

Chapter V

Gram negative coccobacilli

Learning objective:

At the end of this chapter the students will be able to:

- List the medically-important species of Gram negative coccobacilli
- Describe general characteristics of Gram negative coccobacilli
- Recognize diseases caused by Gram negative coccobacilli
- Describe the virulent factor of pathogenic species of Gram negative coccobacilli
- Discuss pathogenicity, clinical manifestations, laboratory diagnosis, prevention & control of members of the Gram negative coccobacilli

Genus Haemophilus

- Gram negative rods, coccobacilli
- Non motile, non-spore forming, variable Catalase rxn
- Microaerophilic, humid enriched environment
- Requires medium supplemented with growth stimulating factor (X and/or V factor)
- Causes Respiratory, genitourinary, and CNS infection
- Species with prefix Para – require V factor only for growth

Consists the following species

- *H. influenzae*
- *H. parainfluenzae*
- *H. haemolyticus*
- *H. parahaemolyticus*
- *H. ducreyi*
- *H. aegypticus*
- *H. aphrophilus*
- *H. segnis*
- *H. paraphrophilus*
- Some are capsulated
- Natural habitats
 - URT, GI, % UGT

Haemophilus influenzae

- A slender , short, non motile, non-sporing, non acid fast gram negative coccobacilli sometime occurring in pair or short chains
- Long thread-like and pleomorphic forms may be seen in c.s.f. (with pus cells), or following culture.
- H. influenzae occurs in capsulate and non-capsulate form
- Based on polysaccharide capsule type, capsulated H. influenzae can be classified in to six serotype: a, b, c, d, e, and f.
 - b serotype is highly virulent
- Non capsulated (Non-typable) H. influenza are less pathogenic

Virulence factor

- Capsular polysaccharide has antiphagocytic activity
- Adherence (fimbriae)
- Membrane lipooligosaccharide may be responsible in bacterial attachment invasiveness, and paralysis of the ciliated respiratory epithelium
 - Outer membrane protein contribute in adhesion and invasion of host tissue
- IgA protease
 - Facilitating attachment to the respiratory mucosa

Pathogenesis and Clinical manifestation

- **In developing countries invasive infections with capsular type b *H. influenzae* are major causes of ill health and premature death in infants and young children.**
 - **Infections are usually bacteraemic.**
- ***H. influenzae* type b causes:**
 - **Pyogenic (purulent) meningitis in young children below 5 year.**
 - **Pneumonia and empyema (mainly adults).**
 - **Acute epiglottitis (acute inflammatory swelling of the epiglottis and neighbouring structures) which may cause fatal airway obstruction.**
 - **Cellulitis (orbital), Osteomyelitis, septic arthritis, and occasionally other invasive infections.**
- **The carrier rate for capsulated b strains is about 2-4%.**

- Non-typable *H. influenzae* strains are mainly

Responsible for

- chronic bronchitis (usually in adults),
 - Middle ear infections,
 - paranasal sinusitis
 - conjunctivitis.
- These strains form part of the normal microbial flora of the upper respiratory tract in 50–75% of persons.

- *H influenzae* type b enters by way of the respiratory tract.
- There may be local extension with involvement of the sinuses or the middle ear.
- *H influenzae* type b is one of the most common etiologic agents of bacterial otitis media and acute sinusitis.
- The organisms may reach the bloodstream and be carried to the meninges or, less frequently, may establish themselves in the joints to produce septic arthritis.
- Clinically, it resembles other forms of childhood meningitis, and diagnosis rests on bacteriologic demonstration of the organism.

Lab diagnosis

Specimens:

- These include cerebrospinal fluid (c.s.f.), nasopharyngeal specimens, pus, and blood for smears and cultures.
- Specimens must be cultured as soon as possible and not refrigerated.
- Long thread-like and pleomorphic forms may be seen in c.s.f. (with pus cells), or following culture.
- It is best stained using dilute carbol fuchsin as the counter stain.
- The capsule which surrounds capsulated strains can be demonstrated by using specific antiserum.

Culture

- *H. influenzae* grows poorly anaerobically.
- Growth is best achieved in a moist carbon dioxide enriched atmosphere
- The temperature range of growth is 20–40 °C with an optimum of 35–37 °C.
- Media used to grow *H. influenzae* must contain haemin or other iron-containing porphyrin and nicotinamide adenine dinucleotide (NAD) or its phosphate (NADP).
 - The porphyrin requirement is referred to as growth factor X and
 - the NAD or NADP requirement as growth factor V.

