic tests. While both detect antibodies using recombinant and synthetic antigens, third-generation enzyme immunoassays detect both IgM and IgG whereas the Multispot test detects IgG only. See Fig 65.1 for a schematic of the time to detection for RNA, p24 antigen, and antibodies in the weeks following primary infection.

This patient was tested using an HIV antigen/antibody combination test ("fourth-generation" test), which simultaneously detects p24 antigen and IgM and IgG antibodies. However, the combination test does not differentiate whether antigen and/or antibodies are the source of a positive result. The use of the combination test reduces the window of false-negative results due to acute infection by about 5 to 7 days. Since his combination test was positive but his Multispot test was negative, this indicates he likely had circulating p24 antigen but had yet to develop an antibody response.

Additional testing that should be performed includes a quantitative HIV RNA test (i.e., viral load) and repeat immunologic tests to confirm seroconversion. The HIV RNA

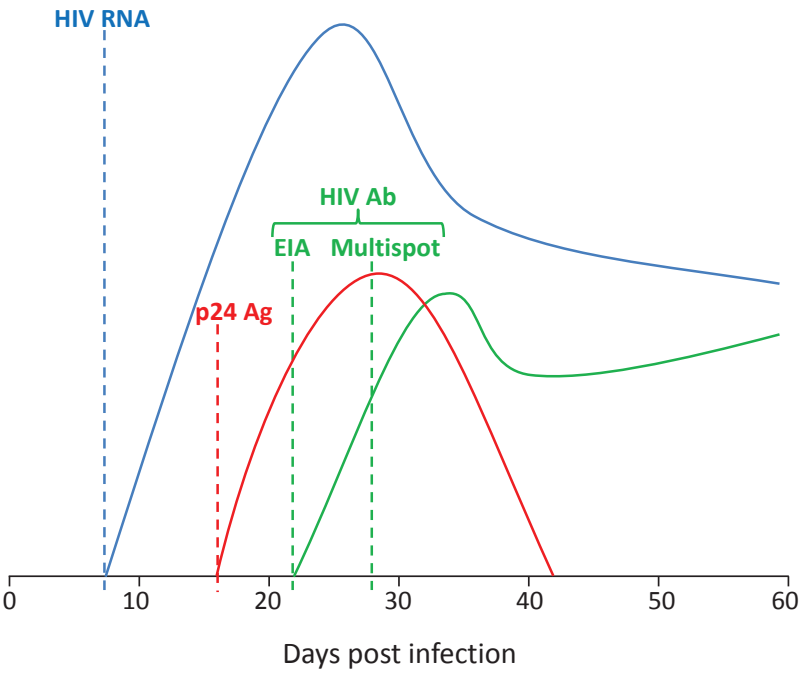


Figure 65.1

test is critical in differentiating acute HIV infection from a false-positive combination test. The results of this patient's additional testing are outlined in Table 65.1. Patients newly diagnosed with an HIV infection should also be tested for other sexually transmitted infections, including syphilis, chlamydia, and gonorrhea.

Note that this patient was also lymphopenic at the time of presentation, which is consistent with his diagnosis. The diagnosis of acute HIV infection is often missed, particularly since patients may present to physicians who are not specialists in managing HIV-infected patients. All physicians need to consider acute HIV infection in persons presenting with a mononucleosis-like illness.

TABLE 65.1 PATIENT'S RESULTS OVER TIME

DAYS FROM PRESENTATION	ANTIGEN/ANTIBODY COMBINATION RESULT	RNA VIRAL LOAD	MULTISPOT RESULT
0	Positive	$>10^6$	Negative
7	Positive	NP[a]	Negative
24	Positive	$>10^6$	Negative
31	Positive	NP	Positive

[a] *NP, not performed.*

4. A variety of populations are at increased risk for acquiring HIV infections. HIV is spread primarily in three ways: sexually, including vaginal and anal intercourse and oral-genital contact; by contact with infected blood; or vertically, from mother to fetus. In the United States, men who have sex with men and men who have sex with men and women have the highest rates of infection, followed by intravenous drug users and their sexual partners. Infection rates are lowest among individuals who are infected through heterosexual activity, but this at-risk population in the United States is developing an increasing incidence of HIV infection, which has been particularly observed in southern states and in the African-American population. This is in sharp contrast to the spread of HIV in other parts of the world, where the major mode of transmission is via heterosexual activity. Although the spread of HIV has slowed in the United States, HIV infection is an increasing public health problem in India, China, Russia, and many countries throughout Southeast Asia. In Russia and China, the increasing prevalence of HIV is largely driven by high rates of infection in intravenous drug users. AIDS continues to be a devastating disease in sub-Saharan Africa, where in 2012 1.2 million people died of AIDS and 1.6 million people were newly infected with HIV. Approximately 70% of the world's HIV-infected persons live in sub-Saharan Africa, including 88% of all infected children. Worldwide, HIV disease and AIDS have surpassed tuberculosis and malaria as the leading fatal infectious diseases, with an estimated 36 million HIV-related deaths. It is estimated that 35 million people in the world are infected with HIV, with ~10% being under the age of 15.

At one time, HIV-contaminated blood product transfusions were an important mode of transmission. At particularly high risk were patients with hemophilia who received pooled clotting factor preparations. Prior to the availability of donor screening tests, methods such as pasteurization were used to kill viruses in the plasma-derived concentrates, which led to a significant decline in HIV infections in this population. Aggressive donor screening using serologic and nucleic acid amplification tests has led to a greatly reduced rate of transfusion-associated HIV infection in the developed world. In areas of the world where screening of blood for HIV antibodies, antigen, or RNA is prohibitively expensive, transfusion-associated HIV infection continues to be a major health problem.

5. Upon infection, large amounts of virus are produced that transiently peak and rapidly decline. At this point, the host's immune response provides some control, which can be measured, in part, by detectable anti-HIV antibodies. The clinical manifestations of acute HIV infection can include gastrointestinal, dermatologic, neurologic, and lymphoid tissue involvement arising from the widespread dissemination of HIV infection. Some individuals can be asymptomatic during the acute stage of HIV infection.

An individual's course of HIV infection and disease is variable and is largely determined by complex interactions between the virus and the host's immune system. The rate at which immune dysfunction occurs determines the rate of disease progression. Advanced HIV disease and AIDS result in complications such as opportunistic infections and malignancies.

Even early in infection, very large amounts of virus are present in lymphoid tissues, semen, and plasma (up to 10^6 particles per ml, with an estimated 10^{10} viral particles generated daily). The concentration of HIV in semen peaks ~30 days after infection (10^4/ml), which is thought to contribute to the high transmissibility of HIV during acute infection. Within 1 year after seroconversion, each infected individual establishes a "steady-state" level of HIV RNA that largely determines the rate at which $CD4^+$ T lymphocytes (the cells HIV infects) subsequently decline. Concurrent use of plasma HIV RNA measurements and $CD4^+$ cell counts can predict disease progression.

$CD4^+$ T cells (T-helper cells) initiate the immune response to infection through the direct interaction with B cells and the release of cytokines that activate cytotoxic T cells and macrophages in response to foreign antigens. As $CD4^+$ cells decline during HIV infection, the host becomes more susceptible to opportunistic infections. Individuals with $CD4^+$ cell counts <200/μl (normal, 500 to 1,500/μl) are at greatest risk for HIV-associated infections including candidiasis, cryptococcosis, cryptosporidiosis, CMV, HSV, histoplasmosis, disseminated *Mycobacterium avium*, tuberculosis, *Pneumocystis* pneumonia, and toxoplasmosis. In addition, the decline in $CD4^+$ cells puts individuals at an increased risk for infection-associated cancers including human papillomavirus-associated cervical and anal cancers, human herpesvirus 8-associated Kaposi's sarcoma, EBV-associated lymphomas, and JC virus-associated progressive multifocal leukoencephalopathy. HIV-positive patients with low $CD4^+$ counts are also at increased risk for non-virus-related cancers, though the mechanism is less clear. Direct oncogenic effects of HIV, chronic inflammation, and long-term exposure to antiretroviral therapy have all been suggested as possible mediators.

6. The patient should be counseled about his HIV infection status, what clinical course to expect, and what behaviors should be modified to avoid transmitting his infection to others. He should be considered for combination antiretroviral therapy. In 1996, combination antiretroviral therapy involving three or more anti-HIV medications, including a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (RTI) plus two nucleoside RTIs, became the standard of care in the industrialized world. These regimens resulted in a substantial reduction in HIV-related deaths in populations that have access to these drugs. Subsequently, three new classes of antiretroviral drugs have been introduced: fusion inhibitors, CCR5 antagonists (or entry inhibitors), and integrase inhibitors. Today's regimens typically consist of two nucleoside RTIs in combination with a non-nucleoside RTI, a protease inhibitor, an integrase inhibitor, or a CCR5 antagonist. Greater viral load reductions and $CD4^+$ cell count increases can result from combination antiretroviral therapy. At the acute stage of HIV infection, the viral population is relatively homogeneous and thus less variable. Treating with more than one antiretroviral agent significantly lessens the risk that drug-induced resistance will develop.

Antiretroviral drug resistance is becoming a critical issue. Some drug-resistant viral isolates can be transmitted. A slowly increasing proportion of patients with primary HIV infection have acquired HIV that is already resistant to one or more classes of antiretroviral drugs. Patient response to therapy is measured by determining the level of HIV RNA

present in the blood by means of a quantitative nucleic acid amplification test (i.e., viral load). The goal of therapy is to achieve and sustain an undetectable viral load. Viral loads are monitored at approximately 3-month intervals. If there is a sudden increase in viral load, there are two common explanations. One is that the patient may have stopped taking his or her medicine. Although current HIV antiretroviral regimens are very effective, they can be complicated to take and may cause significant side effects. The second explanation is that the patient's viral population may have developed resistance to one or more of the agents with which the patient is being treated. The HIV genome is highly mutable, as are most RNA viruses, and mutations leading to resistance are frequent. Antiretroviral resistance testing can potentially benefit patients by allowing individualized treatment regimens based upon the analysis of the genotype and/or phenotype of the patient's viral population.

7. The main approaches to control the spread of HIV infections are education, behavior modification, and vaccine development. First, the Centers for Disease Control and Prevention recommends that everyone between the ages of 13 and 64 be tested for HIV at least once, and high-risk groups should be tested more frequently. Because ~20% of HIV-infected persons are not aware of their HIV status, educating the public about the need to get tested and providing easy access to testing is an important control measure. The FDA has recently approved an in-home HIV test that can be purchased without a physician order. Although access to testing is important, educating persons who engage in high-risk activities is paramount. The patient presented here had been tested for HIV 4 months prior to his infection. Although he was negative, he was in a high-risk category and did not use protection. Educating those at high risk regarding preventative measures such as condom use and needle exchange programs cannot be overemphasized. There have been many attempts to develop a vaccine for HIV, with some candidate vaccines being tested in clinical trials; however, none has been successful to date. HIV vaccine development has been difficult because of the mutability of the virus and our limited understanding of what constitutes protective immunity. A key feature of any successful HIV vaccine will be the capacity to induce mucosal immunity to prevent the acquisition of HIV via sexual activity.

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CASE 66

A 28-year-old woman with no significant past medical history was transferred emergently to a regional hospital from northern California for the management of acute respiratory failure. The patient's job included cleaning of service buildings that had been locked up for the winter. She found mouse droppings in these buildings and in her home, a trailer, as well. Two days prior to admission at an outside hospital the patient developed nausea and vomiting, fever, chills, and joint and muscle aches. On the day of hospital admission she had a cough and shortness of breath.

At the outside hospital, the patient had a temperature of 39.4°C and a platelet count of 20,000/μl, and her chest X ray demonstrated the presence of diffuse bilateral infiltrates. She was emergently intubated and transferred to a regional medical center, where her hemoglobin level was 15.0 g/dl and her hematocrit was 43.5%. Her white blood cell count was 5,700/μl with 53% neutrophils, 34% lymphocytes, and 13% monocytes. Aytpical lymphocytes were not reported.

On arrival following transfer from the outside hospital, the patient had a temperature of 39.7°C, blood pressure of 138/96 mm Hg, and heart rate of 71 beats/min. She was intubated and sedated. Her blood gas at that time showed a pH of 7.33, pO_2 of 55 mm Hg, and pCO_2 of 28 mm Hg. Her lung examination demonstrated coarse breath sounds bilaterally. Her chest X ray demonstrated extensive alveolar opacification bilaterally (Fig. 66.1). Cultures of blood and urine were negative, as were *Legionella* urinary antigen and *Mycoplasma* antibody studies. A subsequent serologic test was positive for antibodies to the viral cause of the patient's illness.

1. This patient was not thought to have an infection due to a common cause of community-acquired pneumonia. Rather, she was thought to have become ill as a result of her exposure to rodent excreta or to rodents. In the United States, what bacterial infections may cause pulmonary infections and can be the result of this type of exposure?

2. Given that the patient had a viral cause of her pulmonary failure, what virus was most likely to be responsible for her illness? What type of virus is this? Historically, in what setting was this virus first detected?

3. Was her clinical course consistent with the organism infecting her? Explain.



Figure 66.1 AP portable chest X ray.

4. Geographically within the United States, where are human infections due to this virus typically found?
5. Viruses in this genus typically cause pulmonary involvement in human cases in North and South America. The viruses in this genus were first identified as human pathogens in Korea and infect people in both Europe and Asia. What are the most common types of clinical involvement in patients who have become ill by viruses within this genus in Europe and Asia?

CASE DISCUSSION



1. Zoonotic infections are infections that are transmitted from nonhuman vertebrates to humans. There are numerous zoonotic infections, and the type of animal exposure often helps the clinician narrow down the possible causes of a patient's illness.

Leptospirosis is a zoonotic infection that is caused by spirochetes within the bacterial genus *Leptospira*. Human infections most commonly occur as a result of cutaneous exposure to infected rodent urine. These exposures most commonly occur after flooding of rat-infested areas, especially in sewer workers, or from recreational exposure to *Leptospira*-contaminated water. Human infection may also occur after cutaneous exposure to the urine of infected dogs, pigs, and other domestic and wild mammals. Pulmonary disease in leptospirosis may occur with or without the more frequently seen hepatic or renal involvement. Pulmonary involvement due to leptospirosis ranges from mild illness with a dry cough to severe and life-threatening illness that may include adult respiratory distress syndrome with or without pulmonary hemorrhage.

A second bacterial zoonosis that is characterized by pulmonary involvement is caused by the Gram-negative bacillus *Yersinia pestis*, the etiologic agent of plague. Plague, which occurs in the American Southwest (with the greatest number of cases occurring in New Mexico) and California as well as in foci in other parts of the world, can be the result of the bites of infected fleas, direct contact with infected animals, inhalation of the organism, and far less commonly, consumption of meat from infected animals (such as camel meat in other parts of the world). Plague pneumonia can be the consequence of the seeding of the lungs during bacteremia from an infection at another site of the body (secondary plague pneumonia), such as an infected lymph node (a bubo), or directly via the inhalation of the organism, which is why *Y. pestis* is of concern as a potential agent of bioterrorism. Inhalation of the organism via droplet spread may occur as a result of exposure to a person who has plague pneumonia, thereby making the infection in the human population one that may spread rapidly from person to person, as was likely the case during the Black Death in the 14th century, when it killed an estimated 30 to 40% of the population of Europe. In the United States, the most common cause of plague pneumonia is exposure of people to cats that have been infected by the organism as a consequence of their contact with infected small, rural, wild mammals (such as squirrels, chipmunks, mice, and rabbits). The last urban epidemic of plague that included person-to-person spread in the United States was during the 1920s in Los Angeles. Although bubonic plague in humans has classically been the consequence of infection from infected rats via the bite of their fleas, this is not the cause of infections in the United States. Infected fleas from small, wild mammals (such as the fleas of squirrels) are the most common cause of cases of bubonic plague in the United States, though rats play a significant role in several other areas of the world.

A third zoonotic bacterial infection that may cause a clinical picture similar to the one seen in this case is tularemia, which is the result of infection by *Francisella tularensis*. This

organism is a Gram-negative coccobacillus that is difficult to recover from clinical specimens because of its nutritional requirement for higher levels of cysteine. High levels of cysteine are typically not found in media used for culture of respiratory specimens in most clinical settings (with the exception of buffered charcoal-yeast agar, a medium used to recover *Legionella* spp.). Unlike human infections due to *Y. pestis* and *Leptospira* spp., *F. tularensis* is more commonly associated with exposure to infected wild (not domestic) rabbits than it is with rodent exposure, though human infections have occurred from a large number of wild and domesticated animals, including rodents, dogs, cats, and sheep. It also may be acquired by exposure to aerosols of the organism from contaminated soil during the mowing of grass. Waterborne infection has also been reported. It is also commonly spread by tick bites and by biting insects (such as deer flies or, in Europe, mosquitoes). It is a potential agent of bioterrorism and is well-known to cause laboratory-acquired infections. As a result, if infection with this organism is being considered, the laboratory should be contacted in order to use culture media that will optimize the recovery of *F. tularensis* and to minimize the risk of laboratory-acquired infection. The diagnosis of tularemia may also be established serologically, which may be of particular importance given that the culture diagnosis may be difficult because of the fastidious nature of the organism due to its nutritional requirements. Of note, commercially available identification systems, including matrix-assisted laser desorption ionization–time of flight mass spectrometry, do not include *F. tularensis* in their databases and the misidentification of the organism has been well documented.

2. The patient's physicians thought that the most likely cause of her illness was infection with Sin Nombre virus. An outbreak of the disease was occurring in the geographic locale, Yosemite National Park, where she was employed. They believed that she was infected as a result of inhaling virus-contaminated rodent excreta found in the building she was cleaning or where she was living. The white-footed mouse, endemic in this region, is a well-recognized reservoir of this virus. Her diagnosis was confirmed by positive IgM and IgG serologic testing, though neither viral isolation nor nucleic acid amplification testing had been performed. This virus is a member of the genus *Hantavirus*, which is in the family *Bunyaviridae*. Hantaviruses are spherical, negative-sense, single-stranded RNA viruses.

The virus that caused this patient's infection was first identified during the investigation of cases of a pulmonary syndrome that occurred in 1993 in the Four Corners region (where New Mexico, Arizona, Utah, and Colorado meet) of the southwestern United States. Early on during the outbreak, before the causative virus was determined, one possibility that was considered for the cause of the pulmonary cases was plague. However, bacterial cultures for plague were negative in settings in which they would have been expected to be positive.

3. Sin Nombre virus is known to cause a biphasic disease with initial flu-like symptoms, including nausea and vomiting, fever, chills, joint and muscle aches, cough, and shortness of breath. The secondary phase includes severe bilateral pulmonary disease. This patient's

blood gas results, showing hypoxemia and respiratory acidosis, were consistent with respiratory failure. Intubation is frequently required in patients infected with Sin Nombre virus. Interestingly, Sin Nombre virus is also associated with thrombocytopenia and hemoconcentration, both of which were noted in this patient's history. Even with improvements in the treatment of the severe pulmonary infection, mortality remains >35%.

4. Although there have been small numbers of cases of hantavirus pulmonary syndrome in a number of states in the eastern United States, the great majority of cases have been reported in patients from the western United States (Fig. 66.2). Relatively large numbers of cases have been reported not only from states with high populations (such as California and Texas) but also from states with low populations (Fig. 66.2) in the western United States. Of note, the Sin Nombre virus also has caused hantavirus pulmonary syndrome in western Canada. Interestingly, cases of human illness due to hantaviruses have not been reported from Mexico, though there have been cases of hantavirus pulmonary syndrome both north and south of Mexico. There has been serologic evidence of human infection with hantaviruses in Mexico, and at least three different hantaviruses have been isolated from wildlife in Mexico. One must consider the possibility that the Mexican public health surveillance for human hantavirus infections is inadequate.

5. Globally, members of the genus *Hantavirus* include (as of 2010) a total of 21 viruses that have been documented to cause human infection. With rare exceptions, the viruses that cause human disease are transmitted from rodents to humans. In one case the reservoir of

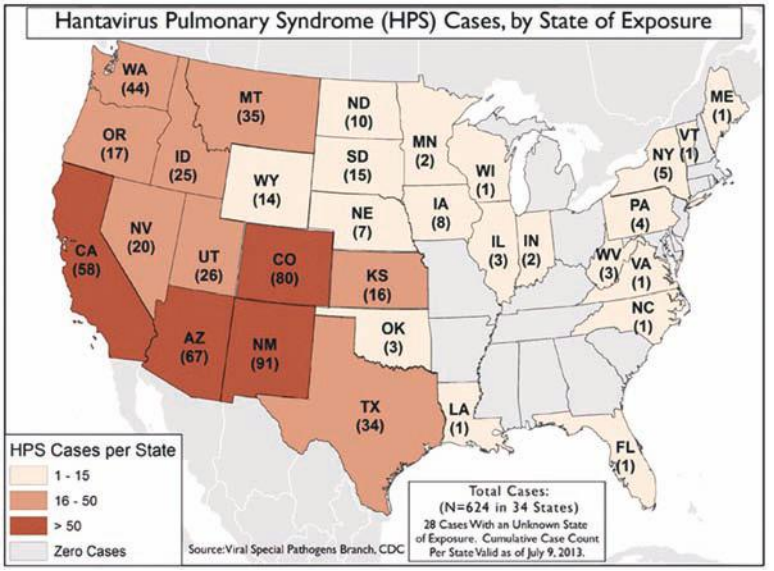


Figure 66.2 Hantavirus pulmonary syndrome cases reported by state, United States, as of February 28, 2013. (Source, Viral Special Pathogens Branch, Centers for Disease Control and Prevention.)

the virus is not a rodent, but an insectivore; other hantaviruses have been found in bats but have not been known to infect humans.

Human infection with a hantavirus was first noted during the Korean War (1950 to 1953) and was due to Hantaan virus, which is the cause of what was referred to as Korean hemorrhagic fever and, more recently, as Korean hemorrhagic fever with renal syndrome. There are a number of hantaviruses in the "Old World" that cause hemorrhagic fever with renal syndrome, and one estimate is that globally there may be as many as 150,000 human cases of hemorrhagic fever with renal syndrome. In addition, in Scandinavia, a less severe illness that involves the kidneys, nephropathia epidemica, has been found to be due to Puumala virus. A relatively small number of cases have also been reported in Europe in which Puumala virus was the cause of hantavirus pulmonary syndrome.

In the Americas, quite a number of hantaviruses have been found to infect humans and to cause hantavirus pulmonary syndrome. There is not normally human-to-human transmission, although in one outbreak there was evidence that a hantavirus, Andes virus, may have been transmitted from human to human.

Hantavirus is considered an emerging disease. Additional hantaviruses are discovered each year, and the number and diversity of these viruses are the subject of active funded research. For example, little is known about the role of hantaviruses in much of sub-Saharan Africa, as a consequence of a poor public health infrastructure.

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CASE 67

The patient was a 57-year-old man admitted for treatment of graft-versus-host disease (GvHD) secondary to a matched unrelated allogeneic peripheral stem cell transplant (PSCT) done 8 months previously for acute myelogenous leukemia. At admission he had severe diarrhea and dehydration attributed to the combination of GvHD and disseminated cytomegalovirus (CMV) infection. His GvHD was being treated with a combination of corticosteroids and infliximab. In addition, he was receiving tacrolimus for immunosuppression for his PSCT and antifungal prophylaxis with micafungin. His CMV diagnosis was based on positive colon biopsy and documented CMV viremia, for which he was being treated with foscarnet after his CMV viral loads rose over the past month while he was on valganciclovir.

On admission he had a temperature of 38.7°C, heart rate of 105 beats/min, respiratory rate of 20 per minute, and blood pressure within normal limits. Over the past month his absolute neutrophil count had gone from 7,500/μl to 1,300/μl. It subsequently reached a nadir of 800/μl on the third hospital day. In addition to his diarrhea and dehydration on admission, he noted that he had sinus stuffiness on the left. A computed tomography scan of the paranasal region noted diffuse left-sided sinonasal disease. Biopsy of the left sinus is shown in Fig. 67.1. On physical examination he was noted to have hemorrhagic, nodular lesions on his extremities and trunk (Fig. 67.2). The physician was concerned that the skin lesions were the result of disseminated infection, and blood cultures were ordered, which grew the organism seen in Fig. 67.3 and 67.4. When results of the sinus biopsy were reported, the patient was begun on liposomal amphotericin B and voriconazole. Despite the combination antifungal therapy, the patient deteriorated, and when the patient developed loss of vision, the family made the decision to withdraw support and the patient died on the ninth hospital day.

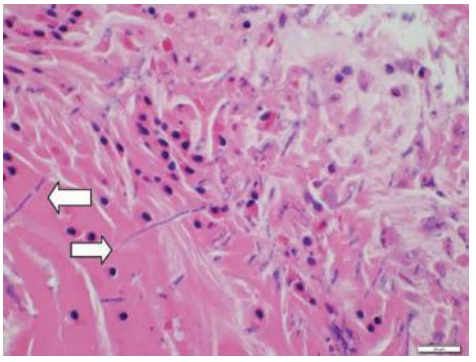


Figure 67.1 Sinus biopsy.

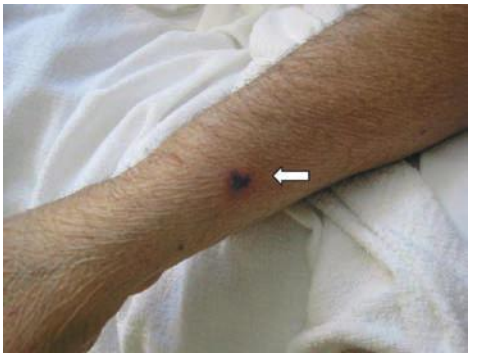


Figure 67.2 Skin lesion at time of positive blood culture.

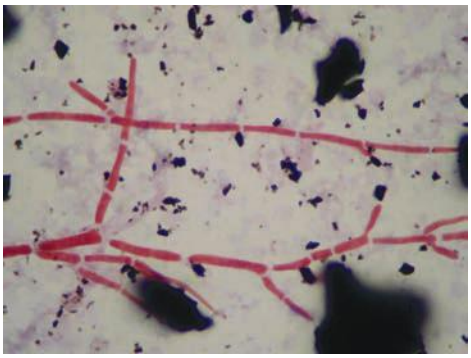


Figure 67.3 Positive blood culture.

