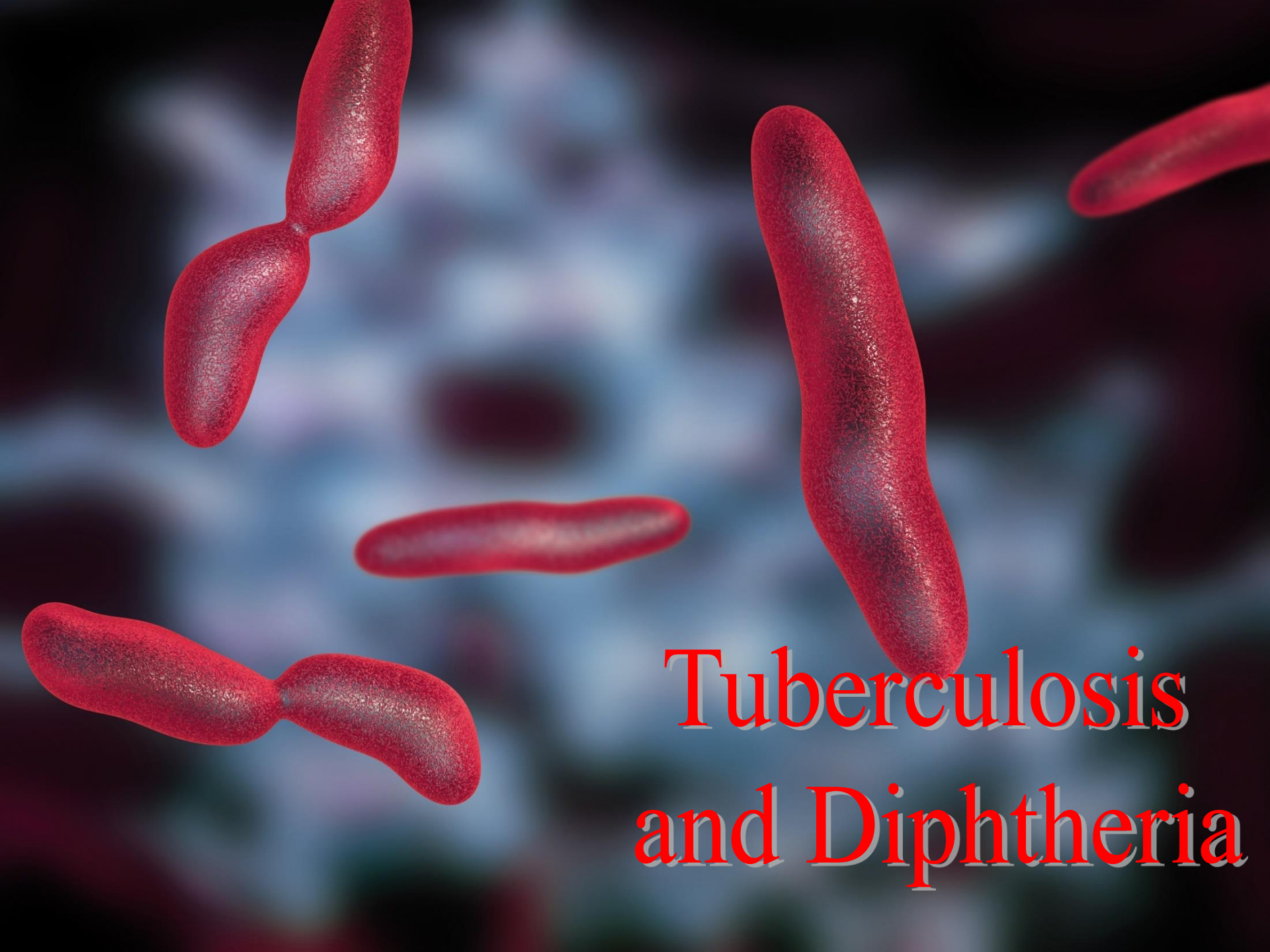
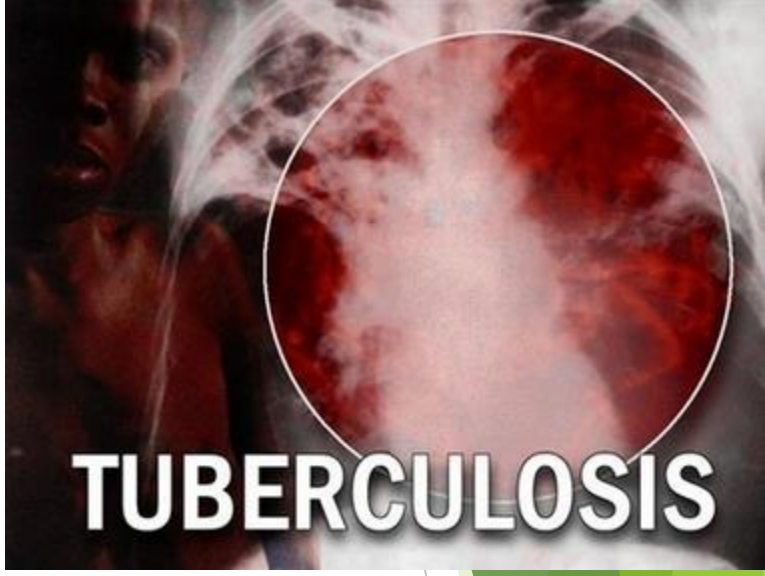
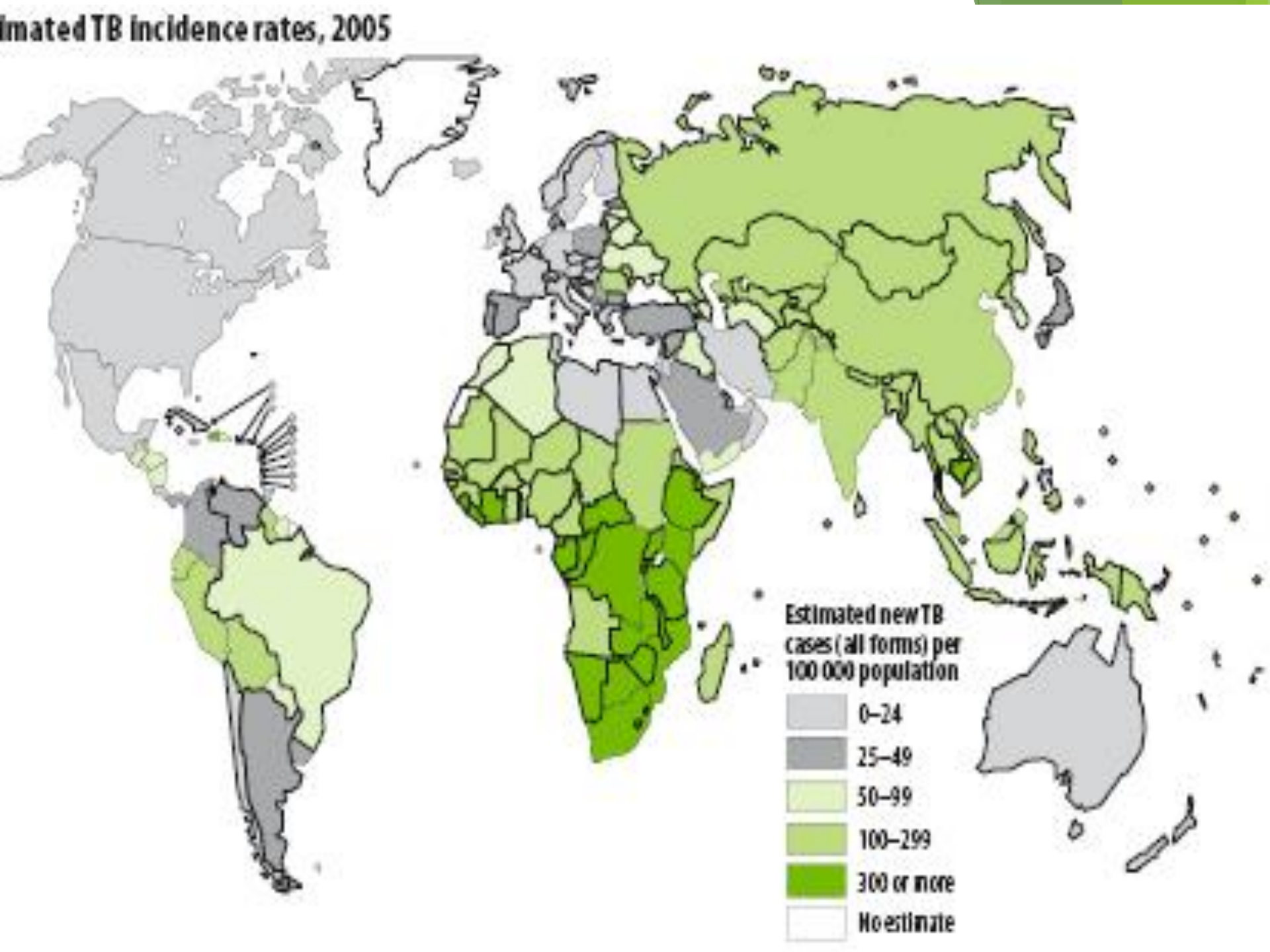

Tuberculosis
and Diphtheria

Historical significance



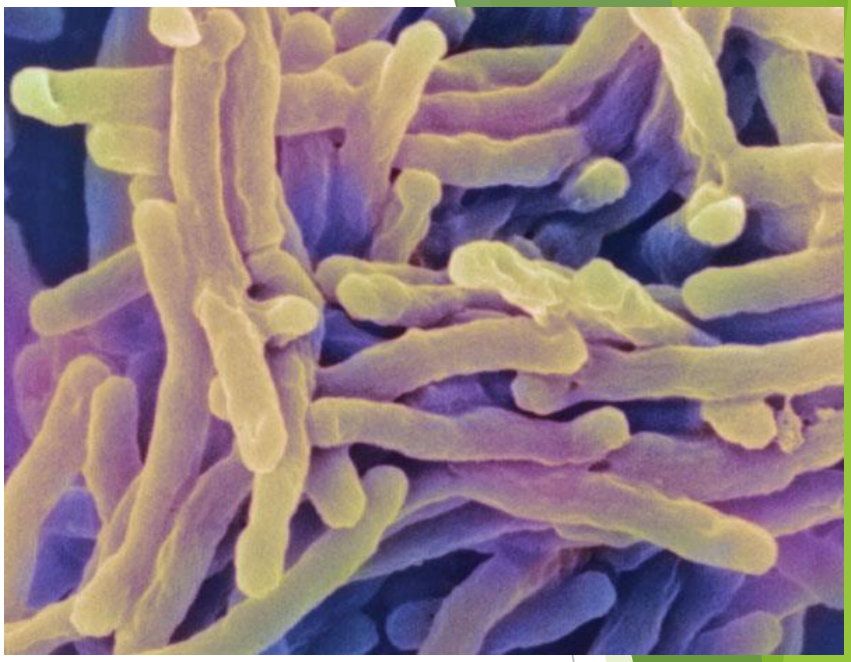
Mycobacterium tuberculosis (M.TB) was the cause of the "**White Plague**" of the 17th and 18th centuries in Europe. During this period nearly 100 percent of the European population was infected with M.TB and 25 percent of all adult deaths were caused by M.TB.

Today, M.TB infects about one-third of the world population, especially in Africa, Asia and the Indian subcontinent.


imated TB incidence rates, 2005
Estimated new TB cases (all forms) per 100 000 population
0–24
25–49
50–99
100–299
300 or more
No estimate

Tuberculosis

Family Mycobacteriaceae
Genera Mycobacterium
Species M. tuberculosis
M. bovis
M. africanus
M. avium.



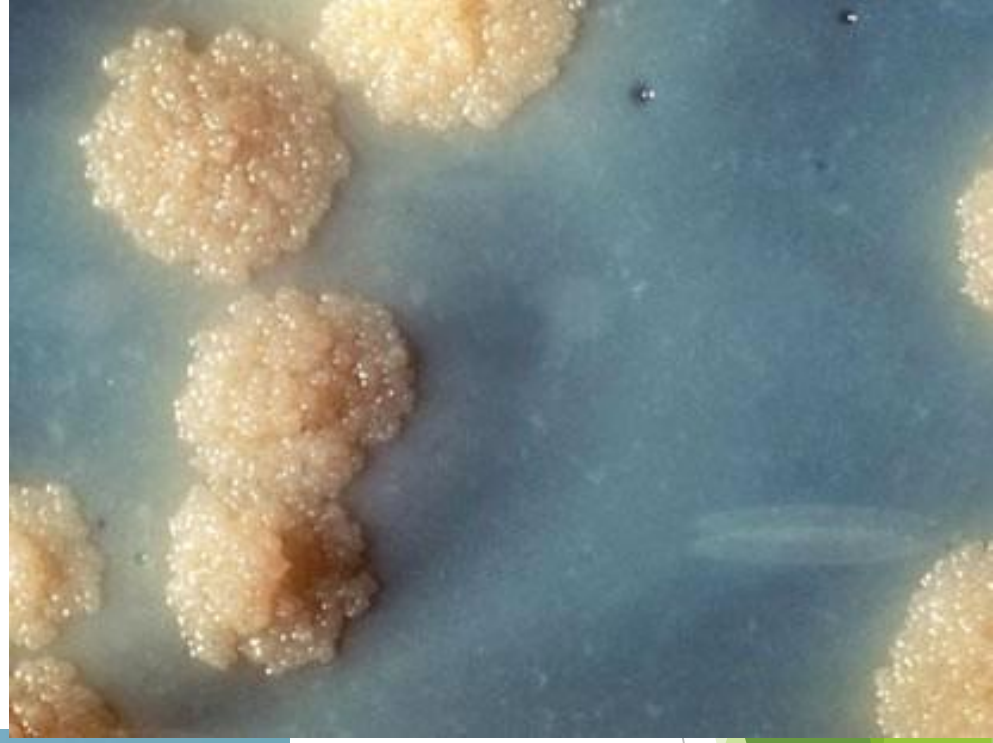
- **Morphology:** *Mycobacterium tuberculosis* is a slender, fairly large nonmotile rod-shaped bacterium. Non-sporing. The rods are 2-4 μm in length and 0.2-0.5 μm in width.
- **Staining:** Ziehl-Neelsen stain: bacilli stained with concentrated acid carbol fuchsin, heated, and then decolorized with 20% sulphuric acid and alcohol: the bacilli retain a **bright red** colour. Nowadays, tubercle bacilli are usually detected under fluorescent microscopy, with auramine stain.

