UNIT THREE
SYSTEMIC BACTERIOLOGY

- The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism.
- This interaction is affected by the host immune status and microbial virulence factors.
- Signs and symptoms vary according to the site and severity of infection.
- Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

Specimen collection, storage and transportation

- **Types of specimen**
- Specimen includes:
  - **Cerebrospinal fluid (CSF)**
  - **Feces**
  - **Sputum**
  - **Swabs of Various Fluids**
  - **Blood**
  - **Urine, etc**

Microbiologic Examination

1. **Direct Examination**

- Provides the most rapid indication of microbial infection.

Eg. microscopic, immunologic, and hybridization techniques

2. **Culturing (cultivation)-** is the growing of the isolated microbe on a specific media into large number

Culture media – is a growth media which support the growth of microorganisms or cells in the laboratory

- culture media can be classified based on
  - **physical states**
  - **functional properties**
  - **chemical composition**

A. Physical state

I . Liquid media

- ✓ Do not contain any solidifying agent
- ✓ Used for propagation of large number of organisms, fermentation studies

e..g. nutrient broth, citrate broth, glucose

II. Solid media

- Contain solidifying agent such as agar, silica gel or gelatin

e. g. Nutrient agar, Blood agar and Sabouraud's agar

- used for developing surface colony growth of bacteria and moulds

Gelatin as solidifying agent

- Koch used gelatin as a solidifying agent to grow *Bacillus anthracis*
  - Drawbacks – it melts at 25 0 c
    - some microbes used as a source of protein

Agar as a solidifying agent – produced from sea weed

- has a number of Advantages
  - ✓ Has melting point above 100 oc
  - ✓ Solidify at 45 oc
  - ✓ It is totally inert with no nutritive value and indigestible by microorganisms
  - ✓ Allow clear observation of microbial colony

I I I . **Semisolid media**

- ✓ Fall between liquid and solid media
- ✓ Useful in determining weather certain bacteria are motile or not

B . **Culture media classification based on chemical compositions**

I . Synthetic media

- Type of media in which all constituents are chemically defined

  e. g. Nutrient agar

- Used to study specific nutritional requirements of micro organisms

e. g. E. coli can grow on minimal media since it can synthesize the remaining nutrients

I I . Complex media

- type of media in which there is one or more nutrients that is chemically undefined
- Usually extracted from biological materials e. g. Beef extract, peptone (casein) extract, yeast extract
- Satisfy the growth of most microorganisms

I I I . **Natural media**

- Naturally available and used as growth media with out modification

  e .g. milk

C. Culture media classification based on their functional property

I . Simple media

- Allow the growth of all micro organisms e. g. nutrient agar

I I . Differential media

- Used to differentiate a specific micro organisms from a given mixed population

e .g. Blood agar serve as a substrate for some microbes – S. pyogens which lysis blood

Eosin methyline blue (EMB) agar differentiate lactose fermenters from non lactose fermenters of enteric bacteria

I I I . Selective media

- Allow the growth of some bacteria and inhibit others

e .g. Bismuth sulfite agar, bile salt etc

IV. Selective - differential media

- ✓ Differentiate and select a specific microorganism from a given mixed population

e. g. mac Ckoneky's agar

- ✓ Selectively grow gram negative bacteria and inhibit gram positive bacteria
- ✓ Differentiate lactose fermenters from non lactose fermenters

V . Enrichment medium

- ✓ Unlike the above types of media, this medium may involve chemical, physiological, nutritional and environmental factors
- ✓ Is used to enrich the required microbe

E.g. suppose you want to grow thermopiles, then incubate the culture at high temperature

. Nitrogen fixing bacteria can get nitrogen from the atmosphere

. Suppose a sample is contaminated with fungi, bacteria, algae and protozoa, if pencilliin is added to the medium bacteria will be eliminated

V I . Transport medium

- Used to transport samples (urine, swab, sputum, blood) to laboratory
- It helps the organism to survive until used for diagnosis especially the pathogens

e. g. -Glycerol saline – used for stool especially for dysentery bacilli test

- Bile peptone used to transport *Vibrio cholerae*

- Cary- Blair medium for faeces specimen – *Salmonella, Shigella, Campyliobacter*

VII. **Anaerobic media** – used to grow anaerobic microorganisms

e. g. Brewer's anaerobic agar

- solid media for culturing anaerobic bacteria in Petri dish

. Gas Pack anaerobic jar – generate hydrogen that removes oxygen from the media

VIII. **Mycobacterium culture media**

Bacterial staining technique

- Stains are generally salts in which one of the ion is coloured and the other is colourless
- Stains are chemicals containing chromophore, groups that impart colour - e.g, methylene blue

Types of stain (based on their charges)

1. Basic stain

- The cation is positively charged
- React with negatively charged materials such as the cytoplasm of bacteria

  e.g. crystal violet (cv), safranin, methylene blue, basic fuchsine

2. Acid stain

- ✓ Have negatively charged chromophore
- ✓ Used to stain the background of the cell and leave the cell transparent

e.g. Nigrosine, Congo red

3. Neutral stains

- ✓ Neither the positive charge nor the negative charge has chromophore

e.g. Indian ink

Staining types (function)

1. Simple stains

- Non specific stain
- React with all microbes in identical fashion
- Consists of only one dye
- Used to increase contrast to that morphology, size, etc

e.g. MB, CV, Safranin

2. Differential stain

- Is specific
- More than one dyes used

e.g. Gram's stain, spore stain, capsule stain, flagella stain, Acid fast stain

Gram`s Staining

- Described in 1884, Christian Gram , a Danish bacteriologist
- It divides bacteria in to two groups:- the gram positive and gram negative bacteria
- Four stains used – CV, GI ,Alcohol, Safranin
- **Principle:-** gram positive bacteria have thick cell well which do not decolorized by alcohol whereas gram negative bacteria have thin cell well which easly decolorized

Procedure for gram staining

1) Place the air dried and fixed smear on a staining rack and apply crystal (gentian) violet. Allow to act for one minute and wash with clean water.
2) Apply Gram `s iodine (lugol`s iodine) solution. Allow it for 30-60 seconds and wash with clean water(tape water can be used)
3) Apply decolorizing (95% ethanol or acetone). Allow to act for 15 to 30 seconds. Then wash with clean water. Time of decolonization could be prolonged up to one minute for thick smears to decolorize it effectively.
4) Apply safranin (counter stain). Allow to act for 30-60 seconds and wash with clean water.

- Air dried the slides or using blotting papers.
- The slide is now ready for examination.
- Use the oil immersion objective and examine all the elements of the smear changing your field until you exhaust the whole area of the smear.

Report of gram stained smear includes

- The number of bacteria present (many ,moderate, few, scanty)
- The gram reaction of bacteria (Gram positive or gram negative)
- The morphology of bacteria (cocci ,diplococcic, streptococci, rods or coccobacilli) and other
- Location of the bacteria (as extracellular or intracellular)
- The presence and number of pus cells
- The presence of yeast cells and epithelial cells

Note:* During gram staining the following elements are gram negative in their reaction(red or pink color)

- Gram negative bacteria
- Nuclei of pus cells
- Epithelial cells

- Yeast cells and gram positive bacteria are gram positive during gram staining(blue color)

Results of Gram stain

- Gram positive…… Dark purple/blue
- Gram negative……pink to dark red
- Yeast cells……dark purple/blue
- Nuclei of pus cells……red
- Epithelial cells……pale red

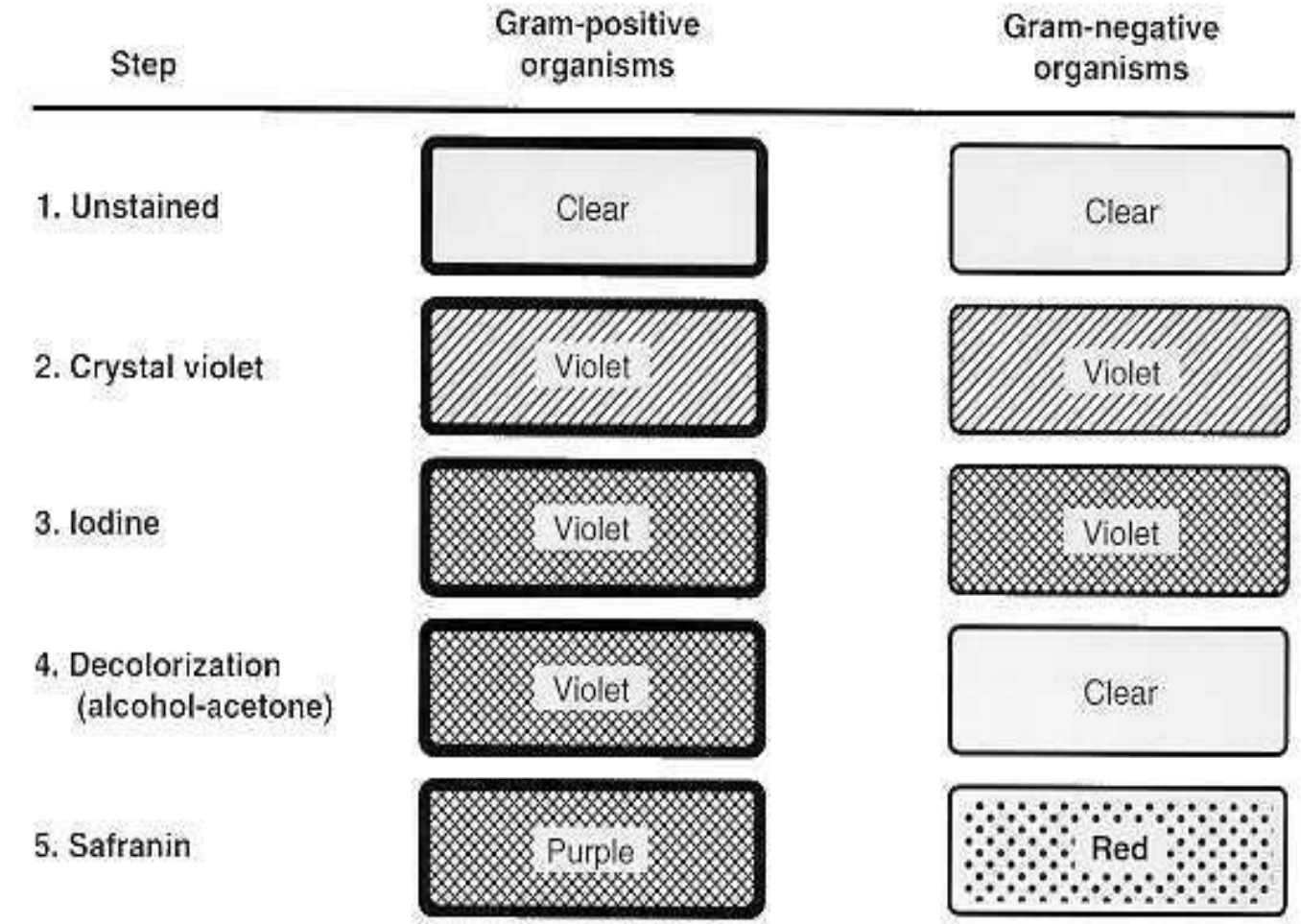
- Gram-positive and Gram-negative bacteria are chiefly differentiated by their cell wall structure.

GRAM POSTIVE BACTERIA

- **Gram-positive** bacteria are those that are stained dark blue or violet by Gram staining
- Retain the crystal violet stain b/c of the high amount of peptidoglycan in the cell wall

GRAM NEGATIVE BACTERIA

- **D**o not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counterstain (commonly safranin) is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color.
- The pathogenic capability of Gram-negative bacteria is often associated with certain components of Gram-negative cell walls, in particular, the lipopolysaccharide layer (also known as LPS or endotoxin layer)


Step
Gram-positive organisms
Gram-negative organisms
1. Unstained
Clear
Clear
2. Crystal violet
Violet
Violet
3. Iodine
Violet
Violet
4. Decolorization (alcohol-acetone)
Violet
Clear
5. Safranin
Purple
Red

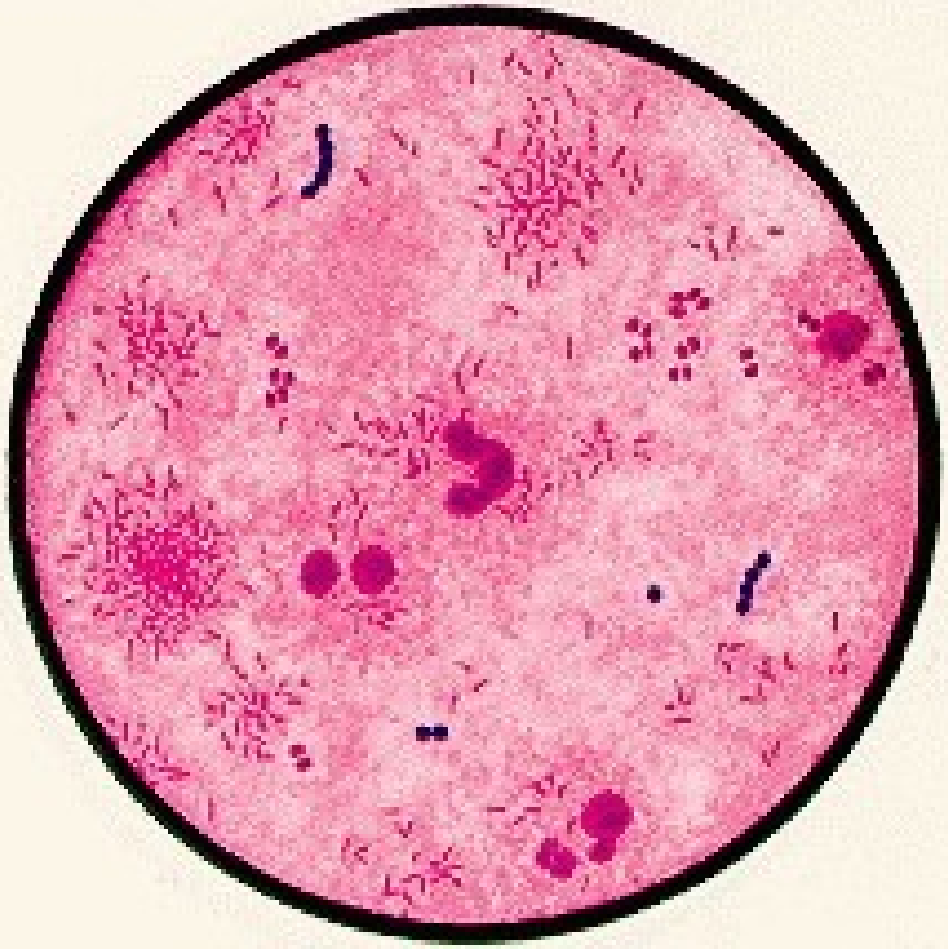
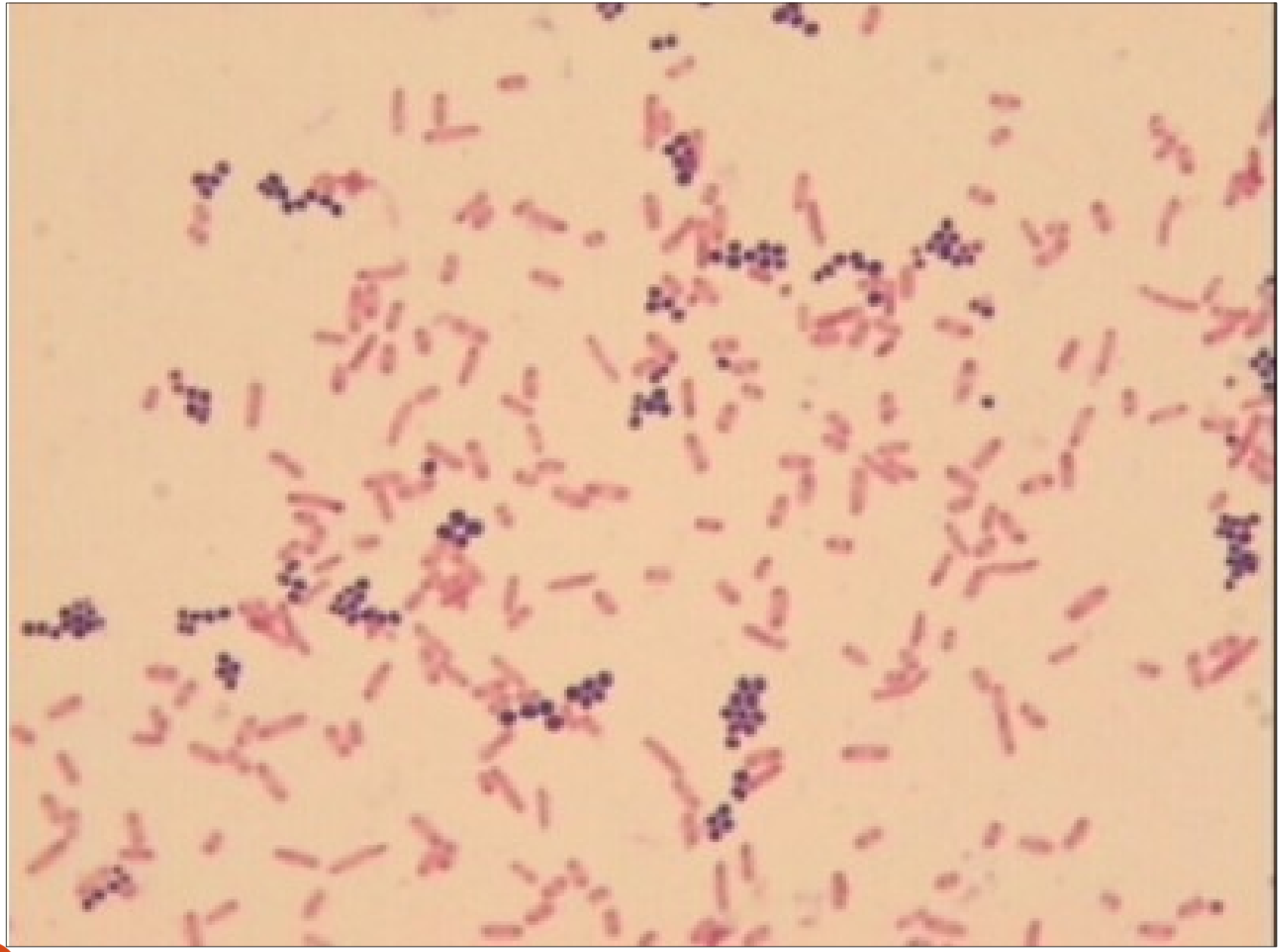


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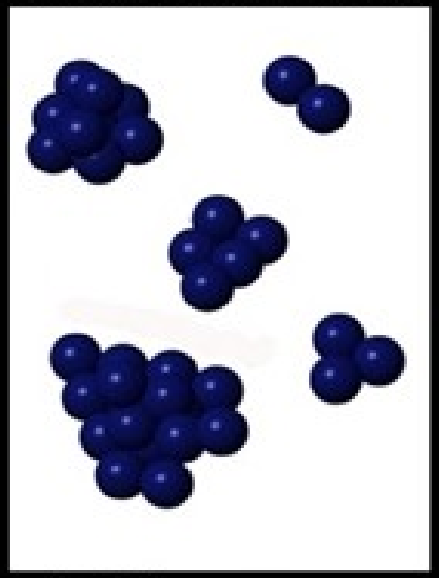
× 1000. *Gram's stain.*

Film of sputum showing Gram-negative **Hæmophilus influenzæ (Pfeiffer's bacillus)**, **Diplococcus catarrhalis** and a Gram-positive streptococcust Note the small size of H. influenzæ and arrangement in clumps.

