Lecture 5

Gram Negative rods related to the respiratory tract

Two medically important gram negative rods associated with respiratory tract

Haemophilus influenzae
Bordetella pertussis

Haemophilus

Haemo= blood , philus= loving ie. Bacteria which like blood in their growth, these are heterogenous group of bacteria.

Small gram –ve rods called coccobacilli, some time are pleomorphic, facultative anaerobic which grow on enriched media, non motile, non spore forming. Require X and V factors for their growth.
Some of them are pathogenic while others are non-pathogenic, the group of coccobacilli include Bordetella and Brucella. Haemophilus can be divided into:

**Haemophilus**

- **Beta- haemolytic**
  - XV
    - H. haemolyticus
    - H. parahaemolyticus
  - V
- **non-haemolytic**
  - XV
  - V
  - X
  - H. influenzae
  - H. parainfluenza
  - H. ducreyi
  - H. aegyptius
  - H. suis
  - H. gallinarum
  - H. haemoglobinophilus
  - H. aphrophilus

*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 immunofluorescent test. So the m.o. with capsule are virulent and form mucoid smooth colony and these without capsule form rough colony.

![Image of bacteria](image)

**Antigenic structure:**

1) Capsule: composed of polyribose ribitol phosphate (PRP) so *Haemophilus influenzae* can be typed into 6 types from A-F. The most important one and pathogenic is type B, while the others are rarely pathogenic.

2) Somatic Ag which are proteins and one of two types M,P

3) Endotoxin.
Cultural Characteristics:
Media used:

- Brain heart infusion agar with blood, colonies are small, rounded, iridescent and dew like appearance.
- Chocolate agar: contain a substance called isovital ex.
  Haemophilus influenzae requires X and V factors.
  X haemin, heat stable needed in the synthesis of respiratory enzymes like cytochrome oxidase, catalase and peroxidase.
  V heat labile, can be provided by a substance called NAD (nicotinamide adenine dinucleotide). Needed for oxidation-reduction system.

Satellite phenomena

Other characteristics:
Poorly ferment CHO, need 5-10% co2 in their growth, 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.

Pathogenesis:

Haemophilus 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. Pathogenesis involves the antiphagocytic capsule and 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. This may be due to decline in maternal IgA and inability of child to generate Ab against the polysaccharide capsular Ag.

Diagnosis:

1) Direct examination of naso- pharyngeal swab, swab, blood, CSF by immunoflourescent technique

2) Quelling reaction for typing and counter currant electrophoresis.
**Immunity:**

Infants below 3 months are usually immune because of the antibodies from the mother (natural passive immunity), after that sub clinical infection will induce Abs, thus in children 3-5 years have Abs are resistant to infection. Adult after 25 years of age the immunity becomes low against *Haemophilus influenzae* so they need vaccination.

**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.

Immediate treatment is essential in order to prevent the late neurological complication, however patient with meningitis which does not improve very well remain a source of infection, so that people in contact with the patient if they are adult then no treatment but in children give prophylactic dose of rifampicin for 9 days.

**Notes:**

Type A causes chronic sinusitis, type E,F are important post operatively.

*Haemophilus aegyptius* important in acute conjunctivitis which is highly infectious called Koch week bacilli.
**Haemophilus haemolyticus** normally presenting throat and it is important in acute and chronic R.T.I (respiratory tract infection)

**Haemophilus parainfluenza**: present in the throat, causes acute, chronic R.T.I and sub acute bacterial endocarditis.

**Haemophilus ducreyi**: causes chancroid i.e. soft chancher which is STD (sexually transmitted disease) with irregular ulcer on genitalia, swelling, tender, lymphadenopathy, it should be differentiated from other STD e.g Syphilis, herpes simplex.

**Diagnosis**: - scraping from ulcer, culture.

**Treatment**: - cotrimoxazol, erythromycin.

**Haemophilus aphrophilus**: found in normal flora of mouth, it is important in endocarditis and pneumonia.

**Bordetella**

**Bordetella pertussis**: which is the commonest type

**Bordetella parapertussis**: causes a disease similar to whooping cough but mild and subclinical.

**Bordetella brochiseptica**: which is small gram –ve bacilli that found in the respiratory tract of canines.
**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.

*Bordetella pertussis* is a small gram –ve coccobacilli, strictly aerobic, encapsulated, the capsule can be identified by using immunoflorescent 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.

**Cultural characteristics:** Grow on enriched media

1) Bordet- Gengou agar: composed of blood- potato- glycerol) and penicillin G (0.5 Mg/L)
2) blood- choclate agar.

The m.o. grows at 37c and need 3-7 days for their cultivation in moist environment. The colonies are 1-2 mm. in diameter and appear like mercury drop or a pearl colony, and iridescent with narrow zone of haemolysis. No need for X and V factors.
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:**

1) *Bordetella pertussis*: serotyped on the basis of K- agglutinogen three serotyping are known:
   - Type 1,2
   - Type 1,2,3
   - Type 1,3

2) Cell wall contain lipopolysaccharide

3) 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**

**d- Heat labile toxin:** found in the protoplasm of the cell.

**Pathogenesis:**

*Bordetella pertussis* is a pathogen only for humans transmission is by the respiratory route from early cases and carriers. The organisms attach to the ciliated epithelium of the URT but do not invade the underlying tissue. It causes decreased ciliated activity followed by death of the ciliated epithelial cells.

Factors which play a role in the pathogenesis:-

1) Attachment of the organism to the cilia of the epithelial cells mediated by protein called filamentous hemagglutinin.

2) Pertussis toxin: stimulate adenylate cyclase and help the addition of ADP ribose to form AMP, the toxin also mediates its
binding on the receptors of the epithelial cells, pertussis toxin also causes lymphocytosis in blood because it causes failure of the lymphocytes to enter the lymphoid tissues.

3) The organism synthesize adenylate cyclase which inhibit the phagocytic activity.

4) Tracheal cytotoxin is a fragment of bacterial peptidoglycan leading to damage of the ciliated cells.

**Laboratory diagnosis:**

1) Throat swab taken during paroxysmal stage
a- direct examination: by immunofluorescent technique which can give false (+ve) result.
b- culture: on appropriate media, then the identification by slide agglutination test with specific antisera or fluorescent antibody stain which is more useful than direct examination.

2) Cough modified plate method.

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

Prevention:

Two vaccines:

1) a cellular vaccine contain purified proteins from the organism. (pertussis toxoid).
2) Killed vaccine contain inactivated *Bordetella pertussis* organism (pertussis toxin)

* The acellular vaccine consists of five Ag, purified from the organism. The main immunogen in this vaccine is inactivated pertussis toxin (toxoid). This vaccine has been inactivated genetically but retains its antigenicity.

The acellular vaccine has few side effects than the killed vaccine.

The killed vaccine is usually given combined with diphtheria and tetanus toxoid (DTP) in three doses beginning at the 2 months of age, a booster dose at 12-15 months of age and another at the time of entering school.

The killed vaccine is no longer recommended because it’s suspected to cause side effects including post vaccine encephalopathy.

**Treatment:**

1) the m.o. are susceptible to many antibiotics however erythromycin used in catarrhal stage and can be used as prophylaxis for the contact individuals because it reduces the number of organism in the throat and decrease the risk of secondary complication.

2) Sedation used to prevent convulsinon.

3) O2 also used.

4) Suction of mucus during paroxysmal stage especially in infants.