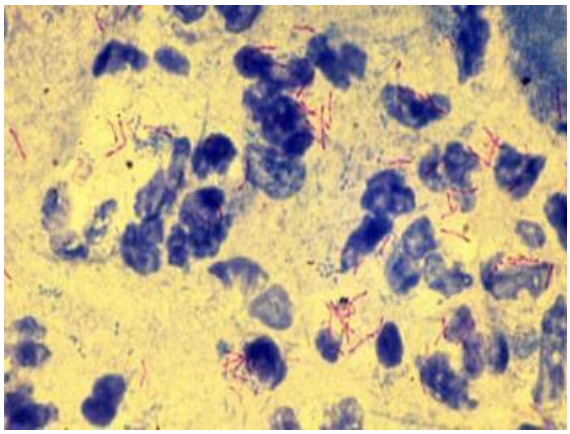
T U B E R C U L O S I S
(M. tuberculosis)

Tuberculosis is chronic infectious diseases which caused by pathogenic Mycobacteria which can defeat all the organ systems and tissues especially the lungs. Mycobacteria belong to order Actinomycetales, family Mycobacteriaceae, species Mycobacterium (discovered by R. Koch in 1882).



Morphology: There are several types of pathogenic Mycobacteria for human organism: **1.** M. tuberculosis (90%), **2.** M. bovis (5%). **3.** M. africanus (3%) **4.** M.avium. These types are different by their morphology, cultural properties and pathogenicity. The organisms are slender, straight or slightly curved rod, 1-4 mμ in length and 0.3-0.6 mμ in breadth. **M. tuberculosis** is long and slender organism. **M. bovis** is a plump and short rod. The organisms are non-motile, gram-positive, pleomorphous (rod-like, thread-like, branching, granular, coccoid) and do not form spores and capsule.

They are aerobic, **acid-fast** bacilli. Mycobacteria contain high concentration of lipids (40-60%): fatty acids, phosphatide, sulfatides, glycolipids, trehalose dimycolate, tuberculostearic acid, mycolic acids, wax D, which contribute to the organism's acid-fastness. They are stained poorly by the dyes used in Gram's stain (they have been described as Gram positive). They are stained by the **Ziehl-Neelsen method.** Spheroplasts are formed when grown in presence of lysozymes. L-forms are also seen. In cytoplasm metaphosphate inclusions can be obtained (Much granules), which have differential diagnostic meaning.

Cultivation: M. tuberculosis is an obligate aerobe. The organisms grow on selective media, which contains egg, potato, milk, coagulated serum (Soton's synthetic media, Petrangnini's media, Dorset media). More often **Loewenstein-Jensen's** media (egg, potato, salt, glycerine, aspargine, malachite green which acts as a selective agent for inhibition of other bacteria) is used. This media is without starch. The optimal temperature for growth is 37º C, pH is 6.4-7.0. Mycobacteria grow slowly, the generation time in vivo being 14-15hours. Colonies appear in about 2-8 weeks. Growing on a solid media they produce R-form dry, brittle, with a wrinkled surface, yellow colonies. On broth they produce a thin delicate film in 10-15 days, which later becomes thick brittle, wrinkled and yellow, which sinks to the bottom of the tube; the broth remains clear. Scarcely visible growth appears in 8-10 days after inoculation. During micro-cultivation (cultivation in citrated rabbit or sheep blood) growth becomes visible in 3-6 days, they form microcolonies and in the smears prepared from these colonies the mycobacteria are arranged in parallel chains. This phenomenon is named "cord" factor, which is trehalose dimycolate.

Biochemical activity: They possess urease, nicotinamidase activity. M. tuberculosis form niacin (they produce nicotinic acid) when grown on an egg medium; reduces nitrates to nitrites. Tubercle bacilli are catalase negative (weakly catalase positive).

Antigenic structure: Antigenic structure of M. tuberculosis depends on proteins, lipids, and particularly large amounts of phosphatides and polysaccharides, which are in the cell wall, tuberculin. The tuberculin is a protein and possesses high antigenic property. It is revealed in complement fixation and agglutination reactions.

Ecology: Tuberculosis is an ancient disease. Evidence of spinal tuberculosis has been found in some Egyptian mummies. Tuberculosis has been for many centuries the most important of human infections, in its global prevalence, devastating morbidity and massive mortality. It has been called the "white plague". In nature **M. tuberculosis** causes tuberculosis in human and other primates (experimentally, it is highly infectious for guinea pigs, non-pathogenic for rabbits). **M. bovis** cause tuberculosis generally in cattle. They cause tuberculosis in man rarely (experimentally, it is highly pathogenic for rabbits, guinea pigs). **M. avium** causes natural tuberculosis in birds and rarely in humans. It is non-pathogenic for laboratory animals (guinea pigs, rabbits). **M. africanum** spread in African countries (They show properties intermediate between M. tuberculosis and M. bovis). Infection with tuberculosis takes place through the respiratory tract by the droplets and dust and sometimes alimentary through contaminated food, and by contact through skin and mucous memb-ranes. Tubercule bacilli are more resistant to external effects compared with other non-

spore forming bacteria because of their high lipid content (25-40%). The organisms survive in dried sputum for 2 months, on the pages of books, on bed - clothes for 3 months, in water - 1year, in soil - 6 months, in butter and milk - 200-250 days. They are easily rendered harmless at temperatures from 100 to 120º C.