Cultural characteristic

Mycobacterium tuberculosis is an obligate aerobe.
Does not grow on ordinary media.
Two media are used to grow M.TB. **Middlebrook's medium** which is an agar based medium and **Lowenstein-Jensen medium** (contain egg, asparagine, glycerol and, to inhibit contaminants, malachite green). M.TB. colonies are small and buff colored when grown on either medium. Both types of media contain inhibitors to keep contaminants from out-growing M.TB. It takes 4-6 weeks to get visual colonies on either type of media. Incubation at 37^{o}C.

Cultural characteristics on Löwenstein-Jensen medium:
M.tuberculosis - rough, dry, yellow colonies, slow grow
M.bovis and **M.africanus**- white, smooth colonies (inhibited by glycerol) slow grow

Colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen medium.



Biochemical activity

- Catalase is positive.
- Lipase is positive.
- Phosphatase is positive.
- Dehydrogenase is positive.
- Proteolitic activity (fermentation of proteins).
- Ferments an alcohol, glycerin.

Classification and Antigenic Types

The mycobacteria are classified into two broad categories members of the *M. tuberculosis* complex *(M. tuberculosis, M. bovis, M. microtii, M. africanum*) and nontuberculous mycobacteria (virtually all other species), which often are described based on their growth rate and pigmentation with and without exposure to light. There are antigenic differences among species on the basis of serologic reactions to carbohydrate moieties in the glycolipids. Modern molecular biologic techniques have revealed a remarkable conservation of genes coding for the immunodominant antigens of all mycobacteria.

Virulence Factors

The cell wall is composed of **mycolic acids**, **complex waxes**, and **unique glycolipids**.

The mycolic acids containing extremely long (C60 to C90) side

chains are joined to the muramic acid moiety of the

peptidoglycan and to arabinogalactan. The mycobacterial cell

wall is acid-fast. Other important wall components are

trehalose dimycolate (so-called **cord factor**, as it is thought

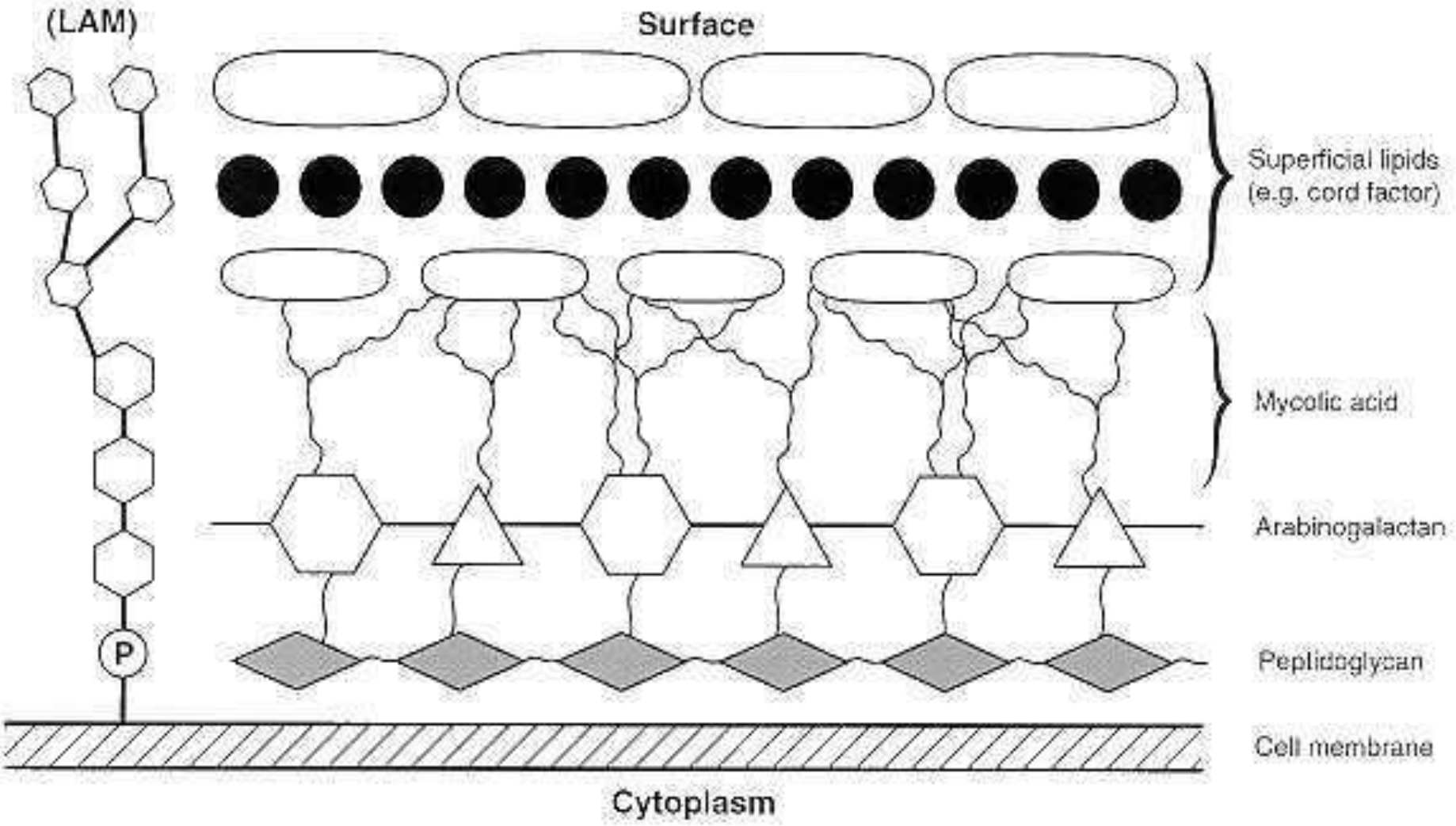
to induce growth in serpentine cords on artificial medium)

and **mycobacterial sulfolipids**. Another unique constituent

is **lipoarabinomannan** (LAM). **Hemolysins** and **lipases**

are produced by *M. tuberculosis*.

Complex cell wall structure of mycobacteria



Pathogenesis

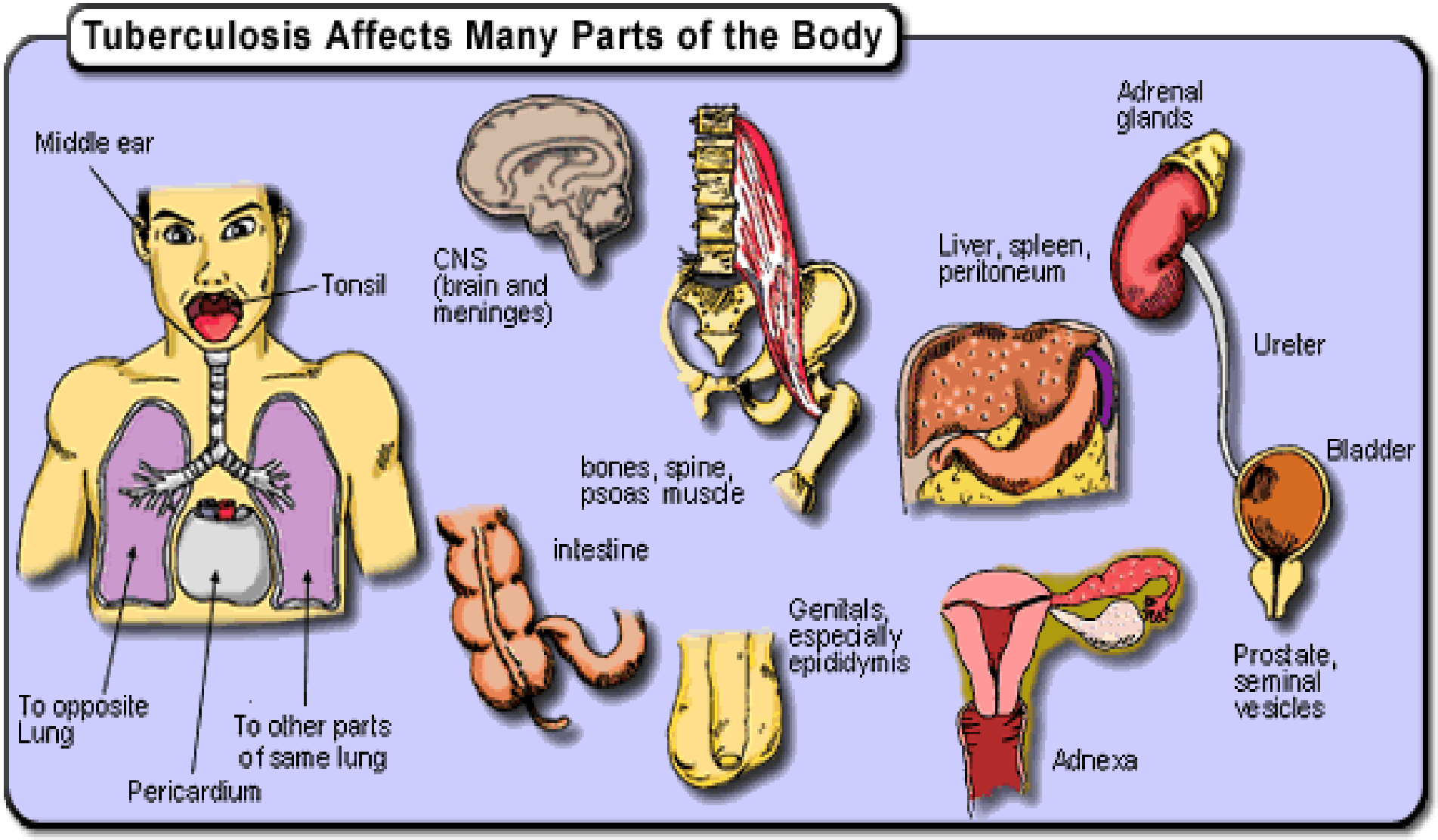
M. tuberculosis infections occur by **airborne transmission.** The bacilli are deposited in the alveolar spaces of the lungs, where they are engulfed by alveolar macrophages. A portion of the infectious inoculum resists intracellular destruction and persists, eventually multiplying and killing the macrophage. The ability of virulent mycobacteria to survive within phagocytes justifies their designation as facultative intracellular pathogens. There are some mechanisms of intracellular survival: *M tuberculosis* can prevent phagosome-lysosome fusion; virulent mycobacteria can prevent acidification of the phagolysosome, perhaps by modulating the activity of a membrane proton pump; cord factor may be directly cytotoxic to macrophages.

Most of the tissue destruction associated with tuberculosis results from cell-mediated hypersensitivity, however, rather than direct microbial aggression.

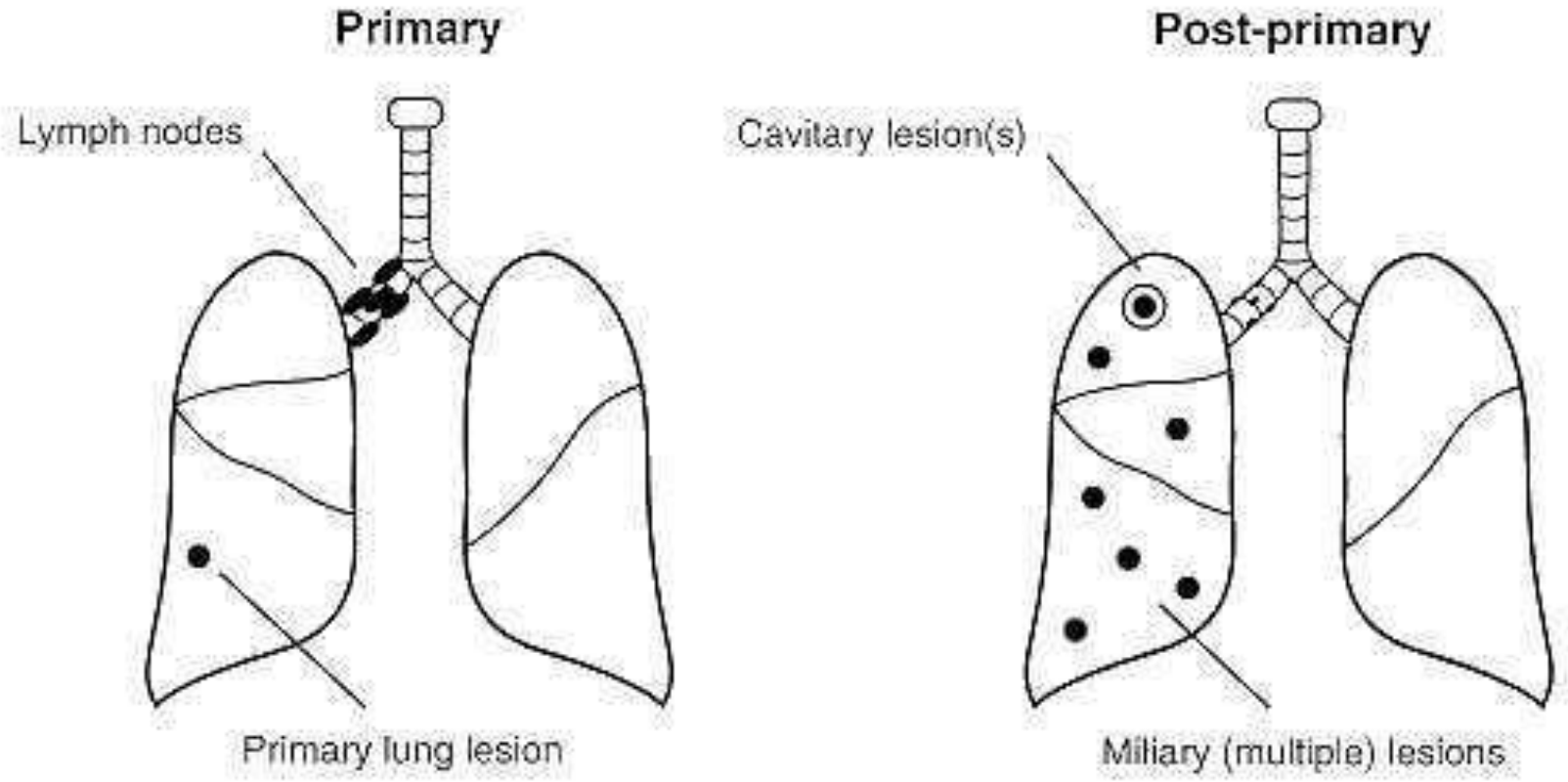
The accumulating mycobacteria stimulate an inflammatory focus which matures into a granulomatous lesion characterized by a mononuclear cell infiltrate surrounding a core of degenerating epithelioid and multinucleated giant (Langhans) cells. This lesion (called a tubercle) may become enveloped by fibroblasts, and its center often progresses to caseous necrosis. Liquefaction of the caseous material and erosion of the tubercle into an adjacent airway may result in cavitation and the release of massive numbers of bacilli into the sputum. In the resistant host, the tubercle eventually becomes calcified.

