Gram-Negative Rods Related to the Respiratory Tract

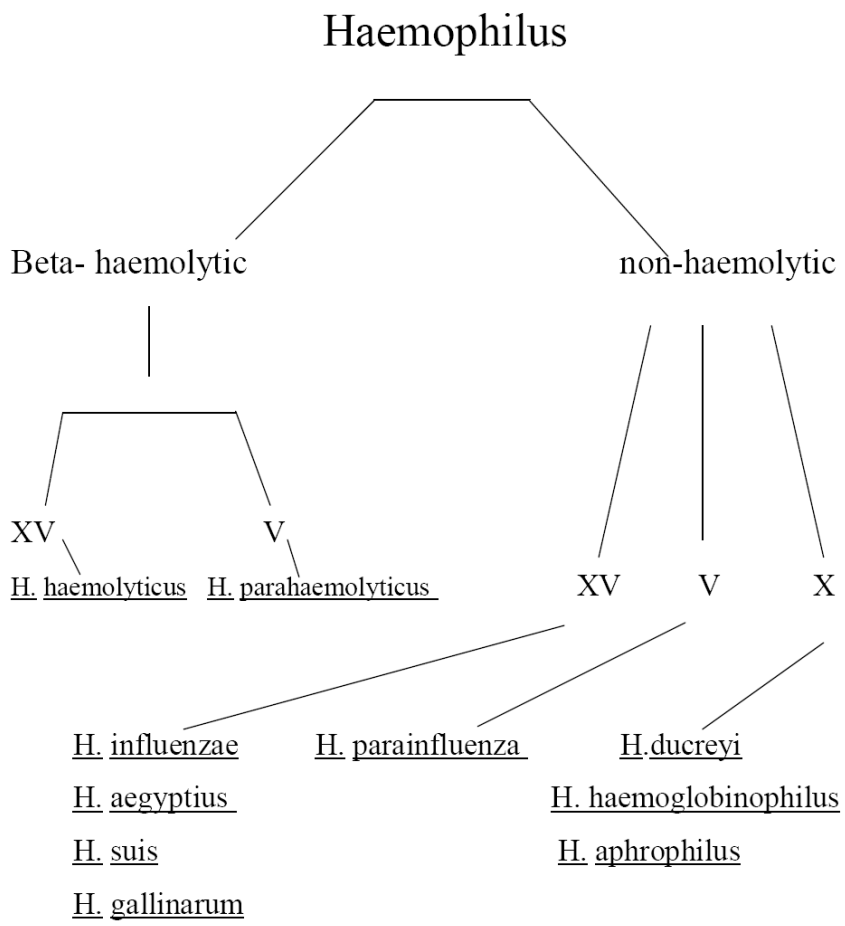
There are three medically important gram-negative rods typically associated with the respiratory tract:

H. influenza, *B. pertussis* and *L.pneumophila*

H. influenzae and *B. pertussis* are found only in humans, whereas *L. pneumophila* is found primarily in environmental water sources.

Haemophilus **(**Haemo= blood , philus= loving)

Small gram –ve rods called coccobacilli, sometime are pleomorphic, facultative anaerobic which grow on enriched media, non motile, non spore forming, catalase positive and oxidase positive. Require X (hematin) and V (nicotinamide adenine dinucleotide) factors for their growth. *Haemophilus* can be divided into:



Haemophilus influenzae called (pfeiffer's bacilli)

Morphology: G-ve, short coccobacilli. In young culture i.e. after 6-8 hour, the m.o. will be capsulated, in old culture i.e. after 18 hour, the m.o. becomes long, filamentous and lose the capsule, so the m.o. described as pleomorphic m.o.

The capsule is important in typing by a reaction using antisera similar to quelling reaction, also the capsule can be detected by (CCE) counter current electrophoresis or by immunoflourescent test.
The m.o. with capsule are virulent and form mucoid smooth colony and these without capsule form rough colony.
six serotypes, type b causes most of the severe diseases such as meningitis and sepsis.

what we mean by X, V, Satellite phenomenon.

H. influenzae will grow in the hemolytic zone of *S.aureus* on blood agar plates. The hemolysis of erythrocytes by *S. aureus* releases nutrients vital to the growth of *H. influenzae* (NAD, factor V). The NAD diffuses into the surrounding medium and stimulates the growth of *H. influenzae* in the vicinity of the *staphylococcus. H. influenzae* will not grow outside the hemolytic zone of *S. aureus*. This is known as satellite phenomenon.

Pathogenesis:

H. influenzae infect only human and there is no animal reservoir, it enters the body through the URT, resulting in either asymptomatic infection or infection as otitis media, sinusitis and pneumonia.
The m.o. produce **IgA protease** that degrade secretory IgA, thus facilitate attachment to the respiratory mucosa.
After establishment in the URT, the organism enters the blood stream and spread to the meninges causing meningitis.
Meningitis caused by **encapsulated** strain 95% of which posses type B capsule (antiphagocytic)
Endotoxin No exotoxin is produced.
Most infection occurs in children between the age of (6 months - 6 years) with a peak between 6 months – 1 year (**why??).** This may be due to decline in maternal IgA and inability of child to generate Ab against the polysaccharide capsular Ag.
The m.o. are able to transform DNA extract from one generation to another in order to transfer the resistance to antibiotics, e.g penicillin, chloramphenicol and this is by plasmid.