Mycobacteria are sensitive to exposure to sunlight. They aren't sensitive to disinfectants. It is necessary high concentrations and prolonged exposure of disinfectants for killing these bacteria.

Pathogenicity: Mycobacteria don't produce toxins: exotoxins and endotoxins. Pathogenic factors depend on the components of microbial cell:

1. **Lipids** (wax D, muramin dipeptide, phtionic acids, sulfatides), which causes specific granuloma and destruction of tissues.
2. **Cord factor** (virulent strains of tubercle bacilli form microscopic "serpentine cords", in which bacilli are arranged in parallel chains), which possesses the following actions:
   a) Cord factor destroys the mitochondria of the cells of the infected body and causes disorders in respiration and phosphorylation.
   b) It inhibits migration of leukocytes, causes chronic granulomas, and can serve as an "immunologic adjuvant".
   c) Has antiphagocytic action.
3. **Tuberculin,** it plays the main role in pathogenesis of the infection. **Tuberculin.** R. Koch is the first scientist, who excretes a substance known as tuberculin from the tubercle bacillus (a 6-8 week culture filtrate of the bacillus grown in 5% glycerol broth) and this tuberculin named "Alt tuberculin Koch" (old tuberculin - OT). Koch employed OT in the treatment of tuberculosis but it also caused a serious illness because it contained heterologous (waste) substances. In 1937 Seibert excreted a clear preparation new tuberculin "PPD-Purified Protein Derivative" (tubercle culture grown in synthetic medium). This protein is used for skin-allergic reactions (Mantoux and Pirquoe).

Pathogenesis: M. tuberculosis causes natural infection in humans, other primates, dogs, and some other animals which have close contact with humans. The source of infection is usually an open case of pulmonary tuberculosis. Other forms of tuberculosis are of much less importance in public health. In 90% cases the infection is caused by M. tuberculosis, in 10-18 % -M. bovis. The other mycobacteria rarely cause infection. The risk of infection and disease is the highest among socioeconomically disadvantages people, who have poor housing and poor nutrition. Tuberculosis is social infection.

The duration of incubation period in tuberculosis comparatively is long, from several weeks to several years. The M. tuberculosis is transmitted by respiratory aerosol, and its initial site of infection is the lung. On the site of entering Mycobacteria cause caseous necrosis of the tissues, sensitization, and rising of specific inflammatory processes lymphadenitis and lymphangitis. Inflammatory focus occurs: **primary affect** or infectious granuloma. *Primary affect is included: infectious granuloma, lymphangitis and lymphadenitis.* The parenchymal exudative lesion and the draining lymph nodes together are called a **Ghon complex**. (During alimentary infection the same processes occur in the intestine). Primary lesions usually occur in the lower lobes. The primary complex can get either acute or chronic form. When body resistance is low and conditions of work and life are unfavourable, the organisms may leave the site of primary localization and spread throughout the body, causing a generalized infection involving urogenital organs, bones, joints, skin, and eyes (the tuberculosis bacterium can infect all organs and systems except muscles). Since the tubercle bacillus can involve every organ system, its clinical manifestations are protean; fever, fatigue, night sweats, and weight loss are common. Pulmonary involvement gives rise to chronic cough and hemoptysis. Scrofula is myco-bacterial cervical adenitis that presents as swollen non-tender lymph nodes, usually unilaterally. M. tuberculosis causes scrofula. Miliary tuberculosis is characterized by multiple disseminated lesions, which are resembled to millet seeds. Tuberculous meningitis and tuberculous osteomyelitis, especially vertebral osteomyelitis are important disseminated forms.

The development of the primary tuberculous foci takes a benign course if the conditions of the life are favourable and there are no aggravating factors present. This stage usually terminates with resorption and healing of the caseous foci, which become calcified and enclosed in a dense connective-tissue capsule **(Ghon complex).** The organisms survive in the lymph nodes and other tissues and organs of the primary focus for many years and sometimes even for life. People infected in such a way acquire, on the one hand, relative immunity and, on the other hand, a potentially latent form of tuberculosis which may become active under the influence of a number of infectious diseases and psychic and physical traumas.

Immunity: Man is naturally resistant to tuberculosis, this property being hereditary. On the basis of the allergic reaction, X-ray examination, and patho-anatomical changes it has been shown that in a great number of cases infection does not result in the disease. There are approximately 80 per cent of adults over

20 years of age among infected persons and no more than 10 per cent of them become ill, and only 5 per cent immediately after infection.

After recovery from the primary infection, resistance to the organism is mediated by cellular immunity but not phagocytosis, because phagocytosis in this case incomplete. Primary role belongs to **T-lymphocytes.** The changes in the T-lymphocytes indices (significant) are adequate to the illness. Experimentally suppressing T- lymphocytes causes the development of infection processes. Circulating antibodies are also formed, but they play no role in resistance and are not used for diagnostic purposes.

In the course of primary infection, the host also acquires hypersensitivity to the tubercle bacilli. This is made evident by the development of a positive tuberculin reaction. Tuberculin sensitivity can be induced by whole tubercle bacilli or by tuberculo-protein in combination with the chloroform-soluble wax D of the tubercle bacillus, but not by tuberculo-protein alone. Hypersensitivity and resistance appear to be distinct aspects of related cell-mediated reactions.