Mycobacteria may spread distally either indirectly through the lymphatics into the blood stream, or directly into the circulation by erosion of the developing tubercle into a pulmonary vessel. Extrapulmonary hematogenous dissemination results in the seeding of other organs (e.g., spleen, liver, and kidneys) and, eventually, reinoculation of the lungs. The resulting secondary lung lesions may serve as the origin of reactivation of clinical disease years or decades later owing to the persistence of viable tubercle bacilli.

Primary disease is usually characterized by a single lesion in the middle or lower right lobe with enlargement of the draining lymph nodes. Endogenous reactivation is often accompanied by a single (cavitary) lesion in the apical region, with unremarkable lymph nodes and multiple secondary tubercles.


Tuberculosis Affects Many Parts of the Body
Middle ear
Tonsil
To opposite Lung
Pericardium
To other parts of same lung
CNS (brain and meninges)
bones, spine, psoas muscle
intestine
Genitals, especially epididymis
Adrenal glands
Liver, spleen, peritoneum
Ureter
Bladder
Prostate, seminal vesicles
Adnexa

Radiologic differences between primary and post-primary tuberculosis



Epidemiology

- **TB Infection**

When a person breathes in Mtb-contaminated air, the inhaled TB bacteria reach the lungs. This causes an Mtb infection. However, not everyone infected with TB bacteria becomes sick. This is called latent TB infection. People who have latent TB infection do not get sick and do not spread the bacteria to others. But, some people with latent TB infection eventually do get TB disease.

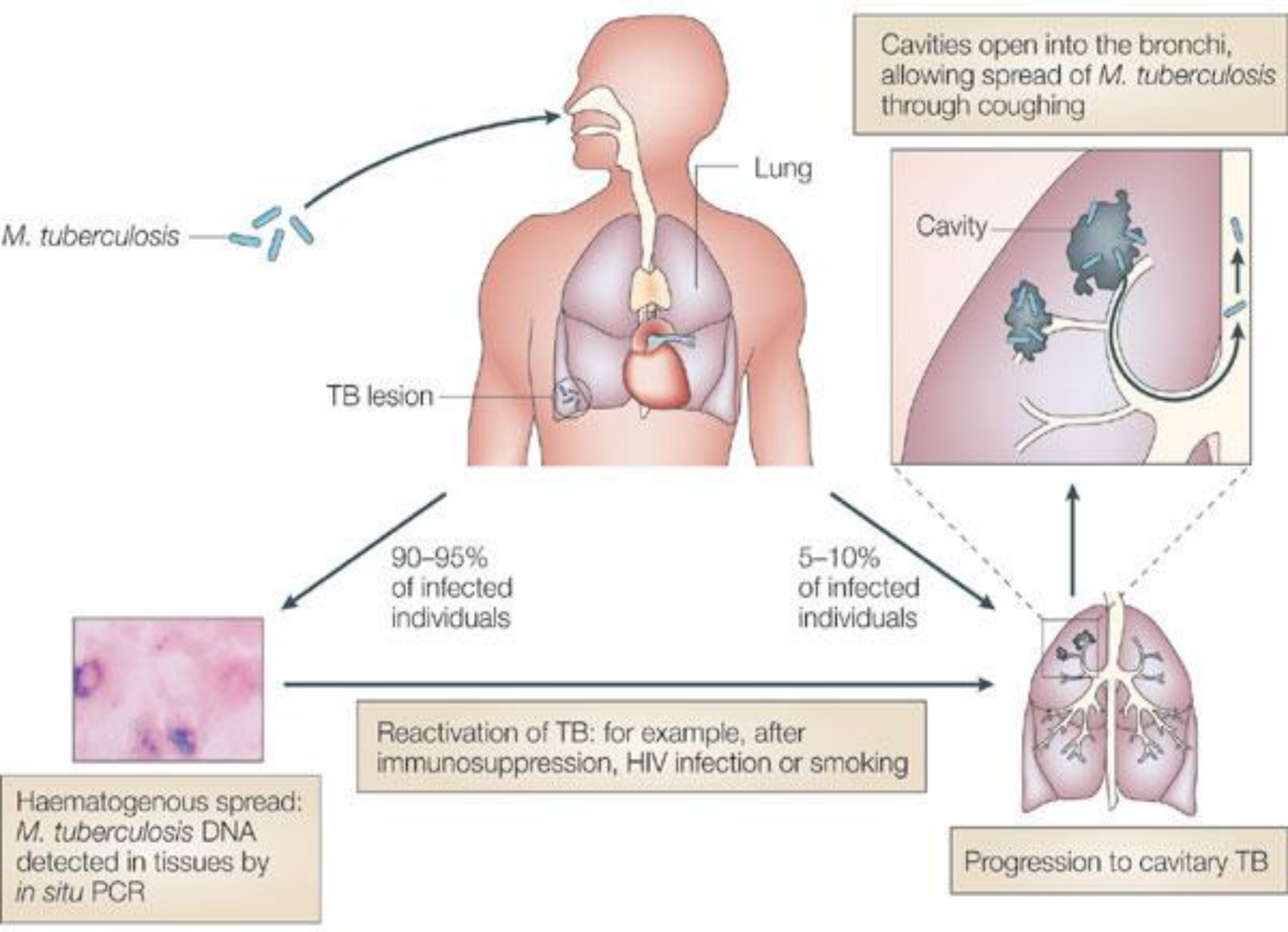
TB Disease

For someone to develop active TB disease, the following two events must take place:

1. The bacteria enter the body and cause an Mtb infection.
2. The immune system cannot stop the TB bacteria from growing and spreading after the initial infection.

One in ten people infected with TB bacteria develop active TB disease at some point in their lives.

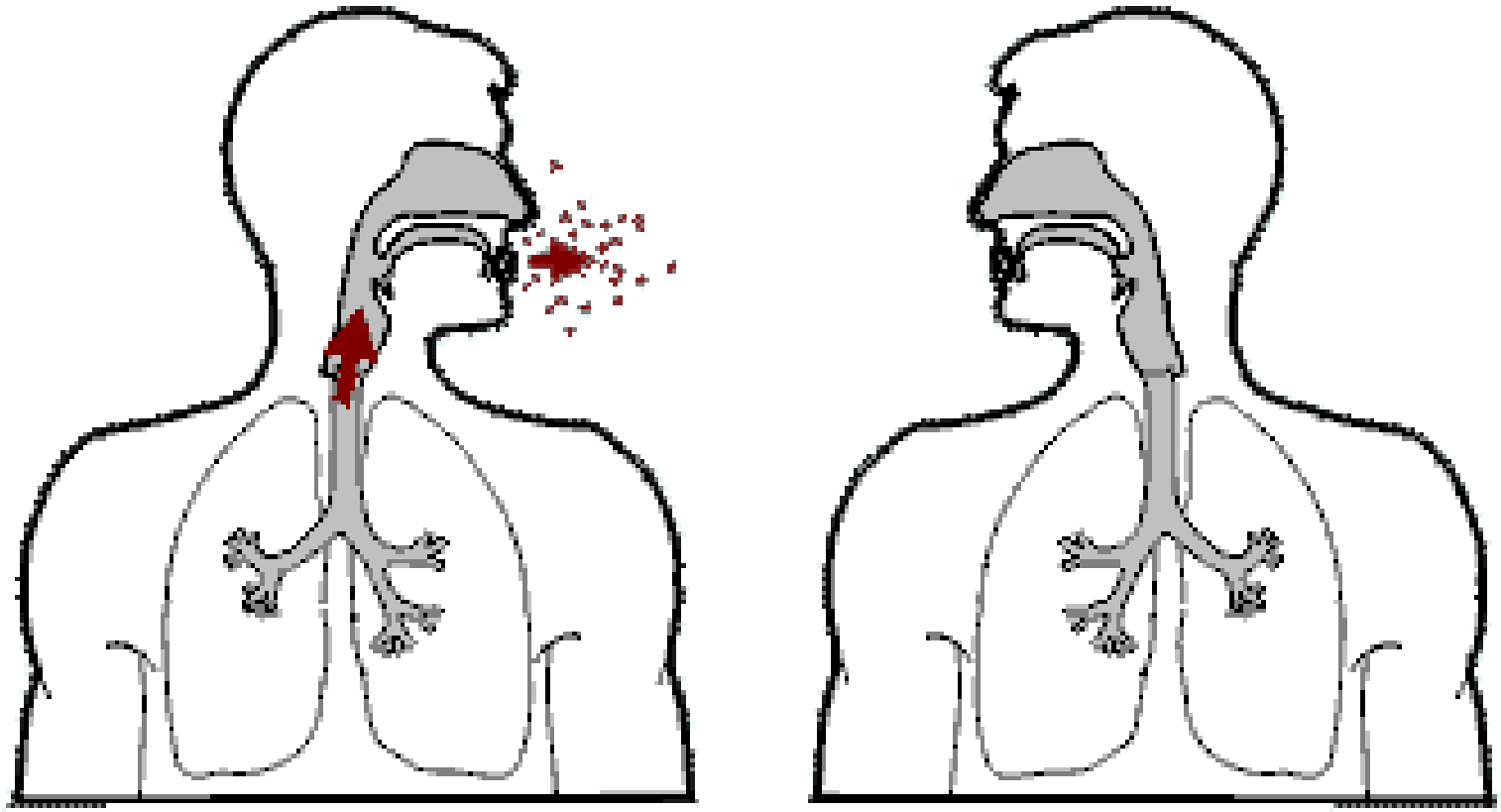
Tuberculosis is particularly common in groups such as the elderly, the chronically malnourished, smokers, alcoholics, and the poor. The most significant factor influencing the incidence of mycobacterial disease has been the HIV epidemic. HIV-infected individuals have a high incidence of tuberculosis, characterized by frequent extrapulmonary disease.


Cavities open into the bronchi, allowing spread of M. tuberculosis through coughing
Lung
M. tuberculosis
TB lesion
Cavity
90–95% of infected individuals
5–10% of infected individuals
Reactivation of TB: for example, after immunosuppression, HIV infection or smoking
Haematogenous spread: M. tuberculosis DNA detected in tissues by in situ PCR
Progression to cavitary TB

Transmission

TB is primarily an airborne disease. The bacteria are spread from person to person in tiny microscopic droplets when a TB sufferer coughs, sneezes, speaks, sings, or laughs. Only people

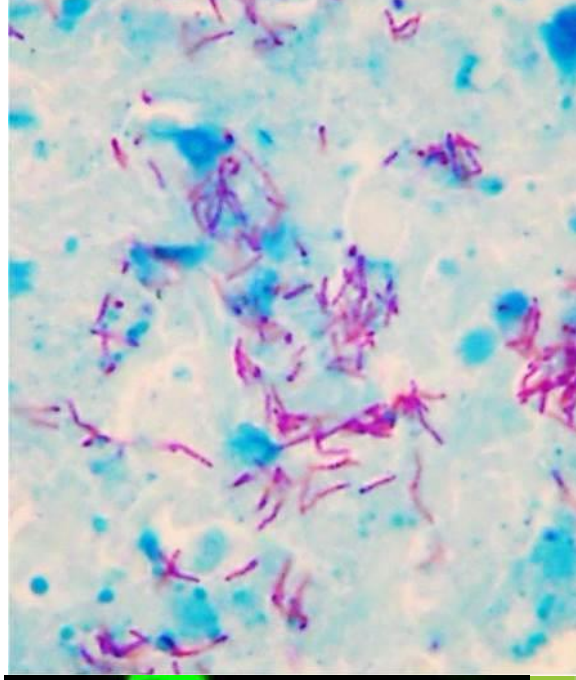
wit

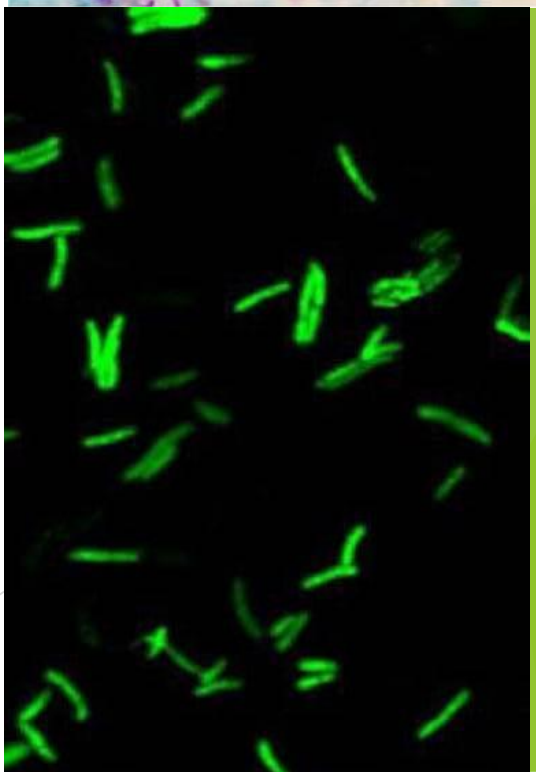


Clinical Disease

Clinical signs and symptoms develop in only a small proportion (5-10 percent) of infected healthy people. These patients usually present with pulmonary disease; prominent symptoms are chronic, productive cough, low-grade fever, night sweats, easy fatigability, and weight loss. Tuberculosis may present with or also exhibit extrapulmonary manifestations including lymphadenitis; kidney, bone, or joint involvement; meningitis; or disseminated (miliary) disease. Lymphadenitis and meningitis are more common among normal infants with tuberculosis, and all extrapulmonary manifestations are increased in frequency among immunocompromised individuals such as patients on chronic renal dialysis and elderly, malnourished, or HIV-infected individuals.

