

Gram-negative rods related to respiratory tract:
-----**Hemophilus:**

H.influenzae is most species that cause serious diseases in human. In 1892 was first isolated by Pfeiffer from patient of influenza and was erroneously considered as causative agent of this disease.

- Small and pleomorphic rod.
- It may encapsulated or unencapsulated.
- Non-motile.
- It is aerobe.
- It need for certain growth factors; X (heme) and V (NAD) for adequate energy production because inability of the organism to synthesize these factors.

Antigenic structure and virulence factors

- Capsular polysaccharide is used for serotyping *H.influenzae*. encapsulated strains contain one of six types(a,b,c,d,e,f). The type-b is most common pathogen for human.
- Somatic antigen consists of outer membrane proteins.
- IgA protease produced by this organism degrades secretory IgA, facilitating colonization of URT.
- It produce endotoxin , and it produce no exotoxin.
- It has attachment factor (pili) .

Transmission

The organisms are found on mucous membrane of URT in human as normal flora, mostly are not encapsulated. Humans are only natural host, there is no animal reservoir. *H.influenzae* is transmitted by respiratory droplets.

Pathogenesis and clinical findings

H.influenzae infect only human(obligate human parasite). It enter the body through URT , resulting in either asymptomatic colonization or infection.

Encapsulated strains are found only in small numbers in URT. *H.influenzae* type-b(HIB) can spread from primary site in URT , enter blood stream and disseminate to distant sites. It is cause meningitis, pneumonia , epiglottitis,cellulites, septic arthritis.

H.influenzae is most common cause of bacterial meningitis in children age 5 months to 5 years. Hemophilus Meningitis may associated with neurologic morbidity (sequelae) that range from hearing loss to mental retardation. Clinical features of meningitis such as fever, headach, and

stiff neck with drowsiness. Mortality rate of untreated meningitis may be up to 90%.

Fulminating obstructive laryngotracheitis with swollen, cherry-red epiglottitis develop in infants (obstructive air way is life-threatening disease) and requires prompt tracheostomy or intubation as lifesaving procedure. Sinusitis and otitis media cause pain in affected area, opacification of infected sinus, and redness with bulging of tympanic membrane.

Lab. Dx

- Isolation of the organism from clinical material (such as CSF, blood, throat swab, sputum) on heated blood agar (chocolate agar) enriched with two growth factors X and V.
- A capsule swelling (quelling test) test with specific antiserum is analogous to quelling test of pneumonia.
- Capsular antigen may be detected in CSF or other body fluid using immunologic tests such as latex agglutination.

Treatment;

- Drug of choice is cefotaxime. Many strains are sensitive to ampicillin, and amoxicillin-clavulanate.
- Vaccine conjugated to diphtheria toxin or to carrier protein. The vaccine is more effective in children.
- Rifampin is given to individuals who close contact with patients, which used to decrease respiratory carriage of organism, thereby reducing transmission of these organisms.

Bordetella:

- Small gram-negative coccobacilli .
- Non-motile.
- Encapsulated rod.
- It is aerobe.
- It is unable to survive outside its host for long time.

Most cases of whooping cough (95%) are caused by *B.pertussis* , whereas 5% of cases are caused by *B.parapertussis*.

Transmission ;

It is found in nasopharynx and respiratory tract mucosa. Transmission is by direct contact , by droplets from respiratory tract , and by freshly contaminated fomites. Most cases occur in children under age 5 years, and most deaths occur in first year of life. It is highly communicable and cause highly morbidity and mortality.

Antigenic structures and virulence factors;

1. Attachment of organism to ciliated epithelial cells of URT is mediated by protein on pili called filamentous hemagglutinin (Fha).
1. Pertussis toxin(Ptx) is A-B toxin, consist of 2 chains ,A-5B (A single subunit ,while B composed of 5 non-identical subunits). Ptx has two effects , one is stimulate adenylate cyclase in manner of cholera toxin. This result in edema and other changes in respiratory tract, leading to cough. Second effect also cause lymphocytosis in blood by inhibits signal transduction by chemokine receptor, resulting in failure of lymphocytes to enter lymphoid tissue(spleen and lymph nodes) , therefore is increased in blood. Inhibition of signal transduction by chemokine receptor is also caused by ribosylation of G protein.
2. Cytolysin is bacterial adenylate cyclase toxin that can inhibits bactericidal activity of phagocytic cells. Hemolysin is associated with virulent strains in pathogenesis.
3. Tracheal cytotoxin is peptidoglycan fragment appears to act in concert with endotoxin to induce nitric oxide ,which kills the ciliated epithelial cells. LPS in cell wall may also be important in causing damage to epithelial cells of URT.

Pathogenesis and clinical findings;

The organism adheres to and multiplies rapidly on ciliated epithelial surface of trachea and bronchi and interferes with ciliary action. The blood is not invaded. The bacteria liberate toxins and substances that irritate surface cells, causing coughing and marked lymphocytosis. Necrosis of part of epithelia may occur. The combined action of at least three toxins inhibits normal respiratory clearance mechanisms. The organism is cause ciliolysis (damage ciliated cells of respiratory tract). Decreased cilia activity followed by death of ciliated epithelial cells is important aspects of pathogenesis.