Chocolate agar:

- After overnight incubation at 35–37 °C in a moist carbon dioxide atmosphere, capsulated *H. influenzae* strains produce mucoid colonies,.
- Cultures have a distinctive smell.
- *H. influenzae* grows well on chocolate agar because it contains factors X and V.
- Heating blood agar to 75 °C inactivates serum NADase and releases extra factor V from the red cells.
- Addition of bacitracin (300 mg/litre) provides a selective medium to recover *H. influenzae* from sputum.
- This is not needed when culturing c.s.f.

Satellitism test

- It is a staphylococcus streak technique that can be used to recover *Haemophilus* spp from clinical specimen.
- This is a routine test in clinical bacteriology for the identification of *H. influenza*
- an organism such as *S. aureus* is streaked across a plate of Blood Agar and Nutrient Agar on which a specimen containing *H. influenza* has been inoculated,
- after overnight incubation, the colonies of *H. influenza* will be large and well developed along side the streak of *S.aureus* and smaller farther away.
- *H. influenzae*: shows growth on the blood agar plate but not on the nutrient agar plate,
- If satellite colonies are present on both plates the organism is probably an *Haemophilus* species that requires only factor V, such as *H. parainfluenzae*.

Treatment

- susceptible to ampicillin
- all strains are susceptible to the third-generation cephalosporins.
- Cefotaxime given intravenously gives excellent results.
- Prompt diagnosis and antimicrobial therapy are essential to minimize late neurologic and intellectual impairment.
- surgical drainage of a localized subdural accumulation of fluid in meningitis

Prevention and control

- Contact with patients suffering from *H influenzae* clinical infection poses little risk for adults
 - but presents a definite risk for nonimmune siblings and other nonimmune children under age 4 years who are close contacts.
- Prophylaxis with rifampin is recommended for such children.

Haemophilus ducreyi

- *Short, ovoid, Gram negative bacilli, may show bipolar staining.*
- *They may occur in end to end pairs or short chains in smears*
- Require both X but not V factor for growth

Pathogenicity and clinical manifestation

- *H. ducreyi* causes chancroid, or soft sore.
- It is sexually transmitted and a common cause of genital ulceration.
- The ulcers are painful, shallow and tend to be ragged with marked swelling and tenderness and it bleed easily.
- Often there is also painful swelling of inguinal lymph nodes, and abscesses (buboes) may form.
- Chancroid increases the risk of infection with HIV and facilitates transmission of the virus.

Lab diagnosis:

- ***Specimens:***

- Specimens should be collected from the base and margins of ulcers following cleansing with a saline swab (exclude necrotic tissue).
- Specimens for culture must be delivered to the laboratory with the *minimum* of delay.
- When this is not possible the swab should be placed in Amies transport medium and delivered the same day to the laboratory or sent in an insulated cool box to reach the laboratory within 48 h.

Culture

- *H. ducreyi* is difficult to isolate.
- It is grown best from scrapings of the ulcer base on
 - chocolate agar containing 1% IsoVitaleX and vancomycin, 3 µg/mL, and incubated in 5-10% CO₂ at 33-36 °C for 2-3 days.
 - GC agar base with added haemoglobin, vancomycin, and fetal calf serum.
- The organism grows slowly, producing small grey-yellow or brown colonies usually within 2-4 days.

Biochemical tests

- Slowly oxidase positive (colour develops after 15-20 seconds).
- Catalase, urease, and indole negative.
- Ornithine decarboxylase (ODC) negative.
- *delta*-Aminolevulinic acid (ALA) negative

Treatment

- There is no permanent immunity following chancroid infection.
- Treatment with intramuscular ceftriaxone, oral trimethoprim-sulfamethoxazole, or oral erythromycin often results in healing in 2 weeks.

Haemophilus aegyptius

(H. influenzae biotype C)

- causes acute and highly infectious conjunctivitis often referred to as 'pink eye'.
- In acute conjunctivitis, *H. influenzae* biogroup *aegyptius* can be isolated from eye discharge and in Brazilian purpuric fever (BPF) from blood culture.
- The organism closely resembles other *H. influenzae* organisms.
- It also requires factors X and V for isolation but it grows more slowly.
- Addition of 1% Isovitalex to chocolate agar improves its growth.