Diagnosis of TB





Bacterioscopic method:

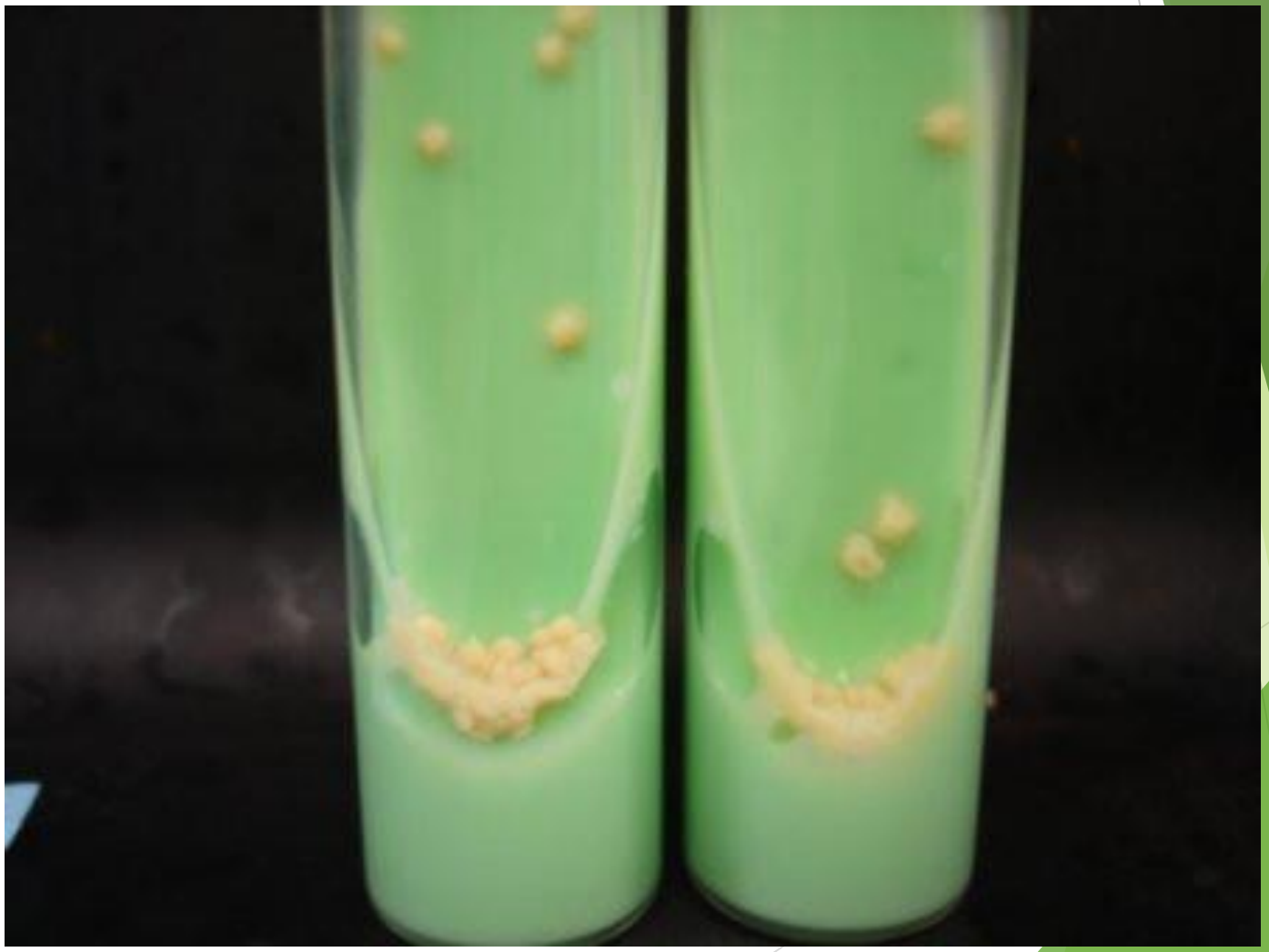
- Smears may be prepared from specimen like sputum, laryngeal swab, pleural fluid, peritoneal fluid, cerebrospinal fluid, pus, urine, gastric lavage, feces and other infected material. The smear is stained by **Ziehl Neelsen** technique. Acid fast bacilli are seen as pink brightened rods, while background is blue.

- Where several smears are to be examined daily, it is more convenient to use fluorescent microscopy. Smears are stained with auramine phenol or auramine rhodamine fluorescent stain. They are examined under ultraviolet light and bacilli appear bright rods against dark background.

Bacteriological investigations:

- The organisms must then be **cultured from sputum.** First, the sputum sample is treated with NaOH. This kills other contaminating bacteria but does not kill the M.TB present because M.TB. is resistant to alkaline compounds by virtue of its lipid layer.
- The media used for growth and the the resulting colony morphology have been described previously. However, methods of culturing can take 4-6 weeks to yield visible colonies. As a result, another method is commonly used call the **BACTEC System**. The media used in the BACTEC system contains radio-labeled palmitate as the sole carbon source. As M.TB multiplies, it breaks down the palmitate and liberates radio-labeled CO2. Using the BACTEC system, M.TB growth can be detected in 9-16 days vs 4-6 weeks using conventional media.

Culture on Lowenstein-Jensen medium revealed typical dry, from yellow to buff-colored colonies of *Mycobacterium tuberculosis*.

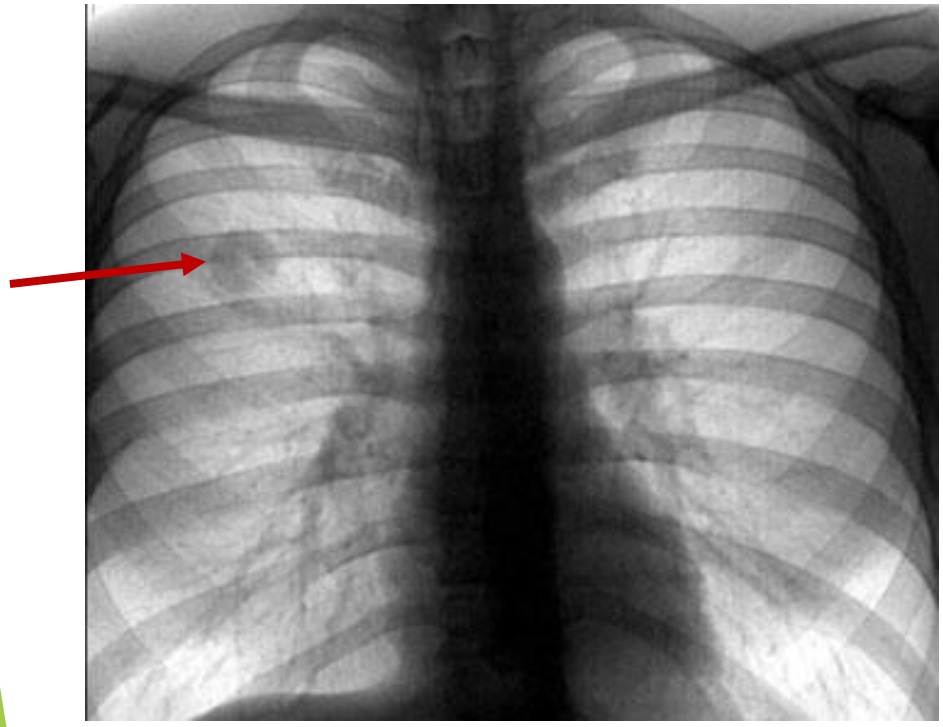


Allergological method

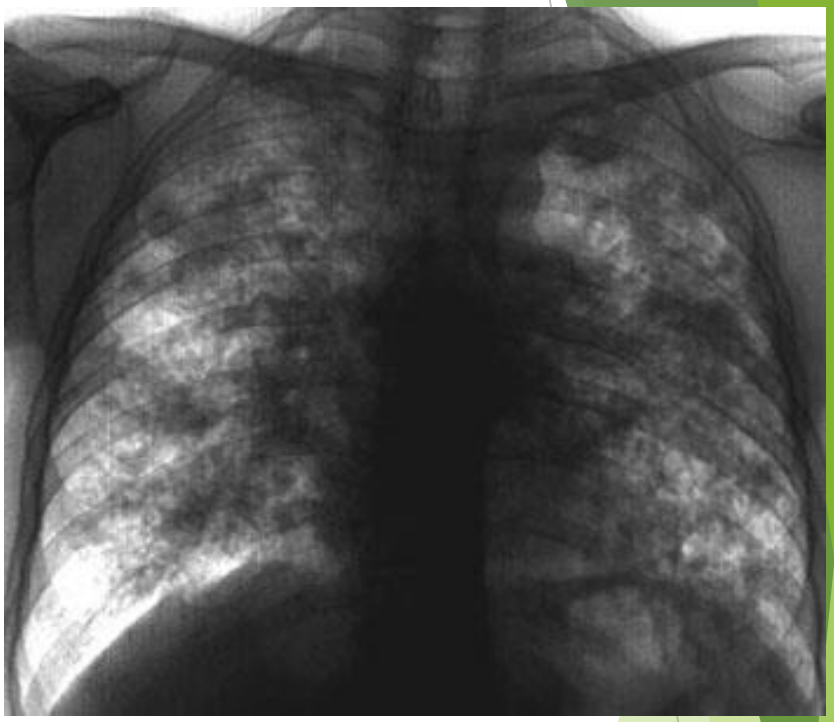
Skin Testing is performed as the **tuberulin** or **Mantoux test**. **PPD (purified protein derivative)** is employed as the test antigen in the **Mantoux test**. PPD is generated by boiling a culture of M.TB, specifically Old Tuberculin (OT). 5 TU (tuberculin units), which equals 0.0001 mg of PPD, in a O.1 ml volume is intracutaneously injected in the forearm. The test is read within 48-72 hours.

- Old tuberculin (O.T.) consist of filtrate of glycerol broth culture of bacilli concentrated to 1/10th of volume by evaporation on water bath.
- Purifed protein derivative (PPD) is prepared by precipitation of tubercle bacilli culture grown in synthetic medium with trichlor acetic acid. PPD is superior to O.T. because it is stable, and constant in activity. Tuberculin prepared from bovine type is as active as tuberculin prepared from human type.

X-ray examination



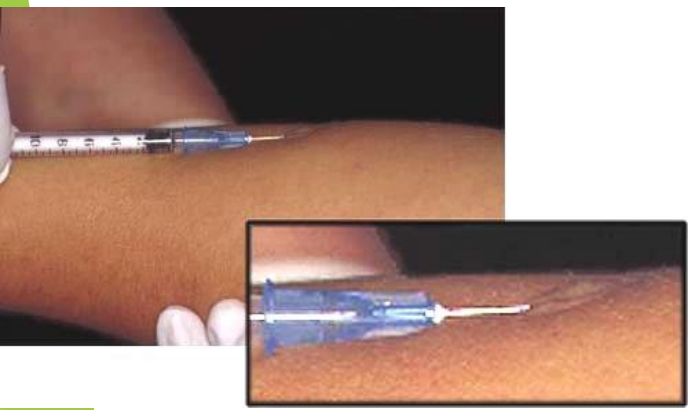
Tuberculoma of the upper lobe of the right lung

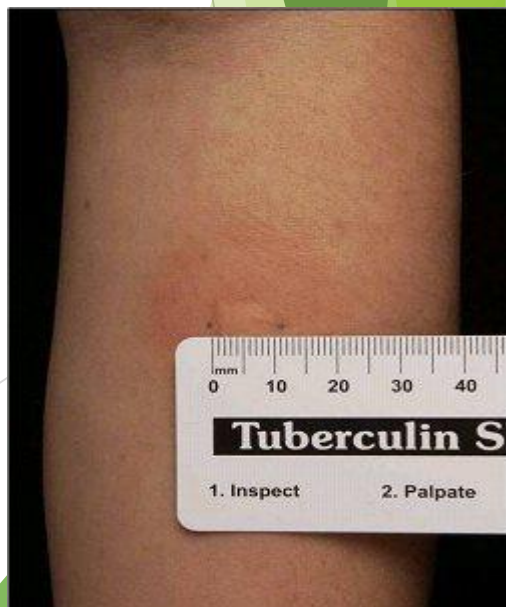


Disseminated pulmonary tuberculosis

Administering the Mantoux test

- The test is considered positive if the diameter of the resulting lesion is 6-10 mm or greater. The lesion is characterized by erythema (redness) and swelling and induration (raised and hard).
- False positive tests usually manifest themselves as lesser reactions. These lesser reactions could indicate prior exposure or infection with other Mycobacteria or vaccination with BCG.
- False negatives are more rare than false positives but are especially common in AIDS patients. Other conditions such as malnutrition, steroids, etc., can rarely result in a false negative reaction.