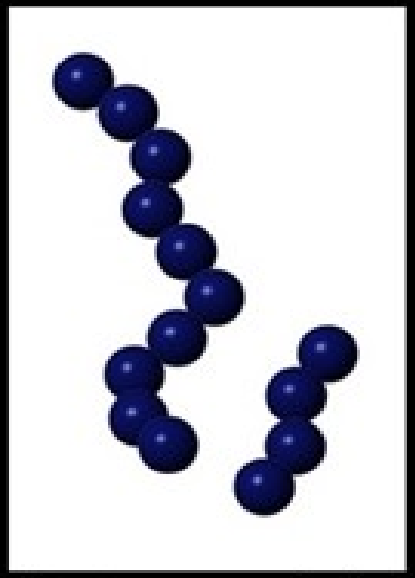


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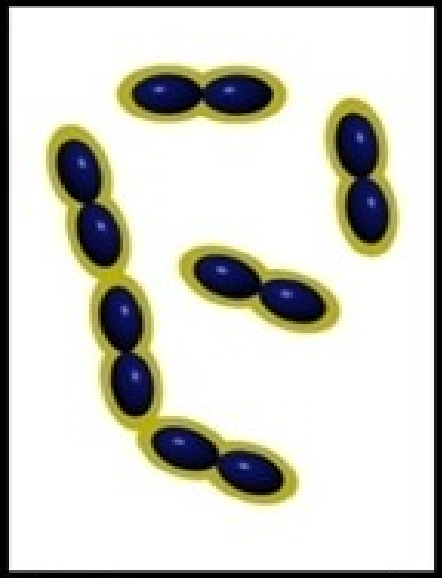
GRAM - POSITIVE



Staphylococcus aureus



Streptococcus agalactiae

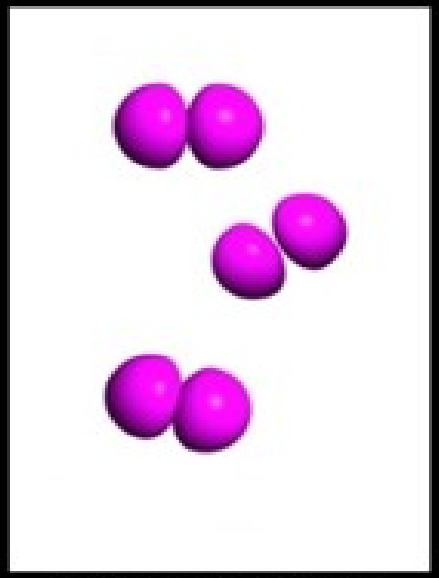


Streptococcus pneumoniae



Listeria monocytogenes

GRAM - NEGATIVE



Neisseria meningitidis



Haemophilus influenzae



Klebsiella pneumoniae



Escherichia coli

The spore stain

- Spore are produced by some bacterial genera such as Bacillus and clostridium
- Spores allow the bacteria to resist adverse environmental conditions- desiccation, extreme heat, chemicals etc
- Spores are difficult to be stained by ordinary dyes
  - Stained by hot dye but can not easily decolorized

Capsule stain

- staining the back ground with acidic stain and the cell with basic stain in which the capsule remains unstained and transparent

Flagella stain

- Flagella are fine thread like organells of locomotion
- Flagella stain provide information about the presence and location of flagella

- great value in bacterial classification

e.g. tannic acid, sodium chloride as primary dye and MB as counterstain

Acid fast stain

- Developed by France Ziehl and Friedrich Neelsen
- Acid fastness is a property of the *Mycobacterium tuberculosis* and related organisms (Cryptosporidium)
- Carbolfuchisin is a primary dye and Methylene blue is a counter stain
- Acid-fast bacteria take stains poorly but once stained retain it strongly
- They are not easily decolourised by acid-alcohol
- The acid fastness is due to the high lipid content (mycolic acid) in the cell wall
- Acid fast stain organism appear – red and non acid fast stain organism appear- blue

Ziehel Neelsen staining (Acid fast staining)

- This enables to differentiate between acid fast bacteria such as Tubercle bacilli and leprous bacilli from non-acid fast bacilli.
- Resistance for decolonization with acid and termed as acid-fast
- This acid fastness property is due to the presence of high lipid in their cell wall (mycolic acid)

Principle:-

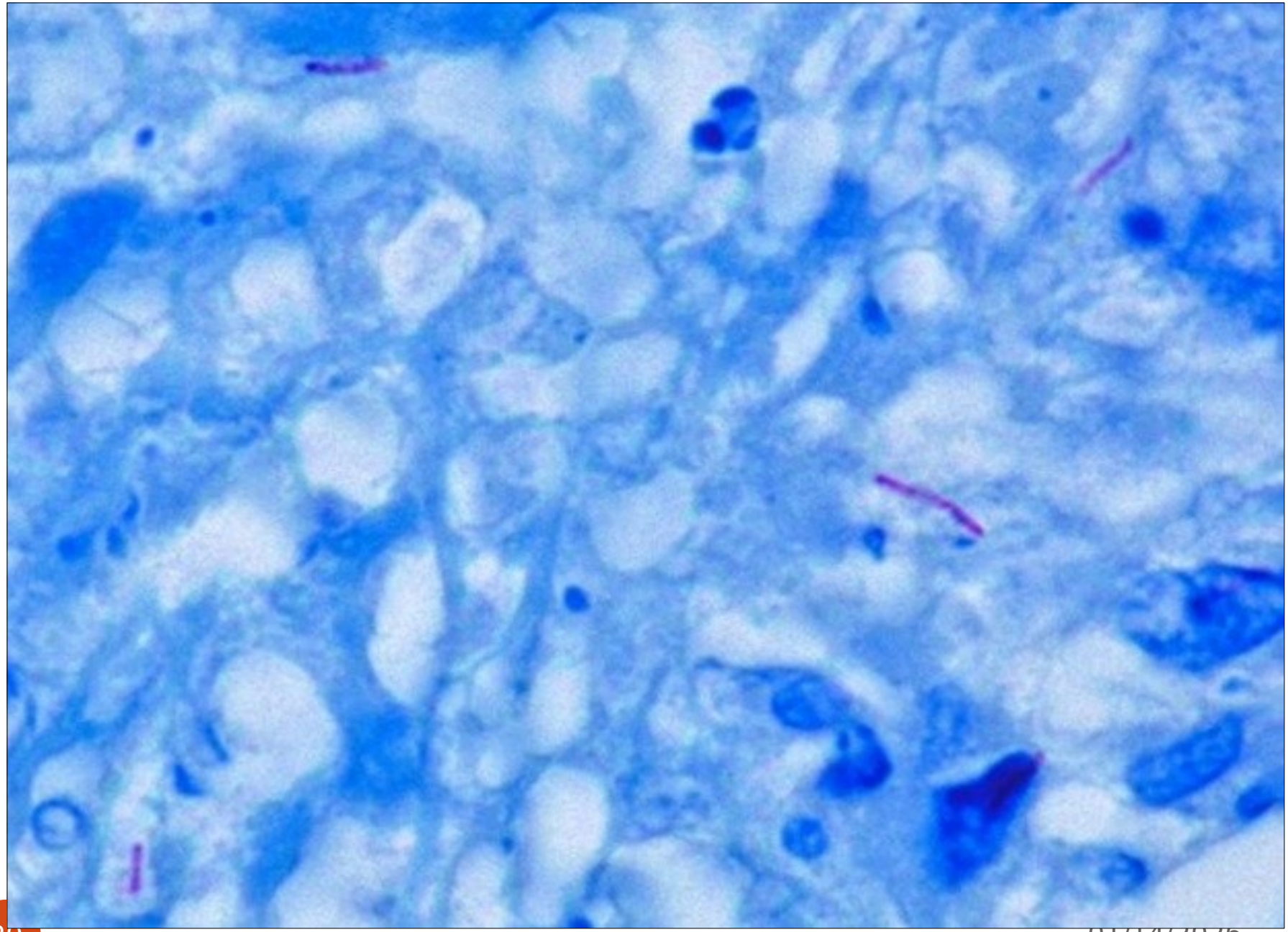
- The application of primary dye, carbol fuchsin, will stain all the cells uniformly pink.
- During the application of acid –alcohol (decolorizing agent) after washing with distilled water decolorizes all bacteria except cid fast bacteria.
- The application of counter stain (methylene blue), stains those bacteria decolorized by acid alcohol appearing blue.

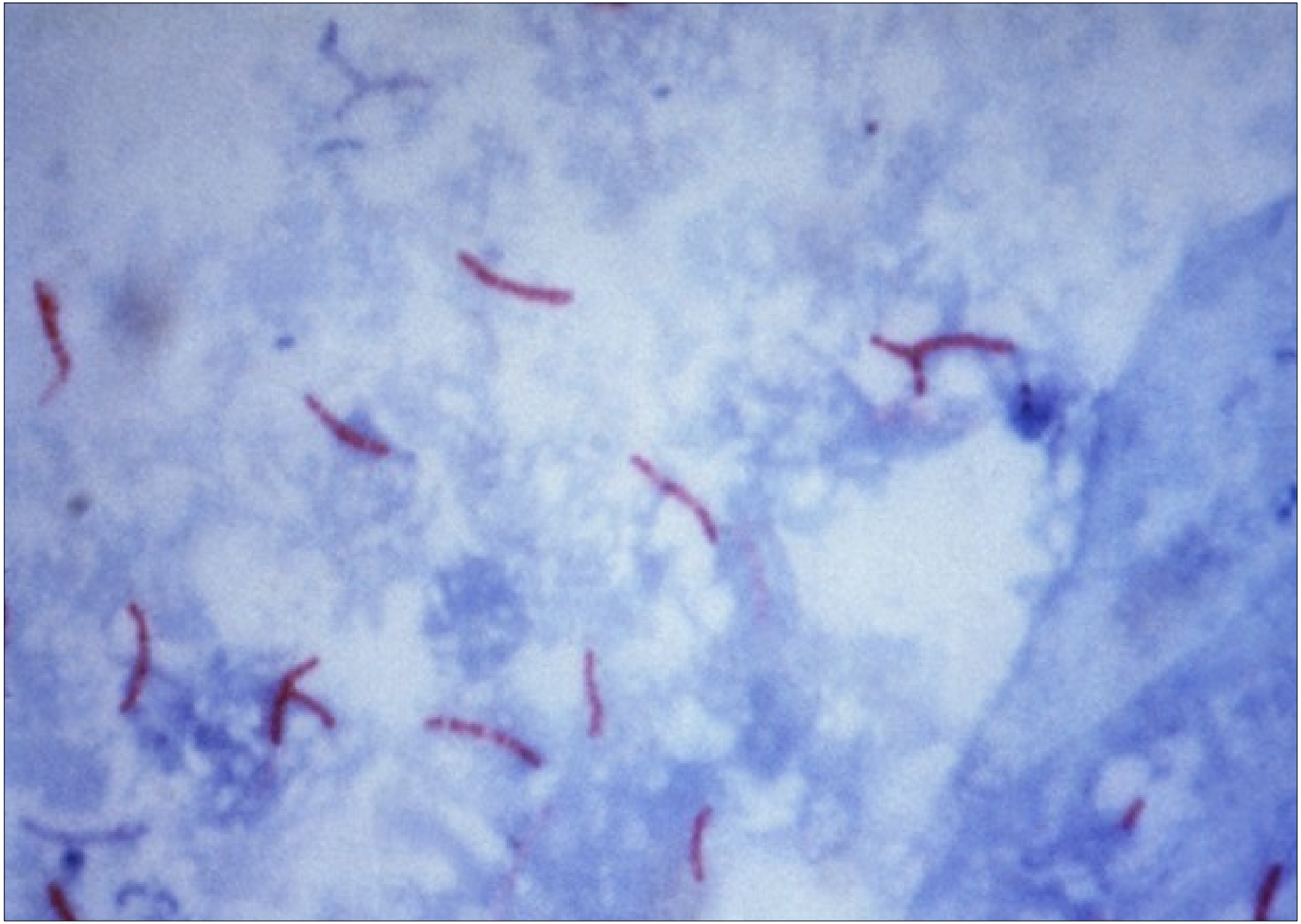
Procedure for acid fast staining

1) Place the air dried and fixed sputum smear on a staining rack and cover the slide with basic fuchsin
2) Flame cotton soaked in alcohol and heats the slide from below till you see a steam coming out of the slides use low and intermittent heat to maintain steaming for 5 minutes
3) Cool and wash with distilled water until a clean water runs off
4) Decolorize by applying 3% Hcl in 95% ethanol for 3 minutes if 3% Hcl is not available use 20% H_2SO_4 as follows. Apply the 20% H_2SO_4 for one minute and wash with water repeating this for ten minutes. Wash the slide well and apply 95% ethanol for 2 minutes and wash with water
5) Counter stain by applying methylene blue or malachite green (0.2%) for one minute. Wash with tape water for one minute continuously and drain off the water. Leave the slide to air dry. The slide is now ready for examination of using oil immersion objective

Reporting of results for acid fast stained, sputum smear

- If you observe one or two AFB, request further specimen from the patient
- If not AFB is seen after examining the whole area of the smear ,report it as No AFB seen .Don`t report Negative
- Number of AFB found
  Report
- More than 10 AFB/ 20 field .....3+
- 1-9 AFB /50 field.....2+
- 10-100 AFB /100 field .....1+
- 1- 9 AFB/100 field .....exact number seen





Microscopy: - M. leprae bacilli are non – motile, non – sporing striaght or slightly curved organisms

- They are typically seen tightly packed in macrophage cells thought single extracelluar ones are also seen. The packed bacilli are referred to as **globi**
- the leprosy bacilli are acid fast but less so than the M. tuberculosis and so a weaker solution of acid is used for decolorisation
- ideally, two smears should be stained by the ZN method, the one with a strong acid decolouriser (e.g. 20%H_2SO_4) and other with a weak acid decolouriser (5% H_2SO_4)
- M. leprae may thus be distinguished from M. tuberculosis as it will be acid fast only in the 5% H_2SO_4

Reporting of smears: - the method of reporting M. leprae smears is to record the total number of bacilli in smears as a bacterial index (BI)

- The BI is used to monitor progress and treatment of the patient

Technique for BI: - the number of bacilli in a globus may be difficult to be accurately counted, so it is usually assumed that:

- A large globus contains about 100 organisms
- A medium globus ontains about 60 organisms
- A small globus contains about 30 organisms

1) if bacteria are found in almost every oil immersion field estimate the number of bacilli in each field and record as follows
   - >1000 per field = BI-6
   - 100 – 1000 per field = BI-5
   - 10 – 100 per field = BI-4
   - 1- 10 per field = BI-3
2) if bacteria are not found in almost every oil immersion field systematically examine the smear and record as follows
   - 1 – 10 bacilli per 10 fields = BI-2
   - 1 – 10 bacilli per 100 fields = BI-1
   - No bacilli in 100 fields = BI-0

GROUP CHARACTERISTICS

- Have a marked tendency to form clusters (from the Greek staphyle, bunch of grapes)
- Some single cells, pairs, and short chains are also seen
- Typical Gram-positive cell wall structure.
- Are nonflagellate, nonmotile, and non-spore-forming
- Grow best aerobically but are facultatively anaerobic
- In contrast to streptococci, produce catalase
- Three are of major medical importance: *S. aureus, S. epidermidis, and S. saprophyticus*
- The ability of *S. aureus to form coagulase separates it from the* other, less virulent species

- **Grouping for Clinical Purposes**

1. Coagulase positive Staphylococci

- **Staphylococcus aureus**

2. Coagulase negative Staphylococci

- **Staphylococcus epidermidis**
- **Staphylococcus saprophyticus**

Staphylococcus aureus

- *S. aureus* are uniformly Gram-positive and regular in size,
- On aging become more variable in size, and many lose their Gram positivity
- Optimum temperature of 37°C
- Facultative anaerobe
- Withstands high salt - grow on media containing 5%-7.5% NaCl (e.g. Mannitol Salt Agar)
- Resist extreme pH, and temperatures
- Colonies are white or golden and hemolytic
- It is coagulase posetive and produces a fibrin clot
- Carried in nasopharynx and skin
- Produces many virulence factors

TABLE 22–3. *Staphylococcus aureus* Virulence Factors

Virulence Factors	Biologic Effects
Structural Components	
Capsule	Inhibits chemotaxis and phagocytosis; inhibits proliferation of mononuclear cells; facilitates adherence to foreign bodies
Peptidoglycan	Provides osmotic stability; stimulates production of endogenous pyrogen (endotoxin-like activity); leukocyte chemoattractant (abscess formation); inhibits phagocytosis
Teichoic acid	Regulates cationic concentration at cell membrane; binds to fibronectin
Protein A	Inhibits antibody-mediated clearance by binding IgG_1, IgG_2, and IgG_4 Fc receptors; leukocyte chemoattractant; anticomplementary
Cytoplasmic membrane	Osmotic barrier; regulates transport into and out of cell; site of biosynthetic and respiratory enzymes
Toxins	
Cytotoxins (α, β, δ, γ, P-V leukocidin)	Toxic for many cells, including leukocytes, erythrocytes, macrophages, platelets, and fibroblasts
Exfoliative toxins (ETA, ETB)	Serine proteases that split the intercellular bridges in the stratum granulosum epidermis
Enterotoxins (A–E, G–I)	Superantigens (stimulates proliferation of T cells and release of cytokines); stimulates release of inflammatory mediators in mast cells, increasing intestinal peristalsis and fluid loss, as well as nausea and vomiting
Toxic Shock Syndrome Toxin-1	Superantigen (stimulates proliferation of T cells and release of cytokines); produces leakage or cellular destruction of endothelial cells
Enzymes	
Coagulase	Converts fibrinogen to fibrin
Catalase	Catalyzes removal of hydrogen peroxide
Hyaluronidase	Hydrolyzes hyaluronic acids in connective tissue, promoting the spread of staphylococci in tissue
Fibrinolysin	Dissolves fibrin clots
Lipases	Hydrolyzes lipids
Nucleases	Hydrolyzes DNA
Penicillinase	Hydrolyzes penicillins

Virulence factors of *S. aureus*

1. Structural components

- **Capsule or polysaccharide slime layer**: This inhibits chemotaxis and phagocytosis
- **peptidoglycan** interspersed with molecules of a ribitol-teichoic acid, which is antigenic
- **Protein A** is a cell wall component *that binds to the FC portion of IgG molecules*
  - prevents "correct"binding of opsonizing antibodies, thus hindering phagocytosis

2. *Toxins*

a. **α - toxin-** is heterogeneous protein

- a potent hemolysin

β- toxin- is a sphingomyelinase which damages membranes rich in this lipid including human red blood cells

- **δ (delta) toxin-** disrupts biological membrane
- γ (gamma) **-toxin and leukocidin -**are two-component protein toxins
  - capable of lysing white blood cells of human and rabbits

b. Exfoliative toxins: epedermolytic toxin

- are two antigenically distinct forms of the toxin, ETA and ETB
  - ETA- is a chromosomal gene product and is heat stable
  - ETB- is a plasmid mediated and heat labile

- causes the scalded skin syndrome in neonates
- Dissolving the mucopolysaccaride matrix of the epidermis

C. Enterotoxins – different serotypes A-E,H, G and I

- are super antigens
- are heat stable and resistant to the action of gut enzymes
- cause of food poisoning
- are produced when *S. aureeus* grows in carbohydrates and protein foods
- Ingestion of 25µg resulting vomiting and diarrhea
- Vomiting is stimulated by neural mechanism

d. Toxic shock syndrome toxin 1 (TSST-1)

- is a super antigen
- stimulate the release of cytokines

3. **Enzymes**

- **Catalase** – converts hydrogen peroxide into oxygen and water
- Coagulase –enzyme like protein that clots plasma
  - ✓ binds to prothrombin and initiates fibrin polymerization
- **Hyaluronidase** – digests connective tissue
- **Staphylokinase** – digests blood clots
- **DNase** – digests DNA
- **Lipases** – digest oils; enhances colonization on skin
- **Penicillinase** – inactivates penicillin
- ❖ **Clumbing factor** – is a surface S.aureus compound

  - is responsible for adherence of the

Epidemiology and Pathogenesis

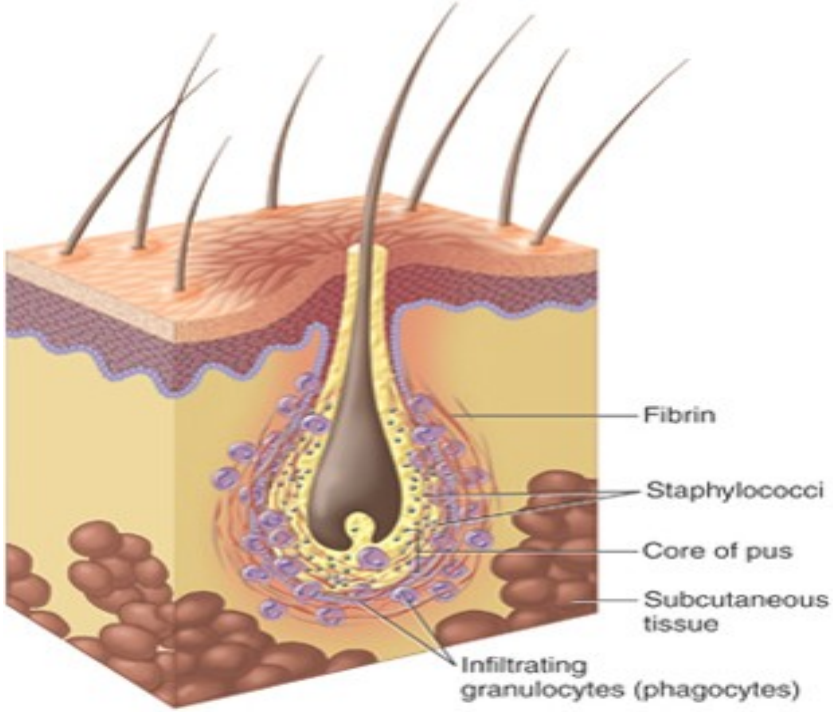
- Present in most environments frequented by humans
- Readily isolated from fomites
- Carriage rate for healthy adults is 20-60%.
- Carriage is mostly in anterior nares, skin, nasopharynx, intestine.
- Predisposition to infection include: poor hygiene and nutrition, tissue injury, preexisting primary infection,immunodeficiency.
- Increase in community acquired methicillin resistance - MRSA

Staphylococcal Disease

Range from localized to systemic

- **Localized cutaneous infections** – invade skin through wounds, follicles, or glands
  - **folliculitis** – superficial inflammation of hair follicle; usually resolved with no complications but can progress
  - **furuncle** – boil; inflammation of hair follicle or sebaceous gland progresses into abscess or pustule
  - **carbuncle** – larger and deeper lesion created by aggregation and interconnection of a cluster of furuncles
  - **impetigo –** bubble-like swellings that can break and peel away; most common in newborns

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(a) Sectional view of a boil or furuncle, a single pustule that develops in a hair follicle or gland and is the classic lesion of the species. The inflamed infection site becomes abscessed when masses of phagocytes, bacteria, and fluid are walled off by fibrin.