Genus Bordetella

- Minute strictly aerobic, non motile ,gram negative coccobacilli that appears singly or in pairs
- non-spore forming, Catalase +ve
- Fastidious organism (require blood supplemented medium)
- Bordetella species of clinical significance include
 - B. pertussis, B. parapertussis, B. bronchoseptica, ...

- *Bordetella pertussis*, a highly communicable and important pathogen of humans, causes whooping cough (pertussis).
- *Bordetella parapertussis* can cause a similar disease.
- *Bordetella bronchiseptica* (*Bordetella bronchicanis*) causes diseases in animals such as kennel cough in dogs and snuffles in rabbits, and only occasionally causes respiratory disease and bacteremia in humans, primarily in immunocompromised hosts.

Bordetella pertussis

- *B. pertussis* is a small, non-motile, capsulated Gram negative coccobacillus.
- It may occur singly or in chains
- With toluidine blue stain, bipolar metachromatic granules can be demonstrated

Virulence factor

- **Adhesins**

- **Most important adhesin is filamentous haemagglutinin**
- **Mediate adhesion to ciliated epithelial Cells**

- **Toxin**

- **Pertussis toxin by only B. pertussis**
- **Adenylate cyclase toxin**
- **Tracheal cytotoxin**
- Dermonecrotic toxin
- Endotoxin LPS

- **Agglutinin**

- **Outer Membrane Protein (OMP)**

Pathogenicity and Clinical manifestation

- *B. pertussis* causes whooping cough, an infection of the mucosa of the upper respiratory tract.
- Toxin from the organisms causes the secretion of mucus which leads to irritation and the spasms of coughing associated with the disease.
- There is a marked leucocytosis with an absolute lymphocytosis.
- Complications of infection include lung damage with emphysema, secondary infection leading to bronchopneumonia, bronchiectasis, convulsions and occasionally brain damage.
- *Note: B. parapertussis* causes a milder form of whooping cough.

- After an incubation period of about 2 weeks, the "**catarrhal stage**" develops, with mild coughing and sneezing.
 - During this stage, large numbers of organisms are sprayed in droplets, and the patient is highly infectious but not very ill.
- During the "**paroxysmal**" **stage**, the cough develops its explosive character and the characteristic "whoop" upon inhalation.
 - This leads to rapid exhaustion and may be associated with vomiting, cyanosis, and convulsions.

- The "whoop" and major complications occur predominantly in infants;
- paroxysmal coughing predominates in older children and adults.
- The white blood count is high (16,000–30,000/L), with an absolute lymphocytosis.
- After 3-4 weeks the disease enters **Convalescent stage**
 - **Frequency and severity of the coughing gradually decrease**
 - **But secondary complications can occur**

Lab diagnosis

- *Specimens:*
 - Preferably nasopharyngeal secretions collected by aspiration or a correctly taken pernasal swab.
 - A saline nasal wash is the preferred specimen.
 - cough droplets expelled onto a "cough plate" held in front of the patient's mouth during a paroxysm are sometimes used but are not as good as the saline nasal wash.

Culture

- The saline nasal wash fluid is cultured on solid medium agar
- The antibiotics in the media tend to inhibit other respiratory flora but permit growth of *B pertussis*.
- Organisms are identified by immunofluorescence staining or by slide agglutination with specific antiserum.

Culture

- For the isolation of *B. pertussis* must be cultured as soon as possible after they are collected.
- A selective and enrichment medium such as charcoal cephalixin blood agar is recommended for the primary isolation of *B. pertussis*.
- Small, convex, smooth colonies

Charcoal cephalalexin blood agar:

- Widely used for primary isolation which has largely replaced Bordet-Gengou medium
- When incubated for 2–6 days at 35–37 °C in a moist aerobic atmosphere *B. pertussis* produces small pearly-grey, shiny (mercury-like), usually mucoid colonies.

Bordet-Gengou media (Potato-blood-glycerol agar)

- is selective media containing a high percentage of blood (20-30%), to inactivate inhibitors in the agar.