Different tests

- Commercial chemiluminescent DNA probes, gas-liquid chromatography, high-performance liquid chromatography, and thin-layer chromatography allow identification of a few species of mycobacteria within hours after sufficient growth is present on solid or in a liquid medium.
- In the future, nucleic acid amplification methods may prove useful for detection of mycobacteria directly in clinical material within 24 hours or less of specimen receipt.

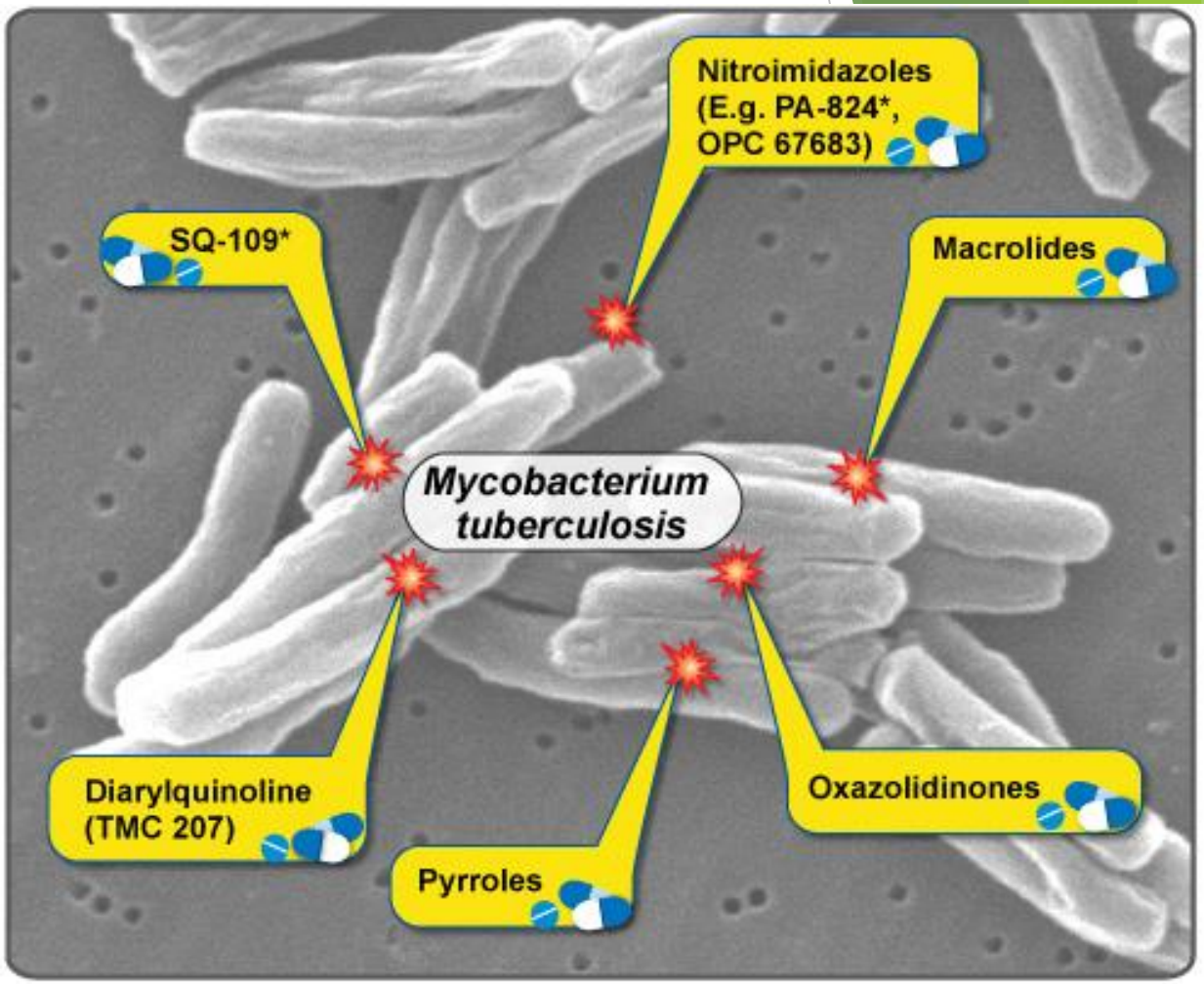
Treatment

M.tuberculosis is sensitive to a wide range of drugs. But resistant variants arise readily, therapy should always comprise a combination of drugs.

First-line drugs	Second-line drugs
Isoniazid	**Streptomycin**
Rifampicin	**Capreomycin**
Pyrazinamide	**Cycloserine**
Ethambut	**Thiacetazone**
Ftivazid	**Ethionamide**

New TB Drugs Under Development

Several new types of TB drugs currently under development are shown here. NIAID has supported the development of two of these compounds, SQ-109 and PA-824, which are denoted by asterisks above.



Prophylaxis

BCG (bacilli Calmette-Guerin)

- This is a bovine strain of tubercle bacillus rendered completely avirulent by culturing repeatedly (M.bovis maintained for 13 years by 239 subculture on glycerine potato medium). The strain is used in inducing active immunity to tuberculosis. The vaccine contains live avirulent bacilli and is administered to tuberculin negative individual.
- The vaccine is given intradermally over deltoid region. It confers 60% protection in India and 80% protection in USA and UK. Immunity lasts for 10 to 15 years. After BCG vaccination a positive tuberculin test may last for 3 to 7 years.

Robert Koch (1843-1910) described the aetiology of tuberculosis in 1882 but failed to develop a subunit vaccine against tuberculosis in 1890. **Albert Calmette (1863-1933)** and **Camille Guérin (1872-1961)** described the first attenuated vaccine against tuberculosis in 1921.

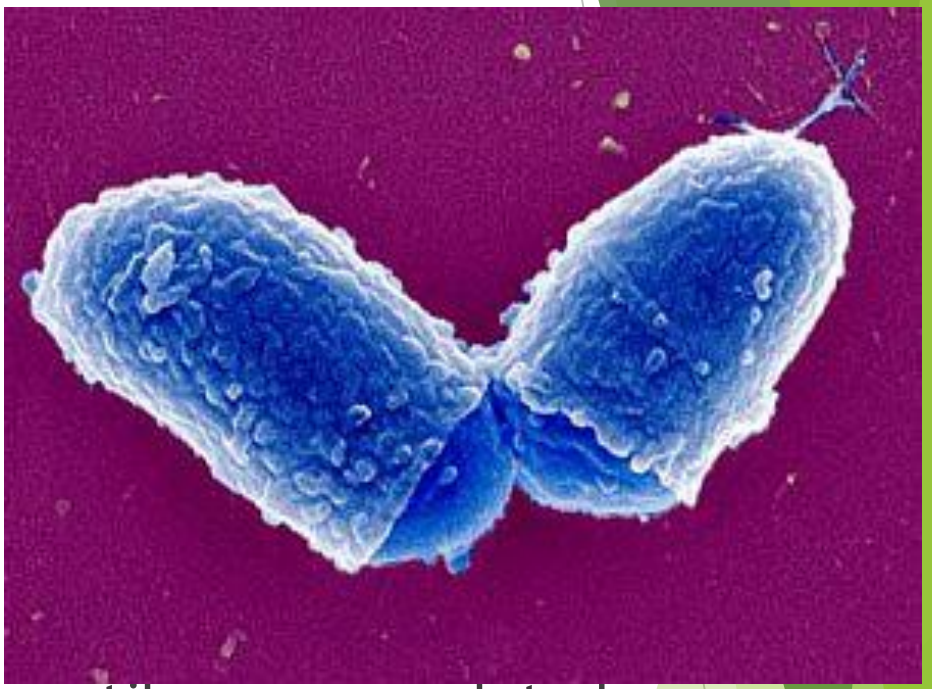
Robert Koch

Albert Calmette

Camille Guérin

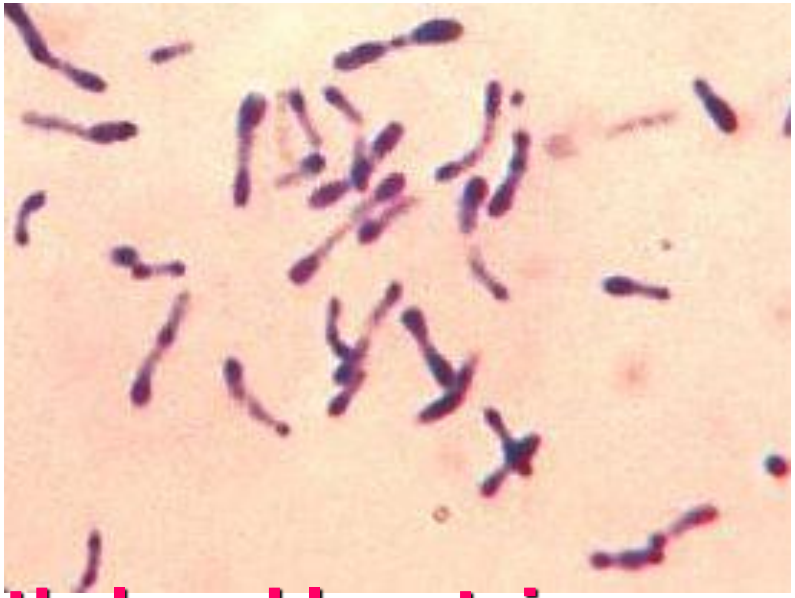
Diphtheria

Family Actinomycetacae
Genera Corynebacterium
Species C. diphtheriae



Morphology: C. diphtheriae is nonmotile, noncapsulated pleomorphic Gram-positive rods (3 x 0.3 μm) or clubs. These divide by “snapping fission”, so that adjacent cells lie at different angles to each other forming V-, L- and W-shapes – a so-called *Chinese-character* arrangement. Adjacent cells may also be parallel to one another, in *palisades*.

Albert's stain

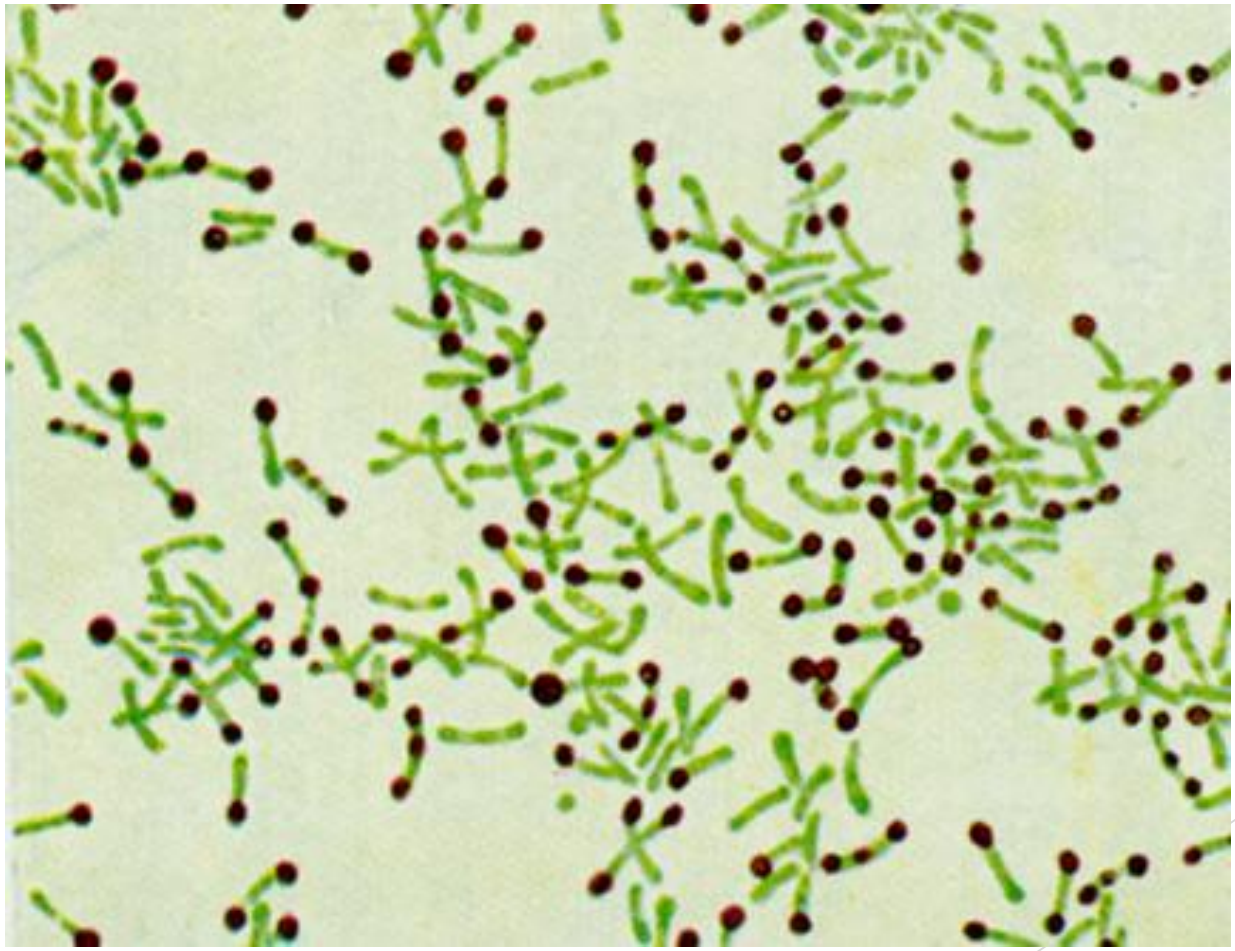


Methylene blue stain

Staining: Some strains stain irregularly due to the intracellular deposition of polymerized phosphate – forming the metachromatic or volutin granules (Babes-Ernst granules) characteristic of, but not exclusive to, C. diphtheriae.