(b) A furuncle on the back of the hand. This lesion forms in a single follicle.
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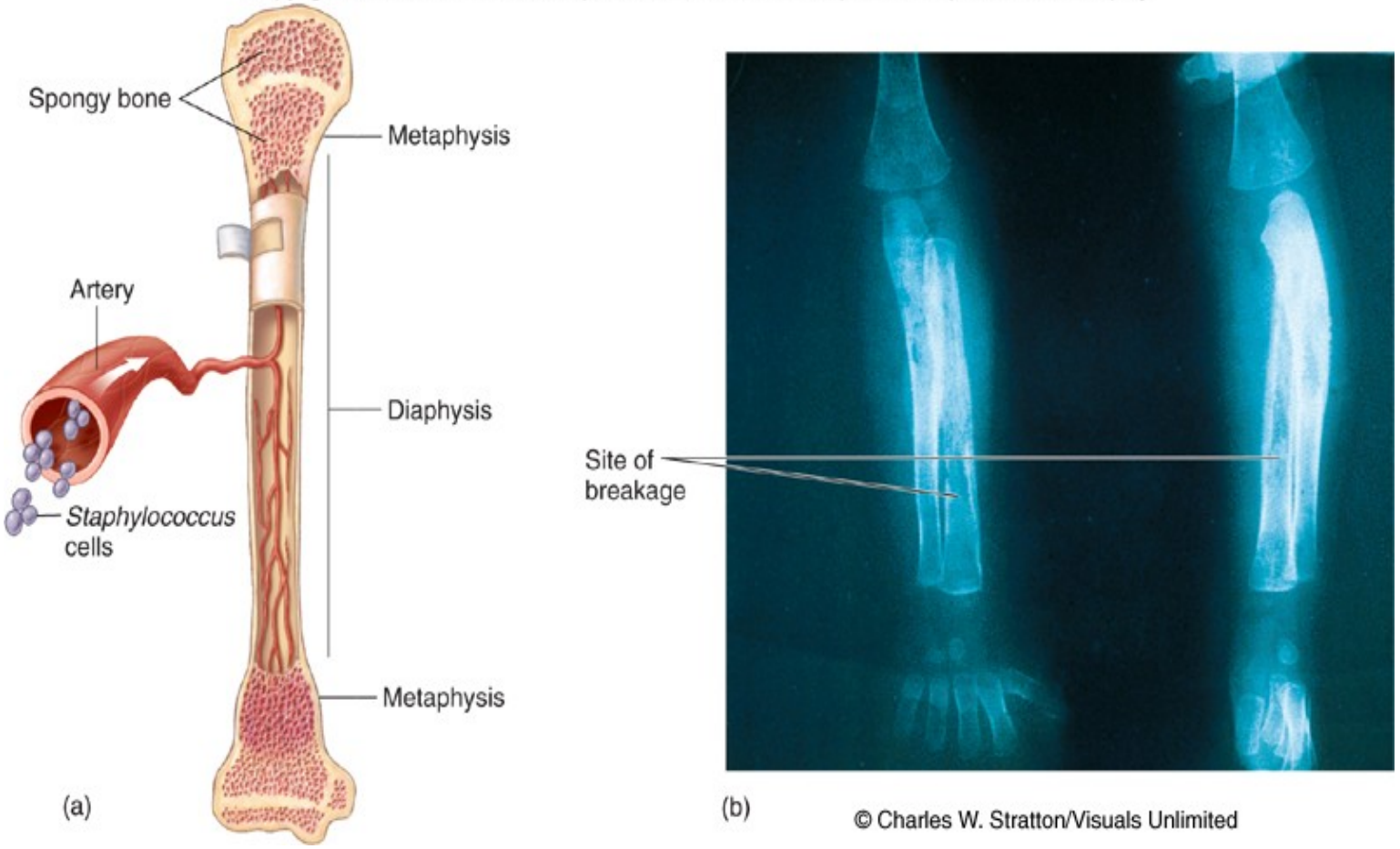
(c) A carbuncle on the back of the neck. Carbuncles are massive deep lesions that result from multiple, interconnecting furuncles. Swelling and rupture into the surrounding tissues can be marked.
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Furuncles in a patient with type 2 diabetes mellitus.

- **Systemic infections**
  - **osteomyelitis** – infection is established in the metaphysis; abscess forms
  - **bacteremia -** primary origin is bacteria from another infected site or medical devices; endocarditis possible

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- **Toxigenic disease**
  - **food intoxication** – ingestion of heat stable enterotoxins; gastrointestinal distress
  - **staphylococcal scalded skin syndrome** – toxin induces bright red flush, blisters, then desquamation of the epidermis
  - **toxic shock syndrome** – toxemia leading to shock and organ failure

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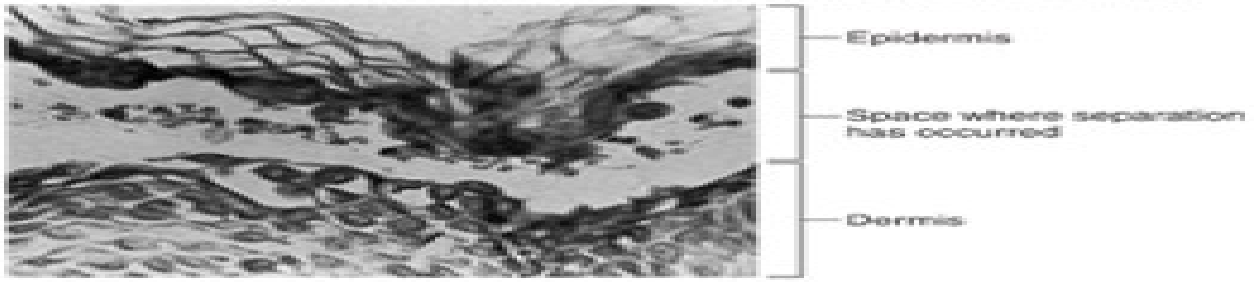
(a)

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(b)

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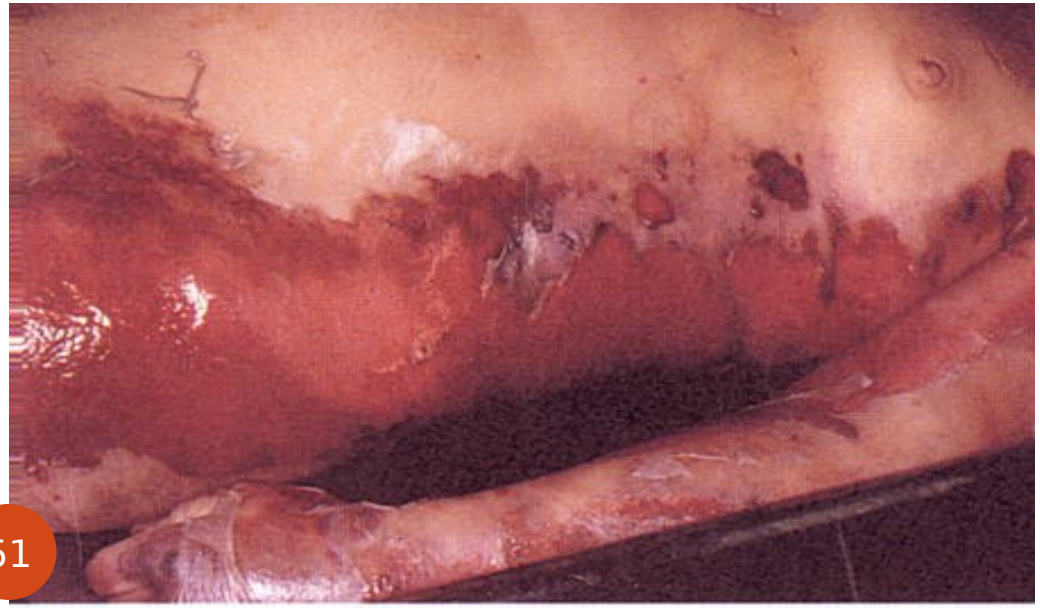
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Other Staphylococci

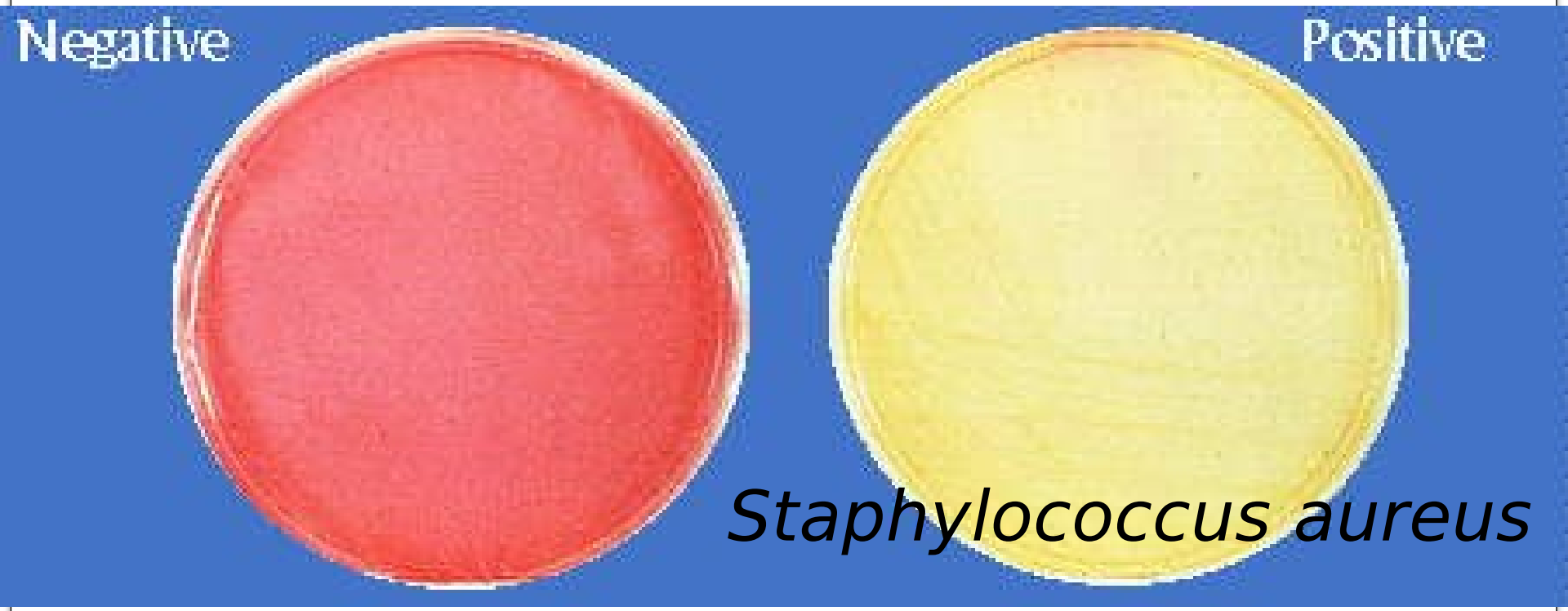
Coagulase-negative staphylococcus; frequently involved in nosocomial and opportunistic infections

- *S. epidermidis* – lives on skin and mucous membranes; endocarditis, bacteremia, UTI
- *S. hominis* – lives around apocrine sweat glands
- *S. capitis* – live on scalp, face, external ear
- All 3 may cause wound infections by penetrating through broken skin.
- *S. saprophyticus* – infrequently lives on skin, intestine, vagina; UTI- in sexually active woman

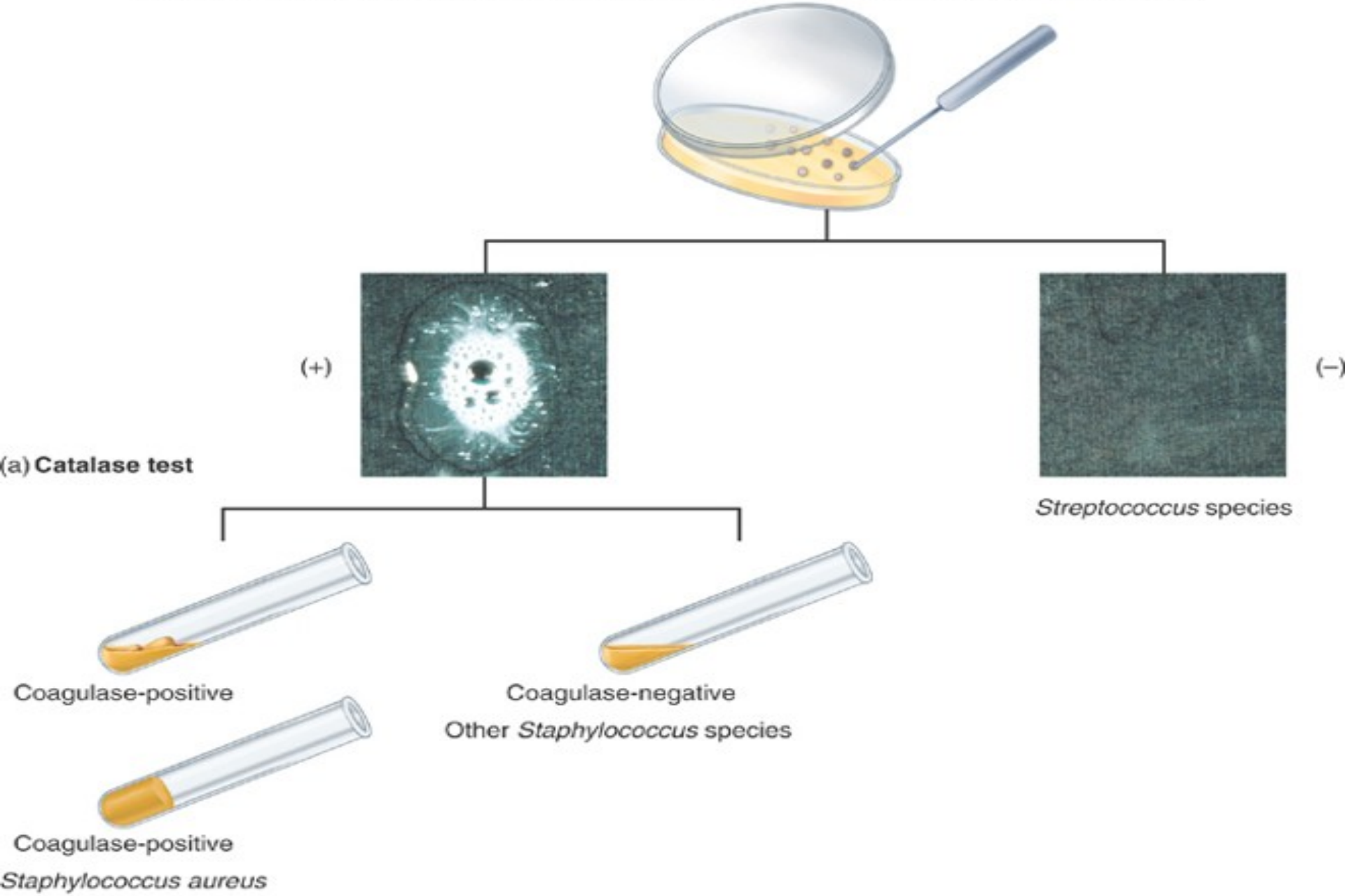
Identification of Staphylococcus in Samples

- Frequently isolated from pus, tissue exudates, sputum, urine, and blood
- Microscopy, Cultivation, catalase, coagulase
- Microscopy- Smears from staphylococcal lesions reveal gram-positive cocci in grapelike clusters.
- Culture- On blood agar inoculation of S. aureus typically yields golden-yellow colonies that are usually beta-haemolytic

Mannitol Salts Agar (MSA)



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Clinical Concerns and Treatment

- 95% have penicillinase and are resistant to penicillin and ampicillin.
- current antibiotic of choice are oxacillin and for oxacillin – resistant strains, vancomycin can be given
- Most strains produce β - lactamase and many are methicillin/ nafcillin – resistant
- Abscesses have to be surgically perforated.
- Systemic infections require intensive lengthy therapy.

Prevention of Staphylococcal Infections

- Universal precautions by healthcare providers to prevent nosocomial infections
- Hygiene and cleansing
- Eg. Clothes and bedding that may cause reinfection should be washed at a sufficiently high temperature to destroy staphylococci (70°C or higher) or dry-cleaned.

Genus Streptococcus

- Gram positive cocci
- Occur characteristically in short chains, but also in pairs and singly
- Non-motile
- Some strains are capsulated
- Grow as small gray colonies on sheep blood `agar
- May exhibit alpha-, gamma or beta-hemolysis, or they may be non-hemolytic.
- Show more robust growth under anaerobic than aerobic conditions
- Are catalase negative (the enterococci are catalase negative or weakly positive).

- Obligate anaerobes and the facultative anaerobes
- Traditional classification depends on the type of haemolysis produced on blood agar media
- Three type of haemolysis are recognized
  - Alpha (α) haemolytic streptococci: partial haemolysis(green discoloration)
  - Beta (B) haemolytic streptococci: clear zone of complete haemolysis
  - Gamma (γ) or non haemolytic streptococci: no discoloration

Lance field groups of streptococci

- In 1933 lance field classified based on the nature of specific carbohydrate antigen or C-substance on the bacterial cell well
- This antigen can be identified using group specific antiserum
- The carbohydrate identification enables a strain to be placed in one of the 20 lance field groups designated A-V excluding I and J
- **Normal Habitat: -** streptococci are commonly found in water, dust, vegetation, milk and milk products. Upper respiratory tract, intestinal tract, and female genital tract.

Pathogenicity

- **S. pyogens (group A):-** causes:-
  1) Respiratory infections
     - Acute sore throat (tonsillitis and pharyngitis)
     - peritonslar abscess
  2) Ear infections:- otitis media
  3) Skin infections
  4) Puerperal sepsis (genital infections)
  5) Septicemia –bacteria/toxins/ destroy tissue
  6) Endocarditic
  7) Abscesses of organs such as brain lung liver and kidney

Virulence Factors

i) Streptolysin toxin(exotoxin):- lyses RBC

- **Streptolysin S** – active anearobically. It is non-antigenic
- **Streptolysin O** – heamolyses RBC under anearobic conditions. It is antigenic, stimulating the production of antistreptolysin O antibody (ASO)

ii) M-Protein (antigen): antiphagocytic , inhibits opsonization by the alternative complement pathway by binding to host complement regulators

iii) Lipotheichoic acid: facilitates adherence to pharyngeal epithelial cells.

iv) NADase(nicotinamide adenine dinucleotidase) – kill leucocytes

v) Leukocidin: kills leucocytes

vi) Erythrogenic toxin/ Streptococcal pyogenic exotoxin A (SpeA) and C(SpeC): responsible for rash seen in scarlet fever and associated with toxic skock syndrome.

vii) C5a peptidase:- cleaves a potent neutrophil chemotaxin called C5a

viii) Streptokinase (fibrinolysin); cause fibrinolysis

ix) Hyaluronidase (antigenic): facilitates spread in the tissue by destroying hyaluronic acid.

x) Deoxyribonuclease (DNAase)/ Streptodornase: causes DNA break down

xi) M protein, lipoteichoic acid and pretein F are also used for adherence

S. agalactiae (Group – B)

- Cause bovine mastitis, cattle. In humans, they cause the following disease
- **virulence factor**: *S. agalactiae's* polysaccharide antiphagocytic capsule is its main virulence factor
- Causes:- Septic abortion and puerperal or gynaecological sepsis, occasionally UTI, neonatal septicaemia and meningitis.

Viridians Streptococci

- This group of streptococci produces alpha haemolysis on blood agar
- Members of the group include:- S. salivarius, S. mutans, S. mitis, & S. milleri
- They are part of the resident normal flora of the throat and mouth
- Though normally non pathogenic ,they are capable of causing of sub acute bacterial endocarditic

Identification test for S. Viridians

- Optichin sensitivity test => resistant

S.Mutans infection

- Certain oral streptococci (S. mutans) are the main culprits in tooth decay.



Laboratory Diagnosis of Streptococcus

Specimens: - include throat swab, pus, blood, ear swab, wound swab, CSF (from neonates), urine or any other specimen depending on the site of infection.

Stained smears: - gram positive cocci in short chains, in pairs or singly

Culture: - majority grow aerobically and anaerobically in optimum temperature of growth is 37^0c the colonies generally irregular in outline

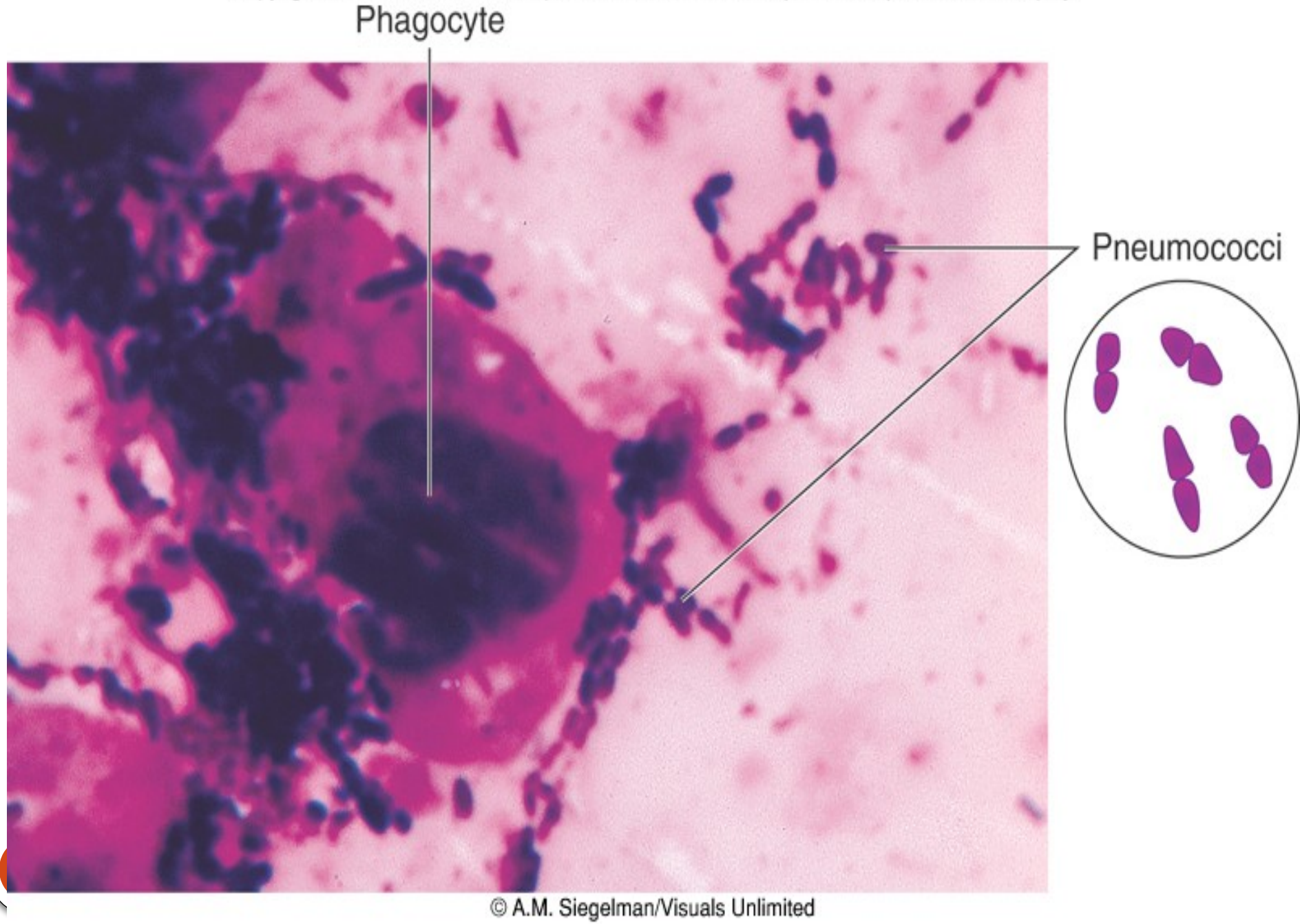
Antibiotic Sensitivity

- Penicillin and erythromycin are very active against S. pyogens and S. agalactiae. Entrococcus are sensitive to ampicillin but resistant to penicillin, erythromycin and co-trimoxazole.