Treatment: The primary treatment for mycobacterial infection is specific chemotherapy. The most widely used antituberculosis drugs: **isoniazid, rifampin**, ethambutol, and pyrazinamide, streptomycin. These are the first line antituberculosis drugs. The second-line antituberculosis drugs include streptomycin, kanamycin, ethionamide, cycloserine and ciprofloxacin.

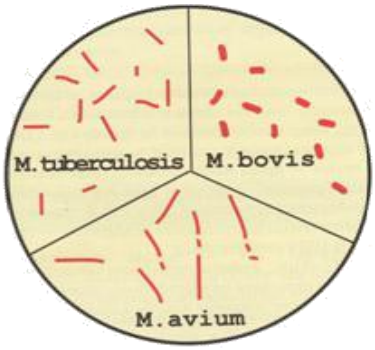
Prevention and control:

1. Prompt and effective treatment of patients with active tuberculousis and careful follow-up of their contacts with tuberculin tests, X-rays, and appropriate treatment are the mainstays of public health tuberculosis control.
2. Drug treatment of asymptomatic tuberculin-positive persons in the age groups most prone to develop complications (eg, children) and in tuberculin – positive persons who must receive immunosuppressive drugs greatly reduces reactivation of infection.
3. Individual host resistance: Non-specific factors may reduce host resistance, thus favouring the conversion of asymptomatic infection into disease. Such factors include starvation, gastrectomy, and suppression of cellular immunity by drugs (eg, corticosteroids) or infection. HIV infection is a major risk factor for tuberculosis.
4. The eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced M. bovis infections.

Immunization: Living avirulent (attenuated) tubercle bacilli, particularly **BCG** (bacillus Calmette-Guerin, attenuated bovine organisms) **vaccine,** have been used for specific prophylaxis. It is given in a single injection to newborn infants. Revaccination is carried out at the age of 7, 12, 17, 23, and 27-30 years. Postvaccinal immunity is produced within 3 or 4 weeks and remains for 1-1.5 to 15 years. Revaccination is given to persons, whose Skin-allergic test (PPD) is negative.

Laboratory diagnosis:

1. **Microscopic:** Microscopy of smears from sputum, pus, spinal or pleural fluid, urine, faeces, lymph nodes, etc., stained by the Ziehl-Neelsen method. For concentration of the organism, the sputum is subjected to enrichment methods: homogenization and flotation.
2. **Bacteriological:** Isolation of the pure culture and differentiation of mycobacteria (table 1): Pryce's micro-culture method is the most effective.
3. **Biological method**.
4. **Tuberculin (allergic)** tests are used for detecting the infection in children with tuberculosis and for tuberculosis diagnosis.
5. **Complement fixation reaction** (positive in 80 per cent of cases with chronic pulmonary tuberculosis)
6. **Indirect hem-agglutination reaction:** Sheep erythrocytes, on which polysaccharides of M. tuberculosis or tubeculin are adsorbed, are agglutinated in serum of tuberculosis patients.

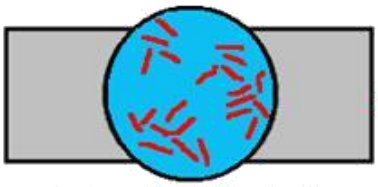


METHODS

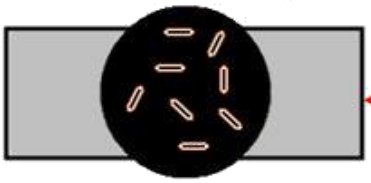
MICROSCOPIC
BACTERIOLOGICAL
BIOLOGICAL
ALLERGIC

MICROSCOPIC

Direct microscopy
(Ziehl-Neelsen method)



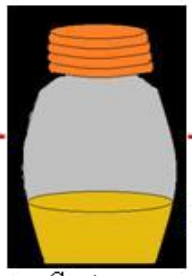
Microscopy after flotation
(Ziehl-Neelsen method)