The granules, usually two and three per cell, show up with special stains – bluish-black by **Albert's method,** deep blue with **Neisser's method**, **Loeffler's methylene blue**.

Stained *Corynebacterium* cells. The "barred" appearance is due to the presence of polyphosphate inclusions called metachromatic granules.

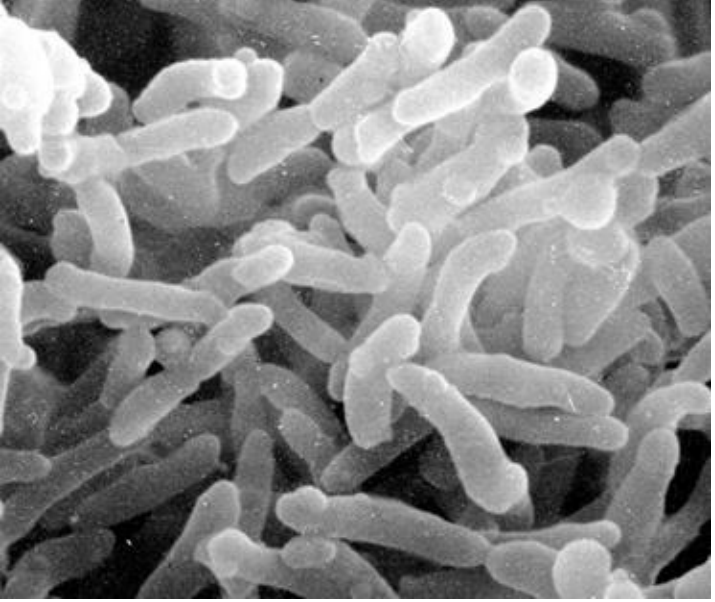


Cultural characteristic

- Culture: An aerobe and facultative anaerobe; optimum temperature 37°C. Does not grow well on ordinary agar – media containing blood or serum are required. Selective media are necessary for isolation from clinical specimens.

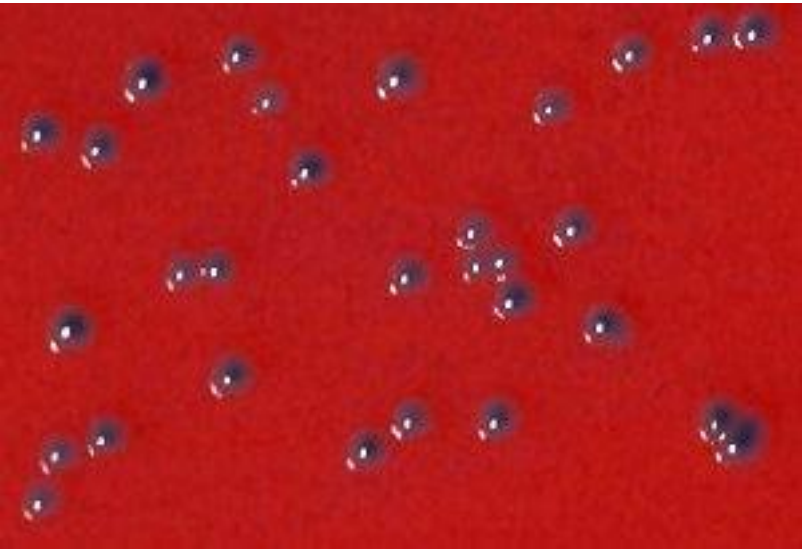
- *Selective media:*
- Loeffler's serum medium: C.diphtheriae grows rapidly – faster than other upper respiratory tract bacteria present in clinical material. The morphology develops particularly well, and smears made as soon as 8 hours after inoculation may show a typical appearance.
- Blood tellurite agar (e.g. Hoyle's or McLeod's medium): after 48 hours incubation, corynebacteria produce characteristic grey-black colonies due to their ability to reduce potassium tellurite to tellurium.

Corynebacterium colonies on blood agar.

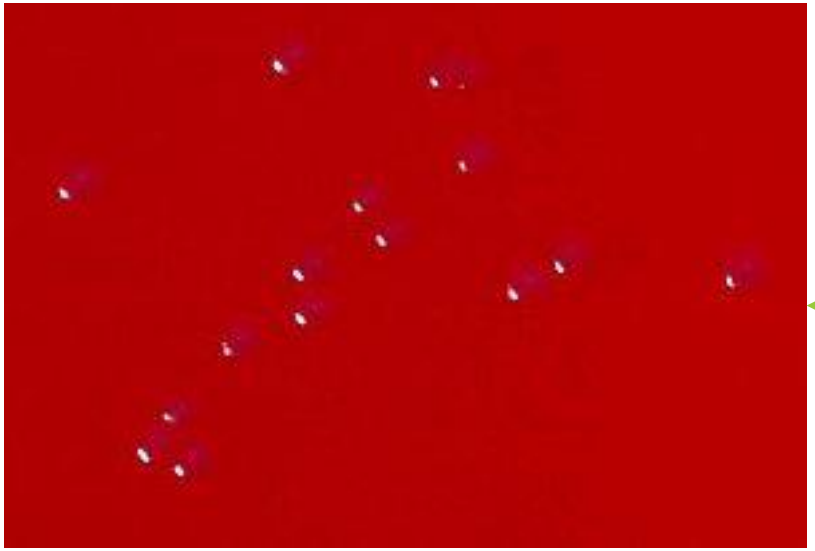


Corynebacterium diphtheriae
is classified into biotypes
(mitis, intermedius, and gravis)
according to cultural properties,
biochemical activity,
antigenic structure etc.

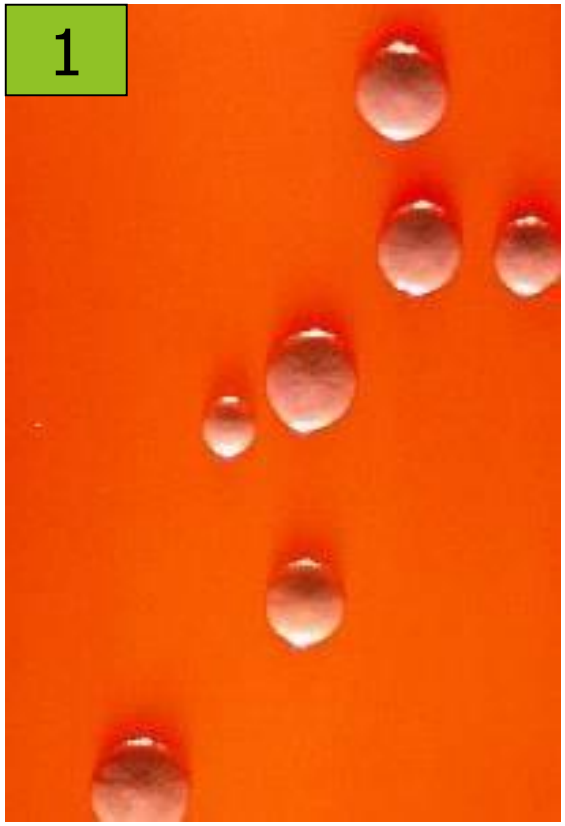
Gravis	Intermedius	Mitis
1. Morphologically they are short uniformly stained with few granules	Long, irregularly barred, very pleomorhphic and poor granulational	Long curved, pleomorhphic with prominent granule
2. Colony is 1 to 2 mm on tellurite blood agar having greyish black centre and semi transluscent periphery with commencing crenation of edge (daisy head colony).	Size 1 mm having dull granular centre with smooth and glistening periphery carrying lighter ring near the edge (frog egg colony).	Colonies are shiny black, flat with central elevation (poachedegg colony).
3. Hemolysis is variable	Non hemolytic	Hemolytic
4. Consistency is brittle and breaks readily, not emulsifi-able	Intermediate between gravis and mitis	Soft, butyrous and emulsifi-able easily
5. Growth in broth is granular deposit, no turbidity and surface pellicle	Turbidity in 24 hour which is cleared in 48 hour having granular deposit	Diffuse turbidity with pellicle later on
6. Glycogen and starch fermentation positive	Negative	Negative
7. Antigenic structure – 13 types	4 types	40 types