Streptococcus pneumoniae: The Pneumococcus

- Gram positive diplococci
- Small, lancet-shaped cells arranged in pairs and short chains; possessing capsule
- 84 capsular types have been identified
- On blood agar they produce α–hemolysis.
- lysed by bile or deoxycholate and their growth is inhibited by optochin.
- Causes 60-70% of all bacterial pneumonias
- Culture requires blood or chocolate agar.
- Growth improved by 5-10% CO_2
- Lack catalase and peroxidases – cultures die in O_2

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- The predisposing factors that lower resistance of persons to pneumococcal infection are
  - Viral and other respiratory tract infections that damage surface cells
  - Alcohol or drug intoxication which depresses phagocytic activity
  - Abnormal circulatory dynamics
  - Malnutrition , sickle cell anemia ,nephrosis

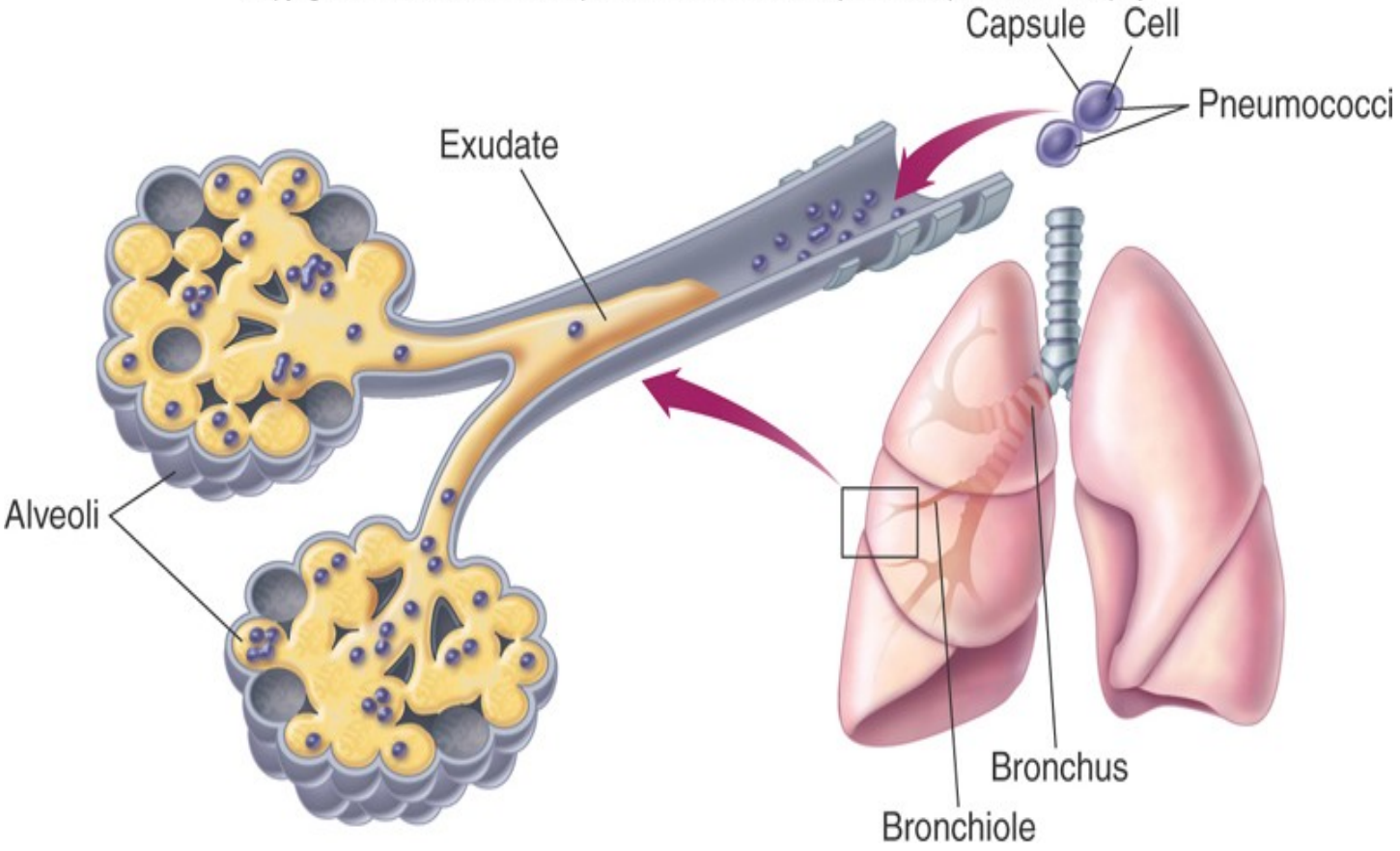
Virulence factors

Virulence factor	Biologic effect
1. Colonization and migration	
Surface protein adhesions	Binds to epithelial cells
Secretary IgA protease	Disrupts secretary IgA mediated clearance
Pneumolysin	Possibly destroys ciliated epithelial cells
2. Tissue destruction	
Teichoic acid and Peptidolglycan fragments	Activates alternative complement pathway
Pneumolysin	Activates classic complement pathway
Hydrogen peroxide	Allows reactive oxygen intermediates to cause damage
Phosphorylcholine	Binds phosphodiesterase activating factor, allowing bacteria to enter host cells
3. Phagocytic survival	
Capsule	Antiphagocytic
eumolysin	Suppresses phagocytic oxidative burst

Epidemiology and Disease

- ***Epidemiology:*** Humans are the natural hosts for pneumococci; there is no animal reservoir.
- 5-50% of all people carry it as normal flora in the nasopharynx; infections are usually endogenous
- **Diseases:** Pneumococci cause pneumonia, bacteremia, meningitis and infections of the upper respiratory tract such as otitis media and sinusitis.

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Cultivation and Diagnosis

- Gram stain of specimen – presumptive identification
- α hemolytic; optochin sensitivity
- Quellung test or capsular swelling reaction

(polyvalent anti-capsular antibodies are mixed with the bacteria)

Treatment and Prevention

- Traditionally treated with penicillin G or V
- In penicillin – allergic patients, erythromycin or azithromycin can be used
- Vancomycin can be used for penicillin resistant pneumococci
- Two vaccines available for high risk individuals:
  - capsular antigen vaccine for older adults and other high risk individuals- effective 5 years
  - conjugate vaccine for children 2 to 23 months

Differentiations of streptococci

	S. pyogens	S. agalactiae	S. Pneumonia	Viridians streptococci	E. faecalis	S.miller
Haemolysis	Beta	Beta	Alpha	Alpha	Beta. /gama	Alpha /gama
Bile solubility	-	-	+	-	-	-
Bacitracinsensitive	+	-	-	-	-	-
Optochin –sensitive	-	-	+	-	-	-
Heat resistance	-	-	-	-	+	-
Growth on 1%bile	-	+	-	-	+	v
Growth 10%bile	-	+	-	-	+	v
Growth 40% bile	-	+	-	-	+	v
Inulin fermentation	-	-	+	-	-	-
Lance field group	A	B	-/O	OK	D	ABFG
Nitro furantion resistance	-	-	-	-	-	+

Aerobic Gram positive rods

- Are Corynebacterium, Listeria, Lactobacillus, & Bacillus

A. Corynebacterium

- Some are useful in industry and others can cause human disease.
- Several species form part of the common microbiota of the human RT and other mucous membranes, the conjunctiva, and the skin.
- *C. diphtheriae* and related organisms collectively coryneforms
- Non-pathogenic species are called "diphtheroids"; two species commonly found in humans are *C. xerosis* and *C. pseudodiphtheriticum*
- Pathogenic species is *Corynebacterium diphtheriae,* which produces a potent exotoxin and causes diphtheria in humans
- Corynebacteria possess capsular (K) and somatic antigens (O)

Morphology & Physiology:

- Small, nonmotile, irregularly staining pleomorphic Gram-positive rods with club-shaped swelled ends but no spores; may be straight or slightly curved.
- The bacteria group together in a characteristic way, which has been described as the form of a "V", "palisades", or "Chinese letters".
- They may also appear elliptical
- May also contain inclusion bodies, known as metachromatic granules.
  - Internal metachromatic granules densely stain ruby red while the rest of the bacillus stains blue
  - Cells appear to be banded or beaded with irregularly staining granules

- Aerobic or facultatively anaerobic
- Fermentative metabolism (carbohydrates to lactic acid); form acid but not gas
- Fastidious; Slow growth on enriched medium
- Catalase, and nitrate positive
- Cell wall containing unusual lipids: meso-diaminopimelic acids; arabino-galactan polymers; short-chain mycolic acids (member of CMN (*Corynebacterium, Mycobacterium, Nocardia)* group)
- *Corynebacterium urealyticum* strongly urease positive

Corynebacterium diphtheriae:

- Causes Diphtheria (respiratory or cutaneous) occurs worldwide
- Carried asymptomicatically in the oropharynx of immune individuals
- Transmitted by respiratory droplets or skin contact
- produces a potent exotoxin and causes diphtheria in humans
- The diphtheria toxin gene is encoded by a bacteriophage found in toxigenic strains, integrated into the bacterial chromosome.
- Four varieties (subspecies)of *C. diphtheriae* colonies may be:
  - *C. diphtheriae mitis*,
  - *C. diphtheriae intermedius*,
  - *C. diphtheriae gravis*, and
  - *C. diphtheriae belfanti*

- Diphtheria toxin can also lead myocarditis, neurologic problems such as paralysis of the soft palate, vision problems, and muscle weakness.
- Diphtheria is acute and contagious infection

Pathogenesis

- Pathogenic type species is *Corynebacterium diphtheriae* produces a potent exotoxin and causes diphtheria in humans
  - Diphtheria A-B exotoxin interrupts peptide formation at the ribosomal level
  - Phospholipase D increases vascular permeability, thus allowing *C. diphtheriae* to spread through tissues of the naso-pharyngeal area

Culture

- Blood agar, hoyle's tellurite medium and Loeffler's serum slope, although the organisms do grow on other media

Identification Tests

- Biochemically, C. diphteriae ferments glucose, and maltose, and is catalase and nitrate positive
- All the strains are urease negative except C urealyticum

Antibiotic sensitivity

- C. diphtheriae is usually sensitive to penicillin, erythromycin and lincomycin. Antitoxin is used in therapy to neutralize C. diphtheriae toxin

B. Genus Listeria

- Type species:- L. monocytogenes
- L. monocytogenes is considered the only pathogen in the genus listeria

Characteristics

- These organisms are short, motile, non – sporing, non –capsulated rods
- It is aerobic and facultative anaerobic grows between 2 -40^{0}c and motile at room temperature

Habitat

- It is found in soil, vegetable s, sewage and in the faeces of humans and animals
- It is naturally pathogenic to a number of animals

Pathogenicity

- Causes listeriosis in man either as a case of occupational hazard among animal handlers or directly from human carriers
- The disease is most common in neonates, pregnant women and those with immunodeficiency
- Intrauterine infection causes abortion, still birth or premature delivery of a neonate with pneumonic septicemia and meningitis
- In elder immuno–compromised patients causes meningoencephalitis often with septicemia, endocarditis and brain abscesses

Laboratory Diagnosis

Specimens

- Blood ,CSF
- Gram stain:- small straight or curved rod or cocco bacillus
- Motility test: show characteristic tumbling motility peptone water incubate at 20 –25^{0}c (room temperature) for six hours.

Culture

- grows well aerobically and an aerobically on media such as blood or serum agar at 2 – 40^{0}C with optimum at 35^{0}C.

Identification Tests

- Produce acid, but no gas
- Lactose and sucrose are fermented after 3 -10 days
- Other test are:- catalase (+), indole (-), oxidase (-), & urease (-)
- Characteristic motility are sufficient criteria for diagnosis

Treatment

- A combination therapy of ampicillin and gentamicin is most effective.

C. Genus Bacillus

- Gram-positive rod-shaped bacteria
- Species can be obligate aerobes or facultative anaerobes,
- Test positive for the enzyme catalase.
- Includes both free-living and pathogenic species.
- Produce oval endospores - widespread dispersal - dust, water, plants, animals fur
- Degrades complex macromolecules - exoenzymes – antibiotics

Bacillus anthracis

- **Morphologic and Physiologic Description** - gram-positive, spore-forming(central)' rod, non-motile, aerobic, encapsulated, ferments carbohydrates
- Host Range - cattle, sheep, horses, swine, other species less frequently. Man is susceptible
- Source - soil, contaminated carcasses and certain animal feed products (bone meal)
- **Cause**: - anthrax
- Based on the site of infection and the source three types:

1) Cutaneous Anthrax - Lesion lump (papule) -black, enlarging, eschar
2) Pulmonary Anthrax (woolsorter's disease)
   - lungs - germinates exotoxins - septicemia –
   - death in a few hours - capillary clots, shock
3) Gastrointestinal Anthrax - rare - contaminated meat

Virulence Factors:

- capsule(polypeptide)
- exotoxin: composed of three components:

Components

* edema factor (EF)
* protective antigen (PA) factor
* lethal factor (LF): produces edema and shock

Bacillus cereus

- Morphologic and Physiologic Description is similar to that of B. anthracis

Causes:

- **Food poisoning**
- In immunocompromised individual: pneumonia, bacteraemia, wound infection
- Virulence factor – its toxin (enterotoxin). Toxin is produced when it sporulates in rice and cereals

Laboratory Diagnosis

- **Specimen**: - include pus or fluid from the pustule, sputum, blood, spinal fluid and swabs from cutaneous lesions

Microscopy

- **Gram stain: -** show large gram positive rods with square or round ends and are with out spores.
- Motility test:- B anthracis is non – motile while the other species are motile

Culture

- Grow well on most culture media aerobically at optimum temperature of 35 – 37^0C with in arrange of 12 – 45^0c

Identification Test

- B. anthracis ferments glucose, maltose and sucrose with acid and no gas production.
- It is catalase positive, nitrate positive unlike other species
- B. anthracis is non- motile and capsulated unlike other species

Treatment

- The organisms are sensitive to penicillin, tetracycline, chloramphenicol and cotrimoxazole.

Anaerobic spore forming GP Rods

Clostridium

- Is a genus of Gram-positive bacteria.
- Are obligate anaerobes capable of producing endospores.
- Terminal, swollen spore; catalase negative, rod-shaped

a) Clostridium botulinum

- gram positive spore-forming (Sub terminal spores) anaerobic rod; motile; ferments carbohydrates, non-capsulated.
- Host Range - horses, dogs, cattle, chickens, ducks, turkeys, man, and other animals
- Source - soil, vegetation, digestive tract of animals and man
- Resistance - Spores are resistant in the environment.
- The organism is not particularly resistant to antibiotics.
- Spores of the organism are highly **resistant to heat**, withstanding 100 ^{0}C for at last 3-5 hours

- **Causes:** Botulism –rare but fatal food poising, in which lethal neurotoxin is ingested

BOTULISM

Pathogenesis:

- **Botulism is not an infection**. It is **an intoxication** resulting from the ingestion of **preformed toxin of** *Cl. botulinium* in food.
- The most common offenders are **spiced, smoked, vacuum packed** or **canned alkaline foods** that are eaten without cooking.
- In such foods, under **anaerobic** conditions spores of *Cl. botulinium* **germinate** and **produce toxin**.
- The toxin **blocks the release of acetylcholine** leading to **flaccid paralysis.**
- Death results from respiratory or cardiac failure. There is **no diarrhea, vomiting or fever**.
- **Virulence Factors** - neurotoxins - 6 different types labeled A, B, C, D, E, F

b) Clostridium tetani

- Gram positive, spore-forming anaerobic rod, motile, does not attack carbohydrates; spores are terminal, giving the cells a classical "tennis-racket" appearance.
- **Host Range** - horses, cattle, sheep, goats, pigs, dogs, cats, and man
- **Source** - spores are primarily found in soil which has been contaminated with human or animal feces.
- **Resistance** - spores are resistant; organism will persist in the environment.
- is not highly resistant to antibiotics.
- **Available Biologicals** - tetanus antitoxin, tetanus toxoid

- **Causes:** Tetanus (a serious and fatal disease caused neurotoxin (tetanospasmin)
- The infection remains may be localized in tissues/not invasive/.
- At the site of infection the spores **germinate** releasing the **toxin** that reaches the **CNS** causing **spastic paralysis.**

Generalized tetanus:

- The disease is characterized by convulsive tonic contractions of voluntary muscles including; spasm in jaw muscles leads to **trismus** (**lock jaw**), facial spasm (**sardonic grin**), pronounced arching of the back due to spasm of the muscles of the back
- **Death** occurs due to **respiratory or cardiac failure**.

Neonatal tetanus "tetanus neonatorum"

The infection follows contamination, of the

A soldier dying of tetanus



Virulence Factors

- tetanospasmin - neurotoxin; tetanolysin hemolysin- may aid in the damage of viable tissue near the wound site

c) Clostridium perfringens

- gram-positive spore-forming (**Spores** are oval, **subterminal** and **non-projecting)** rod, non-motile, encapsulated, anaerobic, fermentative
- Host Range - cattle, sheep, horses, swine, dogs, man
- Source - environment, also present in the digestive tract of man and animals
- Resistance - no major antibacterial drug resistance. Spores are resistant in the environment
- Available Biologicals - commercial bacterins and toxoids are available

- Causes;
  - Gas gangrene - anaerobic cellulitis - localized toxin and gas
  - myonecrosis - progressive, toxins diffuse to healthy tissue
  - compound fractures, sores, septic abortions, Puncture, gunshot wounds, crushing injuries with dirt
  - food poisoning

GAS GANGRENE

Pathogenesis :

- The infection occurs when **wounds are contaminated with soil** containing the **organism.**
- The condition occurs in **deep lacerated, devitalized wounds** as in **car accidents** or **war wounds**.
- The presence of **foreign bodies**, **aerobic pyogenic** bacteria and **decreased blood supply** favour **germination** of spores.
- Vegetative cells **multiply** and **ferment sugars** producing large amounts of **gas** which distends the tissues and **interferes with their blood supply** leading to their death (**necrosis and gangrene**).
- Proteolytic clostridia **digest dead tissues leading to change in colour** and **foul odour** of the wound

Virulence Factors:

- Capsule (polypeptide)
- 12 exotoxins are produced which provide a basis for separating the organism into five distinct types: A,B,C,D,E.
- Toxins

d) Clostridium difficile

- Which can flourish when other bacteria in the gut are killed during antibiotic therapy, leading to pseudomembranous colitis (a cause of antibiotic-associated diarrhea)
- Produces two toxins: **Toxin A** is referred to as an enterotoxin because it causes fluid accumulation in the bowel. **Toxin B** is an extremely lethal (cytopathic) toxin.

Laboratory Diagnosis

- Specimen includes:
- pus and infected tissue for gas gangrene and tetanus
- Blood, vomit and left over food for botulism
- Faeces and food for food poisoning

Microscopy:- gram positive rod

Culture

- Obligate anaerobes and grow best at 35 – 37^{0}c
- Media used for the isolation of clostridia include:
  - Gentamycin blood agar
  - Plain blood agar
  - Cooled meat broth
  - Litmus milk
  - Lactose egg yolk milk agar

Treatment

- Clostridium species are usually sensitive to penicillin, metronidazole, clindamycin, tetracyclines and erythromycin.
- Most species are resistant to the aminoglycosides.