Lab dx:

Poorly ferment CHO, need 5-10% co2 in their growth
Media used:
- Brain heart infusion agar with blood, colonies are small,rounded, iridescent and dew like appearance.
- Chocolate agar:*Haemophilus influenzae* requires X and V factors.

Direct examination of naso- pharyngeal swab, swab, blood, CSF by immune-flourescent technique
Quelling reaction for typing and counter currant electrophoresis.

Treatment:

Mortality rate in meningitis due to this m.o. is high up to 90% in young children. The m.o. is sensitive to ampicillin, about 30% of strain may produce B- lactamase enzyme, all strain are susceptible to new generation cephalosporins.

H. influenzae type b capsular conjugate **vaccines** are available for children age 2 months and older.

Bordetella

Bordetella pertussis:

It causes a highly important communicable disease in human being which is pertussis (whooping cough). The disease has duration of 1-2 months, it affects children with a catarrhal inflammation of respiratory tract with a characteristic of paroxysmal cough that end in whoop.

B. pertussis is a small gram –ve coccobacilli, strictly aerobic, encapsulated, the capsule can be identified by using immunoflourescent method. Staining with tolidine blue shows bipolar metachromatic granules

the causative m.o. named bordet- Gengou bacilli according to the scientists which observe the bacilli in the sputum of a patient with whooping cough.

All Bordetella pertussis are alike when they are freshly isolated from the body but when cultivated they will resolve into four phases:-

Phase I: which represent the freshly isolated virulent and encapsulated pathogen.

Phase VI: is the completely non pathogenic form (a virulent stage)

Phase II and III: are intermediate.

Antigenic structure:

Cell wall contain lipopolysaccharide

4 biologically active substances

a- pertussis toxin: (major virulence factor) which is an exotoxin and is responsible for prolonged immunity. It has a histamine sensitizing properties and is responsible for the paroxysmal cough which is the characteristic of the disease.

b- 2 hemagglutinin: one is filamentous haemagglutinin, the other causes leucocytosis particularly lymphocytosis.

c- Adenylate cyclase complex: catalyze the production of cAMP by various types of cells

d- Heat labile toxin: found in the protoplasm of the cell. It is dermonecrotic and lethal in mice.

Pathogenesis:

B. pertussis, a pathogen only for humans, is transmitted by airborne droplets produced during the severe coughing. The organisms attach to the epithelium of the upper respiratory tract but do not invade the underlying tissue.

Bordetella pertussis infects its host by colonizing lung epithelial cells. The bacterium contains a surface protein, filamentous hemagglutinin, which binds to sulfatides that are found on cilia of epithelial cells. Once anchored, the bacterium produces tracheal cytotoxin, which stops the cilia from beating. This prevents the cilia from clearing debris from the lungs, so the body responds by sending the host into a coughing fit. These coughs expel some bacteria into the air, which are free to infect other hosts. Bordetella pertussis has the ability to inhibit the function of the host's immune system. Two toxins, known as the pertussis toxin (or PTx) and adenylate cyclase (CyaA), are responsible for this inhibition. CyaA converts ATP to cyclic AMP, and PTx inhibits an intracellular protein that regulates this process. The end result is that phagocytes convert too much ATP to cyclic AMP, which can cause disturbances in cellular signaling mechanisms, and prevent phagocytes from correctly responding to an infection.

The course of whooping cough is divided into three stages.
1. The catarrhal stage: This phase typically lasts for one to two weeks. Symptoms during this phase resemble that of an upper respiratory illness: runny nose, nasal congestion, sneezing, and occasional cough.
2. The paroxysmal stage: lasts between one to six weeks. It is characterized by a series of repetitive coughs followed by an inspiratory "whoop" as air rushes past the narrowed glottis.
3. The convalescent stage. This can last for weeks or months and is characterized by a chronic cough.

Laboratory Diagnosis
1) Throat swab taken during paroxysmal stage
a- direct examination: by immunofluorescent technique which can give false (+ve) result.
b- culture: Bordetella pertussis is a slow-growing organism that requires specialized conditions for growth. It is the most fastidious species within the genus.

I. Bordet-Gengou1 medium, then the identification by slide agglutination test with specific antisera or fluorescent antibody stain which is more useful than direct examination. (Bordet- Gengou agar: composed of blood- potato- glycerol) and penicillin G (0.5 Mg/L)
II) charcoal agar supplemented with 10% horse (or sheep) blood and cephalexin. Plates are incubated in air without elevated carbon dioxide at 35°C for a minimum of 7 days before being reported as negative (most isolates are detected in 3 to 4 days). Colonies are small, shiny and round. With age they become whitish grey. Repeated subculture of *B.pertussis* leads to loss of fastidiousness and laboratory adaptation to a variety of media.

2) Cough modified plate method. The plate is held about 10-15 cm in front of the patients mouth during spontaneous or induced coughing so that droplets of repiratory exudates impings directy on the medium.

3) Serological diagnosis of little importance because antibodies do not occur until the 3rd week of illness

Treatment
Treatment with macrolide (i.e., erythromycin, azithromycin) is effective in eradicating organisms and reducing length of infectious stage.

Prevention
The most important preventive measure is the active vaccination. Two vaccines:
1) a cellular vaccine contain purified proteins from the organism. (pertussis toxoid).
2) Killed vaccine contain inactivated *Bordetella pertussis* organism