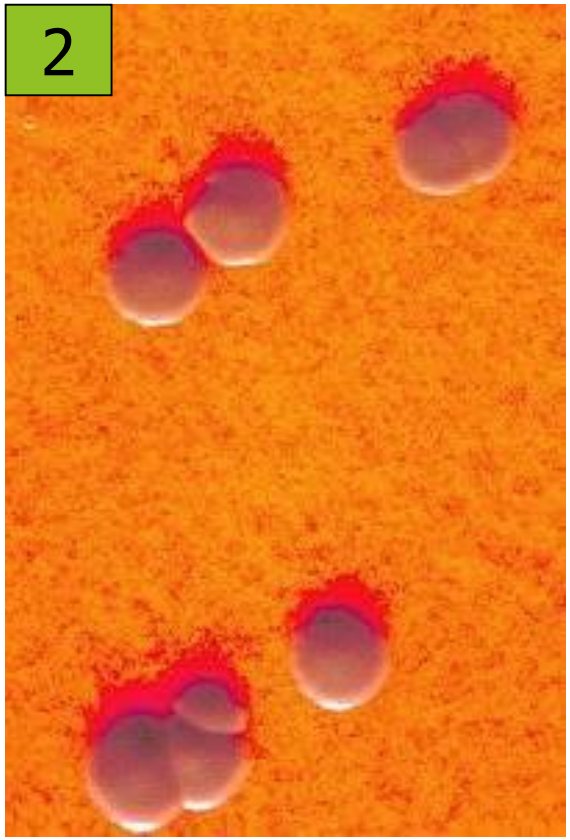


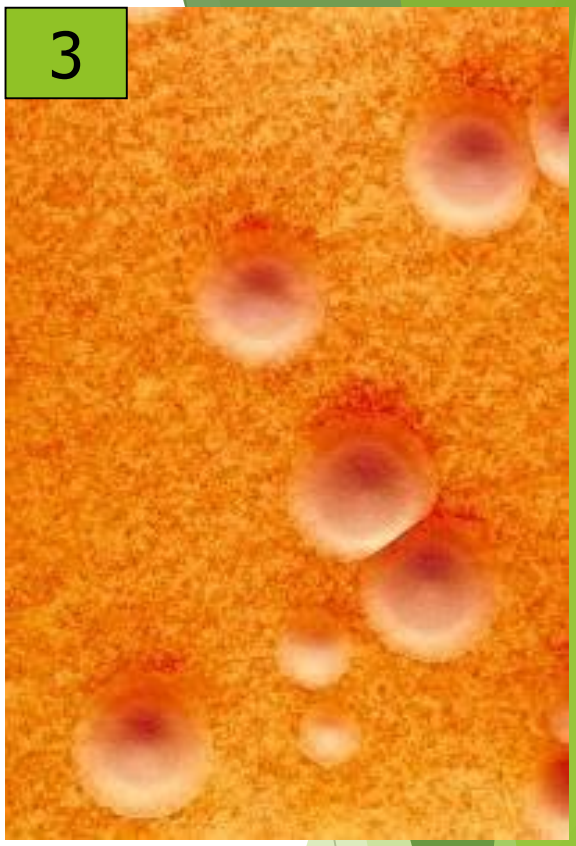
blood agar plate-mitis



blood agar plate-intermedius



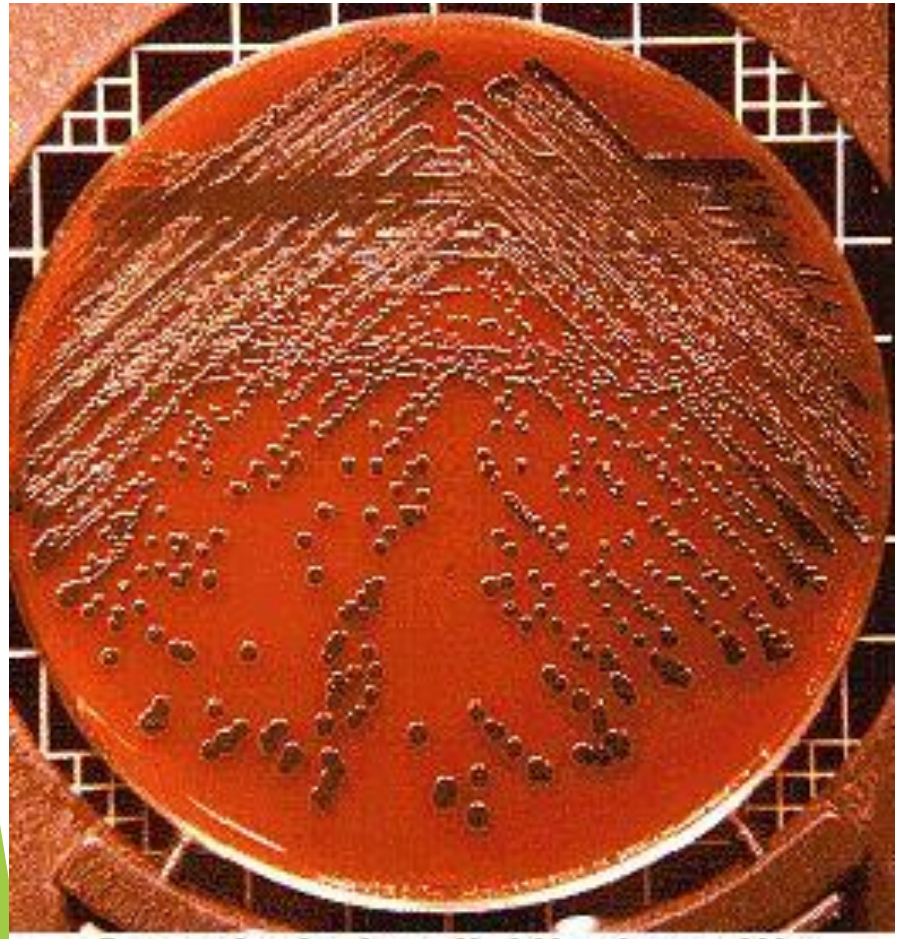




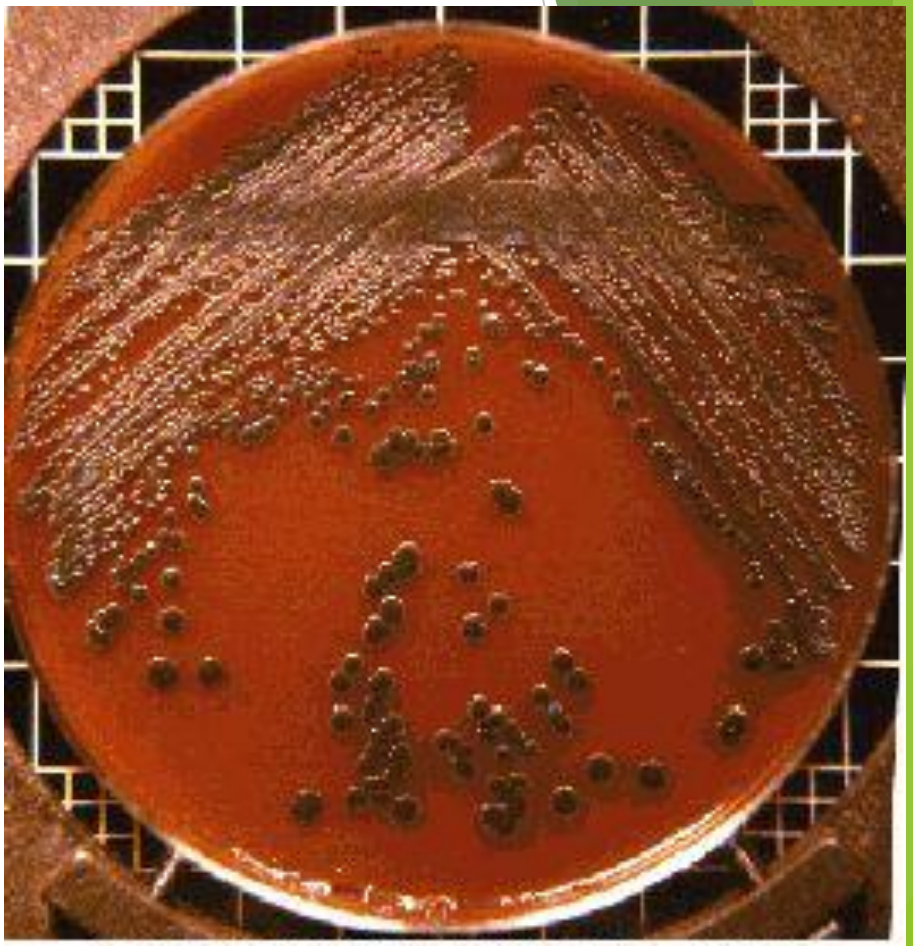
1. McLeod's agar plate-**gravis** biotype.

2. Cystine tellurite plat- **mitis** biotype

3. Cystine tellurite plate-**gravis** biotype



Corynebacterium diphtheriae, mitis
Chocolate tellurite agar



Corynebacterium diphtheriae, gravis
Chocolate tellurite agar

Loeffler slants appear as an off-white agar slant. They are used primarily in the induction of metachormatic granule formation in Corynebacterium diphtheriae but are also used to detect pigment development and proteolysis. This medium is light sensitive and so should be stored in the dark at low temperatures. C.diphtheriae will appear as a yellowish growth on the slant; however, colonies may not be visible.

Biochemical activity

- **Biochemical reactions:** acid production from a range of carbohydrates and other biochemical tests are used to differentiate C.diphtheriae from other corynbacteria. It ferments glucose and maltose with acid production only. Fermentation of starch, glycogen and dextrin is useful for recognation of gravis, intermedius and mitis. Gravis (but not intermedius or mitis) strains ferment starch and glycogen.
- It catalase positive, oxidase negative and do not liquefy gelatin. Urea is not hydrolysed. It is indole negative and do not form phosphatase.

Resistance

Corynebacterium diphtheriae is a relatively resistant organism, susceptible to heat (destroyed in 10 mins at 58°C or 1min in 100°C), chemical disinfectants.

Antigenic structure

Antigenicaly they are heterogenous. Gravis has 13 types, intermedius 4 types and mitis 40 types.

Typing

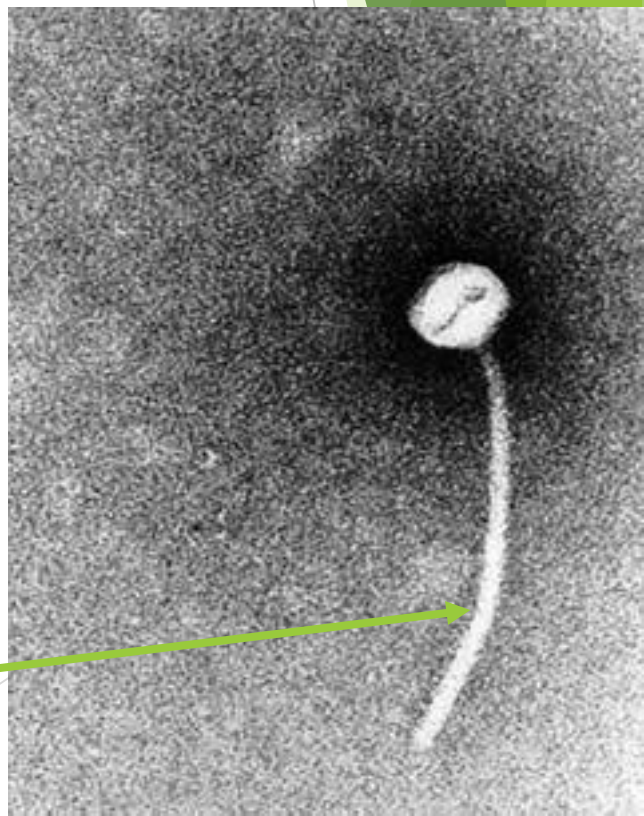
Serotyping, phage typing and bacteriocin typing have all been used to subdivide strains of C.diphtheriae for epidemiological studies.

Virulence Factors

- **Diphtheria toxin** consists of hystotoxin, dermonecrotoxin, hemolisinum;
- **Neiraminidase;**
- **Hyaluronidase;**
- **Factor of necrosis;**
- **Factors of diffusion.**

Toxins

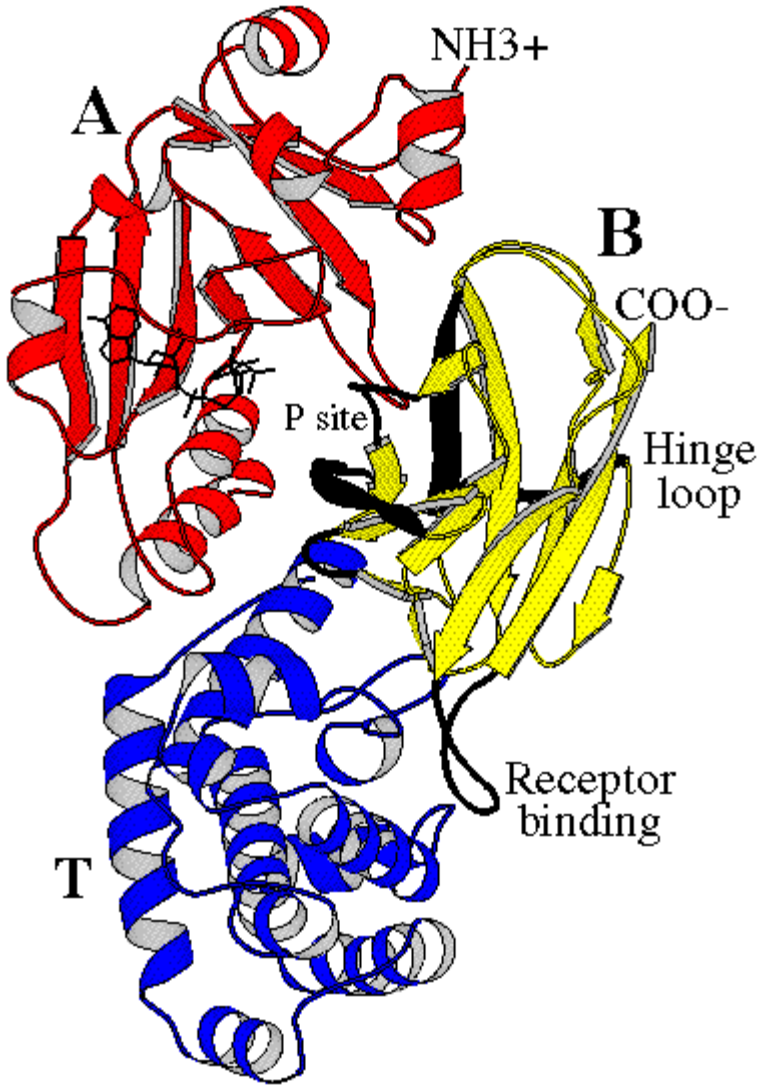
Two factors have great influence on the ability of *C.diphtheriae* to produce the diphtheria toxin: (1) **low extracellular concentrations of iron** and (2) the **presence of a lysogenic prophage** in the bacterial chromosome. The gene for toxin production occurs on the chromosome of the prophage, but a bacterial repressor protein controls the expression of this gene. The repressor is activated by iron, and it is in this way that iron influences toxin production. High yields of toxin are synthesized only by lysogenic bacteria under conditions of iron deficiency.



The Beta phage that encodes the tox gene for the diphtheria toxin.

The Diphtheria Toxin (DTx) Monomer.

A (red) is the catalytic domain;
B (yellow) is the binding domain which displays the receptor for cell attachment;
T (blue) is the hydrophobic domain responsible for insertion into the endosome membrane to secure the release of A.
The protein is illustrated in its "closed" configuration.



The toxin acts locally on the mucous membranes of the respiratory tract to produce a grey, adherent pseudomembrane consisting of fibrin, bacteria, and epithelial and phagocytic cells. After absorption into the bloodstream, it acts systemically on the cell of the myocardium, the nervous system (only motor nerves are affected) and the adrenal glands. The toxin can be rendered non-toxic but still antigenic by treatment with formaldehyde: the toxoid so formed is used in prophylactic immunization.

Pathogenesis

The pathogenesis of *diphtheria* includes two distinct stages:

1. **Invasion** of the local tissues of the throat, which requires colonization and subsequent bacterial proliferation. The bacteria produce several types of pili (adherence mechanisms). The diphtheria toxin, as well, may be involved in colonization of the throat.
2. **Toxigenesis**: bacterial production of the toxin. The diphtheria toxin causes the death eucaryotic cells and tissues by inhibition protein synthesis in the cells.

Epidemiology and clinical features

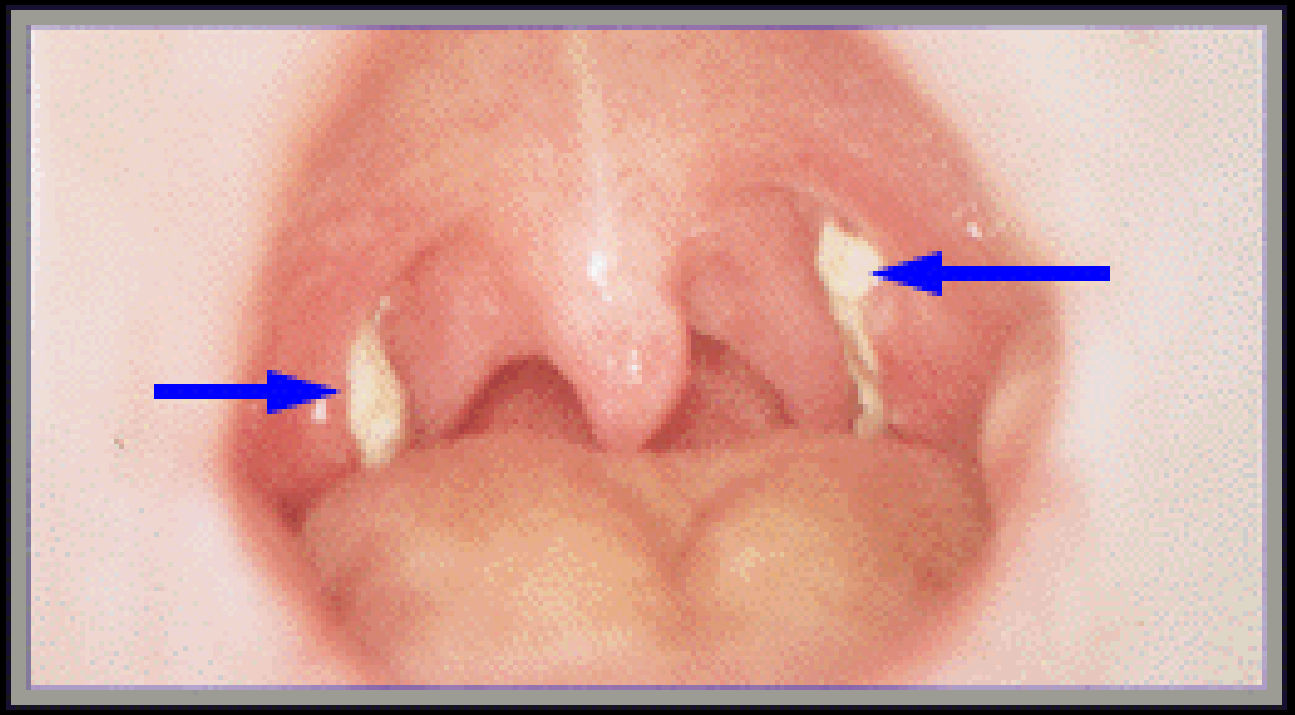
C. diphtheriae is spread by droplets, secretions, or direct contact. The incubation period is a 2-5 day .