Luminiscent microscopy

BACTERIOLOGICAL

Investigated material

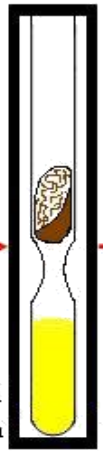


Sputum, excudate

Cultivation of material

ALKALI

ACID

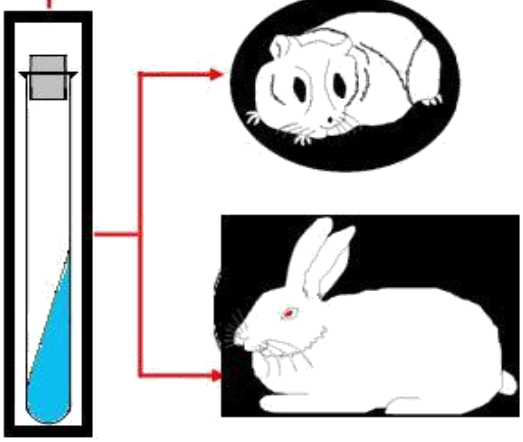


Potato-glycerol media

Lavenstein-Jensen media

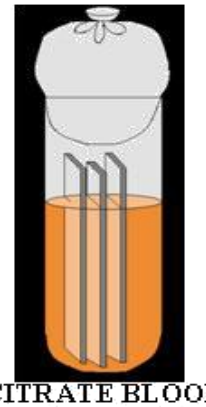
IDENTIFICATION AND DIFFERENTIATION

Examination of microbial sensitivity to drugs



EXPRESS DIAGNOSTICUM

Method of microculture

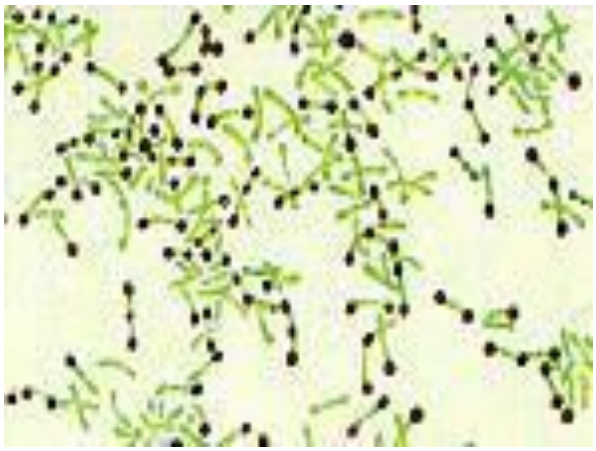


CITRATE BLOOD

CORD-FACTOR

D I P H T H E R I A
(C. diphtheriae)

Diphtheria is an acute infection with fibrinous inflammation in the portal of entry, toxic affecting of cardiovascular and nervous systems. Diphtheria caused by Corynebacterium diphtheriae (the discoverer was Klebs, 1883).



Morphology: C. diphtheriae is a straight or slightly curved rod (Latin coryna-club), 1-8mμ in length, and 0.3-0.8mμ in breadth. Characteristically, they possess irregular swellings at one end that give them a club-shaped appearance. It depends on the granules distributed near the poles (volutin or Babesh-Ernst granules) stained deeply with aniline dyes that gives the rod a beaded appearance. The organisms in stained smears are arranged at various angles to each other, resembling Latin letters L, X, W, Y (this has been called the Chinese letters or cuneiform arrangement).

This is due to the incomplete separation of the daughter cells after binary fission. They are Gram-positive and produce no spores, capsules, non-motile. They are pleomorphous. C. diphtheriae may change into cone-shaped, thread-like, fungi-like, and coccal forms. Normal flora of human organism contains conditionally pathogenic Corynebacteria: C. pseudodiphthericum, C. xerosis which are normal inhabitants of the mucous membranes. They are commonly called diphtheroids and can cause pyo-inflammatory diseases. They are on the skin, conjunctiva and in the throat. Morphologically they are different. Conditionally pathogenic Corynebacteria don't arrange at an angle, volutin granules are spread over the whole surface of the rod.

Cultivation: C. diphtheria is facultative anaerobe. The optimal temperature for growth is 37º C and the pH of medium is 7.2-7.6. The organism grows readily on the media which contain protein (coagulated serum, blood), for growth they require amino acids, mineral elements (magnesium, zinc, cooper, iron), for growth they require carbohydrates too. The **selective media** for C. diphtheria are: **Roux's and Loeffler's** media; **Klauberg's** media (contains potassium tellurite, blood, glycerine). Tellurite inhibits the growth of most other bacteria, acting as a selective agent. On Roux's (coagulated horse serum) and Loeffler's (three parts of serum and one part of sugar broth) media the organisms grow very rapidly; and colonies can be seen in 6-8hours, resembling shagreen leather (dry, crumbling colonies). On Klauberg media (McLeod's, Hoyle's) they form three types of colonies: **gravis, mitis and intermedius.** The names were originally proposed to relate to the clinical severity of the disease produced by the three types: gravis causing the most serious, and mitis the mildest variety, with intermedius being responsible for the disease of intermediate severity.

These three types differ in a number of properties. Corynebacterium of the **gravis biovar** produce large, rough **(R-forms),** daisy-like (camomile) grey colonies. These biovars ferment dextrin, starch, and glycogen; they produce a pellicle and granular deposit in meat broth. They are highly toxic with very marked invasive properties. The colonies produced by the **mitis biovar** on tellurite agar are dark, smooth **(S-forms**), and shining. Starch and glycogen are not fermented, and dextrin fermentation is not constant property. They cause **hemolysis** of erythrocytes and produce diffuse turbidity in meat broth. Organisms of the **intermedius** biovar are intermediate strains. They produce small (RS-forms), black colonies on tellurite agar. Starch and glycogen are not fermented. Growth on meat broth produces turbidity and granular deposit.

Fermentative properties: They all reduce nitrates to nitrites, catalase positive. They possess cystinase activity they ferment cystine with H_2S formation (Pizzu reaction). They don't have urease activity (this property have conditionally pathogenic Corynebacteria). They ferment glucose with acid formation. They don't ferment saccharose.

Antigenic structure: Corynebacteria forms superficial protein microcapsule, which contains K-antigen. By K antigen they are subdivided into 10 serotypes. They contain group-specific O antigen, which cross reacts with Mycobacteria and Nocardia. Serologic tests are not usually employed in identification, because they all produce the same exotoxin. The cell wall of these organisms contains specific lipids

(corynemicolic and corynemicolenic acids), glycolipid (trehalose dimicolate or cord factor), mannose, and inositol phosphates.