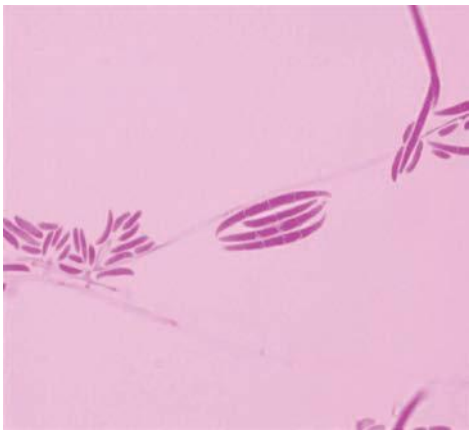


Figure 67.4 Macroconidia of organism infecting this patient.

1. What is GvHD? Why is it of such concern in patients who receive bone marrow transplant? Patients with GvHD require potent immunosuppressive drugs that result in increased risk for disseminated fungal infections. Why are they at risk for these infections?
2. What is infliximab? Why was it used in this patient? What are the risks associated with its use? In what other patient populations is it used?
3. What molds most commonly cause infections in PSCT transplant recipients? Name the four most common genera/groups. Based on Fig. 67.3 and 67.4, you should be able to identify the organism that caused this patient's infection.
4. This patient had a positive blood culture for the organism infecting him. Is that an unusual finding in invasive fungal infections? How useful is a negative blood culture in ruling out an invasive fungal infection?
5. Briefly explain the progression of this patient's infection. Why do you think he developed infection despite receiving antifungal prophylaxis with micafungin and then failed antifungal therapy when it was changed to amphotericin B and voriconazole?
6. Molds are ubiquitous in nature. When they cause infection, they have to be treated with antifungal agents, which can be quite toxic to the host. What criteria should be used to establish that a patient has an invasive fungal infection? Were those criteria met in this patient?
7. The fungus infecting this patient has been associated with a global outbreak. What was that outbreak and how was this outbreak disseminated?



CASE DISCUSSION

1. In a bone marrow transplant such as the one done in this patient using HLA-matched unrelated allogeneic peripheral stem cells, the cells of the host immune system are destroyed by a combination of radiation and drugs prior to transplant. This creates a setting in which the transplanted stem cells can reconstitute the immune system by a process called engraftment. Unfortunately, mature T cells, a component of the transplant, may recognize minor HLA antigens in 50% or more of these recipients and attempt to "reject" the foreign cells of the host in a process called graft-versus-host disease (GvHD). These transplanted T cells target epithelial cells in the skin, liver, and gastrointestinal tract, leading to apoptosis. Patients with GvHD often have diarrhea, which may be bloody, as a manifestation of this process, as was seen in this patient.

The treatment of GvHD is to further suppress the graft immune response using drugs such as corticosteroids and infliximab that inactivate T cells from the PSCT to reduce damage to the host cells. This increase in immunosuppression puts patients at even greater risk for infection, especially with those organisms that are part of their indigenous microbiota or environmental organisms such as molds. We commonly inhale as many as 1,000 mold conidia per hour, and although PSCT recipients are initially placed in rooms with special air handling to limit exposure to airborne organisms, once there is engraftment of the PSCT, patients are returned to an environment where they are readily exposed to mold conidia. PSCT recipients with GvHD receiving additional immunosuppression have an increased risk of fungal infection because T cells play an important role in immunity against this group of pathogens.

2. Infliximab is a mouse-human chimeric monoclonal antibody with a broad range of activity against T cells, inhibiting granuloma formation and the migration and maturation of inflammatory cells such as neutrophils and monocytes. The monoclonal antibody specifically targets tumor necrosis factor-α (TNF-α), resulting in the neutralization of all of its biologic effects as well as causing monocyte and T-cell apoptosis. Because of the central role of TNF-α in the pathogenesis of GvHD, infliximab is widely used to treat the condition.

TNF-α is important in granuloma formation and T-cell activation of innate immunity, processes important in controlling *Mycobacterium tuberculosis*, other environmental mycobacterial infections, indigenous fungal infections (*Histoplasma*, *Coccidioides*), and opportunistic fungal infections (especially environmental molds such as *Aspergillus*). Because infliximab neutralizes the biologic activity of TNF-α, patients receiving it are at increased risk for infection with these agents. Infliximab has become a standard treatment for the autoimmune inflammatory diseases ulcerative colitis and rheumatoid arthritis, putting these groups of patients at increased risk for infections with *M. tuberculosis* and other organisms controlled by cell-mediated immunity. With the increasing numbers of

monoclonal antibodies being developed to treat increasingly complex disease processes, it is incumbent on caregivers to maintain current knowledge of these biologics and their associated risks.

3. The four most common molds associated with invasive disease in PSCT patients are *Aspergillus*, the zygomycetes, *Fusarium*, and *Scedosporium*. *Aspergillus* is by far the most common mold found. Zygomycetes are a group of several different genera, the most frequently encountered being *Mucor*, *Rhizopus*, *Absidia*, and *Cunninghamella*. All are considered opportunistic molds, being ubiquitous in the environment but of low virulence in immunocompetent individuals. The organism that infected this patient was *Fusarium*. *Fusarium* is a septated, hyaline mold. In tissue (Fig. 67.1) it cannot be readily distinguished from *Aspergillus* or *Scedosporium*, but it can be distinguished from zygomycetes, whose hyphal elements in tissue tend to be broader, ribbon-like, and aseptate. *Fusarium* is identified in Fig. 67.4 by its very characteristic banana-shaped, multicellular macroconidia.

4. Positive blood cultures in patients with invasive fungal diseases are unusual in large part because most invasive fungal infections are caused by *Aspergillus*. Because *Aspergillus* invades endothelial cells, it rarely causes fungemia even in those patients who have *Aspergillus* endocarditis proven at autopsy (where this diagnosis is frequently made). On the other hand, positive blood cultures are found in ~50% of patients with disseminated *Fusarium* infections. The reason for this is that *Fusarium* produces a "yeast-like" cell when it invades tissue, which can enter the bloodstream and disseminate the organism. Because the sensitivity of blood cultures is only 50% in invasive *Fusarium* disease and blood cultures are rarely positive with invasive aspergillosis, the finding of a negative culture does not rule out disseminated fungal infections.

5. The sequence of events that resulted in the demise of this patient was based on his exposure to *Fusarium*, its relative lack of susceptibility to antifungal agents, and the profound level of immunosuppression needed to treat his GvHD. The disease process started with a localized sinus infection after the patient inhaled *Fusarium* conidia from the environment. In an immunocompetent patient, localized sinus infection (or "some stuffiness," as this patient related) is a minor inconvenience, but in this patient it heralded a fatal infection. Up to 25% of invasive *Fusarium* infections begin as sinusitis. Examination of the tissue section (Fig. 67.1) shows hyphal elements invading intact tissue (see arrows). From there the organism disseminated. A key finding in 70% of patients with disseminated *Fusarium* is the presence of hemorrhagic, nodular lesions, as were observed on the trunk and extremities of this patient (Fig. 67.2). Biopsies of these nodular lesions will reveal the organism by both culture and pathology.

Dissemination of this organism occurred despite the use of micafungin prophylaxis. This is not surprising, because *Fusarium* is intrinsically resistant to echinocandins such as micafungin. Micafungin is recommended as a prophylactic agent to prevent the two most

common agents of invasive fungal infections in this patient population, *Aspergillus* and *Candida* spp. The other reason for dissemination of this infection was the patient's immunosuppressed state. In a patient with GvHD, the need to suppress the T-cell-mediated response in order to blunt GvHD necessarily makes the host even more immunosuppressed. His profound immunosuppression due to GvHD therapy allowed the dissemination of *Fusarium*. The immunosuppression was such that even though the patient had an adequate number of neutrophils throughout most of his disease course, TNF-α (important in activation of antifungal immune response) was neutralized by the presence of infliximab. Further, no antifungal agents are particularly active against *Fusarium in vitro*, including the ones with which he was treated, amphotericin B and voriconazole. In fact, there has been little success in the treatment of disseminated *Fusarium* infections, with mortality rates of >85% in PSCT recipients. Once the patient became blind (due to either dissemination of the *Fusarium* to the eye or CMV retinitis, both of which would have been a function of a severely immunosuppressed state), the patient and his family lost hope and withdrew support. The patient died a day later.

6. The diagnosis of invasive fungal infection must be precise because the consequences of a misdiagnosis can be profound. First, false-negative diagnoses can result in a failure to treat an infection, which can have fatal consequences, while false-positive results can lead to the unnecessary use of highly nephrotoxic antifungal agents, which can result in the loss of vital kidney function.

Criteria have been established to classify invasive fungal infections as either proven or probable. In proven infections, the fungus can be visualized in a tissue section (as was seen in this patient) or a cytopathology slide, it can be recovered from a normally sterile body site such as blood (as was seen in this patient), or cryptococcal antigen can be detected in cerebrospinal fluid. Probable diagnosis is based on the finding of a fungal pathogen primarily in respiratory tract or wound infections or the presence of *Histoplasma* antigen in urine or bronchoalveolar fluid. These definitions at first glance seem very straightforward, but the reality can be somewhat more complex. We have certainly seen instances in our laboratory where one or two colonies of an environmental mold are found on the edge of a plate and we question whether this represents a true positive or a laboratory contaminant. At times of high rainfall or construction, the likelihood of contamination increases. In another example, a patient may grow a probable fungal pathogen from a sputum specimen, but without lung tissue that is both cultured and examined histologically the diagnosis is not definitive. Unfortunately, obtaining lung tissue is not always feasible, especially in PSCT patients who have low platelet counts that preclude any kind of biopsy. This patient's diagnosis was easily established, but that is not always the case with many of these highly complex cases.

7. *Fusarium* has recently been associated with a global outbreak of keratitis. The outbreak occurred in contact lens wearers who used contact lens solution that was contaminated with the fungus. The largest numbers of cases were reported from the United States and Singapore, but patients from other countries were also found.

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CASE 68

A 21-year-old female was referred to a dermatologist by her primary care physician for evaluation of bilateral leg lesions similar to the ones depicted in Fig. 68.1. The lesions had first appeared 4 months previously, and they came and went leaving a red mark that persisted for about a month. The lesions did not itch, burn, or bleed. She denied fever and had had no other symptoms. No one else in her family had similar lesions.

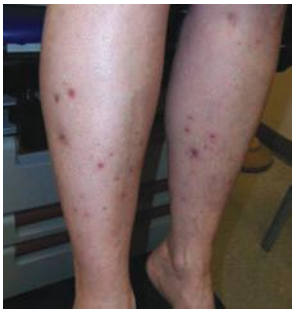


Figure 68.1

Upon physical examination, the lesions were noted to be erythematous to violaceous and firm with no purulent drainage. The lesions were isolated to her lower legs, and there were eight lesions in various stages of development. The patient refused a biopsy and preferred empiric treatment for what was presumed to be arthropod bites. She returned to the dermatologist 6 weeks later because the lesions had become considerably larger. During this visit she denied owning an aquarium, but did recall having a pedicure 2 weeks prior to the onset of lesions. An 8-mm punch biopsy was obtained and sent for histopathology and bacterial, fungal, and mycobacterial cultures. The Gram stain of the biopsy is shown in Fig. 68.2. An organism grew aerobically from both bacterial and mycobacterial cultures. The cultures were noted to be positive after 5 days of incubation. Figure 68.3 shows the organism after subculture to Middlebrook 7H11 agar.

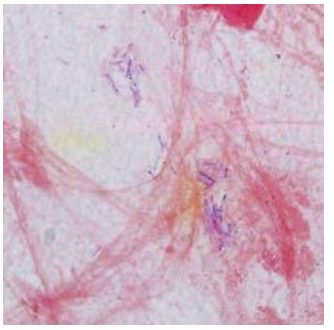


Figure 68.2



Figure 68.3

1. What are the common pathogens that cause skin and soft tissue infections (SSTIs)? To what group of organisms does the agent that caused her infection likely belong?
2. Briefly describe the epidemiology of this group of organisms. What is the significance of the patient's having received a pedicure but not having an aquarium?

3. Several species make up this group of organisms. Does knowing the species help inform therapeutic choices?
4. What other types of infections does this organism cause?
5. Because the organism that caused her infection is an environmental organism, it can be difficult to distinguish colonization from infection, particularly for pulmonary disease. What guidelines exist to aid in this interpretation?



CASE DISCUSSION

1. The most common causes of SSTIs are *Staphylococcus aureus* and *Streptococcus pyogenes*, which can cause a wide spectrum of clinical presentations from uncomplicated folliculitis, furunculosis, and cellulitis to necrotizing infections. Though less common, *Clostridium* spp. (namely, *C. perfringens* and *C. septicum*) can also cause SSTIs and, in particular, necrotizing infections. Had the patient had a history of an animal bite or an animal licking her wounds, organisms such as *Pasteurella* spp. and *Capnocytophaga* spp. would need to be considered. Patients who have exposure to fresh-, brackish, or salt water can have SSTIs due to *Vibrio vulnificus*, *Aeromonas hydrophila*, or *Mycobacterium marinum*. Her clinical course was chronic in nature, as these lesions had been present and progressing over weeks to months. With the exception of *M. marinum*, all of the organisms listed above would have caused more rapidly progressing infections and would have grown from routine bacterial culture within 1 to 2 days. Given that this organism was recovered from both routine bacterial culture media and mycobacterial culture media, it was likely a mycobacterial species or aerobic actinomycete (e.g., *Nocardia*). A fungal infection must be considered based on her clinical presentation, but the Gram stain from the biopsy indicates that this was a bacterial infection. The Gram stain (Fig. 68.2) shows poorly staining, or beaded, Gram-positive rods, which is consistent with mycobacteria. *Nocardia* spp. can also have a similar Gram stain, so culture is needed for definitive identification. Both mycobacteria and *Nocardia* spp. can be cultured from mycobacterial cultures, but the waxy appearance of colonies in Fig. 68.3 is consistent with mycobacteria. *Nocardia* spp. typically have a very white, chalky appearance. An acid-fast stain can be performed to differentiate between mycobacteria (positive) and *Nocardia* (negative); the stain was positive when performed on colonies taken from the plate in Fig. 68.3, indicating that this was a *Mycobacterium* species. Nontuberculous mycobacteria can be separated into two large groups: slowly growing mycobacteria and rapidly growing mycobacteria (RGM). RGM generally grow within 7 days; therefore, this patient's infection was likely due to RGM.

2. RGM are ubiquitous environmental organisms typically found in municipal tap water. RGM have also been isolated from 30 to 78% of U.S. soil samples. Many species can survive in hostile environments, including increased temperatures (45°C). RGM form biofilms and have readily been recovered from pipes supplying water to dialysis centers and hospitals, which have led to local outbreaks. Health care-associated outbreaks have been linked to tap water, ice machines, and water-based solutions. In addition, pseudo-outbreaks have been described due to contaminated bronchoscopes and endoscopes causing positive cultures that were not due to true clinical infections.

During the time period when this patient presented with furunculosis, several other local cases of bilateral lower extremity furunculosis were confirmed to be due to RGM. All patients had a history of getting pedicures, but at different nail salons; thus, eliciting

the history of pedicures was important in narrowing her differential diagnosis. The species of RGM isolated from the cases were not identical, and further investigation found 40 local pedicure-associated RGM infections over a 4-year period. Environmental sampling demonstrated that multiple species of RGM could be recovered from tap water, footbath water, and pipe biofilms from both case and control nail salons. Therefore, it appears that RGM are endemic in this setting and additional factors, such as host susceptibility, play a role in the development of infection. It is known that recent skin trauma such as shaving is a predisposing factor.

Because infections due to *M. marinum* can present similarly, the patient was asked if she had an aquarium. *M. marinum* is found in fresh- and salt water, and infections caused by it are usually associated with aquarium or fish exposure. However, these infections more commonly occur on the hands and arms than the lower extremities. *M. marinum* is a slowly growing nontuberculous mycobacterial species (see case 40 for more details).

3. The most common species of RGM associated with human infections are *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum*. Historically, these species have been difficult to identify in the laboratory, but the use of ribosomal DNA sequencing and mass spectrometry has greatly improved the accuracy of species-level identification. Species-level identification can be helpful in predicting certain antimicrobial susceptibility patterns. For example, *M. fortuitum* is usually susceptible to trimethoprim-sulfamethoxazole and fluoroquinolones, whereas *M. abscessus* and *M. chelonae* are often resistant to both. *M. chelonae* is generally cefoxitin resistant and tobramycin susceptible, and the reverse is often true for *M. abscessus* isolates. Macrolide resistance is of particular concern since this antimicrobial is often used empirically, and resistance can be inducible in some strains of *M. abscessus*. With *M. abscessus*, subspecies-level identification is important for predicting clinical response to macrolide therapy. *M. abscessus* subsp. *abscessus* has a functional inducible *erm* gene (a ribosomal methylase), whereas *M. abscessus* subsp. *bolletii* does not. Extending the incubation time for susceptibility tests to 14 days is needed to detect inducible macrolide resistance, so there is often a delay in final susceptibility results. Although subspecies identification can predict the utility of macrolide therapy, this level of identification is challenging. Most laboratories report these organisms as "*M. abscessus* group" with no further differentiation, which necessitates antimicrobial susceptibility testing. While species-level identification can help guide empiric therapeutic choices, susceptibility testing should still be performed since many of these infections get treated with combination therapy for 6 months to 1 year. This patient's isolate was identified as *M. abscessus* subsp. *bolletii* and was confirmed to be susceptible to clarithromycin and cefoxitin and resistant to trimethoprim-sulfamethoxazole, fluoroquinolones, and tobramycin, as predicted by its identification.

4. RGM have been associated with a wide variety of infections, with pulmonary infections and cutaneous infections being the most common. Cosmetic procedures, in

particular, have been associated with postprocedural RGM infections. RGM can also cause line-related sepsis, particularly in immunocompromised patients. RGM readily form biofilms on indwelling catheters, creating a nidus for continued infection until the catheter is removed. *M. abscessus* is associated with chronic pulmonary infections, particularly in the cystic fibrosis population, as well as posttraumatic and surgical wound infections. It has also been associated with chronic otitis media following tympanostomy. *M. chelonae* can cause posttraumatic wound infections and posttraumatic or postsurgical corneal infections. There has been a particular association of RGM infections with LASIK procedures and punctal plugs used for dry eye syndrome. Like other RGM, *M. fortuitum* can be recovered from cases of posttraumatic and surgical wound infections, but has been more frequently recovered following breast and cardiac surgeries. In addition to causing sporadic infections as described above, RGM have been responsible for a number of outbreaks. Tattooing, plastic surgery, and acupuncture are some procedures associated with repeated outbreaks.

5. The American Thoracic Society and the Infectious Diseases Society of America have published guidelines to assist with the diagnosis of nontuberculous mycobacterial disease. The clinical criteria to establish the diagnosis of lung disease due to nontuberculous mycobacteria, including RGM, are (i) pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan showing multifocal bronchiectasis with multiple small nodules; and (ii) appropriate exclusion of other diagnoses. Microbiologic criteria can also be assessed, particularly when a patient does not have clear disease based on the clinical criteria above. One of the following microbiologic criteria should be met to consider lung disease attributable to RGM (and other nontuberculous mycobacteria): (i) positive cultures from ≥2 sputa, (ii) positive culture from ≥1 bronchial wash or lavage, or (iii) lung biopsy with histopathologic features consistent with mycobacterial infection (e.g., granulomatous inflammation or the presence of acid-fast bacilli in tissue) and a positive culture from at least one pulmonary specimen. If these criteria are not met in a person suspected of having nontuberculous mycobacterial lung disease, continued clinical and microbiologic monitoring may be needed to establish a diagnosis.

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This 43-year-old man with no significant past medical history presented to a hospital on Martha's Vineyard, Massachusetts, with a chief complaint of a sore chest of several days' duration. He had not been feeling well for approximately 1 week, with pleuritic chest pain and a productive cough. Three days prior to presentation he developed progressive shortness of breath and back pain and sought care from a chiropractor, who noted the presence of an abnormality on an X-ray study and referred the patient to the hospital's emergency department for further evaluation.

The patient, who lived alone in a wooded area of Martha's Vineyard, worked as a self-employed house painter and had recently worked cleaning roadside debris. He had no pets.

His vital signs in the emergency department included a temperature of 36.1°C, heart rate of 140 beats/min, blood pressure of 70/50 mm Hg, and respiratory rate of 30 per minute. His oxygen saturation was 78% while breathing room air. The patient had a progressively worse metabolic acidosis on arterial blood gas determinations.

Laboratory studies were otherwise notable for a white blood cell count of 6,900 cells/μl, with a differential of 62% neutrophils and 33% band forms, and significant renal dysfunction, with a blood urea nitrogen level of 90 mg/dl and a serum creatinine level of 7.1 mg/dl. The patient was intubated, given intravenous saline, vasopressors, and intravenous antibiotics, and transferred to a hospital in the Boston area.

Following transfer, the patient's arterial blood gas determination was remarkable for a pH of 6.98, a pCO_2 of 67 mm Hg, and a pO_2 of 78 mm Hg while he received 100% inspired oxygen while on a ventilator. Routine blood cultures and sputum cultures for routine bacteria and *Legionella* were obtained. The patient received broad-spectrum antibiotic coverage and emergency hemodialysis. Despite aggressive intravenous fluid resuscitation and the use of vasopressors, the patient remained hypotensive. A portable chest X ray progressed to complete opacification of the left hemithorax (Fig. 69.1). The patient died the next morning. An autopsy was performed.

Cultures of the patient's blood, sputum, and pleural fluid and a number of autopsy cultures were positive for the growth of a small, Gram-negative coccobacillus that demonstrated small colonies on chocolate agar (Fig. 69.2) and buffered charcoal-yeast extract agar following incubation, but did not grow on either sheep blood agar or MacConkey agar.

1. Are there any infections known to occur on Martha's Vineyard, Massachusetts, that are caused by Gram-negative coccobacilli that do not grow on MacConkey agar and are life-threatening? Where else is this disease noted to occur?

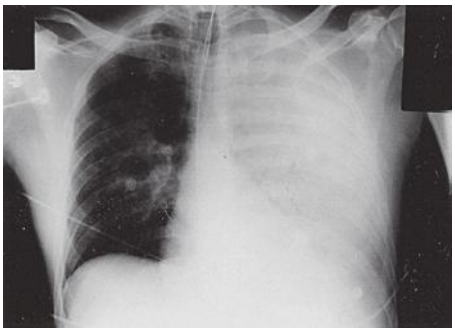


Figure 69.1 Portable chest X ray of patient following transfer.



Figure 69.2 Growth on chocolate agar. (Courtesy Luis de la Maza.)

2. Describe three different syndromes associated with the organism that infected this patient. Which of these did he have?
3. The identification of this organism may be problematic. In fact, it cannot be identified by either commercially available automated or manual biochemical kits because this species is not present in the database of bacteria in these products. In addition, the manipulation of this organism should be minimized as it has caused more than 200 laboratory-acquired infections. How should a laboratory ensure that this organism is accurately identified and that laboratory personnel are not placed at unnecessary risk of infection?
4. This organism is of concern as a potential Tier 1 select agent. What is meant by this term? What other agents are classified as Tier 1 agents?
5. There are a number of different ways to consider zoonotic infections. While the number of zoonoses is certainly greater than 200, the following questions provide three different approaches to think about zoonotic infections. What are the animal reservoirs and the means by which the following zoonoses are transmitted to humans: anthrax, tularemia, salmonellosis, and toxoplasmosis? With what zoonotic infections are each of the following activities or jobs associated: slaughterhouse worker, "Ironman" competitor, veterinary technician, researcher who works with rhesus macaques, seal hunter, and fur trapper? As different animals may transmit different zoonotic infections, which infections have the following animals been established to transmit to humans: bats, cats, reptiles, and rodents?

CASE 69

CASE DISCUSSION

1. *Francisella tularensis*, the etiologic agent of tularemia, is a Gram-negative coccobacillus that does not grow on MacConkey agar, is life-threatening, and occurs on Martha's Vineyard, Massachusetts. Within the United States, cases of tularemia are known to occur in established geographic locations (Fig. 69.3) and occasionally are found in new areas. In Massachusetts, for example, tularemia was essentially unknown until 1937 to 1940, a period when >24,000 cottontail rabbits and >200 jackrabbits from states such as Missouri and Arkansas, where tularemia is endemic, were released by gun clubs in tick-infested areas of southeastern Massachusetts. Tularemia is common among wild rabbits and hares in certain geographic areas. As a result, one of the terms that has been used in the past for the disease is "rabbit fever." The organism is geographically limited to the Northern Hemisphere. There are subspecies of *F. tularensis* that vary in pathogenicity and geography. These include *F. tularensis* subsp. *tularensis* (Type A), which is the most pathogenic for humans, is found in North America, and was the cause of this man's fatal illness; the less virulent *F. tularensis* subsp. *holarctica* (Type B), which is found throughout the Northern Hemisphere; and *F. tularensis* subsp. *mediasiatica*, which occurs in the Central Asian republics of the former Soviet Union. *F. tularensis* subsp. *novicida* rarely causes human disease.

2. The severity of the illness in a patient infected with *F. tularensis* depends in part on the manner in which the infection was acquired. Although six forms of tularemia are described in the literature, the three predominant ones are ulceroglandular, pneumonic (seen in this patient), and typhoidal.

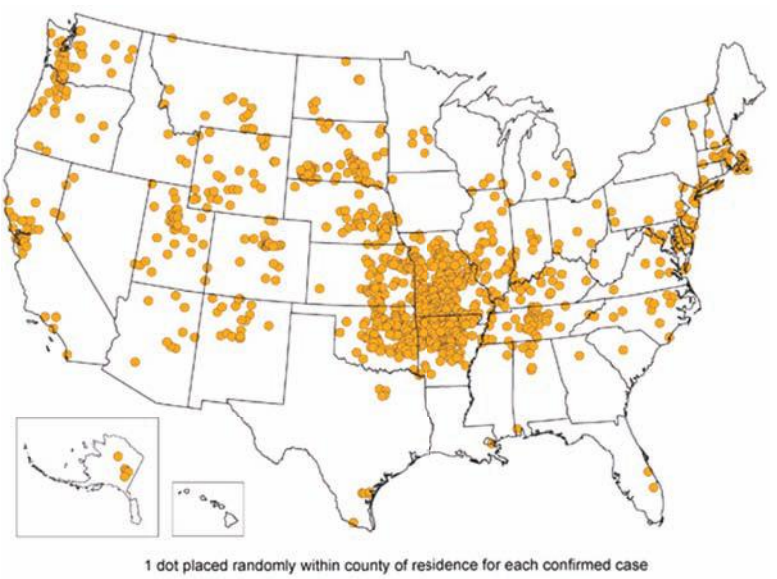


Figure 69.3 Reported tularemia cases, United States, 2003 to 2012. (http://www.cdc.gov/tularemia/statistics/map.html)

In the ulceroglandular form of the infection, the patient may be bitten by an infected tick or cat or may handle an infected animal (such as skinning a rabbit). The patient will have an ulcer and regional lymphadenopathy related to the location of the ulcer. If the lesion is on a hand or an arm, enlarged axillary lymph nodes are seen. If on the legs, inguinal lymphadenopathy will be prominent. Ulceroglandular is the most common form of the disease.