GRAM NEGATIVE DIPLOCOCCI NEISSERIACEAE

- Gram-negative cocci
- Residents of mucous membranes of warm-blooded animals
- Genera include *Neisseria, Moraxella, Acinetobacter.*
- 2 primary human pathogens:
  - *Neisseria gonorrhoeae*
  - *Neisseria meningitidis*

Cont…

- None develop flagella or spores.
- Capsules on pathogens - *N.meningitidis*
- have multiple serotypes Pili
- Strict parasites, do not survive long outside of the host
- Aerobic or microaerophilic
- Produce catalase and cytochrome oxidase

Con…

- Pathogenic species require enriched complex media (chocolate media) and CO_2
- They have endotoxin in their outer membrane
  - lipopolysaccharide (LPS) for *N. meningitidis* and
  - Lipooligosaccharide (LOS) for *N.gonorrhoeae*
  - Both LPS and LOS contain lipid A

Neisseria gonorrhoeae: The Gonococcus

- has no polysaccharide capsule
- marked antigenic variation in the gonococcal pili
- Causes gonorrhea, an STD
- Virulence factors:
  - Pili and the outer membrane proteins (OMP)- responsible for adhesion to cells of urogenital tract
  - Lipooligosaccharide –responsible for resistance of complement and inflammatory tissue reaction
  - IgA protease – cleaves secretory IgA

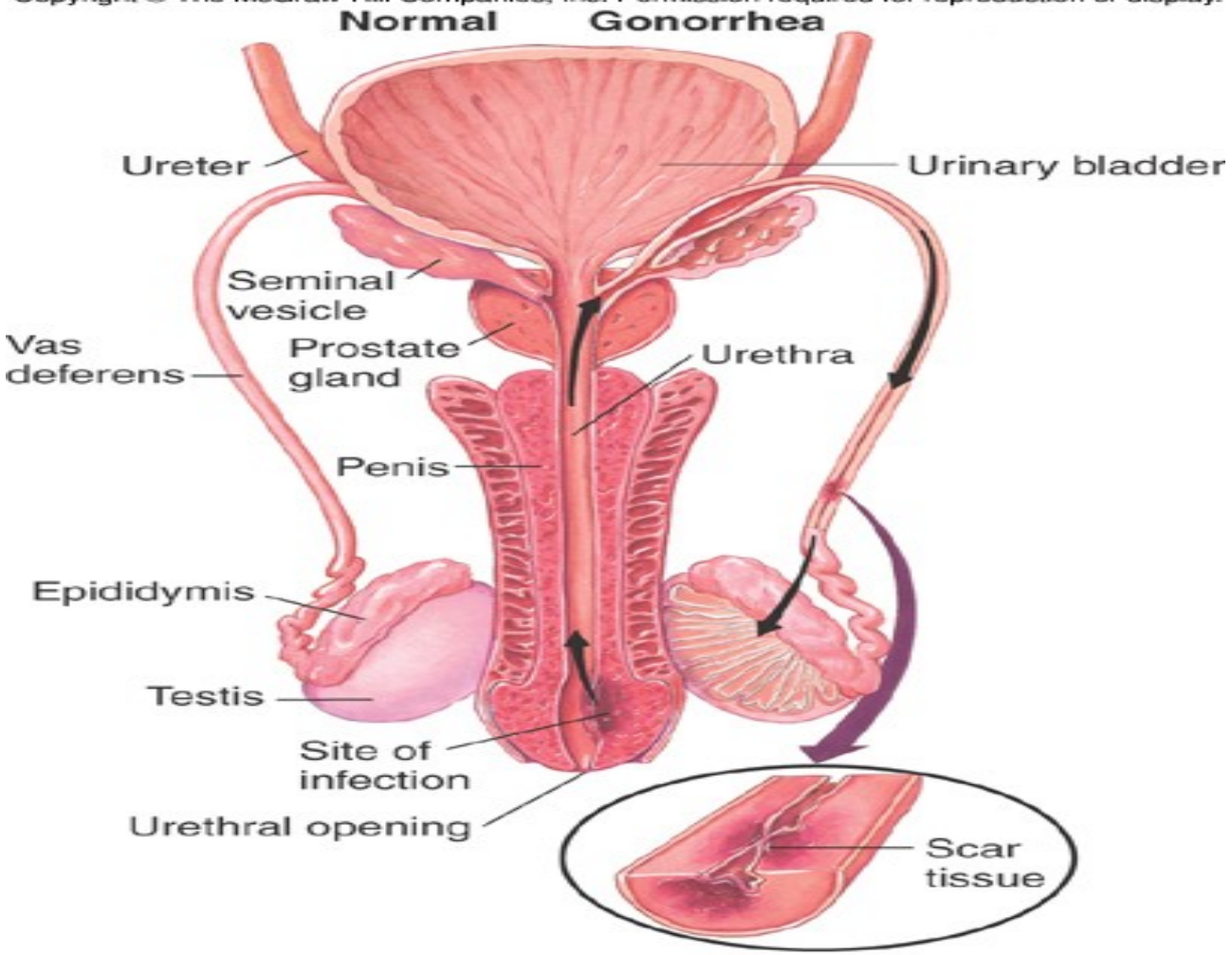
Epidemiology and Pathology

- Strictly a human infection
- In top 5 STDs
- Infectious dose 100-1,000
- Does not survive more than 1-2 hours on fomites
- Gonorrhoea is usually symptomatic in men but often asymptomatic in women
- sources of the organism are genital tract infection (most common), anorectal infection and pharyngeal infection

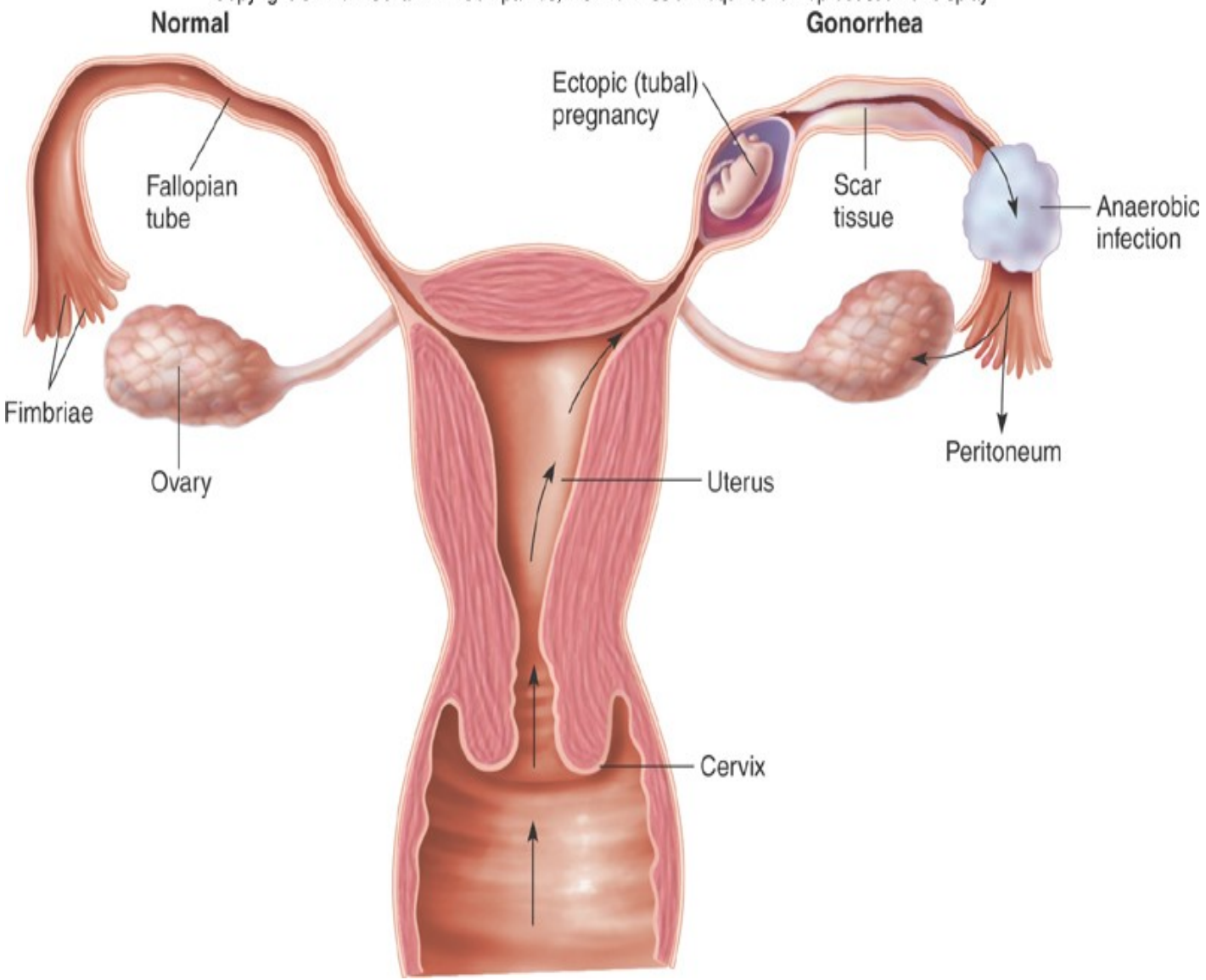
Disease

- Males – urethritis, yellowish discharge, scarring and infertility
- Females – vaginitis, urethritis, infection of the uterine tubes (salpingitis), PID, common cause of sterility and ectopic tubal pregnancies
- Extragenital infections – anal, pharygeal, conjunctivitis, septicemia, arthritis

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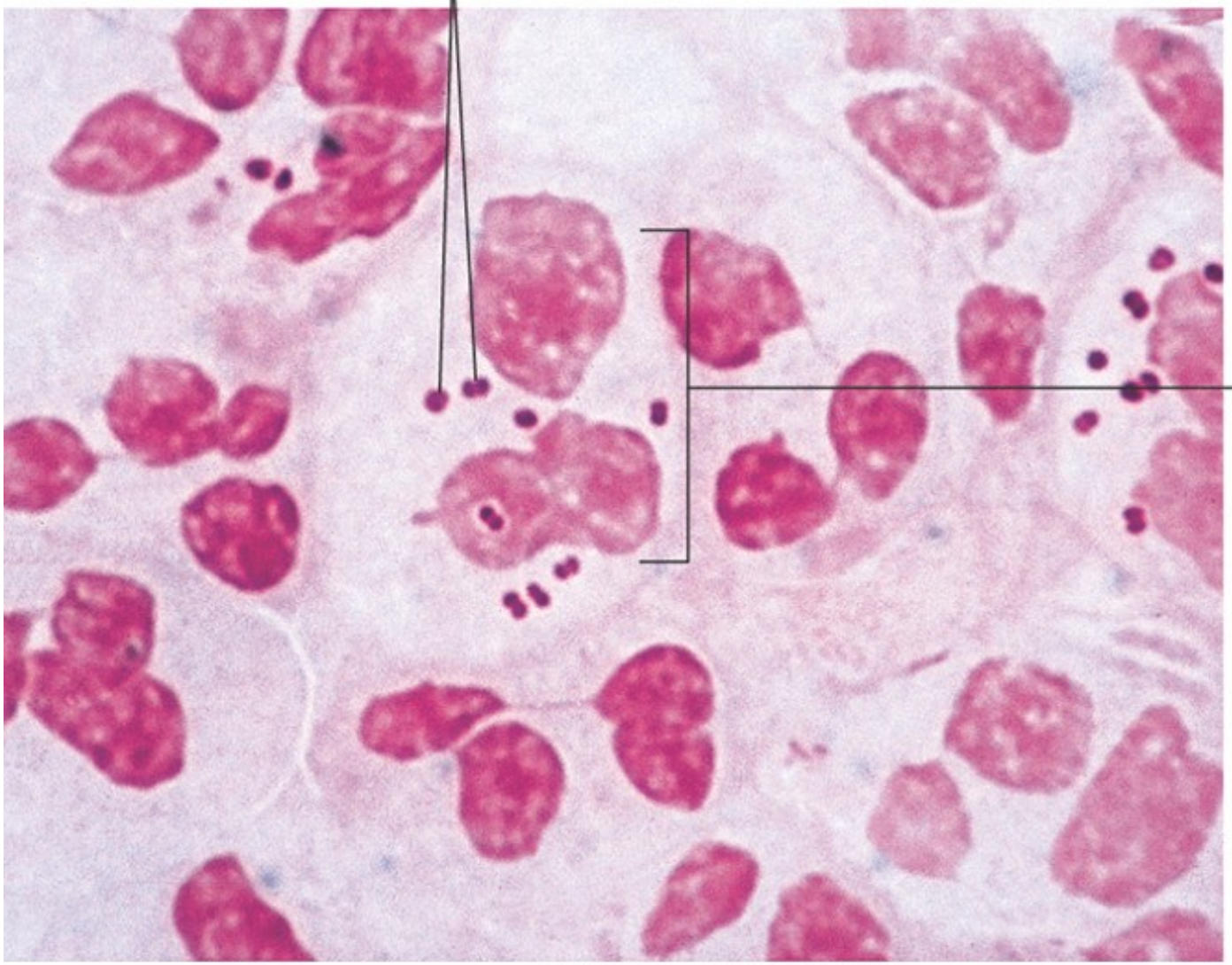
Gonorrhea in Newborns

- Infected as they pass through birth canal
- Eye inflammation, blindness
- Prevented by prophylaxis immediately after birth

Diagnosis

- Gram stain – Gram-negative intracellular (neutrophils) diplococci from urethral, vaginal, cervical, or eye exudate – presumptive identification
- Specimens are cultured on Thayer-martin medium, which is a chocolate agar containing antibiotics
- Specific identification -can be made either by its fermentation of glucose
- rapid tests –ELISA and the DNA probe assay

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Treatment and prevention

- 20-30% of new cases are penicillinase-producing PPNG or tetracycline resistant TRNG
- Ceftriaxone, cefixime, ciprofloxacin, or ofloxacin can be administered in noncomplicated cases
- Combined therapies indicated
- Recurrent infections can occur
- avoiding multiple sexual partner
- Mechanical prophylaxis
- Education
- Screening of people at high risk

Neisseria meningitidis: The Meningococcus

- Gram-negative, coffee-bean shaped cocci
- are frequently pleomorphic
- are non motile and have a polysaccharide capsule
- cultures requires mediums containing blood
- 5–10% CO2 encourages proliferation

Virulence factors:

- capsule
- pili
- IgA protease
- endotoxin (LPS)

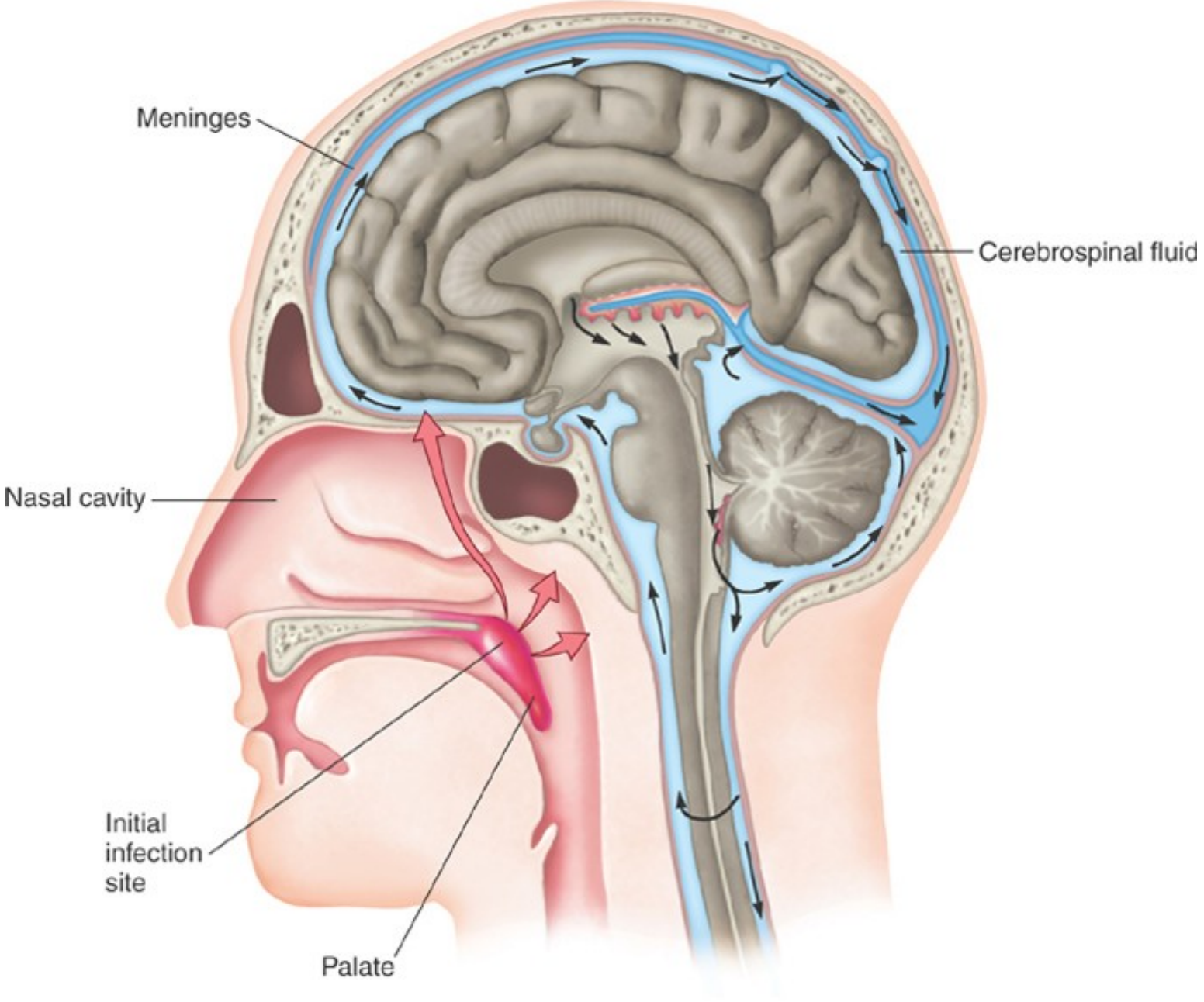
- 12 strains based on capsule chemistry; serotypes A, B, C cause most cases
- Serogroups are divided into serovars based on differences in the outer membrane protein antigens

Epidemiology and Pathogenesis

- Prevalent cause of meningitis; sporadic or epidemic
- Human reservoir – nasopharynx; 3-30% of adult population; higher in institutional settings
- High risk individuals are those living in close quarters, children 6months-3 years, children and young adults 10-20 years.

- Disease begins when bacteria enter bloodstream, pass into cranial circulation, and multiply in meninges
- Hematogenously disseminated pathogens can also infect the lungs, the endocardium or major joints
- Very rapid onset; neurological symptoms (headache, fever, neck stiffness, and severe malaise);
- endotoxin causes hemorrhage (Waterhouse-Friedrichsen syndrome) and shock; can be fatal

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Clinical Diagnosis

- Gram stain CSF, blood, or nasopharyngeal sample
- Culture for differentiation
- ***grows best on blood agar incubated at 37 oC in a 5% CO2 atmosphere***
- meningococci ferment maltose where as gonococci do not
- Rapid tests for capsular antigen
- slide agglutination test is used to determine the serogroup

Treatment and Prevention

- Treated with IV penicillin G, chloramphenicol
- Prophylactic treatment of family members, medical personnel, or children in close contact with patient
- Vaccines exist for group A and C.

Enterobacteriaceae

Classification – more than15 different genera

Escherichia
Shigella
Edwardsiella
Salmonella
Citrobacter
Klebsiella
Enterobacter
Hafnia
Serratia

Enterobacteriaceae

Morphology and General Characteristics

- Gram-negative, non-sporing, rod shaped bacteria
- Oxidase negative
- Ferment glucose and may or may not produce gas in the process (aerogenic vs anaerogenic)
- Reduce nitrate to nitrite (there are a few exceptions)
- Are facultative anaerobes
- If motile, motility is by peritrichous flagella (except Klebsiella, Shigella and Yersinia – non motile)

Enterobacteriaceae

Many are normal inhabitants of the intestinal tract of man and other animals

Some are enteric pathogens and others are urinary or respiratory tract pathogens

Differentiation is based on biochemical reactions and differences in antigenic structure

Selective and differential media are used for initial colony evaluation (ex. MacConkey, HE[1], XLD[2] agars)

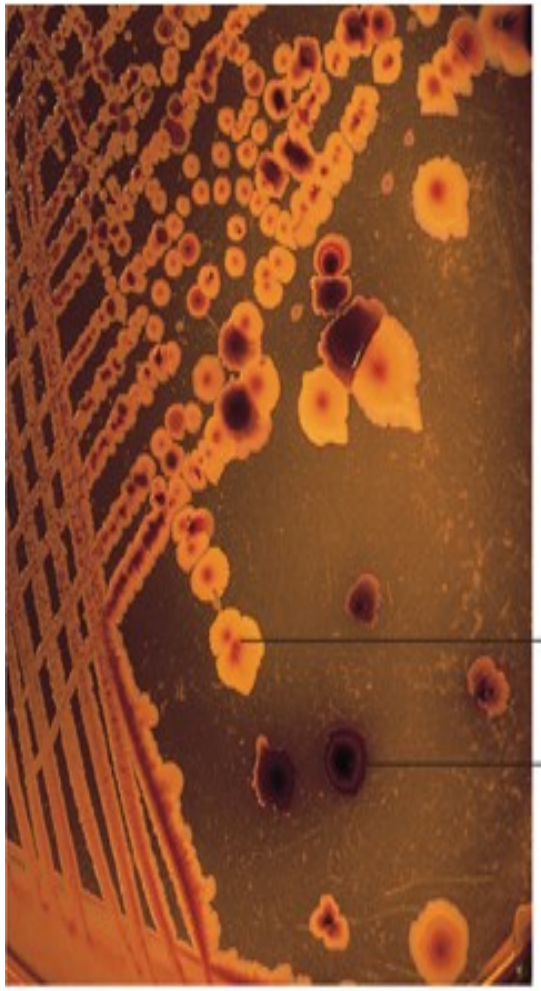
Many are differential on the basis of whether or not the organisms ferment lactose and/or produce H_2S.