Resistance and Ecology: Humans are the only natural host of C. diphtheriae. The sources of infection are patients and carriers. Corynebacteria reside in the upper respiratory tract and aretransmitted by airborne droplets. Transmission by contact route by various objects (toys, bed-cloths, dishes, books, etc.) and foodstuffs (milk, cold dishes, etc.) contaminated with C. diphtheriae is also possible.

C. diphtheria is relatively resistant to harmful environmental factors. They survive for two months at room temperature and for several days on children's toys. They survive in dust about 5 months. They are sensitive to disinfectants. The organisms are killed by a 5% carbolic acid in 1minutes, by 1% phenol solution in 10minutes.

Pathogenicity: The pathogenic factors are:

1 .Adhesion, which occurs by microvilli and microcapsule

2. Colonisation

3. Invasion- the invasive factors are hyaluronidase, neuraminidase, fibrinolysin and protease.

4. Glycolipids, which is the part of cell wall and they destroy the cells in which they multiply.

5. Cord factor (trehalose dimicolate), which destroys mitochondria disturbing the processes of respiratory phosphorylation

6. Toxins (exotoxins): the organisms produce several toxins but in pathogenesis the principle role has **a) Histotoxin,** which is heat-labile polypeptide. This toxin is cytotoxin, which inhibits protein synthesis in the cells and inactivates transferase, the enzyme responsible for the formation of the polypeptide chain (anti-elongated factor). It contains two subunits: A and B. By B toxin the bacteria are fixed on the mucous membrane. Toxin A penetrated into the cell and intoxication occurs. The adrenal gland, kidney, liver, heart muscles are mainly infected The toxin affects all eukaryotic cells regardless of tissue type but has no effect on the analogous factor in prokaryotic cells. C. diphtheriae produces the Histotoxin (diphtheria toxin) only when it is infected by a lysogenic phage carrying the tox gene. C. diphtheriae cells that are not lysogenized by this phage do not produce exotoxin and are non-pathogenic. **b) Dermonecrotoxin,** which causes destruction of tissues. **c) Haemolysins** (posses haemolytic activity).

Pathogenesis: The source of infection is patient or carrier. Children (1-4 years old) are most susceptible to diphtheria. Entering into organism the bacteria are attached on the portal entry, colonized and exotoxin is produced. Diphtheria toxin is absorbed into the mucous membrane and causes destruction of epithelium and a superficial inflammatory response. The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a greyish 'pseudomembrane is formed-commonly over the tonsils, pharynx, or larynx. Any attempt to remove the pseudomembrane exposes and tears the capillaries and thus results in bleeding. The regional lymph nodes in the neck enlarge, and there may be marked oedema of the entire neck. The diphtheria bacilli within the membrane continue to produce toxin activity. This is absorbed and results in distant toxic damage, particularly parenchymatous degeneration, fatty infiltration, and necrosis in heart muscle, liver, kidneys, and adrenals, sometimes accompanied by gross haemorrhage. The toxin also produces nerve damage often resulting in paralysis of the soft palate, eye muscles, or extremities.

Incubation period is 2-10 days. When diphtheritic inflammation begins in the respiratory tract, sore throat and fever usually develop. Prostration and dyspnoea soon follow because of the obstruction caused by the membrane. This obstruction may even cause suffocation if not promptly relieved by intubation or tracheostomy. Irregularities of the cardiac rhythm indicate damage to the heart. Later, there may be difficulties with vision, speech, swallowing, or movement of the arms or legs. All of these manifestations tend to subside spontaneously.

Immunity: Immunity following Diphtheria is antitoxic in character, and the grade of the immunity is depends on the content of antitoxins in blood. Immunity depends on antimicrobial immunoglobulins too. Reinfection in 6-7% cases follows.

Prophylaxis and treatment: Specific prevention: **DPT vaccine.** The carriers treated by erythromycin. For treatment antitoxic serum is used which is effective in early period of diseases (till the toxin is not fixed on the tissue) and antibiotics (penicillin, erythromycin).

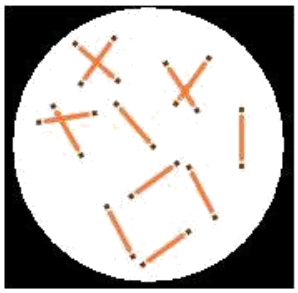
Nowdays pentavaccine is recommended- DPT+HBV+HIb(hemophilus influenzae). Vaccination starts in new born infants at 1.5-2months.

Laboratory diagnosis (picture 1):

1. **Microscopic:** Specimens: Swabs from the nose, throat. Smears stained by Methylene blue or Neisser's method.

2. **Bacteriological:** Isolation of pure culture and identification of diphtheroids by morphology, biochemical activity: fermentation of carbohydrates, cystinase and urease activity(table 1) and determination of **toxigenicity in gel: Auckterloni test** (Elek's test).

3. **Schick test** (the susceptibility test).