Pneumonic disease is obtained via the inhalation of an infectious aerosol. On Martha's Vineyard, which is a hyperendemic region, it is most common in landscapers and individuals who work for lawn services. Mowing lawns and "weed whacking" are believed to create aerosols of the organism, which these individuals inhale. The origin of the organism is unclear, but it has been speculated to be from decomposed animal carcasses or soil contaminated by such carcasses or by animal excreta. As we saw in our patient, patients can have a severe respiratory illness requiring intubation and mechanical ventilation. The organism, which is highly infectious, can be easily aerosolized in a laboratory setting, so laboratorians working with cultures of the organism are at increased risk for infection via both the cutaneous and pulmonary routes.

The most severe form of disease is typhoidal tularemia. This is a disseminated infection, and patients have nonspecific symptoms that may be associated with a variety of systemic infections. These include fever, chills, headache, and myalgias. Patients may also have watery diarrhea and accompanying dehydration. Mortality is significant. Typhoidal tularemia may be obtained from the ingestion of contaminated meat or water, tick and insect bites, inhalation, and exposure to infected animals.

3. *F. tularenesis*, like many fastidious Gram-negative bacilli, is relatively inert biochemically. When attempts are made to identify an unidentified organism using commercially available biochemical identification systems, *F. tularensis* has been misidentified as *Haemophilus* species, *Oligella urethralis*, and in one commercial product, *Aggregatibacter actinomycetemcomitans* (with what the commercial kit designates as a 99% probability). Because of this concern, the identification of small, Gram-negative bacilli recovered from blood or normally sterile sites should be done using protocols that can rule out organisms such as *Francisella* and *Brucella*. Test protocols using easily performed biochemical tests are available to rule out these organisms. Alternatively, laboratories can send these isolates to public health laboratories for identification. *F. tularensis* is well established as a risk to laboratory personnel. Bacterial isolates that meet certain specific criteria consistent with the organism—i.e., small Gram-negative bacilli that do not show satelliting growth around *Staphylococus aureus* on sheep blood agar and are oxidase negative and either catalase negative or weakly positive—should not be further manipulated. Rather, they should be packaged in a manner consistent with regulatory requirements and then shipped to an appropriate public health laboratory that is able to identify *F. tularensis*.

4. Tier 1 agents pose a risk to national security because they (i) can be easily disseminated or transmitted from person to person, (ii) result in high mortality rates and have the

potential for major public health impact, (iii) might cause public panic and social disruption, and (iv) require special action for public health preparedness. The agents that are classified as Tier 1 agents include *Bacillus anthracis* (anthrax), *Clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), variola major (smallpox), *F. tularensis* (tularemia), *Burkholderia pseudomallei*, *Burkholderia mallei*, and agents causing viral hemorrhagic fever, including filoviruses (Ebola virus, Marburg virus) and arenaviruses (including Lassa fever virus and Machupo virus).

One notable property that is shared by these agents, with the exception of smallpox and botulism, is that these are zoonotic agents. Zoonoses are most often defined as infectious agents that are normally found in nonhuman vertebrates and may be transmitted to humans. *F. tularensis* has an extraordinary host range, having been documented in more than 200 different mammalian species. It has been transmitted to humans via tick bites, biting flies, mosquito bites (in Europe), inhalation, contact with infected animals (including skinning of animals), animal bites (most notably those of cats), drinking of contaminated water, and ingestion of infected animals.

Approximately 60% of emerging infections of humans are zoonotic in origin. Zoonotic infections, when they are acquired by humans, often have a very low rate of then infecting other humans due to little if any person-to-person transmission. However, some zoonotic agents may either be easily transmitted or become adapted to the human host over time, resulting in efficient person-to-person transmission. New disease entities may become significant public health problems. One such example is that of HIV-1. Its viral ancestor was transmitted from the chimpanzee, *Pan troglodytes troglodytes*, to humans and was subsequently efficiently transmitted within the human population via sexual transmission, vertically (mother to infant), and by sharing of needles, transfusion of blood products, and (less commonly) organ transplantation. Another notable example is that of influenza A viruses, each of which may infect birds. In some cases, transmission from birds to humans results in inefficient spread among humans, such as has been the case (at the time of this writing) for H5N1, for example. In other cases, however, the efficient spread within the human population may result in a pandemic of a new influenza A virus.

5. Clinically, there a number of ways of thinking about zoonoses (Tables 69.1 to 69.3), and each of these may be helpful in the evaluation and care of a patient. Please note that these tables are not complete but are for illustrative purposes on how to think through clinical issues regarding zoonotic infections. The most common way that zoonoses are taught to medical students is organism by organism. Each infectious agent is described and the reservoir and vector(s) (if any) of the zoonotic pathogens are listed (Table 69.1). This is of little clinical help in the evaluation of an ill patient in whom the diagnosis is uncertain. It is, however, of help to human and veterinary public health workers. Once the agent is detected in a human, the potential zoonotic source can be determined and spread can be limited. Specific activities may be quite useful in determining potential etiologic agents (Table 69.2). A specific history of a type of animal bite, owning a pet reptile, caring for a bird, or working with pigs may immediately generate a more extensive and useful

TABLE 69.1 SELECTED EXAMPLES OF ZOONOSES BY AGENT

ETIOLOGIC AGENT	RESERVOIR	TRANSMISSION
Bacillus anthracis	Sheep, cattle, and other large mammalian herbivores	Inhalation (such as in wool sorting), ingestion, cutaneous contact
Francisella tularensis	Rabbits, hares, rodents, and numerous other mammals	Ticks, biting flies, mosquitoes (in Europe); contact with contaminated water, inhalation of aerosolized contaminated excreta or of animal parts, ingestion of infected animal, inoculation of eye
Salmonella spp. (note that *S. enterica* serovar Typhi, which is the etiologic agent of typhoid fever, is not zoonotic, as its reservoir is humans)	Vertebrates, particularly humans, snakes, turtles, and frogs	Ingestion (fecal-oral); human to human via fecal-oral route
Sporothrix schenckii	Soil	Cutaneous exposure; infected cats have been the cause of human infections, including outbreaks such as the ongoing one in Brazil that has infected hundreds of people
Toxoplasma gondii	Cats (definitive host); occurs in sheep, cattle, and essentially all mammals	Fecal-oral (via cat feces; antibodies to sporozoites are present only when humans or other animals have been infected with *T. gondii* oocysts [which are formed in cats] and are not present when the source of the infection is via ingestion of bradyzoites in meat); ingestion of undercooked meat of infected animals; ingestion of contaminated milk; human to human via transplacental transmission, blood transfusion, or solid-organ transplant

TABLE 69.2 SELECTED EXAMPLES OF EPIDEMIOLOGIC ASSOCIATIONS WITH ZOONOSES

ACTIVITY	ZOONOSIS ASSOCIATED WITH ACTIVITY
Slaughterhouse worker	Brucellosis (due to *Brucella abortus*, *Brucella suis*, and *Brucella melitensis*)
"Ironman" competitor	Leptospirosis from contact with contaminated water
Veterinary technician	Dermatophyte infection commonly due to *Microsporum canis* from cat contact
Researcher who works with rhesus macaques	Macacine herpesvirus 1 (B virus, herpes B virus; formerly Cercopithecine herpesvirus 1) causing potentially fatal encephalitis
Fur trapper	Tularemia (*F. tularensis*)

TABLE 69.3 EXAMPLES OF NONHUMAN ANIMALS THAT MAY TRANSMIT SELECTED ZOONOSES

ANIMAL	ZOONOSIS	ROUTE OF TRANSMISSION
Bats	Rabies; disease clinically indistinguishable from rabies virus infection due to European bat lyssaviruses types 1 and 2 (in selected case reports in Europe) and by Australian bat lyssavirus (case reports in Australia)	Bites, inhalation (rare), possibly contact; human to human via organ transplant from infected human
	Histoplasma capsulatum	Inhalation of bat guano in caves and among bridge workers in appropriate geographic regions
	Severe acute respiratory syndrome coronavirus	Bat transmission to palm civets, followed by transmission from them to humans and subsequent human-to-human transmission
	Nipah virus	Bat transmission via excreta to pigs, followed by transmission from them to humans; human ingestion of tree sap following bats drinking from the vessels containing the sap
	Marburg virus (a hemorrhagic fever virus)	Unclear, but likely has occurred in a cave in Uganda
Cats	*Coxiella burnetii*	Inhalation of infected placental products when cats give birth
	Francisella tularensis	Bites in appropriate geographic region
	Pasteurella multocida	Present in the oropharynx of essentially all felids, not just house cats; bites, scratches, inhalation
	Rabies	Bites; human to human via organ transplant from infected human
	Rickettsia typhi	From fleas of rats, opossums, and cats to humans
	Microsporum canis	Commonly acquired from contact with infected cats
	Toxoplasma gondii	Ingestion of oocysts from cat feces
	Yersinia pestis	Cat bite, close contact with inhalation of cat respiratory aerosols in appropriate geographic region
	Bartonella henselae	Cat scratch, cat bite; present in cat fleas
Reptiles	*Salmonella* of numerous types; *S. enterica* subsp. Arizonae is more common from snakes	Fecal-oral; ingestion of rattlesnake powder has also been associated with *S. enterica* subsp. Arizonae

Rodents	Leptospirosis	Contact with urine-contaminated water; at-risk individuals include sewer workers and people in urban areas of developing countries after flooding due to hurricanes, typhoons, tropical storms, etc.
	Rat bite fever due to *Streptobacillus moniliformis*	Bite
	Rat bite fever due to *Spirillum minus*	Bite
	Lymphocytic choriomeningitis virus	Contact with hamsters, mice, tissue culture cells from hamsters; exposure to rodent excreta; human to human via organ transplant from infected human
	Hantavirus pulmonary syndrome	Inhalation of rodent excreta
	Machupo virus	Inhalation of rodent excreta
	Yersinia pestis	Flea bite from rats, squirrels, and numerous other rodents

differential diagnosis than would otherwise be considered for an individual patient. In some cases, specific infectious agents that simply are poor matches—for example, rabies, in a patient who has bacterial pneumonia following exposure to a cat—need not be considered. Recognize that the diagnosis of an infection associated with rodent or bat exposure means considering well over 1,000 different species of each of these mammals. By contrast, if evaluating an individual with *Trichinella* infection who ate walrus meat (there is a notable association between these), there is only one species of walrus to consider.

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The patient was a 28-year-old female who came to the United States from the Philippines 5 years ago. Two years ago she was diagnosed with systemic lupus erythematosus (SLE). She was treated with cyclophosphamide for lupus nephritis 2 months ago, but therapy was discontinued due to concerns about drug-induced hepatitis. She remained on hydroxychloroquine and prednisone for her lupus. In the past 3 months the patient had two hospital admissions for fever and myositis; she was diagnosed with SLE flares and treated with pulse-dose steroids. Her current admission was directly from the rheumatology clinic for persistent fever. The patient's husband stated that she had had daily fever of 38.3 to 38.9°C for the past 4 weeks. Approximately 4 weeks ago the entire family (patient, husband, and 1-year-old daughter) became ill. The patient and her husband had symptoms consistent with an upper respiratory infection, and the child had tonsillitis but improved. The patient and her husband were put on azithromycin but only her husband improved. In addition, the patient had anorexia with 15 lb of unintentional weight loss in the last few months, as well as nausea, lethargy, and intermittent confusion and dizziness. On physical exam the patient denied neck stiffness, chest pain, shortness of breath, rhinorrhea, abdominal pain, vomiting, diarrhea, arthralgias, myalgias, or rash, though she noted a dry chronic cough.

Her physical examination was relatively benign with the exception of an abnormal chest computed tomography scan showing diffuse, tiny nodules throughout both lungs (Fig. 70.1). The patient was subsequently sent for a bronchoscopy, and lavage fluid was obtained for bacterial, fungal, and mycobacterial cultures and stains. She had a history of negative PPD (purified protein derivative) tests upon entering the United States and prior to starting therapy for SLE 2 years ago.



Figure 70.1 Chest computed tomography scan showing diffuse, tiny nodular lesions.

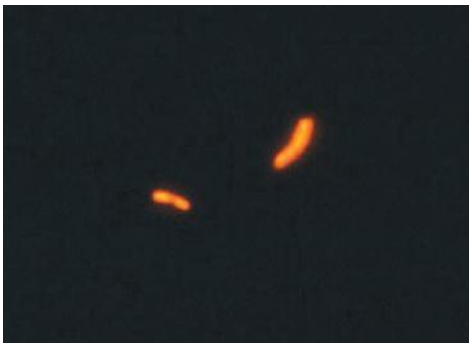


Figure 70.2 Stain of bronchoalveolar lavage fluid.

1. What is the stain in Fig. 70.2? Why do we use it to screen specimens for this group of organisms?
2. What organism was most likely causing her infection? What risk factors did she have for infection with this organism?
3. Describe the natural history of infections with this organism.
4. What are your recommendations for how the patient should interact with her daughter? Can the mother breast-feed her child? Explain.
5. The organism isolated from this patient had the following susceptibility pattern.

Isoniazid	Resistant
Rifampin	Resistant
Pyrazinamide	Resistant
Ethambutol	Sensitive
Ethionamide	Resistant
Capreomycin	Resistant
Ciprofloxacin	Sensitive

   How would you classify this organism? What is the global distribution of these organisms? What is the significance of her being from the Philippines?
6. Briefly describe the changing epidemiology of this disease in the United States in the past 20 years. What treatment strategy was instituted during that time and how has it affected the epidemiology of infection? What change in the microorganism prompted this new treatment strategy?
7. What is a PPD test? What is its value in this patient? What additional tests are available for detecting latent infection with this organism?
8. What recent technical development will, it is hoped, greatly enhance the control of this organism globally?



CASE DISCUSSION

1. The stain in Fig. 70.2 is an auramine-rhodamine fluorescent stain for acid-fast bacilli. The high mycolic acid content in the cell walls of mycobacteria makes the organisms refractory to traditional Gram staining but allows the organisms to be detected directly from patient samples by using stains employing a stringent acid decolorization step; hence the name "acid-fast bacilli." Acid-fast stains include fluorescent stains, such as auramine and auramine-rhodamine, and carbol fuchsin-based stains, such as Kinyoun and Ziehl-Neelsen, which are viewed by light microscopy. Fluorescent stains are preferred for detection of acid-fast bacilli because it is easier to visualize fluorescent rods on a dark background than pink rods on a blue background. Therefore, fluorescently stained smears can be read at lower power (200× to 400×) than carbol fuchsin staining (600× to 1,000×). This allows the microscopist to read more fields of the slide in less time, thereby increasing the sensitivity of detecting acid-fast bacilli while at the same time making the method more efficient. Although less sensitive than culture (50 to 65% versus 80 to 85%), acid-fast smears are performed on direct specimens because the positive predictive value is very high, particularly in respiratory specimens. A positive smear indicates that mycobacteria are present, but not necessarily *Mycobacterium tuberculosis*. In patients with a high suspicion of tuberculosis (TB), a positive smear can be the catalyst to initiate therapy and contact investigations and to place the patient on specific infection control precautions. Persons with smear-positive TB are more infectious because of a higher organism burden than those who are smear negative.

2. The most likely organism was *M. tuberculosis*. Although the acid-fast stain was nonspecific, a nucleic acid amplification test (NAAT) performed directly on the patient's smear-positive respiratory specimen was positive for TB, and the culture grew *M. tuberculosis* 2 weeks later. Those at higher risk for TB infection include recent (<5 years) immigrants from high-prevalence countries; injection drug users; residents or employees of high-risk congregate settings (e.g., homeless shelters, correctional facilities, and nursing homes); organ transplant recipients; oncology patients; and persons with HIV infection, silicosis, diabetes mellitus, severe kidney disease, or malnutrition. The patient's known risk factors included her immunosuppressive therapy for lupus and her immigration from the Philippines.

3. *M. tuberculosis* is spread by respiratory droplets from infected individuals. The droplets are small enough to reach the alveolar space, where they can establish infection. After exposure to TB, only 10 to 30% of people will develop infection. Upon inhalation of *M. tuberculosis*, an individual has four possible outcomes: (i) clearance of the organism, (ii) latent infection with no reactivation, (iii) latent infection with subsequent reactivation, and (iv) primary TB disease. Complete eradication of TB after infection is thought to be rare, but there is evidence suggesting that this may happen. More commonly, disease

progression depends on the balance of the organism and the host immune response. The organisms proliferate until an adequate cell-mediated immune response develops. The macrophages containing TB produce cytokines and chemokines to attract other phagocytic cells and cytolytic T cells. The T cells surrounding the macrophages produce gamma interferon (IFN-γ), which keeps the macrophages activated and able to kill TB. The T cells become surrounded by additional macrophages that form a granuloma, which under normal host circumstances suppresses infection. However, if the balance is upset and organisms kill the macrophages, this produces a caseating granuloma. Further, if TB is able to prevent the formation of a granuloma or replicate out of the granuloma, the activated macrophages release cytokines such as tumor necrosis factor directly into lung tissue, causing tissue damage and cavitary lesions. If necrosis occurs along a blood vessel, a more severe form of infection occurs due to the hematogenous spread of TB, which will cause disseminated disease. This form of TB is called miliary disease because the lesions look like millet seeds, which is how this patient presented (Fig. 70.1). Reactivation occurs in ~5% of infected persons within the first 18 months of infection, and then there is a 5% risk of reactivation over the course of an infected individual's life, usually associated with immunosuppression. Common causes of reactivation-inducing immunosuppression are chemotherapy, chronic diseases, and advanced age. In HIV-positive individuals, the risk of reactivation is increased to 5% per life year.

4. Upon the patient's diagnosis of TB, she was started on four-drug therapy and discharged home. Since the patient was still contagious, she should have been advised to find alternative living arrangements for her 1-year-old child. The child should not stay in the home and should not be breast-fed due to the high risk of the child contracting TB. In addition, the patient should haven been instructed to avoid having visitors or visiting public places and to wear a mask when interactions could not be avoided. Such precautions should remain in place until the health department notifies a patient that he or she is no longer infectious. Patients who have received 2 to 3 weeks of adequate TB therapy, have shown clinical response, and have had three consecutive negative smears collected on 3 separate days are considered noninfectious. Once the mother is no longer infectious, it is safe for her to breast-feed her child, as the first-line antituberculosis drugs do not achieve high enough concentrations in breast milk to pose a problem for the child.

5. The organism isolated from this patient had a drug susceptibility pattern consistent with multidrug-resistant TB (MDR-TB). MDR-TB is defined as being resistant to the first-line agents rifampin and isoniazid. Since standard therapy for TB includes four-drug therapy for 2 months (rifampin, isoniazid, pyrazinamide, and ethambutol) followed by 4 additional months of isoniazid and rifampin therapy, resistance to these two drugs predicts failure of standard antituberculosis therapy. In addition, her organism was also resistant to another first-line agent, pyrazinamide, and the second-line agent capreomycin. Her organism did not meet the definition of an extensively drug-resistant TB (XDR-TB),

which is defined as resistance to rifampin and isoniazid plus resistance to any fluoroquinolone and one of the injectable second-line drugs (amikacin, capreomycin, or kanamycin). Although her organism was resistant to capreomycin, it maintained susceptibility to ciprofloxacin, a fluoroquinolone. According to the World Health Organization (WHO), the prevalence of MDR-TB in the Philippines is increasing; 274 cases were reported in 2005 and 1,148 in 2011. Globally, MDR-TB is most prevalent in the Russian Federation, Kazakhstan, China, India, the Philippines, Eastern Europe, South Africa, and Peru, with a moderate prevalence in Southeast Asia, Brazil, and the Middle East. Since it took several weeks for this patient's culture to grow and for susceptibility testing to be performed on her *M. tuberculosis* isolate, she was inappropriately treated using standard TB therapy initially. Had a rapid diagnostic test been available that, in addition to detecting TB, screened for rifampin resistance, her clinical course might have been altered (see answer to question 8). The patient's disseminated disease caused by a highly resistant organism ultimately led to her death.

6. Following a resurgence of TB in the early 1990s due to the increase of HIV infections, TB cases have been on the decline in the United States. In 2011, there were 3.4 cases per 100,000 persons, which was a ~6% decline from 2010. The number of cases reported is now the lowest since 1953, when national reporting began. The rates are higher in foreign-born persons than those born in the United States, but these rates have also declined over the last 15 years. Due to increasing rates of TB in the 1980s, including the appearance of MDR-TB, directly observed therapy (DOT) was instituted in the United States. The development of MDR-TB was likely due to patient nonadherence, which is influenced by many factors. Effective antituberculosis therapy requires months to complete, which means patients are rarely hospitalized for the duration of treatment. Thus, most antituberculosis therapy is done in the outpatient setting. The treatment regimen is complex and expensive, and the drugs often have side effects. Further, social and cultural factors, including access to health care, contribute to patient noncompliance. Therefore, dosing regimens were modified, public health programs began providing free antituberculosis therapy, and DOT was implemented. In DOT, a reliable individual—anyone from a health care worker to a prison guard to a worker in a homeless shelter—watches the infected individual take his or her medicine to ensure adherence to the appropriate treatment regimen. Public health officials have the option of incarcerating infected individuals who refuse to follow their treatment regimen, to ensure compliance. The thought is that by directly observing a patient take the required treatment, the risk of treatment failure and development of resistance is reduced.

7. A PPD test, also called tuberculin skin test or Mantoux test, is a skin test used to screen individuals for *M. tuberculosis* infection. Tuberculin PPD is a cell-free purified protein fraction obtained from inactivated cultures of *M. tuberculosis*. PPD is injected intradermally in the ventral side of the forearm with a tuberculin syringe. The site of injection

is examined 48 to 72 hours later for evidence of a delayed hypersensitivity reaction, or an area of induration. A positive reaction indicates previous infection with *M. tuberculosis* or other cross-reacting mycobacteria or vaccination with *Mycobacterium bovis* BCG (bacille Calmette-Guérin). An area of induration of 15 mm or more is positive in any person, independent of immune status or risk factors. Individuals with moderate risk factors for TB infection (see answer to question 2) are considered positive with an induration of 10 mm or more. Only a 5-mm area of induration is needed for a positive result in HIV-positive individuals, other immunosuppressed persons, recent contacts of those with active TB, or individuals with chest radiograph consistent with TB. It should be noted that most PPD-positive patients are latently infected with TB and do not have active infection. Immunosuppression may lead to false-negative PPD tests and is common in HIV-positive individuals and patients with underlying chronic diseases and/or on a wide variety of immunosuppressive therapies. Control antigens (usually *Candida*, mumps, and/or tetanus) may be placed to determine anergy, or the inability to mount a local immune reaction to the injected antigens. Although placing control antigens can help determine a false-negative PPD result, they are rarely performed. In addition, approximately half of patients with miliary tuberculosis (disseminated TB) will have a negative PPD. This patient was immunosuppressed by her lupus therapy and also had miliary TB; thus her PPD was falsely negative.

IFN-γ release assays (IGRAs) have come into favor recently as tests to diagnose active and latent TB infection. IGRAs, such as the QuantiFERON and T-SPOT.TB tests, have the advantages of convenience (only one blood draw is needed) and possibly increased sensitivity and specificity over the PPD. There is no cross-reactivity of IGRAs with BCG vaccination, though there is still some cross-reactivity with some nontuberculous mycobacteria (e.g., *Mycobacterium kansasii*, *Mycobacterium szulgai*, and *Mycobacterium marinum*). With IGRAs, whole blood is collected, white blood cells are stimulated with TB-specific antigens, and the resulting IFN-γ response is measured. Patients with TB infection will release IFN-γ in response to antigen stimulation, but similarly to the PPD, IGRAs do not differentiate between active and latent infection. IGRAs include controls that assess the patient's ability to produce IFN-γ to control antigens. Results along the positive threshold can be difficult to interpret, particularly if the test is used serially such as with health care providers. More studies are needed to determine how effectively IGRAs predict those who will progress to active tuberculosis and how the tests perform in children and immunocompromised patients.

8. The availability of commercial NAATs for the detection of *M. tuberculosis* DNA directly from patient samples shows great promise in reducing the global burden of TB disease by offering a rapid, accurate diagnosis. In 2011, there were 8.7 million new cases of TB disease globally and 1.4 million died. Though the global death rate has declined by 41% in the last 20 years, there is still significant disease burden. Because *M. tuberculosis* can take up to 3 weeks to grow in culture, and smears are notoriously insensitive and lack

specificity for TB, the diagnosis of TB can be grossly delayed in an individual who is actively transmitting disease to others. A rapid diagnosis is critical to starting appropriate therapy and preventing disease transmission. There are currently two FDA-approved/cleared NAATs for TB. The WHO endorsed one of these tests (Xpert MTB/RIF; Cepheid, Sunnyvale, CA) in 2010 and has since published policies for its global implementation due to its ease of use in the field, its ability to offer a result while a patient is waiting, and its diagnostic accuracy. The Xpert MTB/RIF test has a time to result of ~2 hours. Its sensitivity in smear-positive respiratory specimens is 98 to 99%, and the sensitivity in smear-negative specimens is 68 to 90% depending on the number of specimens tested. In addition, the Xpert test also screens for the most common rifampin resistance mutations by interrogating the rifampin resistance determining region of *rpoB* in *M. tuberculosis*. In countries with a higher prevalence of MDR-TB, rifampin resistance is a predictor for MDR-TB. This is not necessarily true in the United States, where MDR-TB is uncommon. More data are needed from areas with low MDR-TB rates to determine the positive predictive value of the rifampin resistance screen in low-prevalence settings. As of June 2012, 67 high-burden developing countries had obtained 749 instruments and used over a million Xpert MTB/RIF tests with the help of the U.S. PEPFAR (President's Emergency Plan for AIDS Relief), the U.S. Agency for International Development, UNITAID of WHO, and the Bill & Melinda Gates Foundation. There are other "research use only" commercially available tests for the detection of TB and drug resistance markers, but none are as portable and easy to use in low-resource settings, making their global impact more limited.