The antigenic structure is used to differentiate organisms within a genus or species

1 Hekton enteric

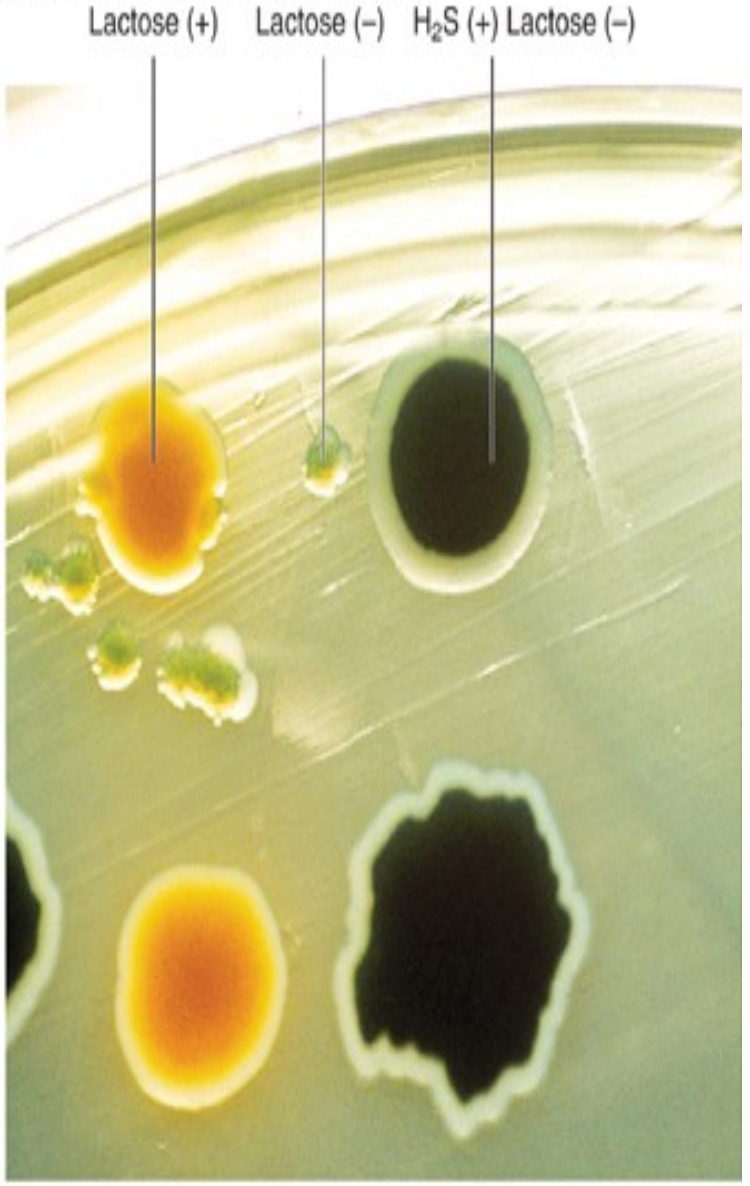
2 Xylose Lysine Deoxycholate Agar

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(a)

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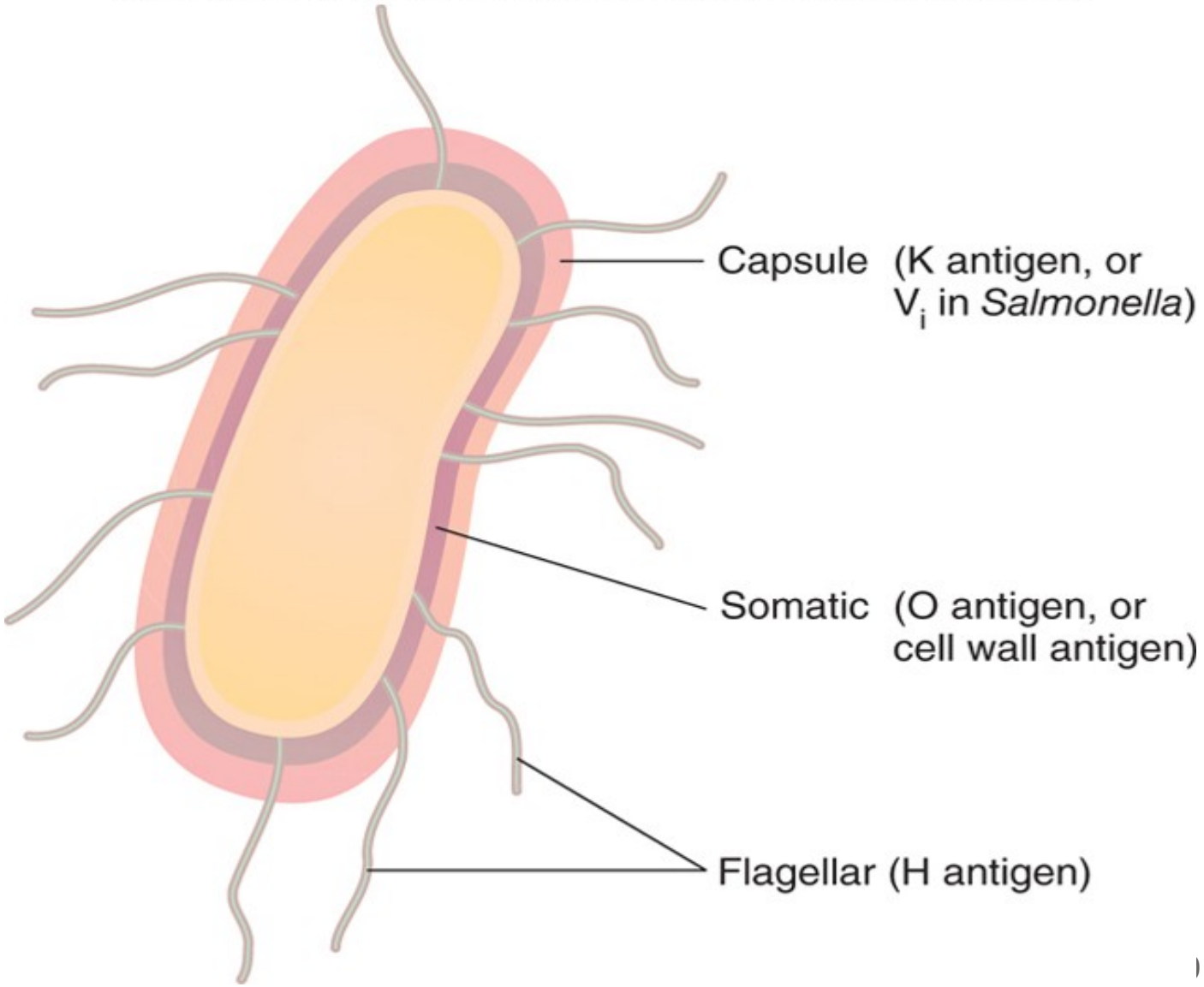
(b)

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Enterobacteriaceae

- Somatic O antigens – these are the heat stable polysaccharide part of the LPS and resistant to alcohol
  - Variation from smooth to rough colonial forms is accompanied by progressive loss of smooth O Antigen
- Flagellar H antigens – are heat labile and denatured by alcohol
- Envelope or capsule K antigens – overlay the surface O antigen and may block agglutination by O specific antisera.
  - Boiling for 15 minutes will destroy the K antigen and unmask O antigens.
  - The K antigen is called the **Vi** (virulence) antigen in *Salmonella typhi*.

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Clinical Significance of Enterics

- Based on clinical infections produced, enterics are divided into two categories:
  - Opportunistic pathogens – normally part of the usual intestinal flora that may produce infection outside the intestine
  - Primary intestinal pathogens – *Salmonella, Shigella,* and *Yersinia* sp.

Escherichia coli

- Most significant species in the genus
- Important potential pathogen in humans
- Common isolate from colon flora
- Some have developed virulence through plasmid transfer, others are opportunists.

Escherichia coli

- Characteristics
  - Dry, pink (lactose positive) colony with surrounding pink area on MacConkey



- Ferments glucose, lactose, trehalose, & xylose
- Positive indole and methyl red tests
- Does NOT produce H_2S or phenylalanine deaminase
- Simmons citrate negative
- Usually motile
- Voges-Proskauer test negative

- Infections
  - Wide range including meningitis, gastrointestinal, urinary tract, wound, and bacteremia
  - Gastrointestinal Infections
    - Enteropathogenic (EPEC) –
    - Bundle forming pili are involved in attachment to the intestinal mucosa
    - primarily in infants and children; outbreaks in hospital nurseries and day care centers;
    - stool has mucous but not blood; identified by serotyping

- Enterotoxigenic (ETEC) – "traveler's diarrhea"
  - The organism attaches to the intestinal mucosa via colonization factors and then liberates enterotoxin.
  - The disease is characterized by a watery diarrhea without blood, nausea, abdominal cramps and low-grade fever for 1-5 days.
  - Transmission is via contaminated food or water
- Enteroinvasive (EIEC) –
  - produce dysentery with bowel penetration, invasion and destruction of intestinal mucosa;

- Enterohemorrhagic (EHEC serotype 0157:H7) –
  - Produce Shiga-type toxin(verotoxin) -is cytotoxic, enterotoxic, neurotoxic, and may cause diarrhea and ulceration of the G.I. tract.
  - Also cause low platelet count, hemolytic anemia, and kidney failure; potentially fatal, especially in young children;
  - does NOT ferment sucrose; identified by serotyping
- Enteroaggregative (EaggEC) –
  - Mucous associated autoagglutinins cause aggregation of the bacteria at the cell surface and result in the formation of a mucous biofilm.
  - The organisms attach via pili and liberate a cytotoxin distinct from, but similar to the ST and LT enterotoxins liberated by enterotoxigenic
  - Symptoms include watery diarrhea, vomiting, dehydration and occasional abdominal pain may persist for over two weeks

- Urinary Tract Infections
  - *E. coli* : is the leading cause of urinary tract infections which can lead to acute cystitis (bladder infection) and pyelonephritis (kidney infection).
  - is due to the formation of pod-like *E. coli* biofilms inside bladder epithelial cells
  - Usually originate in the large instestine
- Septicemia & Meningitis
  - *E. coli* is one of the most common causes of septicemia and meningitis among neonates; acquired in the birth canal before or during delivery
  - *E. coli* also causes bacteremia in adults, primarily from a genitourinary tract infection or a gastrointestinal source

- **Laboratory diagnosis:** can grow initially on blood agar on differential medium such as EMB agar or MacConkey agar
- It produces indole from tryptophan
- It decarboxylates lysine
- It uses acetate as its only source of carbon
- **Treatment**: trimethoprium-sulfamethoxazole or ampicillin, cephalosporin
- **Prevention**-
  - reducing rise of nosocomial infections
  - Maintenance of high hygienic standards

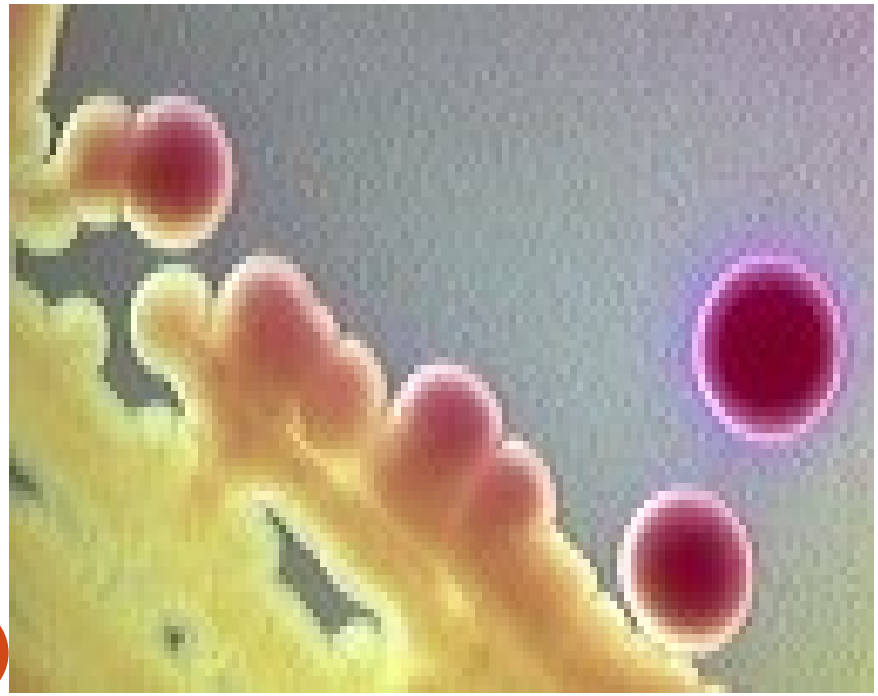
Klebsiella, Enterobacter, Serratia & *Hafnia* sp

Klebsiella species

- Usually found in GI tract
- Four major species
- *K. pneumoniae* is most commonly isolated species
  - Possesses a polysaccharide capsule, which protects against phagocytosis and antibiotics and makes the colonies moist and mucoid
  - Has both O and K antigens
  - Has a distinctive “yeasty” odor
  - Frequent cause of nosocomial pneumonia

Klebsiella species

- Significant biochemical reactions
  - Lactose positive
  - Most are urease positive
  - Citrate + ,VP+, MR-
  - Non-motile





Con…

- Virulence factors
  - Capsule
  - Adhesions
  - Iron capturing ability
- Clinical significance
  - Causes pneumonia, mostly in immunocompromised hosts.
    - Permanent lung damage is a frequent occurrence (rare in other types of bacterial pneumonia)
  - A major cause of nosocomial infections such as septicemia and meningitis

Enterobacter species

- Comprised of 12 species; *E. cloacae and E. aerogenes* are most common
- Isolated from wounds, urine, blood and CSF
- Major characteristics
  - Colonies resemble *Klebsiella*
  - Motile
  - MR negative; VP positive
  - Citrate +
- Clinical significance
  - Nosocomial infections
  - Bacteremia in burn patients

Enterobacter species



Serratia species

- Seven species, but *S. marcescens* is the only one clinically important
- Frequently found in nosocomial infections of urinary or respiratory tracts
- Implicated in bacteremic outbreaks in nurseries, cardiac surgery, and burn units
- Fairly resistant to antibiotics
- Major characteristics
  - Ferments lactose slowly
  - Citrate +
  - Motility +
  - Urea +/-
  - Produce characteristic pink pigment, especially when cultures a om temperature

S. marscens on nutrient agar →



Hafnia species

- *Hafnia alvei* is only species
- Has been isolated from many anatomical sites in humans and the environment
- Occasionally isolated from stools
- Delayed citrate reaction is major characteristic

Treatment

- aminoglycoside (e.g. gentamicin) and a cephalosporin (e.g. cefotaxime)

Prevention

- Changing the site of intravenous catheters
- Removing urinary catheters when they are no longer needed.
- Taking proper care of respiratory therapy devices

Proteus, Morganella & *Providencia* species

- All are normal intestinal flora (except *Providencia)*
- Opportunistic pathogens
- Deaminate phenylalanine
- All are lactose negative
  - Virulence factors
    - Urease – the ammonia produced may damage the epithelial cells of the UT

Proteus species

- *P. mirabilis* and *P. vulgaris* are widely recognized human pathogens
- Isolated from urine, wounds, and ear and bacteremic infections
- Both produce swarming colonies on non-selective media and have a distinctive “burned chocolate” odor
- Both are strongly urease positive
- Both are phenylalanine deaminase positive
- A=> exhibits characteristic
- B=> shows urease positive



Morganella species

- *Morganella morganii* is only species
- Documented cause of UTI
- Isolated from other anatomical sites
- Urease positive
- Phenylalanine deaminase positive

Providencia species

- *Providencia rettgeri* is pathogen of urinary tract and has caused nosocomial outbreaks
- *Providenicia stuartii* can cause nosocomial outbreaks in burn units and has been isolated from urine
- Both are phenylalanine deaminase positive

Citrobacter species

- *Citrobacter freundii* associated with nosocomial infections (UTI, pneumonias, and intraabdominal abscesses)
- Ferments lactose and hydrolyzes urea slowly
- Resembles *Salmonella* sp.



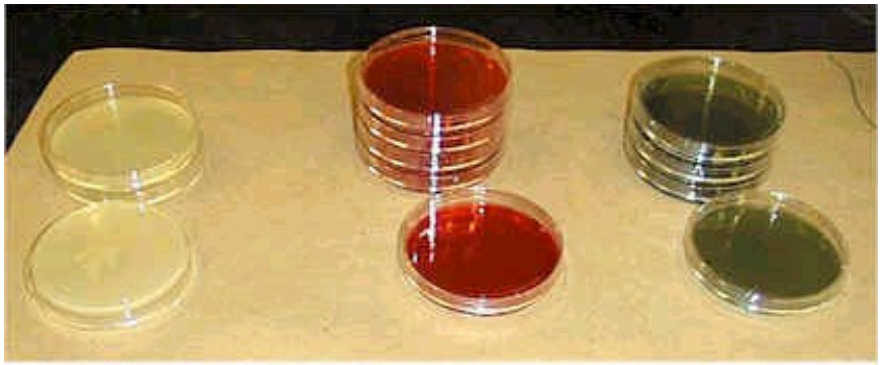
Salmonella

- Salmonella
  - Classification has been changing in the last few years.
    - There is now 1 species: *S. enteritica*, and 7 subspecies: 1, 2 ,3a ,3b ,4 ,5, and 6.
    - Subgroup 1 causes most human infections
    - Clinically Salmonella isolates are often still reported out as serogroups or serotypes based on the Kauffman-White scheme of classification
- Produce significant infections in humans and certain animals
- On differential selective agar, produces clear, colorless, non-lactose fermenting colonies with black centers (if media contains indicator for hydrogen sulfide)

Salmonella (cont'd)

- *Salmonella* on MacConkey





Bismuth sulphite (BS) | Xylose lysine desoxycholate (XLD) | Hektoen (HE)



Bismuth sulphite (BS) | Xylose lysine desoxycholate (XLD) | Hektoen (HE)

Salmonella

- Lactose negative
- Negative for indole, VP, phenylalanine deaminase, and urease
- Most produce H_2S
- Do not grow in potassium cyanide
- three different antigens: O –cell wall (used to group salmonellae in to group A-I), H-flagellar, Vi-capsular
  - Virulence factors
    - Endotoxin – may play a role in intracellular survival
    - Capsule (for *S. typhi* and some strains of S. *paratyphi*)
    - Adhesions – both fimbrial and non-fimbrial
    - Type III secretion systems and effecter molecules – 2 different systems may be found:
      - One type is involved in promoting entry into intestinal epithelial cells
      - The other type is involved in the ability of *Salmonella* to survive inside macrophages

- Outer membrane proteins - involved in the ability of *Salmonella* to survive inside macrophages
- Flagella – help bacteria to move through intestinal mucous
- Enterotoxin - may be involved in gastroenteritis
- Iron capturing ability

- Clinical Significance – causes two different kinds of disease: enteric fevers and gastroenteritis.
  - Both types of disease begin in the same way, but with the gastroenteritis the bacteria remains restricted to the intestine and with the enteric fevers, the organism spreads
  - Transmission is via a fecal-oral route, i.e., via ingestion of contaminated food or water.

Salmonella

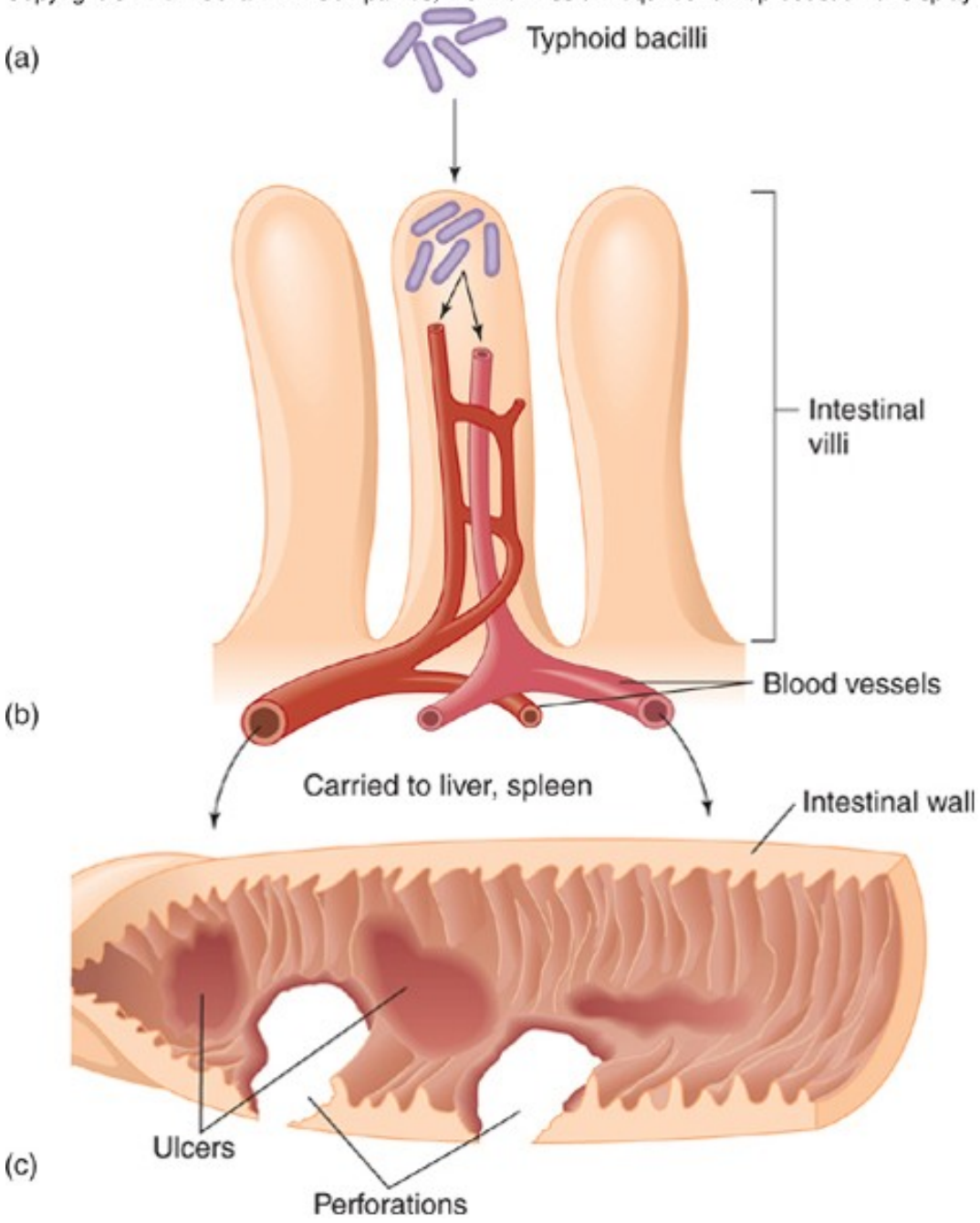
- The organism moves through the intestinal mucosa and adheres to intestinal epithelium

- Effector proteins of the type III secretion system mediate invasion of enterocytes and M cells via an induced endocytic mechanism
- Salmonella multiplies within the endosome.