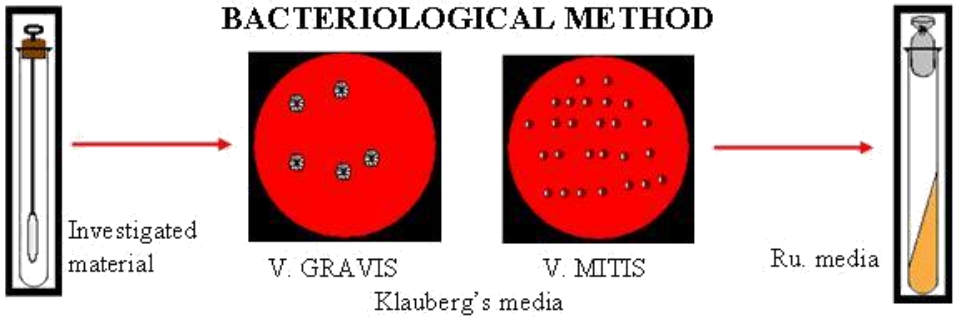


CORYNEBACTERIUM DIPHTHERIAE

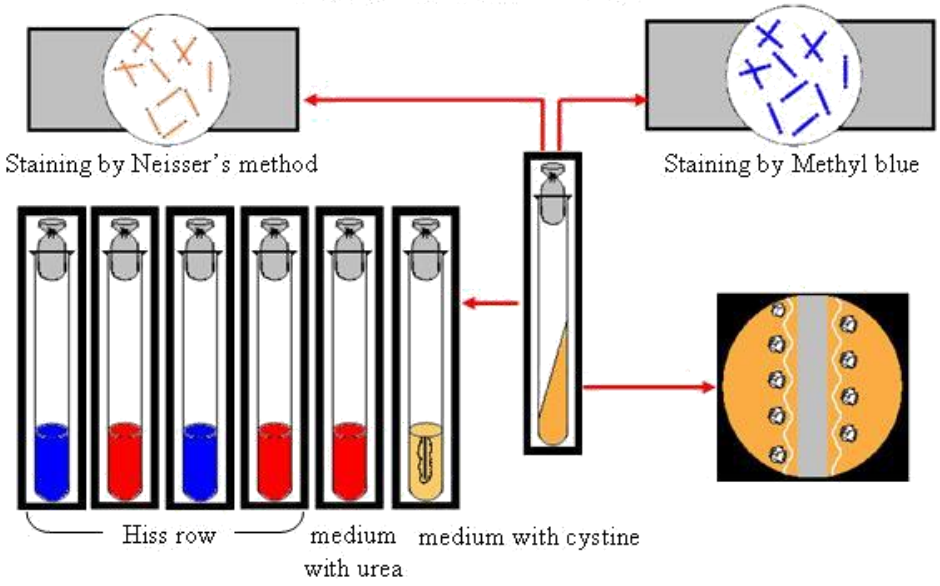
METHODS

Microscopic
Bacteriological

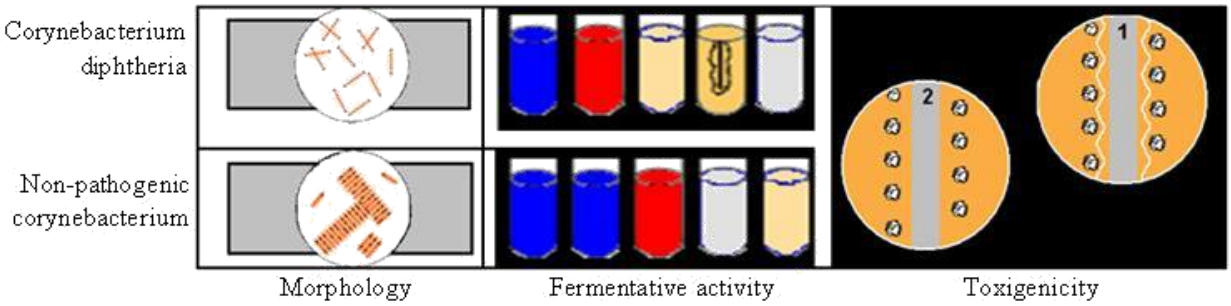
BACTERIOLOGICAL METHOD



IDENTIFICATION



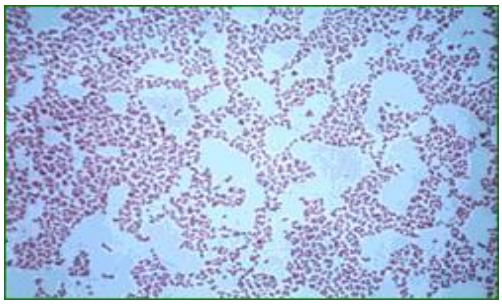
DIFFERENTIATION OF CORYNEBACTERIUM



W H O O P I N G C O U G H
(B. pertussis)

Whooping cough is an anthroponose infection with an acute trachobronchitis, paroxysmal cough, is transmitted by airborne droplets. The causative agents are Bordetellas. To this family belongs **Bordetella pertussis -** the causative agent of Whooping cough, **B. parapertussis -** the causative agent of parapertussis (produce mild infection), **B. bronchiseptica -** the causative agent of bronchitis and pertussis-like illness. They differ by morphology, biochemical activity and by antigenic structure.

Morphology: Bordetella are minute gram-negative coccobacilli, 0.2-0,5 x 1.0-1.2 mμ in size. B. parapertussis is larger (0.6-2mμ). They are arranged single or in pairs. They don't form spore. B. pertussis can form tender capsule, and they are non-motile, except B. bronchiseptica, which is peritrichous. Bipolar metachromatic granules may be demonstrated on staining.