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CASE 71

The patient was a 36-year-old, previously healthy male who 1 week prior to presentation suffered a left eye injury when a tree branch abraded his cornea. He visited his local emergency department 2 days after the injury, where the corneal abrasion was observed. He was given steroid-containing eye drops (concentration not reported) and was discharged. He now presented to the ophthalmology department with complaints of increasing left eye pain, redness, matting, irritation, and a watery discharge. His chief complaint was blurred vision and photophobia. He denied eye itching, eyelid swelling, double vision, and decreased or loss of vision.

His vital signs were within normal limits. His physical examination was unremarkable except for his ocular exam. Figure 71.1 shows the ocular lesion seen at presentation. Examination of his left eye was significant for eyelid edema and erythema. His conjunctiva was severely injected with slight exudate. Corneal abrasion was present with a single medium-sized, circular-shaped area that took up fluorescein dye. Snellen chart eye examination was 20/40 for his right eye and light perception only for his left eye.

A corneal scraping was obtained from the lesion. A calcofluor white examination was performed on this scraping and is observed in Fig. 71.2. The culture grew the organism observed in Fig. 71.3 and 71.4. He was given 1% voriconazole drops four times a day for 14 days. His infection healed on antifungal therapy and his vision returned to baseline.

1. What is a calcofluor white examination? What was learned from that examination?
2. The type of infection that this patient had was keratitis. There are three major forms of keratitis. What are they? Which form did this patient have? What organisms are most likely to be seen in the form of keratitis this patient had? What is the actual organism with which this patient was infected?

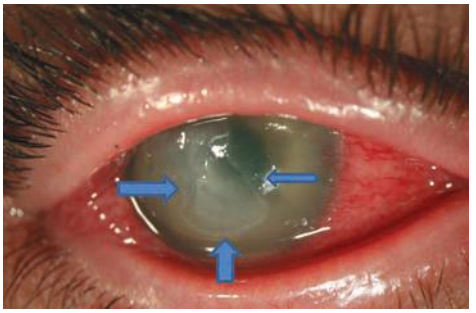


Figure 71.1 Ocular lesion 7 days posttrauma.

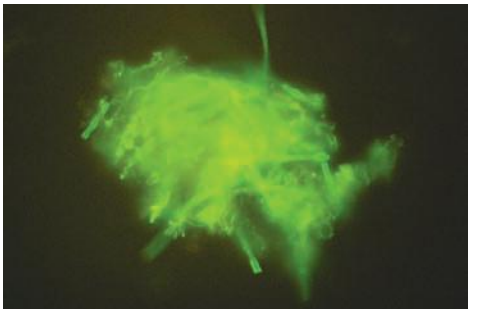


Figure 71.2 Calcofluor white examination of corneal scraping.



Figure 71.3 Organism recovered from corneal scraping at 7 days of incubation at 30°C.

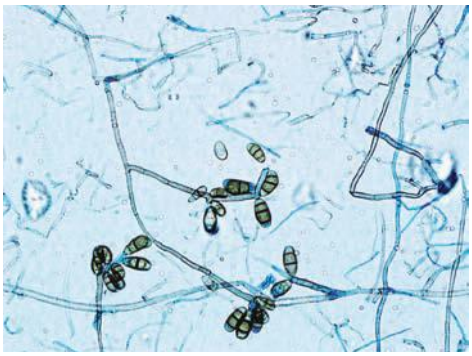


Figure 71.4 Lactophenol cotton blue microscopic view of the organism in Fig. 71.3.

3. Between 2005 and 2007 there was a global outbreak of keratitis. What was the etiologic agent of this outbreak and how did this epidemic likely spread?
4. Keratoconjunctivitis due to two bacterial agents can result in rapid, irreversible vision loss. What are these two organisms and in what clinical situations are they most likely to be seen?
5. What is the most common infectious cause of blindness globally? How is it spread? How can it be prevented?
6. Epidemics of "pink eye" are common in primary schools globally. What is the etiology of pink eye? How is it typically diagnosed in epidemic settings? What is the long-term impact on the vision of infected patients?



CASE DISCUSSION

1. Calcofluor white is a whitening agent often used in clothing that adheres to cellulose and chitin found in the cell walls of fungi. It is widely used to detect fungi in clinical specimens. Because the organisms "fluoresce" when stained with this agent, they can be viewed at lower magnification in a fluorescent microscope, allowing for a more thorough and rapid evaluation of clinical specimens. This results in higher sensitivity than KOH preparations or Gram stains. An added advantage when examining corneal scrapings is that it will also detect the protozoan *Acanthamoeba*, an important agent of keratitis (see answer to question 2 for further details).

2. Keratitis is an inflammation of the cornea. In infectious keratitis, there is destruction of the corneal epithelium and stroma. This patient's clinical presentation and physical findings, including corneal opacity (outlined by arrows in Fig. 71.1), are typical. The lack of purulent exudate may have been due to a combination of a nonbacterial infection and the steroid eye drops he was receiving. There are three common forms of keratitis: (i) cases associated with contact lens use; (ii) keratitis following trauma, including postsurgical infections; and (iii) viral keratitis primarily due to reactivation of the herpesviruses, especially herpes simplex virus 1 and varicella-zoster virus.

Contact lens-associated keratitis occurs as a result of either improper contact lens care or contaminated contact lens solutions (see answer to question 3 for further details). It is the most common cause of keratitis in the industrialized world and is more frequent in females than males because females are more likely to wear contact lenses. Contact lens-associated keratitis is primarily due to waterborne organisms such as the bacteria *Pseudomonas aeruginosa* and *Serratia marcescens* and the protozoan *Acanthamoeba*. The three major sources of this form of keratitis are (i) practicing poor contact lens hygiene; (ii) replacing contact lens solutions with tap water; and (iii) wearing lenses when showering or when swimming in freshwater, swimming pools, or hot tubs.

Trauma-associated keratitis is dependent on the type of injury suffered. If it occurs postsurgically, organisms that are part of the indigenous microbiota of the eye, such as coagulase-negative staphylococci and *Propionibacterium* spp., are most common. Rapidly growing mycobacteria, e.g., *Mycobacterium chelonae* and *Mycobacterium fortuitum*, have also been implicated in postsurgical infections, particularly following LASIK surgery. When patients have trauma associated with foreign objects, fungi take on a more prominent role. These infections are more common in males, who more frequently engage in activities such as landscaping (as was seen in this patient) or metalworking in machine shops. Approximately half of fungal keratitis cases are associated with trauma. The fungi most commonly associated with posttraumatic keratitis are *Fusarium* spp., *Candida* spp., *Curvularia* spp., and *Aspergillus* spp. *Candida* typically is obtained from the patient's own microbiota, while the other fungal agents usually are inoculated into the eye from foreign bodies.

This patient was infected with the mold *Curvularia*. *Curvularia* is a dematiaceous mold (Fig. 71.3), which means its hyphal elements are either brown or black. This can best

be appreciated from Fig. 71.4, where the black color of the hyphae and conidia can be seen. The actual identification of this mold is based on the structure of the conidia seen in Fig. 71.4, but an in-depth discussion of the taxonomy of dematiaceous fungi is beyond the scope of this book. Dematiaceous molds tend to be more resistant to antifungal agents, making them difficult to treat.

3. The largest reported international epidemic of ocular fungal infection was the result of the use of contact lens solution contaminated with the mold *Fusarium*. *Fusarium* is a hyaline (nonpigmented) mold that is highly resistant to antifungal agents. It was believed that the antifungal agent in the solution was heat inactivated during storage, allowing the fungal agent to grow. Approximately 35% of infected patients required a corneal transplant as a result of these infections.

4. Both *P. aeruginosa* and *Neisseria gonorrhoeae* can cause fulminant keratoconjunctivitis, whose most severe outcome is perforation of the globe within 24 hours of the onset of infection. As a result, keratitis in general and keratitis due to these two organisms are considered ophthalmologic emergencies.

P. aeruginosa keratitis is typically associated with contact lens use or trauma. *N. gonorrhoeae* keratitis is associated with ocular autoinoculation by fingers from patients with genital or anogenital gonococcal infections. Neonatal gonococcal ocular infections, ophthalmia neonatorum, occur when the child passes through an infected birth canal. In the industrialized world, such infections are exceedingly rare because the standard of care is the use of prophylactic silver nitrate drops at birth. However, if the drops are improperly administered or are not administered at all, the risk of infection from an infected mother to her infant is ~30%. *Chlamydia trachomatis* may also cause ophthalmia neonatorum and cannot be distinguished clinically from the gonococcal form of infection. Because the treatment of these two infections is different, they need to be differentiated by culture or nucleic acid amplification tests.

Gram stain can be a very efficient means to diagnose exudative bacterial keratoconjunctivitis caused by *P. aeruginosa* and *N. gonorrhoeae*. On Gram stain, *P. aeruginosa* is a long, thin, Gram-negative bacillus, while *N. gonorrhoeae* is a Gram-negative diplococcus that can be found both extra- and intracellularly. These two agents are treated differently, and early therapy can be guided by Gram stain results.

Neisseria meningitidis has a similar Gram stain appearance and cannot be differentiated from *N. gonorrhoeae*. However, *N. meningitidis* keratoconjunctivitis is very rare and when it does occur may also cause an aggressive infection. Both *Neisseria* infections are treated in a similar manner, so differentiating between the two is clinically less important.

5. *C. trachomatis*, the etiologic agent of trachoma, is believed to be responsible for up to 15% of cases of blindness globally. Trachoma is a disease of poverty seen primarily but not exclusively in infants and children living under conditions of poor sanitation and hygiene and overcrowding. The organism is spread by direct contact, typically via the hands; by

fomites such as contaminated towels or clothes used on the face; or by mosquitoes that travel from an infected child's eye to the face of an uninfected one. The disease is most prevalent in rural Africa, particularly in central East Africa, including Ethiopia, Sudan, Kenya, Tanzania, and Niger, but is found globally in rural areas of extreme poverty. The onset of disease in infants and young children is insidious, with the development of chronic follicular keratoconjunctivitis. This disease is characterized by the eyelashes growing inward and rubbing against the cornea, causing irritation and eventual breakdown in the corneal epithelium, leading to corneal scarring and ultimately resulting in corneal opacification and blindness. The disease process is slow and may take decades from the initial infection to the development of blindness.

There is no vaccine for *C. trachomatis*. As a result, the prevention strategy that is used is administration of azithromycin to all individuals in areas of high trachoma endemicity. This is part of the World Health Organization SAFE strategy to treat and prevent trachoma. SAFE stands for "surgery, antibiotics, face washing, and environmental change" (eliminating insect breeding). When trachoma rates rise above 10% in a community, it is recommended that the mass antimicrobial administration be done three times a year.

6. Pink eye is a highly infectious form of conjunctivitis caused by adenovirus. Patients typically have lid edema, subconjunctival hemorrhage, and a watery discharge. Both eyes are typically affected, although this may occur sequentially. Patients complain of eye pain, irritation, itchiness, increased tearing, and a foreign body-like sensation. Patients with this constellation of signs and symptoms are most likely to have adenovirus keratoconjunctivitis. This is typically a clinical diagnosis, but culture or nucleic acid testing can confirm the diagnosis.

Therapy is supportive, including artificial tears or cold compresses to the eye. Some physicians may use topical antibacterial agents to prevent secondary bacterial infections, but clinical trials did not show a benefit to this approach. Patients with this infection usually recover within 14 days without any adverse sequelae.

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The patient initially presented at 10 weeks of age with a history of worsening cough over the previous 6 weeks. This was her third hospital admission in a 4-week period for what was characterized as respiratory distress and bronchiolitis. She also had a history of fevers (38.2 to 38.5°C). She had been treated empirically for pertussis and had multiple negative tests for respiratory syncytial virus. On a previous hospital admission the organism seen in Fig. 72.1 was recovered from bronchoalveolar lavage fluid. At home she was noted to have had multiple "blue spells" with apnea lasting up to 30 seconds. The child also had poor weight gain and apparent formula intolerance. She had many small, foul-smelling stools daily.

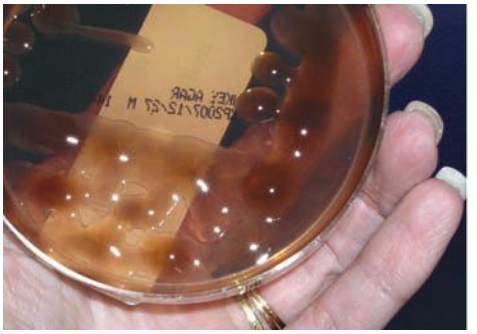


Figure 72.1 Organism growing on MacConkey agar.

On physical examination she was tachypneic with a respiratory rate of 60 breaths/minute. Chest radiograph showed hyperinflation and streaky perihilar densities. Her laboratory findings were significant for a sweat chloride of 106 mEq/liter (normal range, 0 to 39 mEq/liter), a white blood cell count of 30,100 cells/μl with 80% neutrophils, total protein of 4.6 g/dl, and albumin of 2.4 g/dl.

A bronchoalveolar lavage fluid sample was obtained on admission and had a predominance of growth of the organism seen in Fig. 72.1. The child was treated with piperacillin-tazobactam and gentamicin and was begun on chronic pancreatic enzyme replacement therapy. She was discharged after 4 weeks of antimicrobial and supportive therapy. The organism seen in Fig. 72.1 was consistently recovered from her respiratory tract for her entire 8 years of life. She continued to be small for her age and had several additional hospital admissions for antimicrobial treatment of pulmonary infections.

1. What was this child's underlying disease? What clues are present in this case that led you to this conclusion? What diagnostic test confirmed your diagnosis? Explain the physiologic and genetic basis of this disease.
2. Patients with this disease have an abbreviated life expectancy of approximately 30 to 40 years. The organism in Fig. 72.1 is the key pathogen in these patients. Describe the typical sequence of events associated with chronic infections with this organism. Strains of the organism are often refractory to antimicrobial therapy in this patient population. Explain why.

3. Besides antimicrobial therapy, what other strategies can be used to improve pulmonary function in patients with this disease?
4. What infection control practices are required to manage this patient population?
5. Lung transplantation is a proven therapy for improving both the quality and duration of life in this patient population. Chronic pulmonary infection with what bacterial organism is an absolute contraindication to lung transplantation? Explain.
6. Recently molecular techniques have suggested that another group of organisms may play a role in the chronic lung disease seen in this patient population. What is this group of organisms? How might they contribute to the patient's chronic lung disease?



CASE DISCUSSION

1. This patient had the most common inherited disease in the Caucasian population, cystic fibrosis (CF). The disease is much less common in other racial groups but does occur. Approximately 30,000 individuals in the United States and 100,000 worldwide are living with CF. CF is an autosomal recessive genetic disorder caused by mutations in the gene located on chromosome 7 that encodes a transmembrane protein called CFTR (cystic fibrosis transmembrane regulator). Currently >1,500 mutations in CFTR have been described. The most common mutation, found in ~70% of CF patients, is a 3-base deletion of a phenylalanine at position 508 referred to as ΔF508 (This child was heterozygous for this mutation.) The mutation in CFTR results in abnormalities in ion transport across cytoplasmic membranes.

This patient manifested three key features of CF in infancy. First, she had failure to thrive and malnutrition coupled with fatty, foul-smelling stools. The malnutrition seen in this child as evidenced by the low serum protein and albumin was due to blockage of the pancreatic ducts by thickened secretions, which prevents pancreatic enzymes from entering the gut. As a result, CF patients have malabsorption characterized by frequent, foul-smelling stools, as was seen in this child. This manifestation of disease can be ameliorated by the use of pancreatic enzyme supplements prior to meals.

The second manifestation, a highly elevated sweat chloride of 106 mEq/liter, confirmed the CF diagnosis. In the sweat glands of CF patients, there is failure to reabsorb chloride ions across the epithelium, resulting in an elevated salt content in sweat. An elevated sweat chloride level is a highly reliable diagnostic test for CF, with false-positive tests being highly unusual. As we better understand the molecular biology of CFTR, CF patients with borderline or normal sweat chlorides are being recognized but quite infrequently.

The third clue that this patient had CF was the frequent pulmonary infections she was having and the organisms that were detected. In the respiratory epithelium of CF patients, there is increased absorption of sodium, chloride, and water. The end result is thickened respiratory secretions, which adversely affect mucociliary clearance of microorganisms and lead to chronic pulmonary infections of the bronchial airways rather than the alveoli, as is seen in bacterial pneumonia. A key feature of CF chronic pulmonary infections is the limited number of organisms that cause these infections, the most common being *Staphylococcus aureus*, usually seen early in the life of a CF patient, and *Pseudomonas aeruginosa*, typically first seen in late childhood or early adolescence.

Newborn screening for CF is offered in all U.S. states, most provinces in Canada, throughout the United Kingdom, and in several Northern European countries. At the time of this presentation newborn screening was not available, and that is why this diagnosis was initially "missed" in this patient.

2. The critical tipping point in CF lung disease is the establishment of chronic airway infection with an unusual phenotype of *P. aeruginosa* called mucoid (Fig. 72.1). It is estimated that as many as 80% of CF patients have abbreviated life expectancies as a result of

chronic mucoid *P. aeruginosa* infections. The sequence of events in chronic *P. aeruginosa* infection is extraordinarily complex but typically begins with acquisition of this organism from the environment. Often in the early stages of chronic *P. aeruginosa* infection the organism will not be mucoid. During that stage of infection, the organism may be eradicated with aggressive antimicrobial therapy, including the use of aerosolized antimicrobials such as tobramycin or colistin. These same aerosolized antimicrobials may also be used intermittently to attempt to prevent acquisition of this organism.

Progression of chronic lung infection is associated with the evolution from the nonmucoid to the mucoid phenotype. A key step in the emergence of the mucoid phenotype is mutations in genes that regulate alginate production. Alginate is a complex, extracellular carbohydrate polymer. It forms the matrix of the biofilm in which *P. aeruginosa* grows in the CF airway. *P. aeruginosa* organisms growing in biofilms are more refractory to both antimicrobial therapy and to immune clearance. There is solid evidence that the chronic presence of *P. aeruginosa* results in the upregulation of cytokines, leading to increased levels of neutrophils in the CF airway. Abnormal levels of neutrophil-derived proteolytic enzymes are present in the lungs of CF patients chronically infected with *P. aeruginosa* and are believed to make a major contribution to the lung damage seen in this chronic infection.

Pulmonary exacerbation of CF lung disease, the hallmark of chronic *P. aeruginosa* infection, is characterized by increased cough, sputum production, and white blood cell count, as well as fever, malaise, and declining pulmonary function, all of which were seen in this patient. The exact events that lead to pulmonary exacerbation are not understood, but it is most commonly associated with increasing bacterial counts of *P. aeruginosa*. Aggressive antimicrobial therapy, usually with a combination of antipseudomonal β-lactams and aminoglycosides, can reverse these pulmonary exacerbations, but the overall effect is an insidious decline in lung function.

This chronic cycle of periods of well-being followed by pulmonary exacerbation results in an ever downward spiral of lung function loss that eventually results in cardiopulmonary collapse. A key determinant of both the quality and quantity of life in CF patients chronically infected with mucoid *P. aeruginosa* is the ability to extend the length of time between exacerbations and to limit the lung damage during exacerbations.

One of the problems facing physicians caring for these chronically infected patients is that over time the *P. aeruginosa* infecting patients becomes increasingly drug resistant, in part due to hypermutable strains of *P. aeruginosa*, which are found with greater frequency in CF patients. Hypermutability is due to mutations in genes involved in DNA repair. Hypermutability enhances organisms' ability to develop resistance to antimicrobials. The end result is an organism that remains susceptible only to colistin, a highly toxic molecule, among the antimicrobials with activity against *P. aeruginosa*. Pulmonary exacerbations become increasingly difficult to treat because of the dearth of active antimicrobial agents, and patients have acceleration in the decline of their lung function, resulting in cardiopulmonary collapse.

Unfortunately for this child, she was infected very early in life, which is unusual, and continued to have pulmonary exacerbations with this organism. This suggests that the patient will not do well in the long term.

3. Several additional strategies are important in managing CF lung disease. The major strategy revolves around the daily management of airway obstruction. The thick mucus that clogs the airways of CF patients is an ideal environment for chronic infection. Clearance of this mucus is a key step in preventing/managing chronic infection and maintaining pulmonary function in CF patients. Vigorous aerobic exercise that stimulates deep coughing and expectoration of the thick airway secretions is an important means for managing airway obstruction. For those unable to exercise because of age or declining lung function, expelling of airway secretions can be accomplished by percussion of the chest on the front, side, or back followed by coughing up the thickened airway secretions. Percussion can be accomplished either manually or using a mechanical vest designed for this purpose.

Aerosolized recombinant human DNase helps liquefy airway secretions by degrading neutrophil and bacterial DNA, which contribute to the viscosity of these secretions. It is an effective but extremely expensive adjunct to exercise and/or chest percussion.

4. Molecular epidemiology studies suggest that the vast majority of nonsibling CF patients are infected with unique *P. aeruginosa* strains. This is strong evidence that these organisms are typically obtained from the environment, where they are ubiquitous. Although CF patients with *P. aeruginosa* are typically not isolated for infection control purposes, a recent outbreak in a CF center in Leeds, United Kingdom, due to a multidrug-resistant strain of *P. aeruginosa* resulted in a significant rise in mortality in that center. This outbreak has led to a rethinking of that approach and the isolation of patients with multidrug-resistant *P. aeruginosa* in many centers.

Strict infection control practices have been shown to be important with another group of organisms important in CF lung disease, three species belonging to the *Burkholderia cepacia* complex: *Burkholderia multivorans*, *Burkholderia cenocepacia*, and *Burkholderia dolosa*. All three of these organisms have been shown to be spread from person to person. Because significant morbidity and mortality is associated with both *B. cenocpeacia* and *B. dolosa*, strict, perhaps even draconian, infection control measures have been successfully applied to prevent the spread of these organisms in CF populations. The strategy used has been to segregate *B. cepacia*-infected patients from the rest of the CF population both medically and socially. Although effective, it has made *B. cepacia*-infected patients social pariahs in the CF community.

5. Lung transplantation of CF patients with terminal lung infection has proven to be an effective long-term treatment, and given the age at which this patient became infected with mucoid *P. aeruginosa*, it may be needed to prolong her life. Five-year survival of CF patients who receive a double lung transplant is ~60%. However, patients infected with *B. cepacia* complex organisms have been shown to have a statistically significant increase in short-term mortality posttransplant as well as a decreased life expectancy at 1 and 5 years. The reason is that posttransplant infections with strains of *B. cenocepacia* have proven to be highly aggressive, causing bacteremia and septic shock as well as pulmonary and surgical-site infections.

They are usually refractory to antimicrobial treatment in part because the organisms often are resistant to all available antimicrobials. The infections posttransplant have been conclusively demonstrated to be due to the same strain of bacteria with which these patients were infected pretransplant. Because of these poor outcomes and a paucity of transplantable organs, pretransplant infection with *B. cepacia* complex has become an absolute contraindication for lung transplant in essentially all transplant centers worldwide.

6. An interesting frontier in understanding chronic lung infection in CF patients is the potential role of noncultivable bacteria in CF lung disease. Two recent observations suggest that anaerobic bacteria may contribute to the damage that occurs in the CF airway during chronic infection. The first piece of information is that oxygen tension is very low in infected CF airways. *P. aeruginosa* can grow under anaerobic conditions, and that growth may be important in biofilm formation. The second observation is from molecular techniques that can detect microorganisms that cannot be readily grown from CF respiratory secretions. Recent studies suggest that specific anaerobic bacteria and *Streptococcus milleri* group bacteria are frequently detected by molecular methods. These data need to be viewed cautiously for now because they may represent nothing more than contamination of these secretions with upper respiratory tract microbiota, which is rich in anaerobes. However, anaerobes from the upper airways such as *Prevotella* spp. have been shown to produce a variety of proteolytic enzymes that are believed to have a key role in periodontal disease. It is possible that these same enzymes might contribute to airway destruction and declining lung function in the CF airway as well. Additionally, quorum-sensing molecules from noncultivable microbiome members may regulate the production of virulence factors by the predominant members of the microbiome in chronic lung infections such as *P. aeruginosa*.

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The patient was a 21-year-old college student who was referred to the infectious disease clinic from student health services because of anorexia, fatigue, and a 20-lb (9-kg) weight loss. She was also experiencing shaking chills and sweats.

The patient's history was significant for her immigration from Uzbekistan 4 years previously to attend a U.S. university. Her parents were both alive, and she had two healthy siblings. She had a pet dog when she was younger, but there were no other notable exposures. She had had one sexual partner and was not currently sexually active. Seven months previously she had had surgery in Uzbekistan during which a multiloculated cystic lesion was removed from her liver.

She gave no history of nausea, vomiting, diarrhea, or genitourinary tract complaints. On physical examination her vital signs were within normal limits. She had some hair loss, but no rash or skin changes, and her last menstrual period was 2 weeks previously, with no current vaginal bleeding. She had no lymphadenopathy, and her pulmonary examination was unremarkable. Her abdominal examination was significant for diffuse tenderness to palpation that was most pronounced in the left lower quadrant. Laboratory tests, including a white blood cell count and liver function tests, were all within normal limits. An abdominal computed tomography scan demonstrated a 10-by-5.6-cm multiloculated cystic lesion in the rectouterine pouch as well as a 4-by-5-cm multiloculated lesion in the liver.

The patient was taken to the operating room for an exploratory laparoscopy. Two abdominal cysts were removed, one of which was adherent to the posterior abdominal and uterine wall. The other was adherent to the left ovary and fallopian tube. Prior to removal, both cysts were entered, drained, and then filled with 20% saline solution. Figures 73.1 and 73.2 show what was seen on microscopic and histologic examination of cyst material removed from her pelvis.

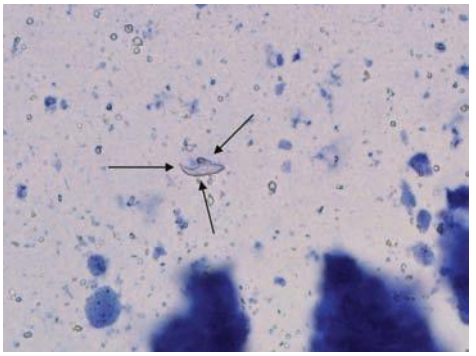


Figure 73.1 Wet-mount examination of aspirate of cystic mass enhanced by methylene blue stain (magnification, ×600).