Obstruction of small bronchioles by mucus plugs results in atelectasis and diminished oxygenation of blood. This problem contributes too frequently of convulsions in infants with whooping cough.

Clinical significance:

B.pertussis is obligate human parasite(infect only human). The incubation period of pertussis (whooping cough) is ranged from 1-3 weeks. The whooping cough is acute tracheobronchitis can be divided in the following stages;

- Catarrhal phase; it begins with nonspecific symptoms(flu-like) such as rhinorrhea(runny nose), mild conjunctival infection, malaise, low fever and then progresses to mild dry ,nonproductive

cough and sneezing. Large numbers of organism are sprayed in droplets, and highly infectious.

- Paroxysmal phase; it is characterized by severe coughing followed inspiratory whoop. Large amount of mucus may be produced. The disease gets its name from sharp intake of air between coughing paroxysms that sounds like a whoop. Leukocytosis is occurring in this phase. Paroxysms may cause cyanosis and /or end with vomiting. (Note; Whooping may not occur in all patients).
- Convalescence phase; it requires at least additional 3-4 weeks. During this period, secondary infection may occur such as pneumonia, otitis media or seizures.

Several agents such as adenoviruses and *Chlamydia pneumoniae* can produce clinical pictures resembling to whooping.

Lab.Dx

1. Saline nasal wash is preferred specimen. Nasopharyngeal swabs or cough droplets expelled onto cough plate.
2. Culture on Bordet-Gengou medium , the initial isolation usually require to 10% CO₂ to enhances the growth of the isolates.
3. Serologic tests have little diagnostic help because arise antibodies does not occur until third week of illness. Agglutination test, fluorescent antibody test and PCR are used in diagnosis of this organism.

Treatment:

1. Drug of choice is erythromycin, it is used to reduce number of organism.
2. Oxygen therapy and aspiration (suction of respiratory mucus) is important especially in infants, and this supportive care may prevent anoxic damage to brain.
3. Two pertussis vaccines are available; acellular vaccine containing purified proteins (such as Fha, ptx) is combined with toxoid of diphtheria and tetanus (triple vaccine; DTP). Killed vaccine containing inactivated organism.

Legionella:

- Gram-negative rod .
- Aerobe and fastidious organism.
- Encapsulated, but in nature is un-encapsulated.
- It is facultative intracellular parasites.
- Motile by polar flagella.
- Catalase-positive and weakly oxidase.

Most cases about 90% of human infection are caused by *L.pneumophila*.

Transmission

Normal habitat is soil and water (fresh water or, including in air-condition system, water-cooling towers and water distribution systems). It found in fresh water especially in warm water, it associated with blue-green bacteria, or perhaps, inside algae and free-living amoebae.

Most infections result from inhalation of aerosolized water or soil particle, but may follow other exposures, for example, swimming in contaminated water.

Antigenic structure and virulence factors

- There are more than 10 serogroups. Serogroup-1,2,3 and 6 are responsible for more than 80% of human disease.
- Major virulence factors are endotoxin and intracellular survival. No exotoxins are produced.
- It is produce several enzymes and proteins such as protease, lipase, and has hemolytic and cytotoxic activity.

Pathogenesis:

The organism gains entry to URT by aspiration of water containing the organism, or by inhalation of contaminated aerosol. Failure to clear the organisms permits them to reach the lungs. The bacteria bind and enter into alveolar macrophage by endocytosis, resulting in inhibition of phagosome to fuse with lysosome. After entry into phagocytic cell, the organism multiplies within alveolar macrophage (intracellular), lead to cell rupture and releasing new crop of bacteria and infection of other macrophages. It causes multi-focal necrosis in lung. Lung damage also results from inflammatory response.

Clinical findings:

It primarily causes RTI. There are two distinctly different presentations; legionnaire disease(LD, legionellosis) and Pontiac fever.

LD is atypical pneumonia occurs in both, community and hospitalized immuno-compromised patients. Factors associated with high risk include smoking, alcoholic men, chronic bronchitis and emphysema, and immuno-compromised as elderly, patients with steroids, renal transplantation, diabetes mellitus, cancer therapy.

Symptoms of LD, after IP 2-10 days include fever, malaise, myalgia, anorexia, slightly dry cough and watery diarrhea in 25-50%. Nausea, vomiting, hematuria and neurologic symptoms(mental confusion) may also occur. Severe disease may progress to shock and respiratory failure within one week of worsening symptoms. Most cases resolve spontaneously in 7-10 days, but in older or immunocompromised patients, the infection can be fatal. Fatality rate for LD range from 5-30%.

Pontiac fever; initially resemble to LD , characterized by influenza-like illness, develop over 6-12 hours, that not result in pneumonia. Dizziness, photophobia, neck stiffness and confusion also occur. Non-fatal infection but recovery completely within one week.

Lab.Dx

- The specimen include; lung aspiration or washing and lung biopsy. Isolation from sputum is more difficult because predominance of bacteria of normal flora.
- The organism fails to grow on ordinary media, but grow best on charcoal-yeast agar which supplemented with cystiene and iron .
- Detection of legionella antigens in urine by using indirect immunofluorescent assay is very important.

Treatment:

- Azithromycin, erythromycin are drugs of choice.
- Reducing cigarette and alcohol consumption, eliminating aerosols from water sources and hospital water supplies by using high temperature and hyperchlorination.
- There is no vaccine.