Culture: They are strict aerobes. Bordetella requires enriched media. **Bordet-Gengou medium** (potato-blood-glycerol agar) that contains penicillin can be used. Blood is required apparently not to be providing additional nutritive factors, but rather to neutralize inhibitory agents like toxic fatty acids. **Casein - charcoal media;** charcoal or ion-exchange resins incorporated in culture media may serve the same purpose. Growth is slow. They grow during 3-7 days. On Bordet-Gengou agar they form small, dome shaped, smooth, opaque, viscid colonies resembling bisected pearls or mercury drops. On blood agar they form a hazy zone of heamolysis. The colonies of fresh culture is S-type, during re-inoculation they form R-type colonies.

Biochemical activity: It is biochemically inactive. It does not ferment sugars, proteins, doesn't reduce nitrates. They possess catalase and oxidase activity.

Antigenic structure: The antigenic structure is compound. They possess 14 antigenic components agglutinogenes. For genus is common **agglutinogene 7**. **Factors 1-6** are specific for B. pertussis. **Factor 12** is specific for B. bronchiseptica, and **factor 14** for B. parapertussis.

Virulence factors are: 1. Adhesion, which depends on capsule, superficial fimbriae, filamentous hemagglutinin. **2. Colonization 3. Penetration**-they penetrate into the epithelium of the respiratory tract and internal medium of macrophages. They produce a number of factors that are involved in the pathogenesis of the disease. Protein on the pilli, which is called **filamentous hemagglutinin (FHA)** plays role in adherence of the bacteria to the ciliated epithelial cells of the upper respiratory tract. Antibody against the FHA inhibits attachment and protects against the disease.

4. They produce protein **toxins: a) Tracheal toxin –cytotoxin** (non-secreted toxin with cytotoxic action) **b) Pertussis toxin** (whooping cough toxin**)** promotes lymphocytosis, sensitization to histamine and enhanced insulin secretion. It causes spasm of bronchial vessels and lead to paroxysmal cough. This toxin is functional blockator and activates ATP system which leads to disturbances in water-salt metabolism. It inhibits phagocytosis. **c) Dermonecrotoxin** is cytotoxin. It acts on myocardic cells by activation of ATP-ase. It promotes lymphocytosis, sensitization to histamine. This toxin causes necrosis of upper respiratory tract's mucous membrane. **d) LPS endotoxin is** heat stable toxin, which damages the epithelial cells of the upper respiratory tract and causes intoxication.

5.They produce **enzymes:** hyaluronidase, lecithinase, coagulase.

6.They form **capsule,** which an antiphagocytic factor (phagocytosis during this infection is incomplete).

Pathogenesis: B. pertussis is a pathogen only for human, is transmitted by airborne droplets. Bordetellas are attached to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue. The organism multiplies rapidly on the epithelial surface of the trachea and bronchi and interferes with ciliary action. Decreased cilia activity and epithelial cell death occur. The infection is limited to the respiratory tract and bacilli do not invade the bloodstream. The bacteria liberate the toxin and substances that irritates surface cells, causing coughing and marked lymphocytosis. Later

there may be necrosis of the epithelium parts and polymorphonuclear infiltration with peribronchial inflammation and interstitial pneumonia.

Incubation period is about 3-14 days. After incubation period the disease takes a protracted course comprising three stages: catarrhal, paroxysmal, convalescence stages.

C**atarrhal stage** develops, with mild coughing and sneezing, malaise, headache. Loss of appetite, nervous irritation occurs. Laryngitis, pharyngitis develops. The temperature is high 39-40° C. This stage continues 11-14 days. During this stage, a large number of organisms are sprayed in droplets, and the patient is highly infectious but not very ill. The next is **paroxysmal stage** (spasmatic cough) stage; the cough develops its explosive character and 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. This stage is prolonged 2-8 weeks. The next is **convalescence** stage which is slow (2-6 months). The disease usually lasts 6-8weeks though in some it may be very protracted. Complications may be:

1. due to pressure effects during the violent bouts of coughing (subconjunctival hemorrhage, subcutaneous emphysema).
2. respiratory (bronchopneumonia, lung collapse)
3. neurological (convulsions, coma). Respiratory complications are self-limited, the atelectasis resolving spontaneously but the neurological complications may result in permanent squeal such as epilepsy, paralysis, retardation, blindness or deafness.

Immunity: High grade humoral immunity acquired after whooping cough is antimicrobial, which is stable and tense in character.

Epidemiology and control: Whooping cough is endemic in most densely populated areas worldwide and also occurs intermittently in epidemics. The source of infection is usually a patient in the early catarrhal stage of the disease. Communicability is high, ranging from 30 to 90%. Most cases occur in children under age 5 years; most deaths occur in the first year of life. Control of whooping cough rests mainly on adequate active immunization of all infants (DPT vaccine).

Prophylaxis: Specific prophylaxis - DPT vaccine.