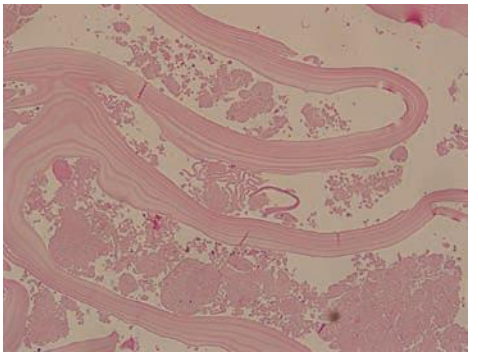


Figure 73.2 Hematoxylin and eosin stain of tissue removed from intra-abdominal lesion.

1. What is your differential diagnosis for an individual with an abdominal pelvic mass?
2. What structures are seen in the figures, and for what organism are they diagnostic? Do you believe her liver and pelvic masses were related? Explain.
3. There are four possible strategies for treating liver and pelvic masses such as these. What are they, and what might the utility of each have been in this patient?
4. What is the natural history of this infection? What in this patient's history explains how she likely became infected?
5. Over the past 20 years the rate of infection with this organism has increased 6-fold in this region of the world. Name two potential explanations for this increase.



CASE DISCUSSION

1. Several things need to be considered in a young woman with a pelvic mass. The most likely explanation is an ovarian mass that is either benign or malignant. Second, if she had been sexually active, ectopic pregnancy would have to be considered, although this patient's lack of vaginal bleeding made this less likely. The third possibility is that the patient has intra-abdominal and liver bacterial abscesses. However, a fourth possibility was considered in this patient for two reasons. First, she had a liver as well as a pelvic mass, and both were multiloculated. This is consistent with a hydatid cyst due to infection with *Echinococcus granulosus*. Second, she lived in a region of the world with a high prevalence of *E. granulosus* infection. Serologic testing for *Echinococcus* that was obtained at the time of the patient's clinic visit was strongly positive, further indicating that the patient had an echinococcal infection. *E. granulosus* is a dog tapeworm for which humans are accidental end hosts (see answer to question 5 for more details).

2. The structures seen in Fig. 73.1 and 73.2 are diagnostic for *Echinococcus*. The structure seen in Fig. 73.1 is a hooklet, which is found in the protoscolex phase of this tapeworm (see answer to question 3 for more details). The ribbon-like material seen in Fig. 73.2 is the laminate layer of the hydatid cyst wall. The laminate layer is an acellular elastic structure made of concentric layers of glycosylated mucins that surround the cyst. It does not elicit an inflammatory response and serves to protect the cyst from the host's immune system. Both of these structures are quite distinctive for *Echinococcus*. The most common location for echinococcal cysts is the liver, followed by the lungs. Infection of the genitourinary tract is unusual. Because this patient had had surgery 7 months previously to remove a liver cyst, a possible explanation for the presence and location of the abdominal cysts was that they were formed after leakage of protoscolices from the original liver cyst.

3. The initial intervention in this woman was the surgical removal of the pelvic cysts. Because she had a positive echinococcal serology prior to this surgery, she was given two agents, albendazole and praziquantel, for 10 days prior to her surgery. The combination of surgery and presurgery antimicrobial therapy was chosen because it has the highest cure rate and the lowest likelihood of relapse. This was of particular concern because it was likely that she had already suffered a relapse after her initial surgery. Presurgical antimicrobial therapy is becoming more common when echinococcal serology is positive or there is a high index of suspicion of the disease, such as in people who have had contact with dogs in areas of the world where there is high disease prevalence. One of the greatest fears in managing echinococcal cysts surgically is the potential for spillage of cyst contents. Fatal anaphylactic reactions to live protoscolices have been associated with this spillage, although it must be stated that this reaction occurs in only about 1 in 750 patients. Treatment with albendazole has been shown to reduce the risk of these reactions and thus is often used prior to surgery.

Second, this young woman was quite concerned about retaining her fertility. The gynecologist caring for her was of the opinion that a surgical approach where the anatomy could be visualized and the cysts dissected away from the reproductive organs was the optimal approach to achieve this. The cysts were wrapped in sponges soaked in 20% saline to kill any protoscolices that might leak in order to prevent the formation of secondary cysts.

A second treatment strategy is to perform a procedure called PAIR. In this method, the cyst is punctured, the contents are aspirated, 20% saline or 85% alcohol is instilled into the cyst, and then the cyst is reaspirated after a 5-minute dwell time that is required to kill the protoscolices; thus the term "PAIR" (puncture, aspiration, instillation, reaspiration). The patients also receive albendazole prior to the procedure and for 15 to 30 days afterwards. This has been reported to have a cure rate of >90% and a relapse rate of <5%. This was the approach that the patient chose for treatment of her liver lesion after the initial failure to cure her liver cyst surgically.

The third approach is to use long-term antiparasitic therapy, including albendazole. The cure rate for this approach is in the range of 70 to 80%, with a relapse rate as high as 25%.

The final approach is to do nothing, as these cysts often grow slowly and may calcify. Because this patient had cysts that appeared to grow rapidly, this final option was not realistic. However, in some individuals, cyst growth is quite slow and it may take as long as 10 years for a cyst to grow as much as 1 cm. Most patients with cysts of <7.5 cm in diameter are asymptomatic, though this depends largely on the location of the cyst.

4. *E. granulosus* is a canine tapeworm for which dogs, wolves, coyotes, hyenas, foxes, and other canids act as definitive hosts. In the natural life cycle of this parasite, an infected dog defecates eggs into an area where ungulates graze. An egg is ingested by an intermediate host (ungulates such as sheep, cows, goats, or pigs), and the ingested egg releases an oncosphere into the small intestine. That oncosphere migrates primarily to the liver or the lung, where it forms hydatid cysts. Protoscolices, the infective form of the parasite for the definitive canid host, develop within the cyst. The life cycle is completed when canids ingest cysts containing protoscolices in infected organs from an intermediate host. This may occur when uncooked viscera are fed to dogs by humans. The protoscolices evaginate and attach to the canid intestinal wall and develop into adult worms that produce eggs, which continues the life cycle.

Humans are an accidental intermediate host. Humans may ingest eggs directly from dogs; the eggs can adhere to fur and can be found on paws and muzzles. Alternatively, humans may ingest eggs from food or water that has been contaminated with dog feces. The natural history of the disease has been closely studied in humans. In most humans, cysts grow slowly or may not grow at all. It takes several months before protoscolices are produced within the cyst, but they may not be produced at all. Such cysts are sterile.

The clinical disease associated with this parasite is based on the size and the location of the cyst. Therefore, the natural history of disease in humans is based on the rate at which the cysts expand. In 90% of patients, the cysts expand at a rate of <1 cm/year.

Because a cyst needs to be ~10 cm to cause symptoms (unless it is in an uncommon site such as the brain), it may be years before infected patients become symptomatic. In 10% of patients, cyst expansion can be quite rapid—as rapid as 15 cm in 1 year. It has been observed that most cysts are seen in patients between 20 and 40 years of age, which is the age of the patient presented here. Some data indicate that the rate of cyst expansion is higher in children and adolescents and then declines with age. This may explain why the disease is most prevalent in early adulthood. The abdominal discomfort that the patient experienced was likely due to the mass effect of the expanding abdominal cysts.

The patient lived in a part of the world with a high and increasing incidence of disease. She also had a pet dog that could have been the source of her infection.

5. When the Soviet Union was dissolved, there was a loss of funding for both veterinary public health and medical services in the former Soviet republics, including Uzbekistan. An important strategy for controlling *Echinococcus* in areas of high endemicity is to give prophylactic anti-echinoccocal agents to domestic dogs. This activity was likely cut back due to lack of funding. A second explanation was the change from a planned to a free market economy. It is likely that animal processing underwent changes that included the opening of smaller operations where strict attention was not paid to disposal of offal that contained fertile hydatid cysts. Feral or even domesticated dogs may have eaten this offal and become infected, thereby serving as a source of infection of humans.

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CASE 74

The patient was a man in his early forties with Crohn's disease. Approximately a week prior to his current admission he was discharged from the hospital after admission for a flare of his Crohn's disease. He was discharged on his standard anti-inflammatory regimen of prednisone, 6-mercaptopurine, and mesalamine, but another anti-inflammatory agent, cyclosporine, was added along with oral trimethoprim-sulfamethoxazole for infection prophylaxis. He reported that his flare had improved slowly over the previous week but that he still had abdominal pain, bloody diarrhea, anorexia, and dizziness on standing. He denied fever, nausea, vomiting, shortness of breath, chest pain, and mental status changes.

Physical examination showed a temperature of 36.1°C, blood pressure of 104/63 mm Hg, pulse of 100 beats/min, and normal respiratory rate and oxygen saturation. Abdominal exam showed mild tenderness to palpation of the left and right lower quadrants. He was unable to tolerate sitting because of abdominal discomfort. His laboratory results were significant for an absolute leukocyte count of 1,100/µl and neutrophils of only 700 /µl, a hemoglobin level of 6.6 g/dl (down from 14 g/dl 2 weeks previously and 8.8 g/dl 4 days ago), and a platelet count of 193,000/µl. His cyclosporine, 6-mercaptopurine, and trimethoprim-sulfamethoxazole were stopped, but his prednisone and mesalamine were continued. He was transfused with 2 units of packed red blood cells. His white blood cell (WBC) count and hemoglobin slowly recovered over the next 9 days. On the 9th hospital day his WBC count was 2,000/µl. Hemoglobin was 10 g/dl, but his platelet count had dropped to 87,000/µl. On that day the surgery service was consulted because the patient had rapidly developing skin changes and left leg edema (Fig. 74.1).

On physical examination of the left leg, crepitance and extensive edema were noted. The patient was emergently taken to the operating room (OR), and exploration of the left calf showed extensive necrosis. The patient had a left above-the-knee amputation. A Gram stain of necrotic tissue is shown in Fig. 74.2. The same



Figure 74.1 Patient's left leg prior to amputation.

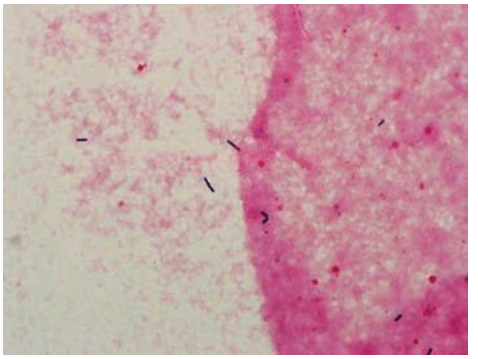


Figure 74.2 Gram stain from a biopsy from the leg of the patient.

organism seen in the Gram stain was also present in two blood cultures obtained prior to the initiation of antimicrobials. The patient was begun on penicillin G. He returned to the OR the next day, where further debridement of the left thigh and now the lower right leg was performed. Healthy muscle was seen under the skin of the left thigh, so further debridement was not needed, but muscle debridement of the right calf was needed. The patient was treated with antimicrobials and hyperbaric oxygen over the next 2 days. He returned to the OR at that time, where exploration of both legs showed healthy muscle. By this time his white blood cell count had peaked at 20,000/μl. Over the next 11 days his wounds were treated with a wound vacuum and antimicrobials. His WBC count slowly trended down to 8,800/μl by discharge, which occurred on the 24th hospital day. He was discharged to a rehabilitation facility to finish his intravenous antimicrobials (4 more days).

1. What is Crohn's disease? Explain why it is treated with immunosuppressive therapy. Why were trimethoprim-sulfamethoxazole, 6-mercaptopurine, and cyclosporine discontinued but prednisone and mesalamine not?
2. What was the infection that he developed? What organism caused it? How did he become infected? The specific species of organism infecting this patient is associated with what type of malignancy? What might explain this predisposition? Do you think that the leukopenia contributed to the development of infection? Explain why.
3. What virulence factor is thought to be primarily responsible for the tissue damage that occurred? Why are no WBCs observed in the Gram stain from the patient's biopsy? Why was it necessary to amputate his leg?
4. Why were hyperbaric oxygen and a wound vacuum used in the treatment of this patient?
5. Why was penicillin chosen for the treatment of this very severe infection?



CASE DISCUSSION

1. Crohn's disease is a chronic inflammatory disease of the ileum and the colon. Normal hosts develop immune tolerance to the organisms that compose the gut microbiome. In Crohn's disease, there is dysregulation of immune homeostasis in the intestinal tract resulting in increased inflammation. This inflammation is mediated by a variety of proinflammatory cytokines, including tumor necrosis factor-α and interleukins-1β, -6, and -12. Inflammation likely targets microbial antigens of the microbiome that cross the intestinal mucosa in part due to disruption of tight junctions between cells of the epithelial gut mucosa and increased intestinal permeability, two well-recognized features of this disease. This inflammatory response is responsible for the pathology associated with this disease, which includes mucosal erosion and ulceration, granuloma formation, and in advanced disease, fistula formation. Common clinical features include bloody diarrhea and abdominal pain, both of which were present in this patient. Since dysregulation of the inflammatory response is the key feature of Crohn's disease, suppression of this inflammatory response is a key therapeutic strategy. This patient had a "flare" of his disease, which means worsening intestinal inflammation often manifesting itself as bloody diarrhea and abdominal pain.

A typical drug regimen for treating this patient's disease included the immunosuppressive agents prednisone, 6-mercaptopurine, and mesalamine. On his previous admission, cyclosporine, a potent T-cell inhibitor used for immunosuppression in organ transplants, was added to his regimen. In addition, trimethoprim-sulfamethoxazole was added for antibacterial prophylaxis. One of the side effects of 6-mercaptopurine, cyclosporine, and trimethoprim-sulfamethoxazole is bone marrow suppression. When he was readmitted, he had evidence of pancytopenia: low absolute neutrophil count, decreased hematocrit, and falling platelets. The decision was made to discontinue these three bone marrow-suppressive agents. Because he still had symptoms of his Crohn's disease, it was necessary to continue some level of anti-inflammatory therapy. The anti-inflammatory agents that were chosen, prednisone and mesalamine, were continued because the physicians caring for the patient judged them to have less bone marrow toxicity.

2. The rapid onset of swelling, crepitance, and hemorrhagic bullous lesion are all characteristics of gas gangrene. Gas gangrene often brings to mind the image of a traumatic dirty wound. Vascular compromise in the traumatized tissue results in loss of blood supply, creating anaerobic conditions. Environmental anaerobic organisms introduced into compromised tissue during a traumatic event find ideal conditions for growth. *Clostridium* spp. are particularly pathogenic in this setting because of the histotoxic virulence factors that they produce and their rapid rates of growth (see answer 3 for further details). A characteristic of gas gangrene is the presence of gas in tissue, which was noted on physical exam as crepitance, meaning in this clinical setting "gas in tissue." Imaging studies also showed the presence of gas in tissue.

However, this patient did not have preceding trauma, so what was the likely sequence of events that resulted in his development of gas gangrene? This patient likely had a syndrome referred to as "atraumatic" gas gangrene. Recent literature suggests that atraumatic gas gangrene is actually more common in the United States than the traumatic kind. In atraumatic gas gangrene, there is no obvious source of infection but gas gangrene is found, typically in an extremity. The most common etiologic agent associated with this syndrome is *Clostridium septicum*, the organism that was recovered from this patient. The development of atraumatic gas gangrene is the result of a bacteremia with seeding of tissue. *C. septicum* bacteremia is most commonly seen in patients who have an intestinal malignancy. This malignancy causes necrosis in the intestinal mucosa, which serves as a portal of entry for the organism into the bloodstream. Why *C. septicum* shows this association much more frequently than other clostridial species is unknown. *C. septicum* bacteremia is unusual in individuals with inflammatory bowel disease even though there is ulceration of the intestinal mucosa creating a potential portal of entry for the organism. However, the high level of inflammation seen in this patient population likely ensures immune clearance of these organisms. It should be noted that this patient was not a typical Crohn's disease patient in that he was leukopenic. His leukopenia may have compromised his ability to clear bacteria that penetrated from the gut to the lamina propria, resulting in the observed bacteremia complicated by gas gangrene.

3. The major virulence factor of all clostridia that cause gas gangrene is pore-forming toxins that induce membrane lysis of a wide variety of cell types, including leuckocytes. The toxin produced by *C. septicum* is called α-toxin. These toxins are histotoxic and are responsible for the massive myonecrosis that is characteristic of this disease process. They also cause leukostasis in blood vessels in areas of tissue destruction, resulting in localized ischemia and further cell death.

Figure 74.2 is a characteristic Gram stain of a biopsy specimen from a patient with gas gangrene. Note that there are large, Gram-positive rods characteristic of *Clostridium* spp. Importantly, there are essentially no leukocytes present in the specimen. There are two possible explanations. First, the absence of leukocytes in the smear was due to the patient's leukopenia; however, he did have a low number of leukocytes. It would be expected that those few leukocytes would be recruited to the area of infection. More likely, the α-toxin had locally destroyed the WBCs that might have responded to the infection. Typically Gram stains of tissue from patients with clostridial gas gangrene do not have leukocytes even if they have a normal or elevated peripheral WBC count.

Amputation to treat gas gangrene infections is a time-honored therapy that was widely used in the American Civil War and the First World War following soil-contaminated wounds caused by high-caliber bullets and artillery shrapnel. With improving wound and surgical care and the advent of the use of antibiotics, gas gangrene was less common during the Second World War. Gas gangrene is now unusual following combat injury because of rapid battlefield evacuation facilitated by helicopters and evolving wound and

surgical care. Unfortunately for this patient, extensive debridement or amputation is common in patients with atraumatic gas gangrene. The reason this is necessary is that clostridia are among the fastest-growing human pathogens. Additionally, α-toxin produced by the organism is histotoxic, causing rapid cell death and massive tissue necrosis. The organism also produces additional toxins including DNase, hyaluronidase, protease, hemolysin (septicolysin), and neuraminidase, which allows the organism to rapidly spread through necrotic tissue. Because of this, removal of all necrotic tissue is the only truly effective therapy, with antimicrobials and other wound care strategies being adjunctive.

4. Hyperbaric oxygen has been used to treat gas gangrene and other anaerobic infections for many years despite the fact that there are no randomized, controlled trials that show its benefit. The theory behind this therapy is that breathing 100% oxygen under high pressure increases dissolved oxygen levels in plasma, which results in higher oxygen perfusion in tissue, enhancing the activity of phagocytic cells and causing direct oxygen toxicity to anaerobic bacteria.

A recent addition to the armamentarium for treatment of wound infection is the wound vacuum. The "wound vac" works by applying negative pressure to the wound bed. The result is that it draws off fluid, reducing localized edema, and removes substances thought to be inhibitory to wound healing, although the exact nature of these substances is not well defined. As with hyperbaric oxygen, the wound vacuum has not been evaluated in randomized, controlled trials although clinical experience has been described extensively in the literature.

5. Penicillin G was first widely used to treat gas gangrene during the Second World War and was recognized as an important reason for the reduced rates of gas gangrene during that conflict. Despite almost 75 years of use in the treatment of clostridial infections, drug resistance in *Clostridium perfringens* and *C. septicum*, the two most common agents of gas gangrene, has not been described in human infections. Animal studies suggest that antimicrobials that inhibit protein synthesis and thus toxin production, such as clindamycin and tetracycline, may be superior in the treatment of gas gangrene. However, because of the broad clinical experience with penicillin G and the lack of drug resistance, it remains the drug of choice for the antimicrobial treatment of gas gangrene. As with surgical treatments, there are no randomized, controlled trials showing the superior efficacy of penicillin for the treatment of gas gangrene.

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GLOSSARY

abscess A cavity of liquefactive necrosis within solid tissue as a result of a localized infection; this can be the result of an acute or a chronic process.

abrasion A shallow denuding of skin, confined to the epidermis.

accidental host A host that harbors a parasite but is not the normal host for that parasite species.

achlorhydria Absence of gastric acid production.

acid-fast Pertaining to a group of organisms that resist decolorization by acid-alcohol; typically associated with *Mycobacterium* and *Nocardia* spp.

acidosis Pathological condition in which the arterial pH drops below the normal value.

acute The initial phase or most severe phase of a disease.

acute specimen A specimen that is collected from the patient during the initial or acute illness.

adenocarcinoma Malignancy of glandular epithelium.

adenopathy An enlargement of a lymph node or lymph nodes in response to some stimulus such as inflammation or infection; can occur singly or in multiple nodes; also referred to as lymphadenopathy.

adhesins Proteins in the bacterial cell surface or cellular appendages which allow bacteria to adhere to a surface or to other cells.

adnexa An appendage to an organ or structure.

adrenalitis Inflammation of the adrenal gland.

aerobic Pertaining to a microorganism that must grow in the presence of oxygen (a "strict aerobe") or may grow in the presence of oxygen (a "facultative aerobe").

aerosol A collection of solid or liquid particles suspended in a gas, such as a liquid that is dispersed in fine droplets through the air.

afebrile Relating to the absence of fever.

agglutination The interaction between a particulate antigen and antibodies specific for that particular antigen; the antigen-antibody complex leads to the aggregation or clumping of the antigen-containing material.

AIDS Acquired immunodeficiency syndrome, a disease caused by one of the HIV (human immunodeficiency virus) retroviruses; the condition is characterized by the depletion of lymphocytes with subsequent failure of the immune system.

airborne precautions Protective measures to prevent the transmission of airborne disease (such as tuberculosis), such as requiring a respirator mask for providers and a negative-pressure room for patients.

allele One of a series of two or more different genes that occupy the same location on a homologous chromosome.

allogenic Not genetically identical.

allograft Tissue transplanted from a non-genetically identical donor.

alopecia Hair loss.

alpha-hemolytic Pertaining to a reaction seen typically on agar medium containing sheep red blood cells, in which the area surrounding a colony has a green hue; the most medically important alpha-hemolytic organism is *Streptococcus pneumoniae*.

alveolar macrophages Macrophages which are found in the alveolus of the lung; part of the innate immune system.

alveolus An air sac in the lung consisting of a single layer of cells surrounded by a network of capillaries also consisting of a single cell layer; gas exchange occurs here.

amastigote A stage of the life cycle for protozoan species within the genus *Leishmania*; the nonflagellated amastigotes survive within macrophages of the host.

ameboma A nodular inflammatory lesion, usually in the wall of the colon, that may develop in chronic amebiasis.

amplification assays Tests for the presence of bacteria or viruses which rely on PCR.

anadromous fish Fish species which live in the sea but return to freshwater to spawn.

anaerobic Pertaining to a microorganism that must grow in the absence of oxygen (a "strict anaerobe") or may grow in the absence of oxygen (a "facultative anaerobe").

analgesics Medications used to decrease pain, such as ibuprofen, morphine, and acetaminophen.

anastomosis A surgical connection between two hollow structures.

anemia A condition in which the number of functional red blood cells is decreased; this is often associated with symptoms such as pallor, fatigue, shortness of breath, and lethargy.

anergy A lack of the ability of the immune system to respond with a delayed-type hypersensitivity reaction to commonly and previously encountered antigens such as mumps and *Candida*; often seen in patients with AIDS.

angioinvasive Characteristic of an infection or neoplasm which grows into the surrounding vasculature.

anicteric Without jaundice.

annular rash A circular rash.

anorexia Decreased appetite.

anterior cervical lymphadenopathy Enlarged lymph nodes in the front of the neck.

anthropophilic A parasite or fungus with a preference for humans.

antibacterials Chemicals which kill bacteria or inhibit bacterial growth.

antigen A substance that is capable of triggering an immune response.

antigenic drift The gradual change in a microorganism's genetic composition after successive generations of reproduction.

antigenic shift A drastic change in a microorganism's genetic composition within one reproductive cycle; typically associated with influenza A viruses in which a new strain is unrecognizable to a previously immune host.

antigenic variation A sudden or gradual change in the expression of an antigen by a microbe; the variation may result in a drastic change in the pathogenicity of the organism or in the host's ability to mount an immune response; antigenic shift and antigenic drift are types of antigenic variation.

antimalarials Chemicals which kill the malaria parasite or inhibit its growth.

antimicrobial susceptibility The degree to which a bacterium can be killed or inhibited by a particular antimicrobial.

antiphagocytic Inhibiting the ability of the phagocyte to ingest bacteria, foreign materials, or other cellular debris.

antipyretics Fever-reducing agents such as aspirin, acetaminophen, and ibuprofen.

antiretroviral therapy Drugs which inhibit HIV.

aortic valve The structure which opens during systole as blood is ejected from the left ventricle into the aorta and closes during diastole as the left ventricular pressure decreases.

apical Near the apex of a structure.

aplastic anemia Decrease in the numbers of all elements in the blood due to the death of their precursor cells in the bone marrow, where the cells usually mature; often associated with specific chemicals or drugs that are toxic to these cells.

apnea Absence of normal respirations.

apoptosis Programmed cell death.

appendicitis Inflammation of the vermiform appendix of the cecum.

areflexia Abnormal absence of involuntary deep tendon reflexes.

arthralgia Severe joint pain, usually characterized as noninflammatory.

arthritis Inflammation or infection of a joint, leading to decreased and painful mobility of the affected joint.

arthroconidium An asexual spore that is budded from the body of some species of fungi.

ascariasis Disease caused by intestinal nematodes of the genus *Ascaris*, usually the giant roundworm species *Ascaris lumbricoides*.

