Bordetella, Corynebacterium

Bordetella pertussis

- The causative agent of whooping cough.
- Pertussis (whooping cough) was a very common childhood illness throughout the first half of the century.
- Although immunizations caused a decline in cases
- Children younger than 6 months are at particular risk because protection is incomplete, even with vaccination.

Bordetella pertussis

Virulence Factors of *B. pertussis*

- Evasion of host response and inactivation of ciliated cells

Pertussis toxin-

A-B toxin, ADP ribosylates Gi:

Gi normally inactivates adenylate cyclase after stimulation

→ adenylate cyclase remains active: higher levels of cAMP

Adenylate cyclase: generates cAMP (co-factor is Calmodulin)

enters host cells, impairs phagocytosis, chemotaxis and oxidative burst

Hemolysin-cytotoxin

Tracheal cytotoxin-cell wall fragment, kills ciliated cells

Type III secretion system-pumps in additional toxins which cause cell necrosis

- Lethal toxin-dermonecrotic toxin
- guanyl nucleo- tide-binding protein (*Gi*)

Bordetella pertussis

Pertussis toxin

- protein exotoxin, secreted during in vivo and in vitro growth;
- five different subunits, designated S1, S2, S3, S4, and S5.
- A subunit - biologic activity
- B subunit - binds the complex to the cell membrane.
- pertussis toxin is capable of adherence
- Pertussis toxins (PT) causes immediate lymphocytosis by prevent migration from the circulating blood pool.

Severe and fatal pertussis has been correlated with degree of lymphocytosis, a manifestation of PT.

Adenylate cyclase toxin

- penetrates the host cells,
- inhibits phagocyte and NK cell functions

Tracheal cytotoxin - peptidoglycan-like molecule

- binds to ciliated epithelial cells,
- interfering with ciliary movement.
- causes ciliated epithelial cell extrusion and destruction

Dermonecrotic (heat-labile) toxin

- vaso-constrictor
- causes ischemia and extravasation of leukocytes
- in association with tracheal cytotoxin causes necrosis of the tracheal tissue.

Filamentous haemagglutinins (agglutinogens) - filament-associated lipo-oligo-saccharides

- binding of the organism to ciliated epithelial cells
- antibodies against these molecules are protective, probably by preventing bacterial attachment.

Pertussis

- **Mode of transmission:** closed contact via respiratory secretion droplets
- **Incubation period:** 6 - 20 days (usually 7-10 days)

- **Clinical manifestation:**
- **Including 3 stages,** which last about 2 weeks for each stage.

- 1. Catarrhal stage
  - low grade fever
  - mild upper respiratory tract symptom, lacrimation, sneezing, conjunctival suffusion
  - contagious stage

- 2. Paroxysmal stage
  - fever minimal or absent
  - cough begins first as a dry, intermittent, irritative hack and evolves into the inexorable paroxysmal
  - post - tussis exhaustion is universal, post-tussis emesis is common
- http://www.youtube.com/watch?v=KZV4IAHbC48

- 3. Convalescent stage
  - Symptom wanes gradually, however with subsequent respiratory illnesses over several months, paroxysmal coughing can recur.

B. pertussis - microscopy

SAMPLING

MICROSCOPY

- *B. pertussis*
- *aerobic*
- very small
- Gram-negative coccobacillus
- appears singly or in pairs.
- Its metabolism is respiratory, never fermentative

Diagnostic tests

- Gold standard is a positive culture from nasopharyngeal aspiration,

- it requires specific culture media eg. Regan - Lowe charcoal agar media
- fresh Bordet -Gengou agar

B. pertussis- cultivation

Bordet-Gengou agar:

- blood
- potato extract – nitrogen and vitamins
- Glycerol - carbon source
- antibiotic (cephalexin or penicillin)
- potato starch absorbed fatty acids present in nasal secretions or collection-swab cotton that inhibited growth

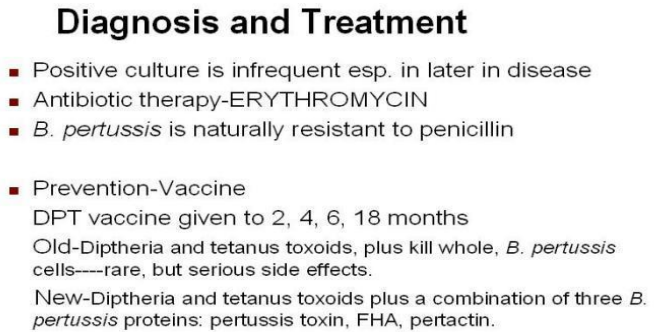
Charcoal horse blood agar:

- cefalexin, beef extract, peptone, and nicotinic
- charcoal and horse blood (10%) to neutralize the growth-inhibiting effects
- plates are incubated in air without elevated carbon dioxide at 35°C for a minimum of 7 days before being reported as negative (3 to 4 days)
- Colonies are small, shiny and round

B. pertussis

Other diagnostic tests are:

- direct testing of nasopharyngeal secretions by **direct fluorescent antibody**
- **enzyme immunoassay (EIA)** for anitbody against components of *B.pertussis*
- **polymerase chain reaction (PCR)**
- **Western blott**



CORYNEBACTERIA

***C. Diphteriae* - DIPHTERIA**

C. pseudotuberculosis

- zoonotic agent
- the cause of caseous lymphadenitis primarily in sheep and goats
- transmitted to humans by contact with diseased animals

C. ulcerans

- cause disease - after contact with milk or farm animals
- transmission between humans and pets
- able to produce diphtheria toxin
- diphtheria-like disease in humans

C. pseudodiphteriae

- respiratory disease, endocarditis, prostheses or wound infections,
- in immunosuppressed hosts

C. diphteriae - Diphtheria

- is an upper respiratory tract illness
- characterized by :
- sore throat
- low fever
- adherent membrane (called a **pseudomembrane** on the tonsils, pharynx, and/or nasal cavity.
- Diphtheria toxin produced by *C. diphtheriae*, can cause myocarditis, polyneuritis, and other systemic toxic effects.
- A milder form of diphtheria can be restricted to the skin

Corynebacteria – Gram, Albert

- pleomorphic rods, Gram-positive, catalase positive,
- non-spore-forming, non-motile,
- straight or slightly curved, with clubbed ends (from the Greek *koryne*, club).
- occur in angular arrangements

(snapping movements just after cell division - characteristic forms - „Chinese-letters“ or „palisades“ or "V" - due to the incomplete separation of the daughter cells after binary fission).

- 2-6 micrometers in length and 0.5 micrometers in diameter

Metachromatic granules (Babès-Ernst body)

- granules found in the protoplasm, usually present in Corynebacteria, representing stored phosphate regions
- it stains a different color from that of the dye used
- composed of complex polyphosphate, lipid, and nucleoprotein molecules (volutin)

Albert´s staining

- Prepare a smear on clean grease free slide.
- Air dry and heat fix the smear.
- Treat the smear with Albert’s stain and allow it to react for about 7 min.
- Drain of the excess stain do not water wash the slide with water.
- Flood the smear with Albert’s iodine for 2 minutes.
- Wash the slide with water, air dry and observe under oil immersion lens.

Cultivation of Corynebacteria

- *Corynebacterium diphtheriae* can grow on media with sheep blood with or without beta-hemolysis.

Tellurite Blood Agar –

- 0.4% tellurite
- diphtheria bacilli reduce tellurite to metallic tellurium - grey or black colour

Tinsdale agar (TIN)

- primary isolation and identification of *Corynebacterium diphtheriae*.
- differentiates between *C. diphtheriae* and diphtheroids found in the upper respiratory tract
- differentiation - based on the ability of *C. diphtheriae* to produce black (or brown) colonies, surrounded by a brown/black halo(due to the production of H_2S from cystine, interacting with the tellurite salt).

Pathogenicity of *Corynebacterium diphtheriae*

- includes two distinct phenomena:

1. **Invasion** of the local tissues of the throat

2. **Toxigenesis** bacterial production of the toxin.

Biochemical characteristics

- Fermentation
- differentiation of *C. diphtheriae* from other corynebacteria isolated from throat
- (*C.ulcerans*, *C. pseudotuberculosis*, *C. xerosis*, *C. pseudodiphthericum)*

	granules	catalase	gelatine	urea	lactose	maltose	glucose
C. diphtheriae	+	+	-	-	-	+	+
C. Pseudodiphthericum	+	+	-	+	-	-	-

Detection of toxigenicity of *C. diphtheriae*

ELEK´s test of toxigenicity

- imunodiffusion of suspension of tested strain and antidiphtheric serum in agar - precipitation

Annimal model:

- application of diphtheria toxin i.d. ……..necrosis
- antidiphtheric serum (i.p. or i.d.) + toxin ……no necrosis

ATB susceptibility

- Susceptibility of Corynebacterium - PNC, ERY - for elimination of bacteria or carriage state - therapy of diphtheria - primarily antitoxic.
- Nondiphtheriae corynebactria - Vancomycin, (Cefalosporins of the 1st generation)