Treatment

- Sensitive to Erythromycin, tetracycline, chloramphenicol

Prevention and control

- adequate active immunization of all infants

Genus Brucella

- Gram negative, short rods, coccobacilli
- Non-motile, non-spore forming, non-capsulated
- Aerobic, require complex media (amino acid, thiamine, nicotinamide)
- Growth enhanced (serum/blood)
- Catalase +ve, oxidase +ve
- Many require CO₂ for growth
- characteristically located intracellularly
- Cause disease primarily in animals
 - Bacteremia and septicaemia in humans

Natural habitats

- Animals – main reservoir
- Transmit from Animal to humans through
 - Milk products
 - Handling of animals and cultures
 - Inhalation of aerosols

- Brucella species and their preferred host
 - B. abortus-cattle, horse, buffalo
 - B. melitensis-goat, sheep, camel
 - B.suis-swine, hare, rodents
 - B. canis-dogs
 - B.ovis-sheep
 - B.neotomae-rodents
- B. abortus, B. melitensis, and B.suis causes human brucellosis

Virulence factor

- Ability to resist phagocytosis
 - Has certain low-molecular substance on bacterial surface
- Ability of intracellular survival

Pathogenesis and clinical manifestation

- Brucellae are intracellular organisms infecting reticuloendothelial cells of the spleen, liver, kidneys and bone marrow. From these sites, the bacteria pass into the blood.
- The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.
- The disease is characterized by fever which may be continuous, intermittent, undulating or irregular.
- Acute infection may resemble severe influenza with headache, sweating (especially at night) and generalized pains associated with fatigue and depression.

- Urogenital symptoms may occur
- Often the patient is anaemic and leukopenic with a relative lymphocytosis.
- Untreated infections can become chronic with musculoskeletal symptoms (back pain, arthritis, arthralgia).

Lab. Diagnosis

- ***Specimens:***
 - Blood, bone marrow aspirates, biopsy specimen (lymph nodes, bone, etc), serum
- In microscopic examination, they do not show bipolar staining but may stain unevenly.
 - tiny gram-negative coccobacilli
- Brucellae multiply faster in bone marrow cultures, and when the patient has received antibiotics, the organism are more likely to be isolated from bone marrow than blood.
- The organisms are more likely to be isolated from the blood in acute brucellosis during times of fever.
 - Isolation is extremely rare in chronic brucellosis.

- *Brucella* species bacteria will grow on commonly used media, including
 - trypticase soy medium with or without 5% sheep blood,
 - brain heart infusion medium, and
 - chocolate agar.
 - Serum dextrose agar
 - Blood culture media (readily grow *Brucella* species bacteria.)
 - Tryptone soya (tryptic soy) diphasic medium (Castaneda)
- Brucellae are aerobic with *B. abortus* requiring a carbon dioxide enriched atmosphere.
- Cultures should be kept for 4 weeks with sub-culturing every few days.
- When sub-cultured on solid agar, colonies usually appear 2–3 days after incubation.
- All cultures should be incubated in 8–10% CO₂ at 35–37 °C with sub-culturing every few days
 - It should be observed for 4 weeks before being discarded as negative;

- After a few days of incubation on agar media, the brucellae form colonies
 - They are nonhemolytic.
 - variety of colonial forms are produced by *Brucella* strains including Small, smooth, convex, mucoid, and rough colonies.
 - They may be colourless or greywhite.
- All further work on such a culture should be done in a biologic safety cabinet.
- A Christensen urea slant should be inoculated and observed frequently.

Biochemical test

- The bacteria are catalase-positive, urease-positive, and usually oxidase-positive.
- They are indole negative and most strains hydrolyze urea.
- Except *B. ovis*, *Brucella* species reduce nitrate to nitrite

Serological diagnosis

- Many cases of brucellae infection are diagnosed by serological test which include the standard agglutination test and an ELISA procedure for detection of brucella-specific IgM/IgG.

Treatment

- Brucellae may be susceptible to tetracyclines or ampicillin.
- For best results, treatment must be prolonged.
- Combined treatment with a tetracycline (such as doxycycline) and either streptomycin or rifampin is recommended.

Prevention and control

- limitation of spread and possible eradication of animal infection,
- pasteurization of milk and milk products, and
- reduction of occupational hazards wherever possible.