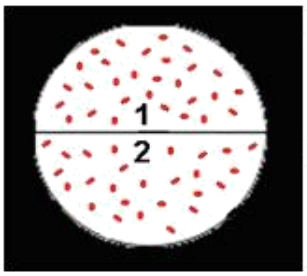
Laboratory diagnosis (picture 2):

1. **Bacteriological**: Isolation of pure culture and differentiation of different species(table 1) a)The cough plate method-A culture plate is held about 10-15cm in front of the patient's mouth during a bout of spontaneous or induced coughing so that droplets of respiratory exudates impinge directly on the medium. This has the advantage that specimens are directly inoculated at the bedside. b)The post nasal (peroral)swab: secretions from the posterior pharyngeal wall are collected with a cotton swab on a bent wire passed through the mouth. c)The per nasal swab: here a swab on flexible nichrome wire is passed along the floor of the nasal cavity and material collected from the pharyngeal wall.
2. **Serological: Complement fixation reaction** (Bordet-Gengou CFR).

3. Direct fluorescent antibody test.

Bordetella parapertussis: This organism may produce a disease similar to whooping cough; this infection is often subclinical.

Bordetella bronchiseptica: is a small gram-negative bacillus that inhibits the respiratory tracts of canines, in which it may cause 'kennel cough" and pneumonitis. It grows on blood agar medium. B. bronchiseptica has a silent copy of the pertussis toxin gene.



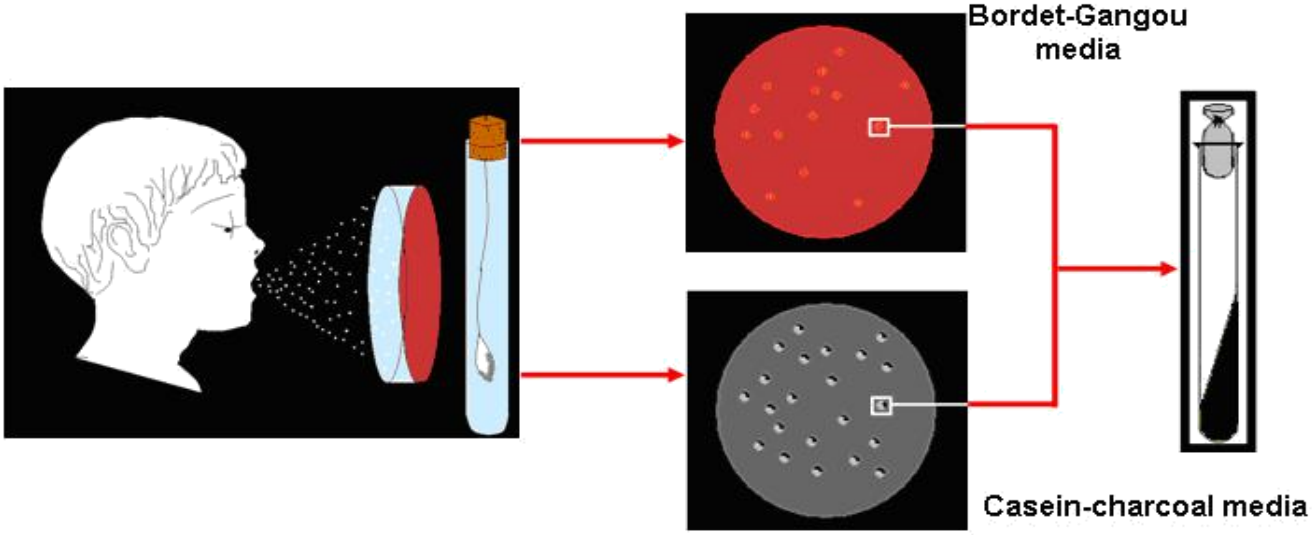
1.BORDETELLA PERTUSSIS

2.BORDETELLA PARAPERTUSSIS

METHODS

Bacteriological
Serological
(CFR)

BACTERIOLOGICAL METHOD



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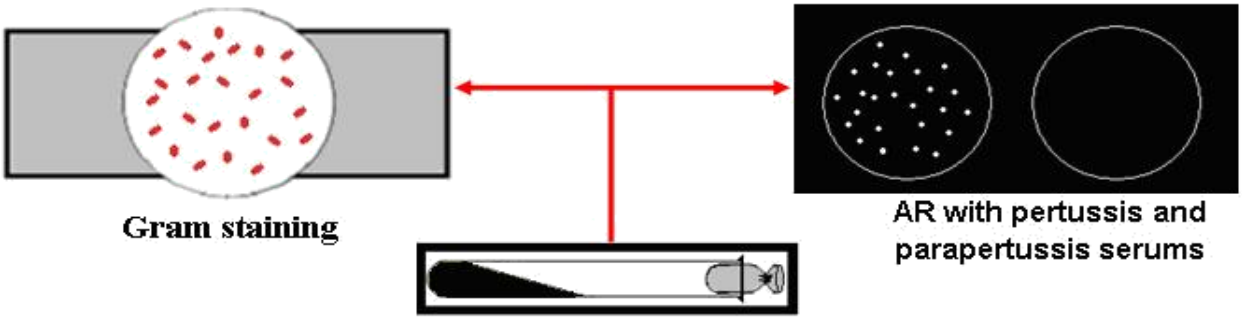

DIFFERENTIATION OF BORDETELLAS

Microbe	Growth on MPA	Rapidity of growth	Growth on thyrozin media	Urease test	Catalase test
B. pertussis коклюша	—	3-5 days	—	—	+
B. parapertussis	+	day	+ Brown pigment	+	+