- The endosome moves to the basal side of the cell and *Salmonella* are released and may be phagocytosed by macrophages

- For gastroenteritis the *Salmonella* multiply and their presence induces a strong inflammatory response
  - The inflammatory response prevents the spread beyond the GI tract and eventually kills the bacteria.
- In enteric fevers (typhoid and paratyphoid) the Salmonella disseminate before they multiply to high enough levels to stimulate a strong inflammatory response so the initial symptoms are only a low-grade fever and constipation.
  - the bacteria move via the lymphatics and blood Stream to the liver and spleen where phagocytosis and multiplication occurs
  - The bacteria re-enter the bloodstream to disseminate throughout the body to all organs causing fever, headaches, myalgia, and GI problems
  - Rose spots (erythematous, muculopapular lesions) are seen on the abdomen. Osteomyelitis, cystitis, and gall bladder infections may occur

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Laboratory Diagnosis

- Stool culture
- Urine culture
- Blood culture
- Widal test -serologically by detecting a rise in antibody titer in the patient's serum

Treatment: ciprofloxacin; chloramphenicol, trimethoprim sulfatethoxazole or a broad-spectrum cephalosporin

Prevention

- Public health and personal hygiene
- Proper sewage treatment,
- chlorination of water supply,
- pasteurization of milk,

Shigella species

- Closely related to the *Escherichia*
- All species cause bacillary dysentery
- *S. dysenteriae* (Group A)
- *S. flexneri* (Group B) developing country
- *S. boydii* (Group C)
- *S. sonnei* (Group D) developed country
- Characteristics
  - Non-motile
  - Do not produce gas from glucose
  - Do not hydrolyze urea
  - Do not produce H_2S on TSI
  - Lysine decarboxylase negative
  - All ferment mannitol except *S. dysenteriae*
  - Fragile organisms
  - Possess O and some have K antigens

- Virulence factors
  - Shiga toxin – is produced by *S. dysenteriae* and in smaller amounts by *S. flexneri* and *S. sonnei.*
    - Acts to inhibit protein synthesis by inactivating the 60S ribosomal subunit by cleaving a glycosidic bond in the 28S rRNA constituents.
    - This plays a role in the ulceration of the intestinal mucosa.
- Outer membrane and secreted proteins
  - These proteins are expressed at body temperature and upon contact with M cells in the intestinal mucosa they induce phagocytosis of the bacteria into vacuoles.
  - *Shigella* destroy the vacuoles to escape into the cytoplasm.
  - From there they spread laterally (Polymerization of actin filaments propels them through the cytoplasm.) to epithelial cells where they multiply but do not usually disseminate beyond the epithelium.

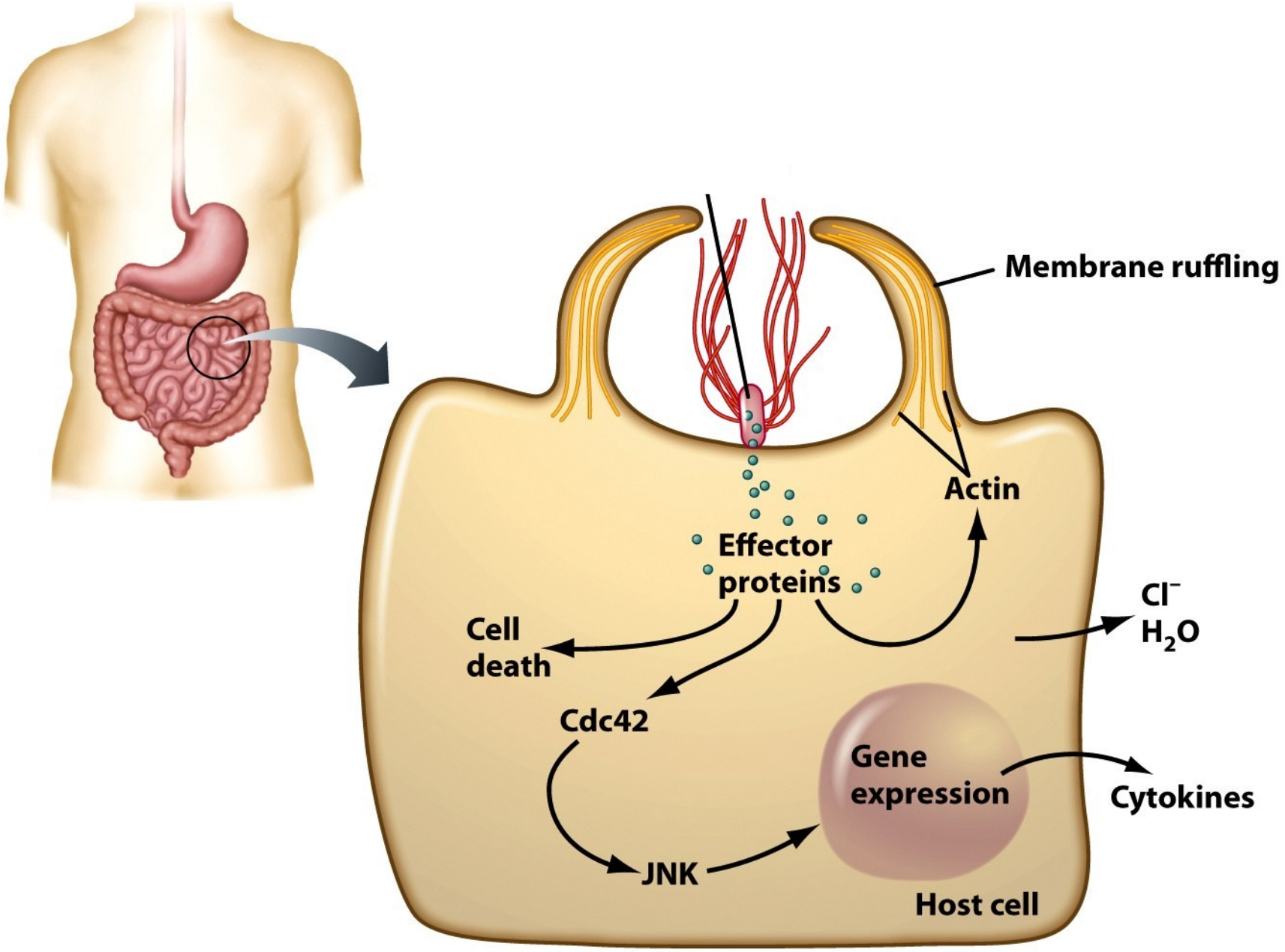


Figure 25.29 Microbiology: An Evolving Science

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Shigella

- **Pathogenesis**
  - Cause shigellosis or dysentery (bloody stools, mucous, and numerous WBC)
    - The severity of the disease depends upon the species one is infected with.
      - *S. dysenteria* is the most pathogenic followed by *S. flexneri*("gay bowel syndrome"), *S. sonnei* and *S. boydii* Oral-fecal transmission
  - There is an incubation of 1-7 days followed by fever, cramping, abdominal pain, and watery diarrhea (due to the toxin) for 1-3 days
  - Fewer than 200 bacilli are needed for infection in health individuals
  - Treatment – fluid replacement and ciprofloxacin and sulfa-trimethoprim

Shigella


Shigella on EMB
Shigella on MacConkey
Shigella on ENDO
Shigella on Heltoen
Shigella on SS agar

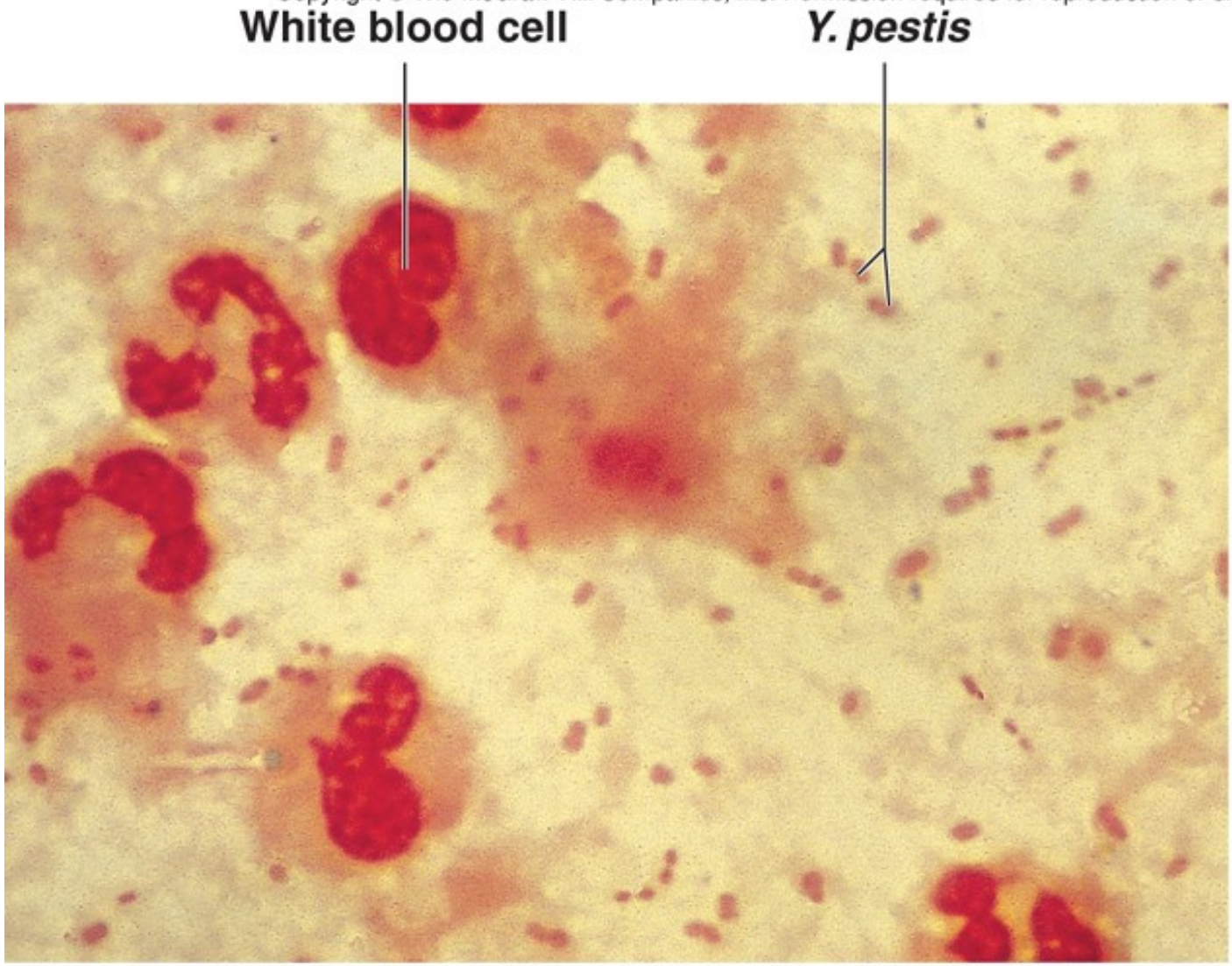
The Enteric *Yersinia* Pathogens

- *Yersinia enterocolitica* – domestic and wild animals, fish, fruits, vegetables, and water
  - bacteria enter small intestinal mucosa, some enter lymphatic and survive in phagocytes; inflammation of ileum can mimic appendicitis
- *Y. pseudotuberculosis* – infection similar to *Y. enterocolitica,* more lymph node inflammation

Plague

- Nonenteric
- Tiny, Gram-negative rod, unusual bipolar staining and capsules
- Virulence factors – capsular and envelope proteins protect against phagocytosis and foster intracellular growth
  - coagulase, endotoxin, murine toxin

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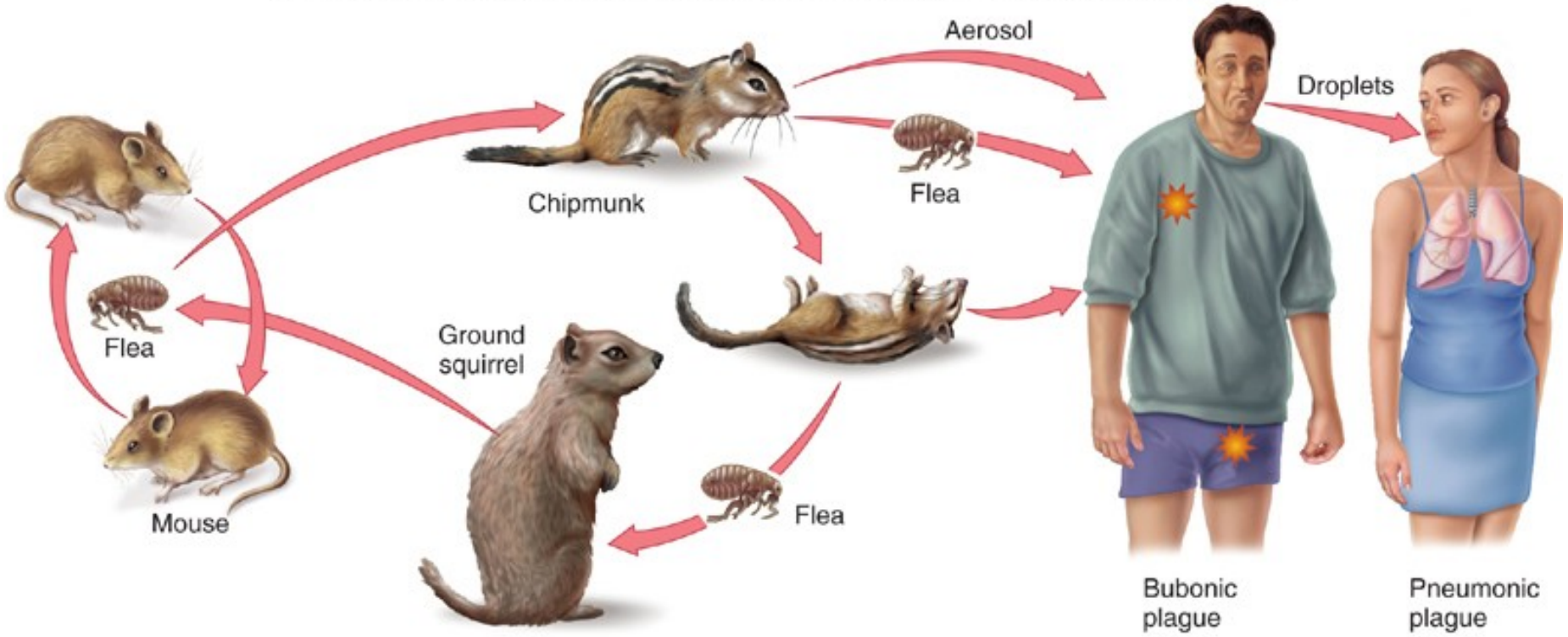


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Yersinia pestis "black death"

- Humans develop plague through contact with wild animals (sylvatic plague) or domestic or semidomestic animals (urban plague) or infected humans.
- Found in 200 species of mammals – rodents, without causing disease
- Flea vectors (*Xenopsylla cheopis)* – bacteria replicates in gut, coagulase causes blood clotting that blocks the esophagus; flea becomes ravenous

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Pathology of Plague

- ID 3-50 bacilli
- Bubonic – bacillus multiplies in flea bite, enters lymph, causes necrosis and swelling called a **bubo** in groin or axilla – non contagious
- Septicemic – progression to massive bacterial growth; virulence factors cause intravascular coagulation subcutaneous hemorrhage and purpura – black plague
- Pneumonic – infection localized to lungs, highly contagious; fatal without treatment

- Diagnosis depends on history, symptoms, and lab findings from aspiration of buboes.
- Treatment: streptomycin, tetracycline or chloramphenicol
- Killed or attenuated vaccine available
- Prevention by quarantine and control of rodent population in human habitats

VIBRIONACEAE

Vibrionaceae family

- Includes the genera *Vibrio; m*ost vibrios of medical importance are enteric pathogens of humans.
- Vibrios are wide spread in nature, mainly in water, only one serovar *V. cholerae*, is the cause of cholera.

- Non-cholera vibrios of diff serovars exist within the spp
- Gram negative curved bacilli.
- Other Spp: *V parahaemolyticus, V vulnificus, V. mimicus*

VIBRIO CHOLERAE

- **Habitat:** water contaminated with pt feces or carriers

VIBRIOS

Morphology:

- G -ve comma-shaped bacilli, arranged in pairs or short chains giving a spiral appearance.
- Actively **motile** by one long polar flagellum, non-capsulated, non-sporing.

Cultural characters:

- Aerobes, grows readily on ordinary media as glistening colonies over a wide temp range (optimum 37^{o}C).
- Growth is inhibited at acid pH (optimal pH for growth: alkaline pH >8.0).

Enrichment medium:

- **Alkaline peptone** water (pH 8.4) promotes the rapid growth of *V. cholera* from mixtures of other bacteria, e.g. fecal samples.

VIBRIOS

Selective media:

1. TCBS medium

- Thiosulphate citrate bile sucrose (TCBS) agar, pH 8.6
- *V. cholerae* produce large yellow sucrose - fermenting colonies after 18-24 hrs incubation.

2. Mansur's medium:

- Gelatin taurocholate tellurite agar pH 8.5 - 9.2.
- *V. cholera* produce large colonies, grey at 24 hrs, with black centre at 48hrs surrounded by a halo due to gelatin liquifaction

VIBRIOS

Biochemical activities:

- Fermentation of sucrose & mannose (acid, no gas) but not arabinose is typical of *V. cholerae* & distinguishing feature from other vibrios.
- All vibrio spp are **strong oxidase positive**.

Cholera red reaction:

- The addition of few drops of sulphuric acid to a 24hr peptone - water culture produce immediate formation of red-pink colour (the reaction is given by many vibrios & is not specific for *V. cholerae*).
- Its mechanism is the production of indole & nitrites in the peptone water results in formation of the coloured substance nitroso-indole.

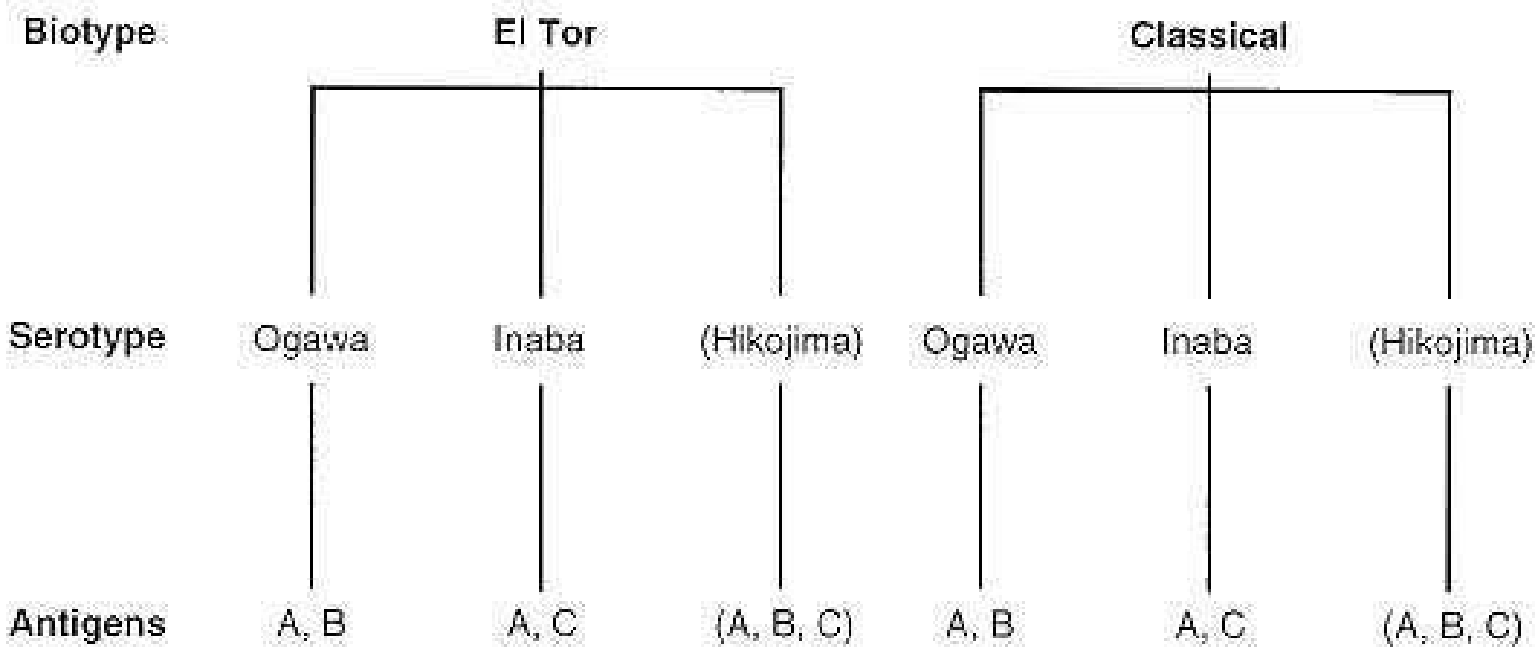
VIBRIOS

Serological identification

- by slide agglutination of suspected colonies with specific antisera.
- **Biotypes**: two biotypes of *V. cholerae*:
    i. Classical,
    ii. El-Tor
- Can be differentiated
- **Biotyping** is the distinguishing of different bacterial strains within a spp

VIBRIOS

V cholera O group 1 Ag



VIBRIOS

Antigenic structure:

- **H** Ags: common to many vibrio spp but of little value in identification.
- **O** Ags: enable the vibrios to be divided into 6 major subgroups.
- Strains of *V. cholerae* are known as **O1** and possess a distinctive O Ag with subdivision into 3 serotypes **Ogawa, Inaba & Hikojima**
- Any serotype can be either classical or El-Tor biotype. **Non- O1** vibrios, deficient in the O Ag 1 are classified as non cholera vibrios.