ASCUS Atypical squamous cells of unknown significance; an intermediate finding on cervical cytology.

aseptate Describes fungi which do not have septa dividing their hyphae.

aseptic meningitis Low number of white cells in cerebrospinal fluid, predominantly lymphocytes, most frequently caused by infection by viruses or fungi. Most causes of acute bacterial meningitis, in contrast, have high numbers of white cells and a predominance of neutrophils.

aspiration Removal of fluid by suction.

asplenia Absence of the spleen, either congenitally (at birth) or later, often seen in persons with long-standing sickling disease. This condition makes the individual susceptible to infections by certain bacteria and parasites.

asterixis An involuntary jerking motion that occurs with various toxic or metabolic encephalopathies, including hepatitic encephalopathy and uremia; most easily demonstrated when the patient is asked to extend his or her arms with the hands pointed up and the fingers extended.

asymptomatic Without outward clinical manifestations of disease.

ataxia The inability to coordinate muscle activity during voluntary movements of the head, limbs, or trunk; often associated with cerebellar dysfunction.

atherosclerosis The stiffening and narrowing of arteries caused by deposition of cholesterol and associated inflammation.

atrophy A process characterized by wasting of specific tissues, organs, or the entire body that can result from a variety of causes.

attenuation Decreased virulence of a pathogen through either natural or experimental means.

atypical lymphocytes White blood cells with an abnormal reactive appearance in response to an antigen.

auramine-rhodamine A stain that fluoresces under a UV microscope; used for visualizing acid-fast organisms.

auscultation A method based on sounds or sound changes, used during a physical examination to gather data on internal organs such as the heart, lungs, and liver; the most common method involves the use of a stethoscope.

autoantibodies Antibodies produced by the immune system which attack the individual's own proteins in specific cells, tissues, or organs.

autochthonous Refers to a process that originated in the place where it is found.

autoimmune Describes a disease caused by an abnormal activation or modulation of the body's immune system toward its own tissues.

autoinfection Process whereby the host is reinfected by the same organism after the organism has undergone a replication cycle.

autosomal recessive Describes a genetic disease, carried on somatic chromosomes, which requires two affected alleles to manifest as disease.

avirulent Describes a microorganism with limited pathogenic potential.

axillus Armpit; the area between the upper arm and chest wall where the two join.

bacteremia The presence of viable bacteria in the bloodstream; may be a transient phenomenon associated with dental care or may be due to a bacterial infection in which bacteria have entered the bloodstream.

bacteriocins Proteins produced by certain bacteria that have a lethal effect on related bacteria; although the effect has a more narrow range than the effect of antibiotics, it is more potent.

bacteriuria The presence of bacteria in the urine.

beta-D-glucans Polysaccharides of glucose linked by beta-glycosidic bonds, found in most fungal cell walls.

beta-hemolytic Characteristic seen on blood-containing medium, where bacteria cause complete hemolysis of red blood cells.

beta-lactamase An enzyme which can degrade beta-lactam, the active component of penicillin.

bibasilar Pertaining to the bases of both lungs.

bilateral Pertaining to both sides of a symmetrically shaped tissue, organ, or the entire body; for example, the right and left lungs are bilateral organs.

biliary tree System of ducts through which bile is transported.

biofilm Organisms which adhere to each other on a surface, often in a protein matrix.

biopsy A procedure that involves the removal of specific tissues from patients for the purpose of diagnosis.

bioterrorism Intentional release or spread of biologic agents, including bacteria, viruses, and toxins.

blanching A temporary lightening of the skin or mucous membranes after direct pressure is applied.

blepharospasm Abnormal contraction of the eyelid.

blood-borne pathogen A pathogen which is spread by contact with blood.

bone marrow biopsy A diagnostic technique which obtains a biopsy of bone marrow along with an aspirate of marrow, generally from the iliac crest.

brackish Describes a mixture of freshwater and salt water.

bronchiectasis Irreversible abnormal dilation of the bronchial tree.

bronchiolitis Inflammation of the bronchioles (conducting airways of less than 1 mm).

bronchitis Inflammation or infection of the airways.

bronchoalveolar lavage (BAL) The instillation of saline into the airways of the lungs so that samples can be removed and the washings (fluids) can be analyzed for malignancy, inflammation, or infection; also done during bronchoscopy.

bronchoscopy The use of a flexible hollow tube to look directly at the trachea, bronchi, and larger airways in the lungs; it is also possible to obtain samples (biopsies, fluids, brushings) through this device.

bronchospasm Episodic constriction of smooth muscles lining the bronchi in response to some kind of irritant or stimulus.

Brudzinski sign A sign of meningeal irritation, elicited when flexion of the neck causes involuntary flexion of the hips.

budding A form of asexual reproduction in fungi.

bulla (pl. bullae) Fluid-filled blister at the junction of the dermis and epidermis, larger in size than a vesicle.

cachexia General weight loss or wasting due to a disease process or emotional imbalance.

calcification Focal area of increased deposition of calcium compounds.

calcofluor white A fluorescent stain which binds to chitin, a component of the fungal cell wall.

candidemia Bloodstream infection with *Candida* species.

capsule A polysaccharide (except for *Bacillus anthracis*, in which it is poly-D-glutamic acid) covering of certain bacteria and yeasts that makes them resistant to phagocytosis by white blood cells; often utilized in vaccines as the antigen.

carbapenemase Beta-lactamase with the ability to hydrolyze penicillin, cephalosporin, and carbapenem antibiotics.

carcinoma Malignancy of epithelial tissue.

cardiopulmonary arrest Cessation of normal blood flow due to the inability of the heart to contract.

caseating granuloma A collection of immune cells with a "cheese-like" necrotic core, characteristic of certain infectious and autoimmune diseases.

catalase Enzyme that catalyzes the production of water and oxygen from hydrogen peroxide.

catarrhal Describing inflammation of the mucous membranes in the nose and throat.

catheter A thin tube introduced to a body cavity or blood vessel. **Permanent and removable intravascular catheters** can be placed in peripheral or central vessels for delivery of fluid, medication, or nutrition and for the removal of wastes through hemodialysis, as well as monitoring of hemodynamic status. **Urinary catheters** can be introduced through the urethra or through the skin overlying the bladder for drainage of urine. **Ventriculoperitoneal** catheters can be placed in the ventricles of the brain to drain into the peritoneal cavity for removal of excess fluid in hydrocephalus.

cavitary Describing destruction of normal lung tissue and replacement with a cavity, caused by particular organisms or disease processes.

CD4-positive ($CD4^+$) cells Subset of T lymphocytes that are characterized by the presence of CD4 receptors on their cell membrane surfaces; they assist in turning on the immune response by activating other T and B lymphocytes; also called T-helper cells. Less than 200 cells per μl is seen in adult AIDS patients.

cDNA (complementary DNA) DNA synthesized from a messenger RNA (mRNA) template.

cell-mediated immunity An immune response that is activated and carried out by T lymphocytes; the counterpart to humoral immunity.

cellulitis Inflammation or infection of the skin and tissues beneath the skin.

cerebellar Pertaining to the cerebellum (the portion of the brain concerning the coordination of complex movements and balance).

cerebellar ataxia A loss of motor coordination as a result of damage to the cerebellum or its pathways; may be manifested as a loss of balance in the entire body when moving or by unsteady movement of the arms or legs.

cerebral malaria Neurologic sequela of infection with *Plasmodium falciparum*.

cerebritis A focal infection or inflammation of the brain tissue.

cerebrospinal fluid Fluid surrounding the brain and spinal cord.

cervical Pertaining to the neck or a necklike portion of an organ.

cervicitis Inflammation of the mucous membranes or deeper structures of the cervix.

Cesarean section A nonvaginal delivery whereby the fetus is surgically removed via an incision through the lower abdominal wall and uterus.

cestodes The class of helminths that includes the tapeworms (e.g., *Taenia* sp.); these flatworms have a rounded head (scolex) followed by a chain of multiple segments (proglottids).

chancre The primary lesion of syphilis; typically dull red, hard, and insensitive with a center that erodes and ulcerates, then heals slowly over a period of 4 to 6 weeks.

chemoprophylaxis The use of chemicals such as antibiotics to prevent the occurrence of disease.

chemotaxis Migration of an organism or cell in response to a chemical gradient.

chemotherapy The use of drugs or chemical substances to treat disease.

chimeric Combining traits from two different species to create an organism with unique characteristics; in the context of bioterrorism, adding new virulence factors to a microorganism. This term is also used in the context of genetically engineered antibodies in which different parts of the antibody come from different animals.

chitin A pliant, strong, glucose-derived polymer which composes the fungal cell wall.

cholangitis Inflammation or infection of the bile ducts.

cholecystectomy Surgical removal of the gall bladder.

cholecystitis Inflammation of the gall bladder.

chorioamnionitis Inflammation or infection of the chorion, amnion, amniotic fluid, and often the placental villi and decidua as well.

chorioretinitis Inflammation or infection of the light-detecting layer (retina) and the underlying vascular tissue (choroid) beneath it in the back of the eye, which can lead to progressive impairment of vision.

chronic obstructive pulmonary disease (COPD) A group of slowly progressive lung disorders that includes emphysema and chronic bronchitis; results in the marked decrease in airflow through the lungs with the main impact on the ability to exhale; the vast majority of cases are associated with cigarette smoke.

cilia Motile organelles found in the respiratory tract and fallopian tubes, which facilitate mucus clearance.

ciliostasis Pathological process, usually induced by viral infections, that causes changes in the cilia such that they do not beat.

cirrhosis Destruction of a tissue or organ with loss of normal structure which is replaced with scar tissue; common in association with alcoholism, where it involves the liver.

clinical care algorithms A series of standardized decision points based on previous test results or signs and symptoms which are designed to optimize efficiency of testing and treatment.

coagulopathy Abnormal platelet aggregation and/or clotting of the blood.

cofactor A non-protein chemical which is necessary, in addition to an enzyme, to catalyze a reaction.

cohort A designated group of individuals to be examined for a particular trait, exposure, or outcome.

coitus Sexual intercourse.

colitis Inflammation of the colon.

colon Portion of the gastrointestinal tract beginning at the cecum and continuing to the rectum; large intestines.

colonization The presence of one or more bacterial species inhabiting an organ, surface, or foreign body.

colonoscopy Introduction of a light and camera through the anus to examine and sometimes biopsy the luminal surface of the colon.

colposcopy Examination of the vulva, vagina, and uterine cervix with magnification.

commensal A relationship between two organisms whereby one benefits and the other neither benefits nor is harmed.

complement Component of the immune system that consists of a set of proteins in the serum; the proteins are activated in a cascade fashion and assist in the process of phagocytosis, direct microbe destruction, and phagocyte recruitment.

compliance The degree to which a patient follows the prescribed regimen of treatment.

computed tomography A series of computer-processed radiographs which construct "slices" of images through the body.

confirmatory test A diagnostic test that is conducted after a positive screening test to confirm the illness or trait; a good confirmatory test should have a low rate of false-positive results.

congenital Pertains to a condition developing at or before birth.

congenital syphilis An infection by *Treponema pallidum* which occurs in a fetus before birth or during birth.

conjugate vaccine Type of bacterial vaccine in which a portion of the bacterial capsule is attached to an inactive toxin or protein to enhance the immune response to the capsule antigen.

conjunctivitis Inflammation of the tissue protecting the front of the eye; often due to infection. Also called "pink eye."

conidia Nonmotile, asexual spores of fungi.

constipation Decrease in frequency or amount of bowel movements or decreased water content of stools.

contact dermatitis Inflammation of the skin caused by superficial contact with an allergen or chemical.

contact precautions Protective measures used to prevent the transmission of diseases spread by contact with skin, mucous membranes, or bodily fluids, which require that providers wear a gown and gloves and practice appropriate hand hygiene.

contaminant An organism identified from culture of a body fluid or wound which is thought to be present because of inadequate aseptic sampling and not because of pathogenic infection.

continuous bacteremia The persistent presence of bacteria in the circulation.

contrast enhancing Describes a structure or lesion which takes up oral or intravenous contrast dye, distinguishing it from surrounding tissues.

convalescence The period of time after a disease process has ended but before the return of optimal health.

convalescent specimen A specimen that is collected from the patient either in the late stages of an acute infectious process or when it has ended.

cornea The clear outer layer forming the front of the eye, continuous with the sclera and covering the iris and pupil.

coryza Acute inflammation or infection of the nasal membranes, leading to a thin watery discharge from the nose, as seen with the common cold.

costal Pertaining to the ribs.

costovertebral angle The area in the back where the last ribs join to their respective vertebrae.

crepitance The crackling sound (resembling the sound that occurs when rubbing hair between the fingers) that is heard in certain disease states.

Crohn's disease An immune-mediated inflammatory disease most commonly observed in the terminal ileum, but which can occur anywhere in the gastrointestinal tract from mouth to anus. Granulomatous inflammation is a hallmark of the disease.

cross-reactivity Binding of an antibody to an antigen other than the one against which it was developed.

croup The difficult, noisy respirations and hoarse cough that characterize laryngeal problems in children; this is often caused by parainfluenza virus.

cushingoid body habitus An increase in adipose tissue (fat) in certain areas of the body, legs, and trunk. Purplish stripes (striae), especially on the abdomen, are also associated with Cushing's disease.

cutaneous Of or pertaining to the skin.

cyanotic Blue in color, associated with decreased oxygen saturation.

cyst An abnormal membranous sac containing gas, fluid, or semisolid material. Also a dormant form of the protozoan life cycle that is capable of resisting destruction by heat or dehydration.

cysticercus Larva of *Taenia solium* that is present in the tissue of the hosts.

cystitis Inflammation or infection of the urinary bladder; also called urinary tract infection (UTI). Associated with symptoms including painful urination, increased urination, and/or malodorous urine.

cystocele A hernia of the bladder; the hernia usually protrudes into the vagina.

cystoscopy Introduction of a light and camera into the bladder for examination or treatment.

cytokine One of a diverse group of chemicals produced by various cell types that regulate the immune response.

cytologic Relating to the study of cytology (the anatomy, physiology, pathology, and chemistry of the cell).

cytolysin A substance or antibody that is capable of directly destroying a cell.

cytopathic Changes in intracellular structures due to disease or toxins, usually leading to the death of the cell.

cytotoxic Destructive or damaging to a cell.

debride To remove devitalized tissue and/or foreign material from a wound.

decolonization Elimination of a potentially harmful microorganism colonizing an individual, regardless of whether it is causing an infection.

decubitus The position of a patient when lying down; lateral decubitus specifically refers to the patient's lying on his or her side. A decubitus wound is a wound that occurs as a result of pressure on an area, such as the heels or buttocks, because of the way the patient is lying.

defensins Cysteine-rich peptides active against a wide range of microbial pathogens; produced by granulocytes and mucosal and epithelial cells.

defervescence The disappearance of fever.

definitive host A host that supports the adult, sexually reproducing form of a parasite.

dehiscence Rupture of a surgically closed wound.

dehydration The loss of water resulting in the increased osmolarity of the body fluids.

dematiaceous fungi A group of fungi which have dark hyphae, typically brown or black, with irregular branching.

dementia A state of decreased mental capacity and orientation without a loss in consciousness; can be caused by toxins or by a gradual loss of normal brain tissue.

dermatome The area of skin that is served by one sensory spinal nerve.

dermatophyte One of the fungal organisms capable of infecting human skin, hair, or nails; includes the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*.

desaturation The state of having unoccupied binding sites or the process of removing ligands from the binding sites; with hemoglobin, refers to a decrease in the amount of bound oxygen.

diaphragmatic hernia An acquired or congenital protrusion of abdominal contents through the diaphragm and into the thorax.

diarrhea Increase in frequency or amount of bowel movements, or increased water content of stools.

differential medium A type of medium used to determine whether an organism demonstrates a specific characteristic.

dimorphic A characteristic of certain fungi to exist in two distinct forms, such as yeast and mold.

direct fluorescent-antibody assay Detection of an antigen by a fluorescently tagged antibody directly, rather than by an untagged primary antibody and subsequently by a fluorescent-tagged secondary antibody.

directly observed therapy A type of therapy in which a responsible individual directly observes the patient taking the prescribed medications; utilized as a way to improve patient compliance.

disequilibrium Unsteady balance.

disk diffusion An assay which measures the distance of clearance around a disk impregnated with an antimicrobial solution placed on a lawn of bacteria.

disseminated intravascular coagulation (DIC) The depletion of clotting elements in the blood, caused by many disease processes. Diffuse, severe hemorrhaging can occur; without treatment, it is often fatal.

dissemination Spread throughout the body.

diverticulitis Inflammation of intestinal diverticula.

diverticulosis Herniation of the intestinal mucosa through weaknesses in the muscle of the bowel, most commonly in the sigmoid colon.

DNA gyrase An enzyme that introduces a negative supercoil into DNA to relieve strain as the DNA is being unwound by helicase.

DNA polymerase A cellular or viral enzyme which synthesizes DNA from nucleotides.

dorsal Referring to the back or posterior aspect of a tissue or organ.

dorsal root ganglion Group of nerve cell bodies outside the spinal cord that convey sensory impulses to the brain.

double vision Also called diplopia, the perception of seeing a single object as two separate images.

droplet precautions Protective measures used to prevent the transmission of diseases spread by droplets, requiring providers to wear a surgical mask.

DTP vaccine Whole-cell vaccine which contains toxoids to diphtheria, tetanus, and pertussis.

DTaP vaccine Acellular vaccine which contains toxoids to diphtheria, tetanus, and pertussis.

dysentery A condition marked by frequent watery stools usually containing blood and mucus; often accompanied by pain, fever, and dehydration; typically the result of amebic, bacillary, helminthic, or viral infections.

dyspepsia Gastric indigestion (upset stomach) due to alterations of gastric function that are caused by various disorders of the stomach.

dysplasia Abnormal cell growth and division which is not malignant but which may evolve into malignancy.

dyspnea The sensation of having difficulty in breathing; also called shortness of breath. Dyspnea on exertion is shortness of breath which begins during strenuous movement.

dystonia Abnormal, involuntary muscle contractions that cause abnormal movements or postures.

dysuria Difficulty or pain on urination.

ecchymosis Purplish lesions of the skin and mucous membranes due to hemorrhage beneath the tissues; greater than 1 cm in size, lesions may be flat or raised.

echinocandins Antifungal drugs which inhibit synthesis of the cell wall polysaccharide glucan.

echocardiogram A real-time, noninvasive study that utilizes ultrasound to evaluate the heart tissue, heart valves, great vessels, and corresponding blood flow.

ectoparasite A parasite which lives on the outside of its host.

ectopic pregnancy Type of pregnancy in which the embryo implants outside the uterus; this usually occurs in the fallopian tube.

eczema An itchy, scaly, blistery, or raised rash often seen in children and associated with irritation of the skin.

edema An accumulation of large amounts of extracellular watery fluid in tissues throughout the body.

effusion The presence of excess fluid in the tissues or a cavity; for example, referred to as pleural effusion when excess fluid is found in the pleural cavity around the lungs, usually as a result of inflammation, infection, or malignancy.

elastase A protease enzyme that breaks down elastin, a component of connective tissue.

electromyography (EMG) Test used to represent electrical currents associated with muscles; used in the diagnosis of neuromuscular diseases.

embolism An object in the bloodstream that becomes dislodged and carried until becoming trapped in a different vessel; may be a thrombus, immune complex of microorganisms, air, foreign object, fat, or amniotic fluid; leads to partial or complete occlusion of the blood vessel.

emesis Vomiting.

empiric Type of therapy that is employed when the causative agent or the antibiotic susceptibility of the organism is not definitively known.

empyema The collection of purulent material in the pleural space.

enanthem A mucous membrane eruption; typically occurs in relation to the skin eruptions that are symptoms of acute viral or coccal disease (*see* exanthem).

encephalitis Inflammation of the brain.

encephalopathic Referring to the pathological condition of encephalitis.

endemic Describes the presence of a disease that persists in a community or group of people.

endocarditis Inflammation or infection of the tissue lining the inside of the heart; usually involves the heart valves.

endocervical swab Collection of cells from the endocervical canal for cytological, microbiologic, or molecular genetic testing.

endocytosis The process of internalizing external substances by fusion with the plasma membrane and formation of an intracellular vesicle; is specifically considered phagocytosis if the internalized substance is solid.

endogenous A property of substances which originate from within an organism.

endometritis Inflammation or infection of the lining of the uterus.

endophthalmitis Inflammation of the aqueous and/or vitreous humor of the eye.

endoscopy Procedure involving the passing of a flexible hollow tube into the esophagus or rectum for the purpose of visualizing portions of the gastrointestinal tract; also useful for obtaining diagnostic samples.

endothelial Referring to the cells lining blood vessels.

endotoxin A lipopolysaccharide on the outer membrane of Gram-negative bacteria; has many possible biological effects on the infected host, including activation of the clotting cascade, shock, or death.

endotracheal tube A tube introduced through the mouth into the trachea to deliver oxygen and to clear carbon dioxide in a patient who cannot maintain his own airway.

endovascular Within the lumen of the blood vessels.

enteric Relating to the intestine.

enterocytes The absorptive epithelial cells that line the lumen of the intestine.

enterotoxin Exotoxin that causes fluid secretion in the gut.

enzootic Referring to a temporal pattern of disease occurrence in an animal population that is marked by predictable regularity with little change over time; for example, the pattern of hantavirus infection in deer mice.

enzyme immunoassay (EIA) A diagnostic test in which a specific antibody is linked to an enzyme; in a positive test, the antibody is attached to the antigen being tested for with a subsequent reaction by the linked enzyme; the enzyme reaction leads to a noticeable change, such as a change in color.

eosinophilia Increased number of eosinophils (a type of leukocyte) in the blood, often associated with parasitic infections.

epidemic An unexpectedly large number of cases of a disease or illness in a community.

epididymitis Inflammation or infection of the epididymis.

epigastric Relating to the area of the abdomen that lies between the margins of the ribs.

epiglottitis Inflammation or infection of the flexible flap of tissue that covers the larynx during swallowing.

episome An extrachromosomal piece of DNA that is capable of being replicated independently of the host chromosome and integrated within the host DNA.

epithelioid Describes a macrophage which resembles an epithelial cell.

epitope The portion of an antigen recognized by an antibody.

epitrochlear The region surrounding the medial trochlea of the ulna.

erythema Reddening, usually of the skin or mucous membranes.

erythema infectiosum A mild facial rash in children that has a "slapped cheek" appearance; usually caused by parvovirus B19.

erythema migrans A circular red rash that has a "bull's-eye" appearance due to the repeating red rings within an expanding outer ring; forms at the site of a tick bite and is indicative of Lyme disease.

erythematous Relating to erythema.

erythrocyte sedimentation rate (ESR) The rate at which red blood cells settle to the bottom of a test tube. A marker of systemic inflammation.

esophagitis Inflammation of the esophagus.

esophagogastroduodenoscopy (EGD) Introduction of a light and camera through the mouth to examine and sometimes biopsy the luminal surface of the esophagus, stomach, and duodenum.

estuary A body of brackish water where freshwater from rivers or streams meets the sea.

ethmoid sinus Air-filled cavity in the ethmoid bone located below the orbit of the eye and beside the nose.

ethmoidectomy Excision of the cells of the ethmoid sinus to create a single large cavity.

etiology The cause of a disease or process.

exacerbation An increase in the symptoms of a disease.

exanthem A skin rash that occurs as a symptom of acute viral or bacterial infection (*see* enanthem).

excoriate To physically scratch or remove the skin.

exocytosis A process whereby intracellular vesicles fuse with the plasma membrane in order to empty their contents into the extracellular space.

exogenous Pertaining to substances which originate from outside an organism.

exotoxins Proteins secreted by bacteria that have toxic effects on mammalian cells.

expectoration Ejection of secretions from the lungs or throat by coughing.

extensor surface The surface of a joint involved in extension or straightening of a limb.

external genitalia Outwardly visible genitalia, including the penis and scrotum in males and the vulva and vaginal introitus in females.

external otitis Inflammation of the external auditory canal and/or auricle.

extradermatomal Not confined to one dermatome.

extradural Referring to the outer side of the dura mater.

extraluminal air Air outside of the confines of the intestinal wall, a sign of bowel perforation.

extramedullary cranial ganglion Outside the medulla oblongata (brain stem), in reference to the cranial nerves.

extraparenchymal Outside of the parenchyma.

exudate Fluid resulting from inflammation or infection; contains an increased number of cells and an increased amount of protein and other cellular debris.

facultative Refers to an organism which can readily grow under either aerobic or anaerobic conditions.

false negative A test result of negative when the true result is positive.

false positive A test result of positive when the true result is negative.

fascia Fibrous connective tissue dividing muscle layers, neurovascular structures, and body cavities.

fastidious Describes a type of organism with complex nutritional requirements; requires special media for laboratory culture.

febrile Relating to fever.

fecal-oral route Mode of transmission.

fetid Foul smelling.

fibrosis Replacement of normal tissue by excess fibrous tissue.

filariform The third larval stage of the intestinal nematodes; this stage is infectious and nonfeeding.

fimbriae Appendages on the surface of bacteria that allow for attachment and infection; same as pili.

fistula An abnormal communication between hollow organs or vessels, or between a hollow organ or vessel and the skin.

flaccid paralysis A loss of muscle function with a resulting absence of muscle tone.

flagellum (pl. flagella) A cellular appendage on some prokaryotic and eukaryotic unicellular organisms that is used for motility and sometimes has a sensory role as well.

flank pain Pain in the posterolateral aspects of the trunk.

flatulence The presence of excessive gas in the stomach and intestines.

fluctuance A wave-like motion felt on palpation, due to underlying fluid content.

fluid attenuated inversion recovery (FLAIR) A technique in magnetic resonance imaging that suppresses cerebrospinal fluid to improve visualization of periventricular lesions.

fluid resuscitation The administration of large-volume crystalloid and/or colloid fluids to maintain adequate perfusion in the setting of shock.

folliculitis Infection of hair follicles.

fomite An object that is capable of transmitting an organism from one location to another, for example, toys, clothing, utensils, or hairbrushes.

fontanel Soft area between the cranial bones of an infant's skull, indicative of areas not yet ossified.

frozen section A tissue sample typically used during a surgical procedure to allow rapid diagnosis during the operation.

fulminant Referring to the sudden occurrence of an intense or severe process.

fungemia The presence of fungi in the bloodstream.

furunculosis Infection involving a hair follicle resulting in a skin boil.

gag reflex The reflex arc mediated by the glossopharyngeal nerve sensing posterior pharyngeal irritation, which stimulates the vagus nerve to elevate the soft palate and contract the pharyngeal muscles.

gait Manner of walking.

galactomannan Polysaccharides of mannose with galactose side chains found in *Aspergillus* spp. Used to diagnose invasive disease caused by this organism.

ganglion A group of nerve cell bodies located in the peripheral nervous system.

gangrene Necrosis due to any cause (for example, obstructed, diminished, or lost blood flow); may be localized or widespread; may be dry or wet.

gas gangrene Life-threatening necrotic infection of soft tissue by gas-forming bacteria, most commonly *Clostridium perfringens*.

gastritis Inflammation of the stomach, usually involving only the lining inside the organ.

gastroenteritis Inflammation of the mucous membrane lining of both the stomach and the intestine.

gene A complete unit of genetic information that encodes a single protein.

genetic probe A DNA sequence which is designed to covalently bond to a target gene.

genitalia Sex organs.

genotype Genetic makeup of an individual or microorganism.

geophilic Pertaining to an organism with a preference for the soil.

gestation The interval between fertilization of a female and birth of the offspring.

glomerulonephritis Bilateral inflammatory changes of the glomeruli that are the result of renal disease.

graft-versus-host disease (GVHD) A complication of bone marrow transplant or transfusion of nonirradiated blood products in which immune cells from the donor destroy host cells.

Gram stain A sequence of dyes and solvents applied to bacteria to enable viewing under the microscope; organisms stain either Gram positive or Gram negative.

granulation Regenerating, vascular tissue growing from the bed of a healing wound.

granuloma A collection of leukocytes, macrophages, and specialized cells of the reticuloendothelial system surrounding a focal area of chronic inflammation or infection; usually forms a nodular mass.

granulomatous Pertaining to or resembling a granuloma.

gravid Pregnant, carrying eggs or a fetus.

guaiac A reagent used to test for occult blood, usually in the feces.

guarding Voluntary or involuntary flexion of the abdominal wall muscles in response to an examiner palpating the abdomen; a sign of peritonitis.

Guillain-Barré syndrome Inflammation of peripheral nerves leading to increasing weakness or paralysis; most often occurs in more distal areas before affecting more proximal portions of the body; may progress to the point where the patient requires support on a ventilator because of weakness of the respiratory muscles.

HAART Highly active antiretroviral therapy, used to treat HIV infection; includes several medications with different mechanisms of action.

hallucinosis A syndrome characterized by hallucinations that are caused by organic substances such as drugs and alcohol; may occur during withdrawal from drugs or alcohol.

halophile Extremophile organism that grows in high salt concentrations.

helminths A group of flatworms and roundworms which derive their nutrients from parasitizing a living host.

hematemesis Vomiting blood.

hematocrit Amount of red blood cells (erythrocytes) in a given volume of blood; usually expressed as a percentage or fraction.

hematogenous Refers to anything produced by, derived from, or spread via the blood.

hematoma A collection of blood within an extravascular space such as an organ, a tissue area, or a potential space; the blood displays various colors and degrees of organization; a bruise.

hematopoietic stem cell transplant The transplant of multipotent hematopoietic cells from donor bone marrow, peripheral blood, or cord blood and administration to a recipient. Transplants can be **autologous** (harvested from the patient and given to the same patient after toxic therapies) or **allogenic** (harvested from a related or unrelated human leukocyte antigen matched donor and given to the patient).

hematuria The presence of blood in the urine.

hemithorax The left or right half of the chest.

hemoconcentration An increase in the number of red blood cells per unit volume of plasma.

hemodialysis Extracorporeal removal of wastes and/or toxins from the blood in states of acute or chronic renal failure, or other medical conditions where rapid clearance is necessary.

hemodynamics The movement of blood through the vasculature, measured for example by blood pressure, heart rate, and contractility; an important component of oxygen delivery to the tissues.

hemoglobinopathies Disorders or diseases that are the result of abnormalities of hemoglobin structure; for example, sickle cell disease and thalassemia.

hemolysins Proteins or lipids that cause lysis of red blood cells in laboratory media, but not necessarily *in vivo*; useful for classifying bacterial species.

hemolysis Destruction or breakdown of red blood cells.

hemoptysis Coughing up (expectoration) of blood or blood-streaked sputum.

hemorrhage Bleeding; can be either external or internal, e.g., intracranial hemorrhage.

hemorrhagic shock Massive blood loss resulting in inadequate delivery of oxygen to the tissues, leading to cell death and multisystem organ failure.

hemorrhoids Swollen veins in the anal canal or outside of the anus which can bleed or thrombose and become painful.

Henoch-Schönlein purpura (HSP) Systemic vasculitis caused by immune complexes of IgA, characterized by cutaneous purpura, arthritis, abdominal pain, and renal disease; most commonly presents in children and is self-limited.

hepatitis Inflammation of hepatic (liver) cells.

hepatocytes Functional cells of the liver.

hepatomegaly Enlargement of the liver.

hepatosplenomegaly Enlargement of the liver and spleen.

herbivore An animal that obtains its energy and nutrient requirements from plants.

hernia Part of an anatomical structure that has partially expanded out of its normal confined area.

herpangina A disease caused by the coxsackievirus group B; results in fever and ulcers of the palate.

heterophile antibodies Antibodies produced by an organism which react to antigens present on cells in another species.

heterosexual Pertaining to interactions of a person with people of the opposite sex.

heterozygous An organism which contains two different alleles for a particular gene.

hilum The area of an organ where the nerves and vessels enter and exit; the lungs, kidneys, lymph nodes, ovaries, and spleen all have hilar areas.

histopathology Microscopic evaluation of tissue.

histotoxic hypoxia Inability of cells to utilize oxygen, in spite of normal oxygen delivery to the tissues.

homeostasis The body's ability to regulate variables so that a stable state is achieved in a wide range of external conditions.

horizontal transmission Passage of disease from person to person or by contact with infected materials.

humoral Referring to substances in the blood; in the immune system, this refers to antibodies to help fight disease rather than the cellular portion which involves leukocytes.

humoral immunity An immune response that is activated and carried out by B lymphocytes and involving specific antibodies; the counterpart to cell-mediated immunity.

hyaluronidase An enzyme that catalyzes the degradation of hyaluronic acid, a component of the extracellular matrix.

hybridization The annealing of a fluorescently labeled DNA probe to a target sequence which can then be detected by microscopy or chemical reactions.

hydatid cyst Possible sequela of *Echinococcus* infection; cysts are most commonly present in the liver or lungs.

hydrocephalus Condition characterized by a large accumulation of fluid in the cerebral ventricles; results in increased intracranial pressure and dilatation of the cerebral ventricles. It can also occur secondarily as a result of loss of brain tissue.

hydrops General term for the accumulation of clear, watery fluid in spaces or cavities throughout the body.

hydrops fetalis Accumulation of fluid in the pleural, pericardial, or peritoneal cavities, as well as in the subcutaneous tissues of a fetus, usually resulting from anemia.

hyperbaric oxygen Delivery of higher than atmospheric pressures of oxygen in a pressurized chamber, used to enhance wound healing and to treat infections.

hypercholesterolemia The presence of abnormally high levels of cholesterol in the blood.

hyperendemic A disease which is persistently present at high prevalence.

hyperimmune globulin Donor plasma with high titers of antibody against a particular antigen.

hyperinfection Infection caused by very large numbers of organisms that results from an immunodeficient state.

hyperinflation Abnormal increased functional residual volume in the lungs.

hypermutable An organism or gene with abnormally frequent mutations.

hypertension Abnormally high blood pressure.

hyperventilation Abnormally increased respiratory rate, or tidal volume, or both, resulting in abnormally decreased dissolved carbon dioxide, increased bicarbonate, and alkalosis if no compensations are made.

hyphae The tube-like structures visible with a microscope that make up filamentous fungi.

hypnozoites A dormant phase of a *Plasmodium vivax* or *P. ovale* infection; hypnozoites are located in hepatocytes and are responsible for late-onset relapse.

hypogammaglobulinemia Abnormally decreased production of IgG.

hyponatremia Abnormally low concentrations of sodium in the circulating blood.

hypopituitary Pertaining to a decrease in the amount of hormones produced by the anterior (forward) portion of the pituitary gland; involves hormones that affect growth, steroid production, thyroid gland function, and reproduction.

hypoplasia The underdevelopment of tissues or organs as a result of either atrophy or a decrease in the number of cells.

hyporeflexic Abnormally decreased deep-tendon reflexes.

hypotension Abnormally low blood pressure.

hypothermia Abnormally low body temperature.

hypotonia Abnormally decreased muscular tone.

hypoxemia Low oxygen content in the blood.

icterus *See* jaundice.

idiopathic cardiomyopathy Dilatation and weakening of the heart muscle with no known cause.

idiopathic thrombotic purpura (ITP) An immune-mediated disease characterized by low platelet counts with normal bone marrow and no other primary conditions associated with low platelets.

ileum The most distal portion of the small intestine, site of absorption of many important nutrients and vitamins.

immune complex Antigen bound by specific antibodies with or without activated complement; may become insoluble and deposit in the vessel walls, including those of the glomeruli (*see* glomerulonephritis) or tissue.

immune modulators Targeted therapies which work to selectively block steps involved in the inflammatory response.

immune reconstitution inflammatory syndrome (IRIS) In patients with significant immune compromise (usually seen in advanced HIV), the recovery of immune function may cause an overwhelming inflammatory response and worsen disease symptoms.

immunization The process of exposing an organism to an antigen with the intent of generating immunological memory.

immunochromatography An assay which uses antibodies to first detect an analyte and then to exhibit a colored stripe to show the presence of the analyte; can be used rapidly with no further preparation, commonly for at-home or point-of-care testing.

immunocompromised A general term indicating an increased susceptibility to infection. May be due to defects in cell-mediated immunity, as occur with AIDS and posttransplant immunosuppressive medications; defects in neutrophil number or function, as occur following cancer chemotherapy; defects in humoral immunity, as occur in immunoglobulin deficiencies; or other immune defects such as deficiencies in splenic function.

immunoglobulin (G, M, E, A, D) Y-shaped proteins, produced by plasma cells, which have unique epitopes that recognize particular antigens and mark them for destruction.

immunomodulatory therapy Treatment of a disease by enhancing, suppressing, or changing the immune system.

immunoprophylaxis Protection from a disease by active or passive immunization.

immunosenescence The decline in function of the immune system with age, leading to increased rates of infection, neoplasia, and autoimmune disorders.

immunosuppression Decreased activity or magnitude of the immune response, caused by illness or drugs.

immunosuppressive Pertaining to an agent or disease that prevents or interferes with the immune system response.

impetigo Infection of previously damaged skin by group A streptococci or staphylococci; lesions usually drain honey-colored fluid.

in utero Inside the womb.

incidental host A host from which a parasite cannot be transmitted to a definitive host.

inclusions Phagocytized pathogens contained within a phagosome which cannot be effectively degraded and excreted.

indolent Nearly or completely painless, sluggish, or inactive; used to describe a disease process.

induced sputum A clinical sample of secretions from the tracheobronchial tree that is produced by having the patient inhale an irritating aerosol; the presence of macrophages or inflammatory cells suggests an adequate sample, whereas the presence of squamous epithelial cells suggests contamination by the upper respiratory tract.

inducer A molecule which inhibits a repressor or promotes an activator, resulting in increased transcription of a particular gene.

inducible resistance Chromosomal resistance genes that are expressed only in the presence of a specific antimicrobial agent.

induction chemotherapy Chemotherapy given as the initial treatment for a malignancy, sometimes followed by surgery, radiation, or additional chemotherapy treatments.

induration Firmness in soft tissue.

infarct Tissue death often due to an interruption in the blood supply to that tissue.

infection The invasion and growth of a disease-causing microorganism within a host.

infectious dose The number of microorganisms inoculated into a host necessary to cause disease.

infertility The inability to produce viable offspring; may be due to dysfunctional germ cells or to a structural abnormality.

infiltration The invasion of the spaces in a tissue by materials not usually found in the tissue, such as tumors, infectious agents, and white blood cells.

inflammation A series of chemical and physical processes within a tissue in response to toxins, foreign antigens, or injury; involves various cells of the vasculature, connective tissue, and immune system.

inhalation The creation of negative intrathoracic pressure which causes air to move into the lungs. Infectious agents may be obtained in this manner.

innate immunity Nonspecific and nonadaptive first line of defense against infection; includes skin and physical barriers, humoral and cell-mediated immunity, and the inflammatory response.

insidious Describes a disease which causes harm in a gradual way.

integrins Transmembrane cell surface proteins which facilitate binding of cells to surfaces or other cells.

interferon alpha A cytokine produced by white blood cells in response to viral infection.

interferon gamma release assay (IGRA) A test which measures interferon gamma released by a patient's white blood cells after exposure to *Mycobacterium tuberculosis* antigens.

interleukins A group of cytokines whose role in the immune response includes cell differentiation, growth, and motility.

intermediate host A host in which a parasite is capable of completing only part of its life cycle.

interstitial Pertaining to spaces between the components of a tissue; in the lungs, refers to spaces between the lung parenchyma.

intertriginous The spaces where the skin of two surfaces may touch.

intoxication The state of experiencing deleterious effects of toxin ingestion or dissemination.

intracranial Within the skull.

intradermal Within the skin.

intrapartum During labor or delivery.

intraperitoneal Within the peritoneal cavity.

intrauterine Inside the cavity of the womb, as for a fetus.

intrinsic resistance Innate ability of a pathogen to resist antimicrobial therapy.

intubation The placement of a tubular device from the oro- or nasopharyngeal cavity into the trachea in order to assist with ventilation.

intussusception The prolapse of a distal part of an organ back into the proximal portion of the same organ; usually refers to a portion of the intestine, where it may result in obstruction.

ischemia Cellular injury due to a decreased delivery of oxygen to a tissue because of either the impairment of blood flow to the tissue or decreased oxygen content of the blood. This may progress to cellular death.

isotonic saline 0.9% or 154 mM solution of sodium chloride used to replete intravascular volume.

jaundice Yellow cast of the skin and mucous membranes due to an increase of bilirubins (bile breakdown products) that occurs when the liver is unable to clear these chemicals from the blood; often due to toxic or infectious hepatitis; also referred to as icterus.

keratinized Pertains to skin which has an outer covering of keratin.

keratitis Inflammation of the cornea.

keratoconjunctivitis Inflammation of the cornea and conjunctiva.

Kernig's sign A sign of meningeal irritation, elicited when flexion of the hip causes involuntary flexion of the neck.

Koch's postulates A series of four criteria which should be met to establish that a microorganism is causative of a disease process: (i) the organism is isolated from hosts with the disease and absent in healthy hosts; (ii) the organism can be grown in culture; (iii) the organism can then be injected in a healthy host and cause disease; (iv) the organism can then be isolated from the newly inoculated host.

Koplik's spots Small red spots with a white center that are located on the inside of the cheek; pathognomonic for measles.

lacrimal duct A small duct that carries fluids (tears) from the lacrimal gland to the surface of the eyeball.

lactoferrin A glycoprotein secreted from exocrine glands which is part of the innate immune system and has antiviral, antibacterial, and antiparasitic properties in addition to a role in iron binding.

lactose intolerance The inability of the body to produce enough lactase to adequately metabolize lactose.

larva Developmental stage of the helminths, resembling a small immature worm.

laryngitis Inflammation of the larynx (voice box).

laryngotracheobronchitis Inflammation of the larynx and larger airways.

laser-assisted *in situ* keratomileusis (LASIK) A procedure which reshapes the cornea with a laser to correct vision.

latent Referring to any organism or disease that is quiescent but is capable of being reactivated.

latent infection Type of infection in which the organism or virus is present but not producing an inflammatory response.

latex agglutination An assay which uses antibody-coated latex beads; the presence of the antigen causes aggregation of the beads.

lethargy Drowsiness or decreased responsiveness.

leukemia General term used to describe a group of malignancies of either lymphoid or hematopoietic origin; progressive proliferation of abnormal leukocytes can be found in the blood, the hematopoietic tissues, and other organs.

leukemoid reaction An increase in white blood cell counts similar to the profile seen in leukemia; can be caused by infections (particularly diphtheria, pertussis, *Clostridium difficile*, and viral mononucleosis), drugs, and other medical conditions.

leukocidin A pore-forming cytotoxin produced by certain bacteria.

leukocyte esterase An enzyme produced by active white blood cells; a sensitive screening test for infection in the urinary tract.

leukoencephalopathy Encephalitis that is restricted to the white matter of the brain.

leukopenia Decreased number of white blood cells.

leukostasis An elevation in white blood cell count above 50–100,000 cells per µl which impairs normal blood flow and causes ischemia and hemorrhage.

lipopolysaccharide (LPS) Found in the outer membrane of Gram-negative bacteria; lost in the decolorizing step of Gram staining so that the Gram-negative cells do not stain with crystal violet.

loop electrosurgical excision procedure (LEEP) A procedure which excises dysplastic lesions of the uterine cervix by using an electrified wire loop.

lumbar puncture A procedure that is used to obtain cerebrospinal fluid for diagnostic purposes, performed by introducing a needle into the lumbar region of the subarachnoid space.

lupus erythematosus An autoimmune disease which can affect a wide variety of structures, including the skin, joints, kidneys, lungs, heart, blood cells and vessels, and nervous system, and can have reproductive consequences.

lymphadenopathy Abnormal enlargement of the lymph nodes.

lymphatics The collection of vessels, lymph nodes, and spleen which composes the lymph system.

lymphocytosis An increase in the number of lymphocytes above normal.

lymphogranuloma venereum A sexually transmitted infection caused by type L1, L2, L2a, or L3 *Chlamydia trachomatis* which causes genital ulcers, inflammation of the inguinal lymph nodes, and inflammation of other structures in the pelvis.

lymphoma A malignant neoplasm of lymphocytes and their precursors which typically presents as a solid tumor.

lymphoproliferative disorder Any one of a group of cancers involving cells from which white blood cells or platelets are derived; includes lymphomas, leukemias, and multiple myeloma.

lysogeny A stable and heritable characteristic of bacteria to produce and release bacteriophage as a result of a prophage within the bacterium; the prophage can be either integrated into the bacterial genome or maintained as a plasmid.

macroconidia Large, multinucleated conidia.

macular Pertaining to lesions that are flat and that are often detected only by a change in color or texture of the lesion compared with surrounding normal tissue.

maculopapular Pertaining to lesions with properties that are both macular and papular.

malabsorption Condition in which substances are not absorbed properly; usually occurs in the intestine, where it results in excessive loss of water or nutrients in the stool.

malabsorptive diarrhea An increase in the total number or volume of stools due to a decrease in the absorption of nutrients (especially fats and fat-soluble vitamins) in the small intestine.

malaise Generalized feeling of discomfort caused by many disease processes.

malaria Clinical manifestation of infection by the mosquito-borne *Plasmodium* genus, characterized by cyclic hemolysis, fevers, and sometimes splenomegaly, hepatomegaly, and encephalopathy.

malignancy Abnormal collection of cells with a tendency to grow in a rapid, unregulated, and uncontrolled fashion.

malignant otitis externa Inflammation or infection of the external auditory canal and/or auricle which is aggressive and difficult to treat.

malnutrition Characteristic of a long-standing diet with inadequate calories, nutrients, or both.

Mantoux test A screening test for tuberculosis in which a protein is injected into the dermis; a type IV sensitivity reaction causes induration of the injection site in a patient who has been exposed to tuberculosis.

mass spectrometry An analytical technique that produces a spectrum of the masses and charges of atoms or molecules in a sample.

mastitis Infection or inflammation of the breast tissue.

matting Inflammation of a collection of lymph nodes which are not individually palpable or mobile.

MDR-TB Multidrug-resistant *Mycobacterium tuberculosis*; characterized by resistance to at least isoniazid and rifampin.

melena Excretion of black, tarry stools containing blood that has been altered by intestinal substances.

meninges Thin, tough tissue surrounding the brain and spinal cord.

meningitis Inflammation of the meninges.

meningoencephalitis Inflammation of the brain and surrounding membranes (meninges).

metaphyseal Relating to the conical portion of long bones that lies between the epiphysis and diaphysis.

metastasis The occurrence of disease at sites distant from and not connected directly with the site where the disease first appeared; this process is seen with malignancies and infections.

MIC Minimum inhibitory concentration; the lowest concentration of a drug needed to inhibit the growth of a microorganism.

microaerophilic Pertaining to an atmosphere with a reduced concentration of oxygen; some microorganisms, called microaerophiles, grow best in such an environment.

microbiome The collective genome of the microorganisms colonizing a host.

microbiota The microorganisms that colonize a host, including the microorganisms in the gastrointestinal tract, genitourinary tract, mouth, and skin for example.

microvasculature The part of the circulation that includes the smallest vessels; typically refers to the capillaries.

miliary disease Disseminated tuberculosis, with small lesions in both the lungs and extrapulmonary organs.

mitral regurgitation Any condition of the mitral valve between the left atrium and left ventricle that allows blood to flow back into the atrium when the ventricle contracts; normally, the valve shuts tightly, allowing no flow of blood back into the atrium; often heard as a heart murmur during auscultation.

mitral valve prolapse A defect in the valve between the left atrium and ventricle caused by a weakening of the tough connective tissue of the valve leaflets, which allows the valve to project back into the left atrium; during normal function, the valve closes tightly during ventricular contraction.

modified acid-fast Describes changes made to a typical Ziehl-Neelsen stain, typically in the acid wash step, to detect acid-fast organisms such as *Isospora*, *Cyclospora*, or *Nocardia*.

monoclonal antibodies Antibodies which are cloned to target a particular epitope.

morbidity Harmful effects of a condition or treatment, excluding death.

morbilliform rash Rash that resembles the flat to slightly raised (maculopapular) lesions seen in measles.

morphology Study of the structure, form, or shape.

motility The ability of an organism to have self-propelled motion.

mucin Highly glycosylated, negatively charged proteins which give secretions lubrication and the ability to bind pathogens and irritants.

mucociliary Pertaining to the movement of a mucous layer resting on a ciliated epithelium, part of the innate immune system of the respiratory system.

mucocutaneous Involving the skin and mucous membranes.

mucoid Pertaining to or resembling mucus.

mucormycosis An invasive infection caused by Mucorales order fungi which most commonly affects the mouth, sinuses, and brain.

mucosal immunity The portion of the immune system which protects the mucosal membranes from pathogens.

multiloculated Describes a collection of fluid in multiple noncommunicating pockets separated by septa or fibrosis.

murmur Normal or abnormal sounds heard on auscultation of the heart or vessels; this is a physical finding that has a variety of causes.

mutation An error in genome replication which can be advantageous, deleterious, or neutral on an organism's ability to grow and reproduce.

myalgia Soreness or aching of muscles.

mycetoma A ball of fungus, commonly *Aspergillus*, found in pre-existing cavitary lesions of the lungs.

myeloma Cancer of well-differentiated immunoglobulin-producing cells; progressive proliferation of plasma cells is seen with this type of malignancy.

myelopathy A disease of the neural tissue of the spinal cord.

myocarditis Inflammation of heart muscle cells.

myonecrosis Necrosis of muscle tissue.

myositis Inflammation of the muscle.

nares Nostrils.

nasopharyngeal Pertaining to the nasal and pharyngeal cavities.

nausea The urge to vomit.

necrosis Death of cells, tissues, or portions of organs that results from irreversible injury; several different types of necrosis can be distinguished.

necrotizing fasciitis A destructive soft tissue infection that causes necrosis of the superficial fascia and surrounding tissues; this is often a fulminant process and one that may be difficult to diagnose.

negative predictive value The proportion of patients who do not have a disease who will test negative by a given assay.

negative-pressure room A confinement room that has a lower atmospheric pressure than the surrounding rooms; a pressure gradient prevents air from escaping into occupied areas; necessary for the containment of airborne pathogens, such as *Mycobacterium tuberculosis*.

nematode The class of helminths that includes the roundworms *Ascaris*, *Enterobius*, and *Strongyloides* spp.

neonate A newborn infant.

nephrosis Degeneration of renal tubular epithelium.

nephrotoxicity Property of a toxin or drug which results in damage to the kidney.

neuralgia A stabbing pain that follows the course of a nerve.

neuraminidase Glycoside hydrolase enzyme on the influenza virus which cleaves sialic acid from cell surface, facilitating release of new virions from infected host cells.

neuropathy Diseases or disorders affecting the cranial nerves or the peripheral or autonomic nervous systems.

neurosyphilis A possible manifestation of tertiary syphilis with a wide range of presentations, including meningitis, tabes dorsalis, Argyll Robertson pupils, cerebrovascular disease, and dementia.

neutralizing antibody An antibody that binds to a particle, usually a virus or toxin, to prevent it from being infectious or hazardous.

neutropenia Abnormally low numbers of neutrophils in the circulating blood.

neutrophil The most common type of white blood cell, neutrophils phagocytize antigens and contain myeloperoxidase-filled granules; part of innate immunity and the inflammatory response.

newborn screen A test performed on dried blood samples 24 hours after birth which screens for serious disorders which can be improved with treatment.

nocturnal Referring to night.

nodular Describing knotlike or raised solid lesions of the skin or other organs.

noncompliant Characteristic of a patient who is unable or chooses not to adhere to prescribed therapy.

noninfectious sequelae Consequences of an infection which are associated with the body's immune response to the organism rather than with the infection itself.

non-insulin-dependent diabetes mellitus (NIDDM) Disease characterized by impaired glucose clearance and insulin resistance; managed with diet or oral medications.

noninvasive Characteristic of infections which do not invade multiple tissues or cause disseminated tissue destruction.

nonpurulent Characteristic of infections or conditions which do not result in the production of pus.

nonsuppurative sequelae Complications, caused by a previous disease status, that do not involve purulence.

nontoxigenic Not producing a toxin.

normotensive Describing the condition of having normal blood pressure; the usual readings seen in adults are between 90/50 and 150/90 mm Hg.

nosocomial Health care-associated; any condition resulting from a person's hospital stay; usually used in the context of an infection acquired as a result of hospitalization.

nuchal rigidity Stiffness of the neck, often associated with meningitis.

nucleic acid amplification test (NAAT) A molecular microbiology technique which uses PCR or other methods of amplification of nucleic acids to identify microorganisms or other genetic targets.

nymph An intermediate life cycle stage in some insects and arachnids, following the larval stage and preceding the adult stage.

obligate intracellular Characteristic of an organism which can only reproduce inside a host cell.

obstruction A block along the path (of the intestines or a blood vessel).

occlusive Serving to block the flow of air or liquid.

occult blood Blood present in body fluids, such as stool, that cannot be detected with the naked eye; the most commonly used test for occult blood is a guaiac test.

ocular Of or pertaining to the eye.

oligonucleotides Short segments of RNA or DNA which can be used to identify genes of interest in PCR, microarrays, and fluorescent *in situ* hybridization assays.

oncogenic The property of having the potential to cause malignant neoplasm.

oncospheres Motile tapeworm embryos that are released from the egg after the egg is ingested by a suitable host.

oocyst A cyst containing zygotes of a parasite which can be transmitted to a suitable host.

opacification An area of clouding or obscuring which decreases the penetrance of light.

opening pressure The pressure of the cerebrospinal fluid measured upon entry during a lumbar puncture.

opisthotonic Describes a spastic state in which the head and heels are arched backward and the torso extends outward.

opportunistic infection An infection caused by an organism capable of causing disease only in individuals whose resistance to infection is lowered.

opsonic Pertaining to an agent (typically an antibody) that, when bound to an antigen such as bacterial polysaccharide capsule, enhances the ingestion of the antigen by white blood cells.

opsonophagocytic Describes proteins, including some antibodies and complement proteins, which tag an antigen for destruction by phagocytosis.

oral rehydration The reversal of dehydration by drinking a solution of water, electrolytes, and carbohydrates.

organomegaly Abnormal enlargement of the organs; visceromegaly.

orifice A normal anatomical opening, such as the mouth.

orolabial Pertaining to the mouth and lips.

oropharyngeal Pertaining to the oral and pharyngeal cavities.

orthostatic hypotension Decreased blood pressure caused by sitting up or by standing erect; often seen in patients who are dehydrated.

osteoarticular Pertaining to the bones and joints.

osteomyelitis Inflammation or infection of bone.

otitis media Inflammation of the middle ear; visualized with an otoscope, which often shows the presence of fluid or pus behind the eardrum.

ototoxic Refers to a substance that has a toxic effect on the ear; some antibiotics, for example, have this property.

palatal Referring to the palate, the bony or soft tissue roof of the oral cavity.

palpation A technique used during physical examination that involves the use of the hands to feel for organs, abnormal masses, pulses, or vibrations.

pancolitis Inflammation throughout the colon.

pancreatitis Inflammation of the pancreas.

pancytopenia A significant reduction in the number of red blood cells, white blood cells, and platelets in the circulating blood.

pandemic Relating to a disease that is affecting the population of an extensive region, country, continent, or the entire world.

papular Pertaining to lesions that are raised and well circumscribed.

paranasal sinuses Four paired sinuses—frontal, ethmoidal, sphenoid, and maxillary—found in the skull.

paraparesis Weakness of the legs.

parasite An organism that lives in or on and derives its nutritional support from a host.

parasitemia Presence of parasites in the blood.

paravertebral Beside a vertebra or the vertebral column.

parenchyma Cells of a gland or organ that are contained within and supported by the surrounding connective tissue network.

parenteral route Describes the means of administering medication, nutrition, or fluids by vein, rather than the digestive tract.

paresis Incomplete paralysis.

paroxysm The abrupt episodic recurrence of disease or disease symptoms; also, a spasm or fit.

pathogen Any microorganism that causes disease.

pathogenesis The mechanism by which a microorganism causes disease.

pathogenicity island A small portion of a bacterial chromosome that includes many of the genes necessary for infection; the physical closeness of these genes suggests that the bacterium obtained infectious capability from a single source or process.

pathogenicity locus The area of the bacterial chromosome that includes the genes required for infection.

pathognomonic Symptoms or lesions characteristic of a single disease process, on the basis of which a diagnosis can be determined.