VIBRIOS

Toxins:

- Endotoxins & exotoxins are recognized.
- The enterotoxin stimulates persistent and excessive secretion of isotonic fluid by the intestinal mucosa
- Cholera is an acute infectious disease characterized by severe vomiting & watery diarrhoea (**rice water stools**) resulting in dehydration & collapse.
- The disease is endemic in the Indian subcontinent & used to occur in world wide epidemics.

Pathogenesis:

- Infection occurs by the oral route through contaminated food or drinks.
- Source of infection is a case or a carrier who excretes the organism in the stools.
- IP is short 1-4 days.
- Infection is restricted to intestine with no blood invasion.
- The organisms attach to the micro villi of the brush border of epithelial cells where they multiply & liberate cholera **enterotoxin** which:
- Binds to ganglioside receptors on the mucosal cells.

CHOLERA

- After a lag period of 15-45 minutes adenyl cyclase is activated & the cAMP concentration inside the intestinal cells increased.
- Increased intracellular cAMP results in the excretion of electrolytes such as chloride & bicarbonate ions along with massive quantities of water.
- Severe diarrhoea & vomiting may lead to dehydration & death.
- Convalescent carriers occur but chronic carriers are rare.

Diagnosis of cholera:

A. First case in a non-endemic area

- Specimen for culture: mucous flecks from stools.

1. Stools are inoculated on alkaline peptone water
2. Subcultures are made from the surface pellicle after 6-8 hrs on TCBS or alkaline agar.

Colonies are identified by:

- A **wet mount** which is examined for **motility**.
- Smears stained show G-ve comma shaped bacilli.
- Biochemical activities including cholera red reaction.
- Agglutination with specific anti 01 cholera sera (polyvalent), anti-Inaba & anti-Ogawa sera.

B. Secondary case during an epidemic

- In an established epidemic, cases can be diagnosed by microscopic examination of stools for comma shaped G-ve bacilli with characteristic motility, which can be immobilized by adding specific antisera.

Treatment:

1. IV fluids to correct the fluid & electrolyte balance
2. Antibiotics have a secondary role in treatment. Tetracyclines are the most effective.

CHOLERA

Prophylaxis:

- Community & personal hygiene to prevent feco-oral transmission.
- Isolation of cases & proper disposal of sewage.
- Chemoprophylaxis by using tetracycline for exposed persons.
- Available vaccine **Koll's Vaccine** which is heat killed suspension of the organism. It is given in 2 sc doses.
- The role of vaccine in protection is questionable as it stimulates anti-bacterial & not anti toxic Abs.
- Attempts are made to develop new oral vaccines which protect against the cholera enterotoxin by the recombinant DNA tech.

VIBRIO PARAHAEMOLYTICUS

- **Halophilic** (i.e. salt tolerant) marine vibrio isolated from shellfish, particularly in countries with warm coastal water, e.g. SE Asia.
- It causes an acute gastroenteritis (enteritis) acquired by ingestion of raw or improperly cooked sea foods
- Vibrios are excreted in large numbers in stools.
- Fecal samples plated on TCBS agar yield large blue-green colonies of *V. parahemolyticus*.

Vibrio vulnificus

- *V. vulnificus* account for most (82%) of **wound**-associated infections
- *V vulnificus* lives in areas where the temperature exceeds 18°C
- Low-to-moderate salinity (15-25 parts per thousand) provides the most favorable growing condition for *V vulnificus,* and, conversely, high salinity (>25 parts per thousand) adversely affects its survival.
- Low seawater temp (<10°C) significantly inhibits the growth of *V vulnificus*.
- Several mechanisms contribute to the virulence of *V vulnificus*. Iron is an important growth factor

CAMPYLOBACTER

- Strictly microaerophilic, causes human or animal diarrhoeal illness are thermophilic,
- Oxidase positive; Grows best at 43^{o}C.
- The main human pathogenic spp: *Campylobacter jejuni* & *C. coli* cause enterocolitis especially in children.

Habitat:

- Various animal including chicken & domestic animals.

Morphology:

- Gram-negative, curved or spiral rods.
- Motile by a single flagellum at one or both poles.

CAMPYLOBACTER

Cultural characters:

- Microaerophilic, grow best in an atm containing a mixture of 5% O_2, 10% CO_2, with the remainder an inert gas, usually N_2 or H_2.
- A simple way to produce the incubation atm. is to place the plates in an anaerobic jar without the catalyst & to produce the gas with a commercially available gas-generating pack or by gas exchange.
- Growth takes place at 37°C but the optimal temp of *C. jejuni* is 43°C

CAMPYLOBACTER

- **Media:** grow readily on simple media, but selective media is necessary for isolation from faeces, e.g. **Skirrow's media** with vancomycin, polymyxin & trimethoprim.
- Incubation for 24-48 hrs at 43°C under microaerophilic conditions.
- Colonies look like spreading fluid droplets.
- Identification is done by Gram-film appearance, motility, growth temp. requirements 43°C
- *C. jejuni & C. coli* : sensitive to erythromycin & nalidixic acid.

HELICOBACTER

HELICOBACTER PYLORI

- Spiral-shaped, Gram negative rod, produce urease, has multiple flagella at one pole
- Found closely associated with gastric mucosa & is associated with antral gastritis, gastric & duodenal (peptic) ulcer dis. & gastric carcinoma.
- It differs in several important biochemical characteristics from campylobacter spp & does not grow at 43^{o}C.

Growth characteristics

- Oxidase & catalase positive, motile

HELICOBACTER

Pathogenesis

- Grow optimally at pH 6 -7 but not at pH of gastric lumen
- Found deep in the mucus near the epithelial surface with pH 7.4
- Produce protease that modifies the gastric mucus
- Produce urease –yield ammonia which buffers acid
- Motility helps it to find its way to the epithelial surface
- Destruction of the lumen & glandular atrophy
- Toxins, LPS & ammonia directly damage the cells

HELICOBACTER

Epidemiology

- Is present in the gastric mucosa of 20% in persons <30 years, but in 40-60% of persons age 60 years
- Developing countries: prevalence about 80% in adults
- Person-to-person transmission is likely

Diagnosis

- Gastric biopsy used for histology used for culture
- Culture – grows in 3-6 days
- Grows optimally at pH 6.0 -7.0 in Skirrow's medium
- Smear: stained with Giemsa or silver

HELICOBACTER

Treatment

- **Triple Chemotherapy (synergism):**
  - Proton pump inhibitor (e.g., omeprazole)
  - One or more antibiotics (e.g., clarithromycin; amoxicillin; metronidazole)
  - Bismuth compound
- Inadequate treatment results in recurrence of symptoms
- **Amoxicillin** or tetracycline - for 14 days eradicates H. pylori in 70-95% of patients.
- Proton pump inhibitors inhibit *H. pylori* are urease inhibitors

Pseudomonas

Structure and Physiology

Gram-negative rods.

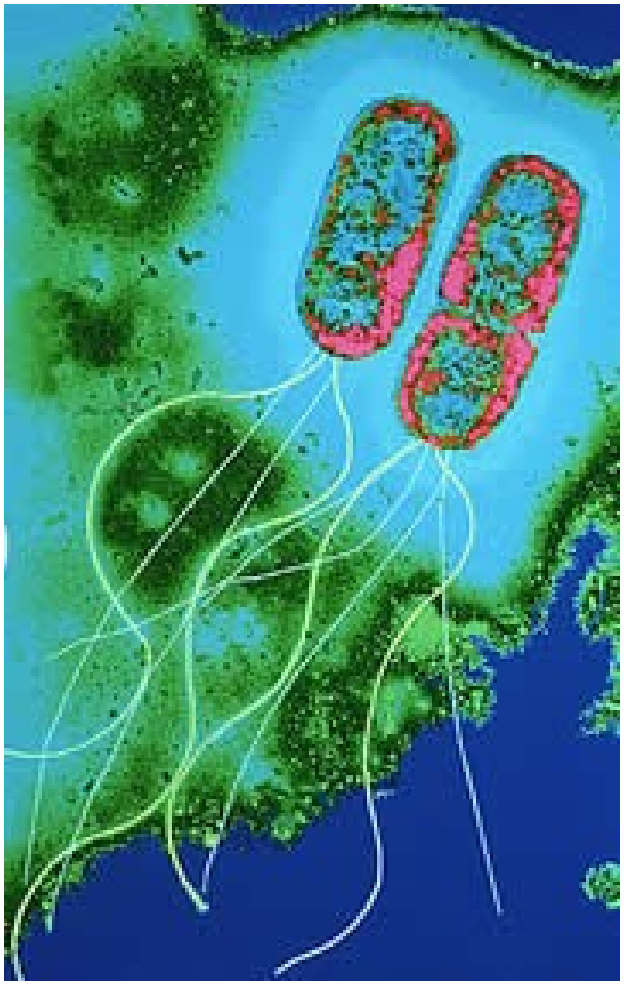
Motile with polar flagella.

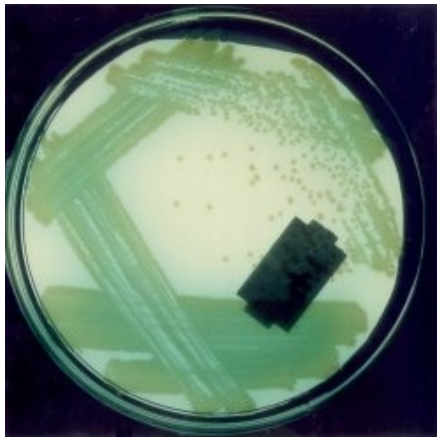
Obligate aerobe.

Oxidase-positive.

Do not ferment carbohydrates.

Resistant to multiple drugs.







P. aeruginosa

Forms round colonies with a fluorescent greenish color, sweet odor, and b-hemolysis.

Pyocyanin- nonfluorescent bluish pigment;

pyoverdin- fluorescent greenish pigment;

pyorubin (dark red), and pyomelanin (black)

Some strains have a polysaccharide capsule.

Identification of *P. aeruginosa* is usually based on colonial morphology, β-hemolysis, oxidase positivity, the presence of characteristic pigments and sweet

P. aeruginosa

Pathogenesis and Immunity

This organism is widely distributed in nature and is commonly present in moist environments in hospitals. It is pathogenic only when introduced into areas devoid of normal defenses, e.g.,

1. Disruption of mucous membrane and skin.
2. Usage of intravenous or urinary catheters.
3. Neutropenia (as in cancer therapy).

P. aeruginosa can infect almost any external site or organ.

P. aeruginosa is invasive and toxigenic. It attaches to and colonizes the mucous membrane or skin, invade locally, and produces systemic diseases and septicemia.

P. aeruginosa is resistant to many antibiotics. It becomes

P. aeruginosa

Pathogenesis and Immunity

Antigenic structure, enzymes, and toxins

Pili and nonpilus adhesins.

Polysaccharide capsules (alginate, glycocalyx): seen in cultures from patients with cystic fibrosis.

LPS- endotoxin, multiple immunotypes.

Pyocyanin: catalyzes production of toxic forms of oxygen that

Elastases: helps bacteria spread and inhibit neutrophil chemotaxis; induces antibodies in chronic infections.

Hemolysins

- Phospholipase C;
- Rhamnolipid, also inhibits ciliary activity.

Exotoxin A: causes tissue necrosis and is lethal for animals (blocks protein synthesis); immunosuppressive.

Exoenzyme S and T: cytotoxic to host cells

Diseases

- **Infection of wounds and burns** (blue-green pus).

  *Verdoglobin or fluorescent pigment detected in wounds, burns, or urine by UV fluorescence
- **Skin and nail infections**
- **Meningitis** (when introduced by lumbar puncture).
- **Pulmonary infection**
  - Necrotizing pneumonia common in cystic fibrosis patients
- **Eye infections**: corneal ulcer

- **Ear infections**
  - Otitis externa: mild in swimmers; malignant (invasive) in diabetic patients.
- **Endocarditis** seen in intravenous drug abusers.
- **Urinary tract infection**
- **Sepsis**: most cases originate from infections of lower RT, UT, and skin and soft tissue.
-

P. aeruginosa

Laboratory Diagnosis

Specimen: skin lesions, pus, urine, blood, spinal fluid, sputum.

Culture: blood agar plate and differential media. Identification of *P. aeruginosa* is described above.

Several subtyping methods, including phage typing and molecular typing, are available for epidemiologic purposes.

Treatment

Combined antibiotic therapy- **aminoglycoside**

P.

Prevention and Control

Control:

1. Patients at high risk should not be admitted to a ward where cases of pseudomonas infection are present.
2. Patients infected with *P. aeruginosa* should be isolated.
3. Sterilize

   all instruments, apparatus, and dressing;

   antimicrobial and other therapeutic substances.

4. Monitor clinically relevant isolates of *P. aeruginosa*

Genus Chlamydia

- Obligate intracellular micro – organism that causes a host of infections in avian and mammalian species, including man.
- Possess both DNA and RNA
- Sensitive to antibiotics
- Reproduce only inside a living cell
- Sensitive to interferon
- Depend on ATP produced by the host cell for their synthetic process
- Have a cell wall, this wall lacks muramic acid and peptidoglycan

- Three stage of development:
  - Elementary bodies:- infective form and can be survive extracellularly
  - Initial body:- metabolically active non – infective fragile form and multiply by binary fission
  - Reticulate (initial) body:- smaller initial or reticulate body.

Characteristics

- Gram negative but are better stained with giemsa
- Produce basophilic inclusion bodies in the infected cell
- Heat labile and can be inactivated at 56^0c with in minutes

Habitat

- C. trachomatis is found in the urogenital tract of man
- C. psittaci is found in many wild and domestic birds.
- Infection in man is by inhaling the organisms in the dried dropping of birds

Pathogenicity

- Human infection caused by:
  - Chlamydia trachomatis
  - Chlamydia pneumonia
- 15 d/t Chlamydia serovars have been identified

I) **C. trachomatis:** causes:

1. Eye disease

- Trachoma (servars A, B, Ba, C)
- Inclusion conjunctivitis (serovars D to K)
- Neonatal ophthalmia (serovars D to K)

2) Genital tract infections (Serovars D- K)

- In Men: - urethritis, which can lead to epididymitis and occasionally prostatitis

-In Women:- cervicitis and endometritis which can lead to salpingitis, pelvic inflammatory disease (PID), and infertility .For pregnant women, increased risk of ectopic pregnancy

3) Lymphogranuloma venereum, or LGV (Sero L_1 - L_3)

- LGV is a sexually transmitted disease. It is also called tropical, or climatic bubo

4) Pneumonia and pneumonitis (serovars D-K)

- Most infection occur in new born infants 2 – 12 week after birth or in immunocompromised persons and those already in poor health.

II) C. pneumonia: Causes:-

- Atypical pneumonia usually a mild respiratory infection with sore throat, cough fever, and symptoms associated with asthma
- Transmitted by inhaling aerosols

III) C. psittaci: cause

- Cause psittacosis which is a disease of parrots and other birds
- Pneumonia flu – like illness
- The disease x-rized by pneumonia, septicaemia, meningoencephalitis, endocarditis and pericarditis.
- Inhalation of infected aerosols by man leads to atypical pneumonia

Laboratory Diagnosis

Specimen: – includes conjuctival scraping, blood, sputum, pus and swabs of urethra, endocervix, conjunctiva ad nasopharynx

Microscopy: - direct smears are stained with Giemsa, with iodine or by immunofluorescence (IF) to detect inclusion bodies.

Culture: - the isolation of C. trachomatis can only be undertaken by a laboratory with facilities for tissue culture

Serological Diagnosis

Antimicrobial Sensitivity

- Sensitive to tetracycline, sulphonamides, chloramphenicol and rifampicin. Topical preparation may also be applied

Genus Mycoplasma

- Consist or three groups of organism mycoplasma, Acholeplasma & ureaplasma

Characteristics

- The smallest free living micro organism and very pleomerphic
- Occur as cocci or long filaments which may appear branched
- True bacteria but resemble virus in their filterable size
- Do no have rigid cell wall
- Inhabited by antibiotic, though not by penicillin
- Ureaplasma urealyticum urea dependent strain
- Type species:-
  - M. pneumoniae
  - M. hominis
  - Ureaplasma urealyticum

Habitat: - are found in soil, plants, sewage and mucous surfaces of animal body

- M. hominis and U. urealyticum can be found in the UGT and URT of humans.

Pathogenicity

M. pneumonia

- Commonest cause of atypical pneumonia and some milder respiratory disease
- Also cause arthritis, hepatitis, cerebral nervous system involvement (meningoencephalitis, cerebral ataxia , etc), muscle inflammation, skin rashes, myocarditis, and gastrointestinal disorder.

M. hominis and U. urealyticum:-

- Cause pelvic inflammatory diseases, salpingitis, tube – ovarian abscesses, pelvic abscesses, septic abortion and puerperal fever.
- U. urealyticum also reported to cause urinary tract infections and arthritis.

- ***Laboratory Diagnosis***
- Specimen: - Respiratory tract, urogenital tract , Eye , warts , synovial fluid , Placental membrane , and Foetal tissues
- **Microscopy: -** gram negative, in coccoid and filamentous forms
- **Culture: -** Mycoplasma grow on solid or liquid media enriched with 20% horse or human serum and yeast extracts
- They show typical "fried egg" appearance colony
- **Antimicrobial Sensitivity**
- Resistant to penicillin and cephalosporins. U.urealyticum is resistant for tetracycline. Some are sensitive to erythromycin

RICKETTSIAE

- Rickettsiae have three Genera:
  - *Rickettsia,*
  - *Rochalimaea*
  - *Coxiella*
- These organisms are small, pleomorphic coccobacilli about 2 µm in length. Their structure is typical of Gram-negative bacteria.
- The *Rickettsia* is Gram-negative, obligate intracellular bacteria that infect mammals and arthropods.
- *Rickettsia* replicate in the cytoplasm and nucleus of their host cell; *Coxiella* replicate only in the phagolysosome.

- *Rickettsiae has the following* Species:
  - *Rickettsia prowazekii* (epidemic typhus),
  - *Rickettsia typhi* (endemic typhus),
  - *Rickettsia rickettsii* (spotted fever),
  - *Rickettsia tsutsuga mushi (*Scrup typhus)
  - *Rickettsia conorii(*Tick typhus)
  - *Rickettsia akari (Rickettsialpox)*
  - *Rochalimaea quintana* (trench fever),
  - *Coxiella burnetii* (Q fever)
- Q fever is found mostly in animals. Humans acquire disease primarily by inhalation of contaminated aerosols.
- Trench fever is transmitted from human to human via the louse.

- Based on disease they cause Rickettsia species are divided into three main groups:
  - Typhus group: those spp that cause epidemic typhus (louse borne typhus) and endemic typhus (murine, or flea borne typhus)
  - Scrub typhus group: contains spp that causes mite-borne typhus
  - Spotted fever group: includes those rickettsia that cause Rocky Mt spotted fever

PATHOGENESIS:

- Trench fever is transmitted via arthropod vectors; Q fever is acquired via inhalation or ingestion of contaminated milk or food.
- Within minutes, the bacteria enter host endothelial cells via an induced phagocytosis. The enzyme phospholipase A may help penetration

- Replication of the bacteria causes lysis of the host cell and consequent spread to other cells.
- Initial replication occurs at the site of entry producing a local lesion. This is followed by dissemination via the vascular system producing vasculitis and a skin rash. These lesions may become necrotic.
- Virulence is probably due to many factors including release of endotoxin, the production of immune complexes and hypersensitivity reactions

- Diagnosis of rickettsial disease is based on serological analysis
- treatment of choice for all ricketsial disease is tetracycline, with chloramphenicol
- **Prevention and control:**
  - reducing exposure to the arthropod vector by wearing protective clothing and using insect repellent
  - personal hygiene and “delousing” with DDT