PCP *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) pneumonia. This abbreviation can also refer to "primary care physician" or to the drug of abuse phencyclidine (phenyl cyclohexyl piperidine ["angel dust"]). Be careful when using abbreviations in medicine.

PCR (polymerase chain reaction) A laboratory technique for rapidly replicating DNA strands in a test tube; can be used for diagnosis when a probe specific for an exact sequence of DNA is used to initiate the replication process.

pelvic inflammatory disease Inflammation of the female reproductive organs, typically due to infection.

peptidoglycan The main component of the bacterial cell wall; composed of carbohydrates and amino acids.

percussion Determining the properties of a structure by tapping over the structure.

percutaneous Through the skin.

perforation An abnormal opening.

perianal The area surrounding the anus.

pericarditis Inflammation of the sac covering the heart.

perihilar Around or near the hila of the lungs.

perinatal The period of time immediately preceding and following birth.

periorbital Around the eye socket (orbit).

periosteum The tough tissue surrounding the surface of any bone.

periostitis Inflammation of the periosteum (the thick fibrous membrane that covers the surface of the bone, except for the articular cartilage).

peripheral neuropathy Pain, numbness, or tingling in the extremities as a result of inflammation of or injury to the peripheral nerves.

peristalsis The alternating waves of contraction and relaxation within the gastrointestinal tract that propel its contents onward.

peritonitis Inflammation of the peritoneum (the thin lining of mesothelium and connective tissue that lines the abdominal cavity).

peritonsillar abscess A collection of pus in the area surrounding the tonsils.

periumbilical Pertaining to the area surrounding the navel (umbilicus).

periurethral Pertaining to the area surrounding the urethra.

petechiae Pinpoint, flat lesions due to hemorrhage of blood into tissues under the skin or mucous membranes.

petechial rash Small, pinpoint, and flat lesions of the skin and mucous membranes associated with hemorrhage beneath the tissue; similar to purpura, except the lesions seen with purpura are larger.

Peyer's patches Lymphatic tissues in the walls of the large intestine.

phagocytosis The process by which cells ingest and digest solid substances such as tissue debris, bacteria, or foreign material.

phagolysosome A phagosome fused with a lysosome for degradation of its contents.

phagosome A vacuole formed upon phagocytosis of a particle, eventually fused with a lysosome for degradation.

phalanx A long bone of the finger.

pharmacogenomics The study of the ways in which a patient's genome affects his or her response to specific drugs. Used commonly in selecting cancer chemotherapeutic agents.

pharyngitis Inflammation of the pharynx, the muscular tube connecting the nose and mouth to the esophagus and larynx; sore throat.

phenotypic Pertaining to the effects of an organism's genes and the environment on its physical appearance, biochemistry, and physiology.

photophobia Abnormal sensitivity to light.

pica The desire to eat inappropriate and nonnutritional substances such as soil.

pigmented Colored.

pili Appendages on the surface of bacteria that allow for attachment and infection; same as fimbriae.

"ping-pong ball" phenomenon Patient becoming reinfected with an infectious agent after treatment because the patient's infected close contacts have not been treated.

placebo A treatment with no active medication, only approved for use in investigating the effects of a novel medication by comparing the test drug to the placebo.

plasma exchange A technique used to treat certain malignancies and autoimmune disorders, in which a patient's plasma is removed and replaced with equal volume of plasma or other colloid.

plasmid A piece of DNA that is physically separate from the host's chromosome and not essential for survival; often a means for bacteria to acquire a new function such as antibiotic resistance or toxin production.

pleocytosis The presence of abnormally large numbers of cells; often refers to the increase in white blood cells that occurs during an infection.

pleuritic pain Chest pain which is caused by irritation or inflammation of the pleura, characterized by worsening pain upon inspiration.

pleuritis Inflammation of the pleura.

pleurodynia Episodic pain due to transient spasms of the intercostal muscles (muscles between the ribs that assist in respiration), caused by irritation of the pleura.

pneumocytes Functional cells of the lung.

pneumonia Infection of the lung parenchyma.

pneumonitis Inflammation of the lungs.

poliomyelitis Inflammation and destruction of the gray matter of the spinal cord, possibly resulting in paralysis; causative agents are the polioviruses.

polyarteritis nodosa An autoimmune disease which causes inflammation of small and medium-sized arteries.

polymicrobial Pertaining to multiple different microorganisms.

polymorphism Variants of a particular gene.

portal circulation The circulation of blood to the liver from the small intestine, the right half of the colon, and the spleen via the portal vein.

portal of entry The site where microorganisms enter a host.

positive predictive value The probability of having a disease given a positive test result.

post ictal Referring to a patient's condition following a seizure; many people are less alert, and transient neurologic findings may be observed.

postpartum The condition or period of time after the delivery of a fetus.

poststreptococcal sequelae Consequences of infection by group A streptococci that appear after the initial infection; these processes are not a direct result of the organism but rather are due to the host immune response to the organism.

posttransplantation lymphoproliferative disorder Hyperplasia, premalignant or malignant polyclonal or monoclonal neoplasms of lymphocytes following solid-organ or stem-cell transplant.

PPD skin test A diagnostic test in which purified protein derivative is placed just under the skin; used to determine if patients are infected with *Mycobacterium tuberculosis*.

precipitin An antibody that binds with its antigen and causes the antigen-antibody complex to precipitate from solution.

predisposing factors Genetic or acquired susceptibilities to a disease.

preeclampsia A condition of the late stages of pregnancy caused by accumulation of toxins in the blood; characterized by high blood pressure, swelling of the hands and feet, and the presence of proteins in the urine. If seizures occur, it becomes known as eclampsia.

preemptive therapy Initiating antiviral therapy for patients who exhibit viremia.

pretest probability The likelihood of a patient having a particular disease before any testing.

prevalence The number of people at a given period of time with a given trait or disease divided by the number of people at risk for the trait or disease.

prion Infectious protein, possibly self-replicating.

proctitis Inflammation of the rectum.

proctocolitis Inflammation of the colon (large intestine) and rectum.

prodrome An early symptom of a disease.

proinflammatory Describes substances such as cytokines which augment the initiation or amplification of the inflammatory response.

promastigote A stage in the life cycle of the protozoans in the genus *Leishmania*; a characteristic finding in host macrophages during infection.

prophylactic Pertaining to agents or procedures that prevent disease processes in susceptible individuals; for example, giving vaccines or antibiotics to prevent an infection before it occurs.

prosthetic Pertaining to a human-made replacement for a missing or defective body part, such as artificial limbs or heart valves.

protease An enzyme which catalyzes the degradation of a protein.

protoscolices Intermediate stage of the *Echinococcus* life cycle; evaginate from daughter cysts upon infection of definitive host, attach to the intestinal wall, and develop to mature adults.

Protozoa A phylum or subkingdom of the animal kingdom; contains many medically important unicellular parasites.

proximal phalanx The part of a digit (finger or toe) that is closest to its attachment to the body.

pruritus Itching.

"pseudo" outbreak A false perception of increase in disease incidence, due to a contaminant or error in the testing process or a change in testing patterns.

pseudohyphae Elongated yeast cells resembling hyphae, but which remain connected and unicellular.

pseudomembranous colitis Inflammation of the mucous membranes of the small and large intestines with the formation of false membranes composed of bacteria, white blood cells, fibrin, platelets, and necrotic material; occurs most frequently as a result of the action of exotoxins made by *Clostridium difficile*.

ptosis Drooping of the upper eyelid.

pulse oximetry A technique that uses light to rapidly estimate the percentage of hemoglobin in the arterial blood which is saturated with oxygen.

punch biopsy A cylindrical core of tissue obtained for histologic analysis.

punctal plugs Plugs placed to occlude the nasolacrimal duct.

punctate Pertaining to lesions or markings that look like points or dots.

purpura Purplish lesions of the skin and mucous membranes due to hemorrhage beneath the tissues; usually less than 1 cm in size, lesions may be flat or raised.

purulent Related to presence, consistency, or formation of pus.

pustular Pertaining to a skin rash with pus in the lesions.

pyelonephritis Infection of the kidney with or without a concurrent bladder infection.

pyoderma An infection of the skin that produces pus; caused by pyogenic bacteria.

pyrogenic Causing fever.

pyuria The presence of pus in the urine.

quadriparesis Weakness of all four extremities.

quorum sensing Coordinated gene expression in bacteria triggered by the release and detection of chemical signals; used in the formation of biofilms, virulence, and antibiotic resistance.

rales Abnormal breathing sounds (crackles) heard by auscultation of the lungs during respiration; classified as dry or moist.

randomized controlled trial A type of research study which randomly assigns patients to either an investigational therapy or no therapy (or placebo).

reactivation The process by which a latent infection can become active after a variable period of dormancy.

reassortment Mixing of genetic material into new combinations.

rebound Pain which is worse upon release of the abdominal wall than upon compression of the abdominal wall, a sign of peritonitis.

recombinant Containing genetic material from more than one organism.

rectocolitis Inflammation of both the rectum and the colon.

rehydration Restoration of body fluids to normal osmolarity by adding fluids.

renal Pertaining to the kidney.

reportable disease Diseases of significant public health importance which prompt registry of the case with the health department upon diagnosis.

reservoir The host, in nature, of an infectious agent. Some of these infectious agents may cause disease in humans after transmission from the reservoir either directly (e.g., a rabid bat bites a person) or indirectly (e.g., a rodent infected with *Leptospira* urinates in water in which a person later bathes).

respiratory alkalosis A decrease in serum pH due to increased minute ventilation, decreased carbon dioxide, and decreased free hydrogen ions.

respiratory tract The anatomical structures involved in respiration, from the mouth, nose, and paranasal sinuses to the alveoli.

restriction fragment length polymorphism (RFLP) The variation in size of certain DNA fragments that exists between homologous chromosomes; the difference in the fragment lengths is due to alterations at the site of cleavage by restriction enzymes; can be used to determine the genetic variation between organisms of the same species.

reticulocyte An immature red blood cell.

reticuloendothelial system (RES) The specialized white blood cells (especially macrophages) and other cells in the lymph nodes, liver, and spleen.

reticulonodular Description of radiographic findings of interstitial lung disease, characterized by "ground glass" appearing opacities.

retinal Referring to the retina.

retinitis Inflammation of the light-detecting tissue (retina) in the back of the eye.

retropharyngeal abscess A collection of pus posterior to the pharynx.

reverse transcription The synthesis of DNA from a single-stranded RNA template.

Reye's syndrome An illness that occurs in children with influenza or varicella; can result in encephalitis, liver involvement, coma, and death; strongly associated with the use of aspirin during the initial viral infection.

rhabditiform The first and second larval stages of the intestinal nematodes; this form is feeding and noninfectious.

rheumatic fever An autoimmune disease of children and young adults that follows infection by group A streptococci; most severe sequelae are the destruction of heart tissue and scarring of heart valves, both of which can lead to heart failure.

rheumatoid arthritis A systemic, inflammatory, autoimmune disease with manifestations commonly in the joints, but also in the skin, eyes, lungs, kidneys, and cardiovascular system.

rhinitis Inflammatory or infectious process involving the mucous membranes lining the nose.

rhinocerebral Describes a disease which spreads from the mouth, nose, and sinuses to the brain.

rhinorrhea Thin, watery discharge from the nose; runny nose.

rhonchi Coarse, low-pitched sounds associated with the presence of secretions or obstruction of larger airways in the lungs during inspiration and expiration.

ribosomal DNA DNA template from which ribosomal RNA (rRNA) is made.

ribosomal RNA (rRNA) Genetic material within a ribosome, essential for protein synthesis.

ring forms Trophozoite form of malaria parasite.

rigors Episodes of rigidity and shaking chills that may be brought on by infections; rigors may precede a fever.

ring-enhancing lesions Lesions in the brain seen on computed tomography (CT) scans, consisting of lucent (less dense to X rays), rounded masses surrounded by a region of increased density (especially with the use of vascular contrast material); often seen in certain infections of the brain.

RNA Ribonucleic acid; a polymer of nucleotides that is produced from a DNA template; has several functions in protein synthesis, including directing the sequence of amino acids and serving as a constituent of the ribosome complex.

salpingitis Inflammation or infection of a tube, usually the fallopian tubes between the ovaries and the uterus.

scarlet fever An illness caused by infection with group A streptococci; most prominent feature is the formation of numerous bright red spots on the skin and mucous membranes that are subsequently shed; before antibiotics, eruptions led to severe sequelae such as sepsis.

scleral icterus Yellow discoloration of the sclera due to elevated concentrations of bilirubin.

sclerosing cholangitis Recurrent or persistent obstructive jaundice due to extensive, destructive fibrosis of the bile ducts; frequently progresses to cirrhosis, liver failure, or portal hypertension; most common in young men.

screening test An inexpensive, rapid test used for asymptomatic persons for a specific condition or trait; the test should be sensitive in order to avoid false negatives; usually followed by a confirmatory test.

sebaceous glands Holocrine glands which excrete an oily substance called sebum, generally found in hair-covered areas.

secretory diarrhea An increase in the total number or volume of stools due to increased secretion or decreased reabsorption of electrolytes, caused by cholera toxin or enterotoxigenic *Escherichia coli*, for example.

selective medium Type of medium that inhibits the growth of some organisms while allowing the growth of others; useful when trying to isolate or detect specific organisms.

selective pressure External factors which favor the growth of certain strains of microorganisms, thereby favoring particular genes such as those that encode virulence factors or antimicrobial resistance and resulting in such strains becoming more frequent in particular locations such as patients or hospital environments.

sensitivity The ability of a diagnostic test to detect an organism if the organism is present; a very sensitive test should miss only a small number of infections; often a characteristic of screening tests.

sepsis The presence of the systemic inflammatory response syndrome as a result of an infection.

septate Having septa; divided into compartments; usually a description of hyphae in fungi.

septic abortion Infection of the mother after the abortion of a fetus; can involve the uterus and become a widespread systemic infection.

septic embolus Clot (blood or other occlusive material) carried by the blood which contains infectious agents; often leads to systemic infection; its occurrence can be sudden.

septic shock Hypotension as a result of sepsis, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities of vital organs. This is a life-threatening condition that results in the increased permeability of the blood vessels, leading to excess fluid escaping into the tissues, abnormalities in cardiac function, and changes in vascular tone.

sequela (pl. sequelae) A condition that occurs as a result of a disease process.

serology The study of a patient's blood to determine the presence of specific antibodies to viruses or other microbes; can be used to determine exposure to a pathogen, to follow the course of a current infection by measuring the antibody level present, or to determine vaccination status.

seropositivity Detection of the presence in the serum of antibodies to a particular antigen.

serotyping Differentiating different species or strains of a particular bacterium, fungus, or virus.

serum sickness An immune complex disease that occurs approximately 1 to 2 weeks after a drug, foreign serum, or protein has been introduced into the body; local or systemic reactions can occur.

siderophores Iron-chelating compounds excreted by some microorganisms.

silicosis A disease characterized by lung inflammation and fibrosis caused by the immune response of alveolar macrophages to silica particles.

sinopulmonary Pertaining to the lungs and paranasal sinuses.

sinusitis Inflammation of the sinus cavities, often leading to headaches or nasal congestion.

solid-organ transplant Transplant of a living- or deceased-donor organ (most commonly kidney or liver) into a patient.

somnolent Drowsy.

specific gravity A laboratory measurement that allows for comparison of the density of a liquid with that of distilled water; frequently used to determine the relative concentration or dilution of urine.

specificity The ability of a diagnostic test to detect the absence of an organism if the organism is absent; a positive test result with a very specific test should "rule in" disease; often a characteristic of confirmatory tests.

splenectomy Removal of the spleen.

splenic Of or pertaining to the spleen.

splenomegaly Enlargement of the spleen.

spondylitis Inflammation or infection of the vertebra.

spongiform Pertaining to a spongelike appearance; when noted in brain tissue, it is indicative of the presence of Creutzfeldt-Jakob disease or another prion disease.

spores The product of sexual or asexual reproduction in fungi; a very hardy latent form that certain bacteria can employ in the presence of environmental stress.

sterile pyuria Urine which shows high numbers of white blood cells but no growth on culture.

stillbirth The birth of a fetus that is dead before delivery.

strabismus Misalignment of the eyes, caused by discrepancies of the ocular muscles.

stridor Type of breathing that is characterized by a high-pitched whistling sound; caused by obstruction of the upper airway.

stridorous cough A characteristic high-pitched cough associated with laryngeal blockage.

stuporous Being in a state of decreased consciousness that is characterized by a diminished sense of orientation and response to the environment.

subarachnoid hemorrhage Bleeding into the area between the arachnoid membrane and the pia mater, usually resulting from a ruptured cerebral aneurysm or traumatic injury.

subcarinal Refers to the area below the ridge that separates the right and left main bronchi at their junction with the trachea.

subclinical Describes a disease which does not show readily identifiable signs and symptoms.

subconjunctival Beneath the conjunctiva.

subcostal retractions Inward movement of the area between the ribs, associated with an increased respiratory effort.

superantigen An antigen that induces a T-cell response by binding to a T-cell receptor in an area outside of the antigen recognition site; usually results in the activation of many T cells.

superoxide dismutase An enzyme found in white blood cells or some bacteria which catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen.

superinfection An infection by organisms because of a previously acquired but ongoing infection (such as a bacterial pneumonia which sometimes occurs during or after a viral pneumonia).

supine Refers to lying on the back with the face directed upward.

suppurative Of or relating to infection and inflammation.

suppurative lymphadenopathy Enlarged, tender lymph nodes from which pus is draining.

surveillance Systematic screening of hospitalized individuals for colonization or infection by an organism of epidemiologic significance at the institution.

syncope Transient, brief episodic loss of consciousness (fainting).

syndrome A group of signs and symptoms that is characteristic of a particular disorder.

synergy When the actions of two or more processes, structures, or agents are combined to yield a result that is greater than the sum of each individually; for example, antibiotics can be synergistic.

synovitis Inflammation of the joints.

systemic inflammatory response syndrome A response to a stressor, especially infection, in which two or more of the following criteria are met: temperature >38°C or <36°C; heart rate >90 beats/min; respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg; and white blood cell count >12,000/mm^3 or <4,000/mm^3, or >10% immature (band) forms.

systolic murmur A sound which can be appreciated between S1 and S2 in the cardiac cycle.

T cells Lymphocytes which mature in the thymus and are the primary components of cell-mediated immunity.

T1 weighted imaging An MRI technique in which fat appears the brightest and water appears the darkest.

T2 weighted imaging An MRI technique in which water appears the brightest and fat appears the darkest.

tabes dorsalis A neurologic disease of tertiary syphilis that results in destruction of the sensory or posterior portions of the spinal cord; when the upper spinal cord is involved, parts of the sympathetic nervous system can be involved.

tachycardia Increased heart rate (>100 beats/min in adults).

tachypnea Increase in respiratory rate (>20 breaths/min in adults).

tachyzoites An intermediate stage in the life cycle of *Toxoplasma gondii*; invades host cells of any tissue type and divides rapidly.

tapeworm Type of flatworm of the class *Cestoda*; infects humans after the larva or eggs are ingested in contaminated food or water; some species may infect only the digestive tract, whereas others may disseminate to the brain, lungs, or liver.

tenosynovitis Inflammation of the tough sheath surrounding a tendon.

teratogenic Pertaining to agents that cause the abnormal development of an embryo, commonly resulting in fetal death or birth defects.

thrombocytopenia A decrease in the numbers of platelets.

thrombosis Clotting within a blood vessel that may lead to infarction of the tissues supplied by that vessel.

thrush An infection of the oral cavity that produces a white plaque; caused by the yeast *Candida albicans* in immunocompromised patients or in patients who have received broad-spectrum antibacterial agents.

tight junction The junction between two cells which is nearly impermeable to fluids.

titer A way to quantify the amount of antibody present through serial dilutions, expressed as a ratio of the dilution.

Toll-like receptors Receptor proteins on sentinel immune cells that recognize a broad range of pathogens and other proteins based on pattern recognition; a part of the innate immune system.

tonsillar Of or pertaining to the tonsils.

tonsillitis Inflammation of one or more tonsils, usually the palatine tonsil.

toxic megacolon Life-threatening acute inflammation and massive dilation of the colon, commonly associated with pseudomembranous colitis due to *Clostridium difficile*.

toxin A poisonous substance which causes damage or death to cells.

toxoid A toxin that has been denatured into a nontoxic form; the toxin retains its antigenic potential and can be used in a vaccination.

tracheobronchitis Inflammation or infection of the trachea and bronchial tree.

trachoma Infection of the conjunctiva and cornea due to *Chlamydia trachomatis*; a major cause of blindness in the developing world.

transbronchial biopsy During bronchoscopy, the removal of a small piece of lung tissue through the walls of a bronchus (airway).

transient bacteremia The presence of bacteria in the bloodstream for a brief period of time followed by rapid clearance; patients with transient bacteremia are asymptomatic.

transillumination Applying a light to the skin overlying a body cavity to detect the presence of air.

translocation The migration of bacteria from the lumen of the gastrointestinal tract to the mesenteric lymph nodes or blood.

transmembrane protein A protein which spans through the entire lipid bilayer of a cell.

transplacental Crossing the placenta; pertaining to any substance that passes from the mother to the fetus or vice versa.

tricuspid valve The valve through which blood flows from the right atrium to the right ventricle.

trismus Spasm of the jaw muscles.

trophozoite The pathogenic or non-cyst stage of certain protozoans.

tropism Movement of an organism toward or away from a source of light, heat, or some other stimulus.

tuberculoma A tumor-like mass composed of *Mycobacterium tuberculosis* and the corresponding phagocytes that are unable to digest the bacteria.

tumor necrosis factor alpha A proinflammatory cytokine which causes fever, cachexia, and apoptosis.

tympanic membrane The eardrum.

tympanostomy An incision made in the ear drum to allow drainage of fluid from the middle ear.

typhoidal Pertaining to or resembling typhoid; disseminated infection without a particular anatomic focus.

Tzanck preparations Staining and microscopic examinations of the cells from a skin vesicle, used to identify cells infected with herpes simplex virus and varicella zoster virus.

ulcer A lesion on the surface of the skin or mucous membranes caused by superficial loss of tissue; often accompanied by inflammation.

ulceroglandular Describes disease resulting in ulcerations of the skin and regional lymphadenopathy.

unicellular Being composed of a single cell.

urease An enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia.

urethritis Inflammation of the urethra.

uropathogen A pathogen which infects the urinary tract.

vaccine Weakened (attenuated) or dead (inactivated) bacteria or virus, inactivated toxin (toxoid), or genetically engineered component of an infectious agent, used to promote immunity against a disease (such as smallpox or diphtheria).

vaccine cold chain Temperature-controlled storage and distribution of vaccines.

vaginitis Inflammation of the vagina.

vasculitis (pl. vasculitides) Inflammation of blood vessels leading to lesions on the skin, mucous membranes, or internal organs.

vasodilation Relaxation of the smooth muscle surrounding blood vessels, resulting in an enlargement of the vessel lumen.

vasopressors Endogenous or exogenous substances that promote vasoconstriction and improved oxygen delivery to tissues.

vegetation A clump of fibrin and platelets on the heart valves; may include microorganisms as a result of endocarditis or may be sterile.

venipuncture A procedure used to draw blood or inject a solution that involves puncturing a vein.

vertical transmission Passage of an infectious agent from the mother to a fetus.

vesicular Pertaining to small, blisterlike lesions filled with clear fluid.

vesiculopustular Pertaining to blisterlike lesions containing pus.

violaceous Violet or purple in color.

viral load A quantitative measurement of the amount of virus present in the bloodstream; used as a means of following the course or severity of a viral infection.

viremia The presence of viable virus in the bloodstream.

virulence factor A trait of an infectious agent that gives the organism the capability to cause disease; may be a toxin, a biochemical component of the organism's outer surface, or other factor.

virulent Refers to the particularly toxic effects of a pathogenic microorganism.

virus A particle composed of genetic material surrounded by a protein capsule; capable of infecting host cells by inserting the genetic material into the host cell and utilizing the host's machinery and energy to replicate.

virus-like particles Particles which contain the structure of a virus but lack viral genome and are therefore not infectious; used in vaccines and potentially in gene therapy.

viscera The organs located within the thorax, abdomen, and pelvis.

wheeze A high-pitched whistling noise made when narrowed airway passages cause difficulty in breathing.

wound vacuum A wound dressing that applies negative pressure to an acute or chronic wound bed to promote wound healing.

zoonotic Pertaining to diseases or conditions usually affecting vertebrates other than humans; however, humans can become infected after contact, either direct or indirect, with infected animals, their tissues, or their excrement.

zoophilic Pertaining to a parasite or fungus with a preference for animals over humans.

zygomycosis An infection caused by fungi belonging to the zygomycetes; typically seen in either diabetics or immunocompromised hosts.

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