There are two types of clinical diphtheria: nasopharyngeal and cutaneous. Early symptoms of pharyngeal diphtheria include a sore throat and fever, but after a few days a bluish-white adherent membrane, the pseudomembrane, forms over the back of the throat and tonsils.

The pseudomembrane is actually a fibrin network infected with multiplying *C. diphtheriae* cells which grows over a necrotic lesion on the epithelial cells on the back of the throat. The consequences of this membrane growth can be severe if the membrane grows to the extent that it blocks the airway in the throat.

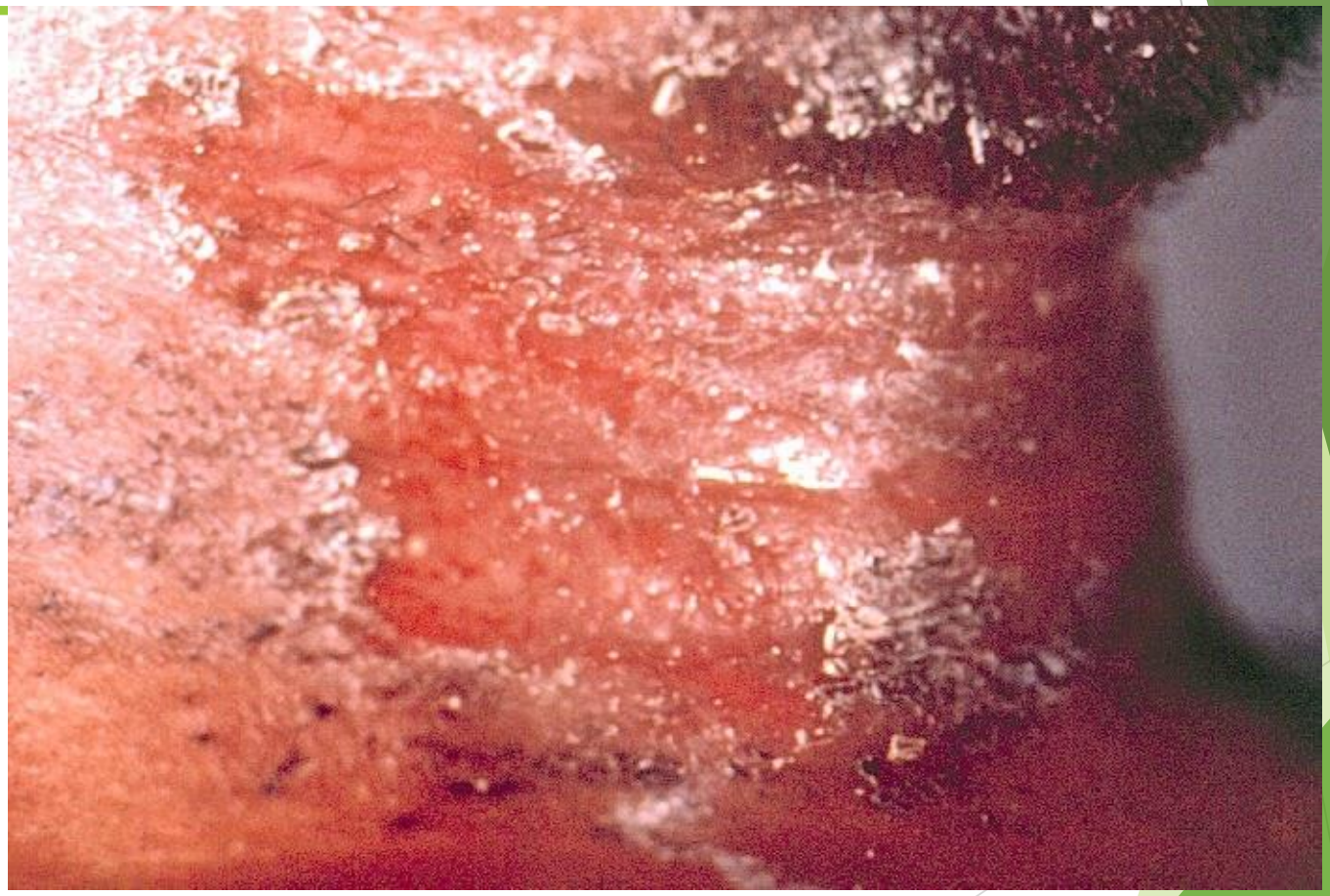
The involvement of **cervical lymph nodes** may cause profound swelling of the neck (bull neck diphtheria), and the patient may have a fever. The skin lesions in cutaneous diphtheria are usually covered by a gray-brown pseudomembrane. Life-threatening systemic complications, principally loss of motor function (e.g., difficulty in swallowing) and congestive heart failure, may develop as a result of the action of diphtheria toxin on peripheral motor neurons and the myocardium.

Diphtheria pseudomembrane

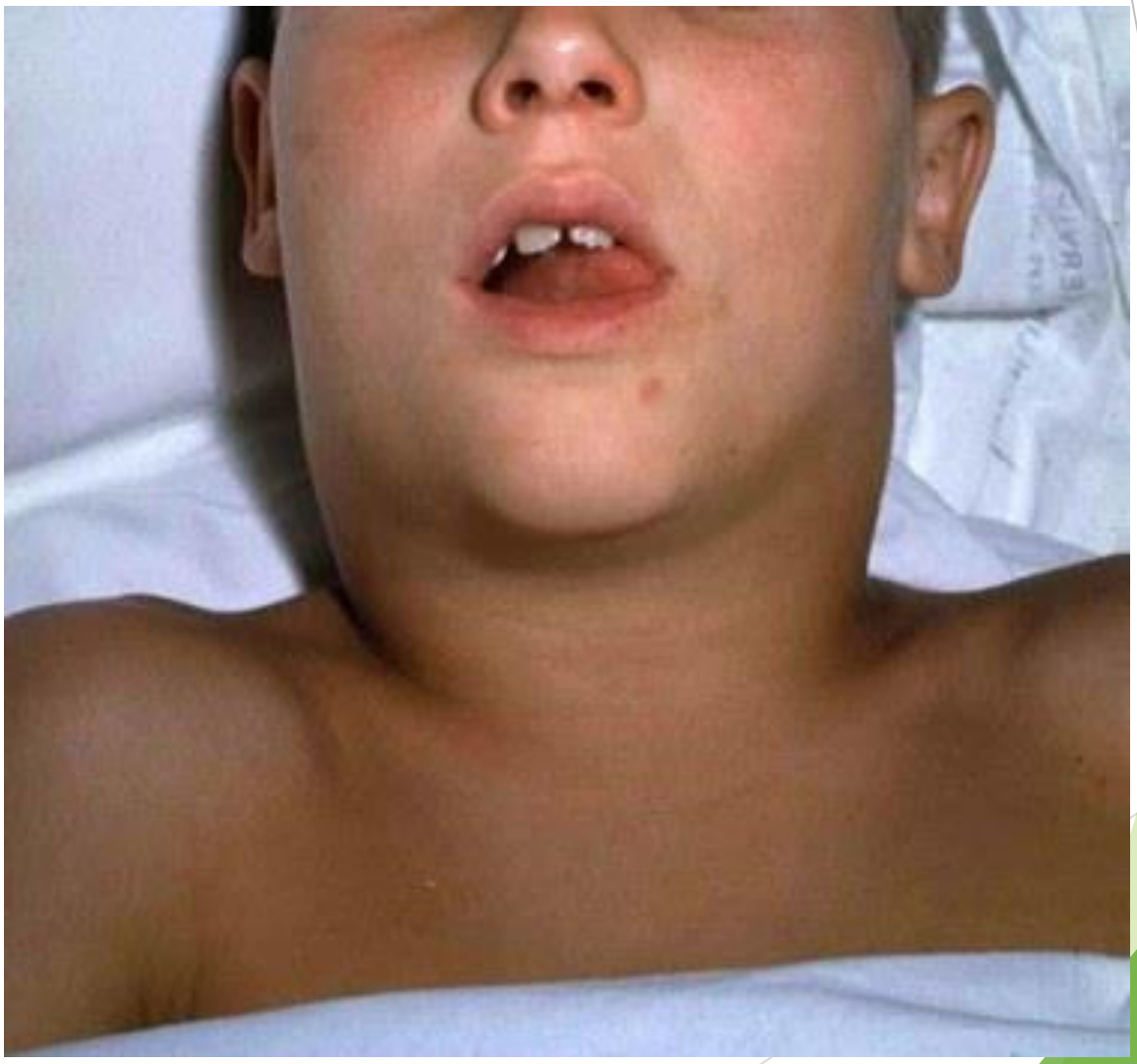


Diphtheria - notice the pseudomembrane in the posterior pharynx. It can become very large and may obstruct the airway.

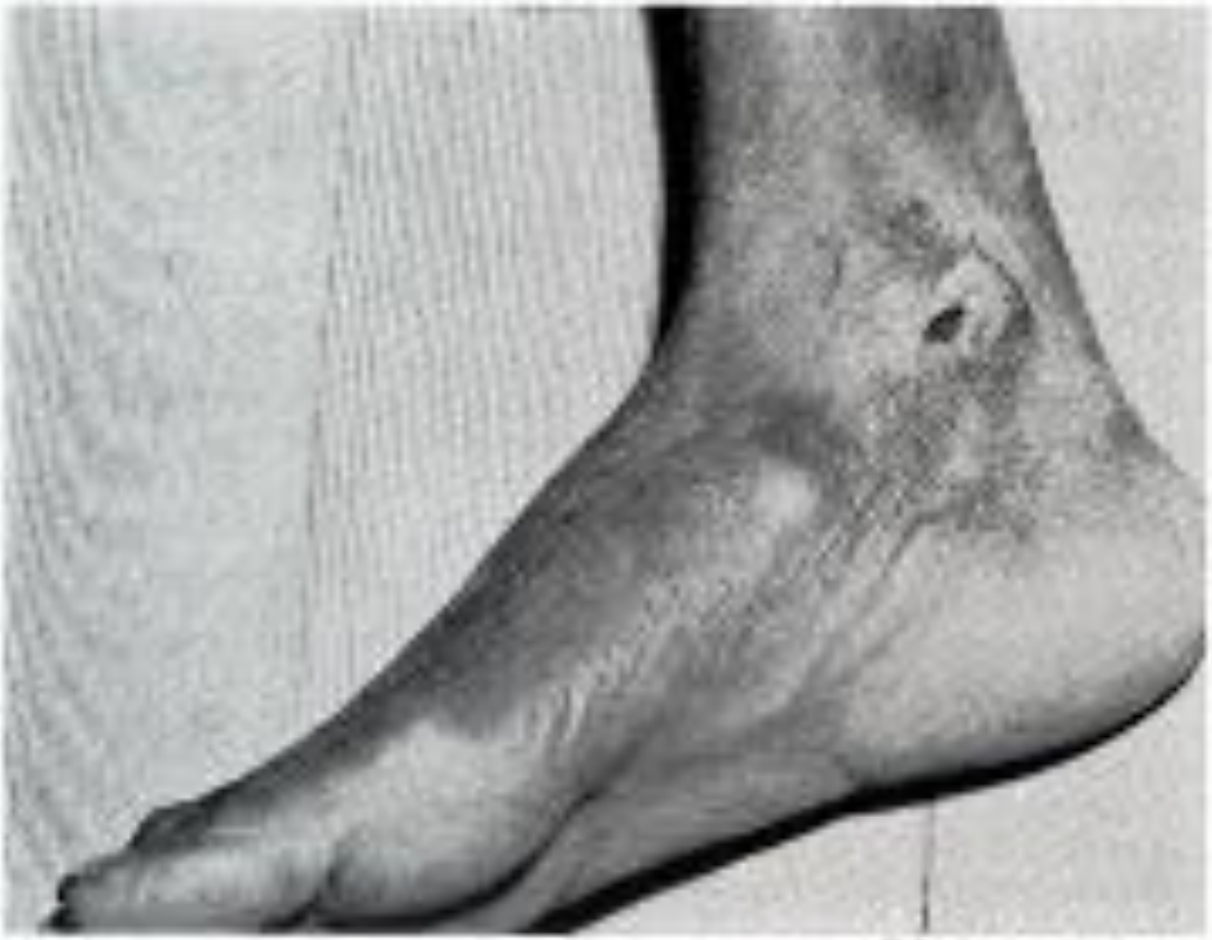
Diphtheria skin lesion on the neck



“Bull neck” appearance of diphtheritic cervical lymphadenopathy



Chronic ulcer of ankle containing C.diphyheriae.

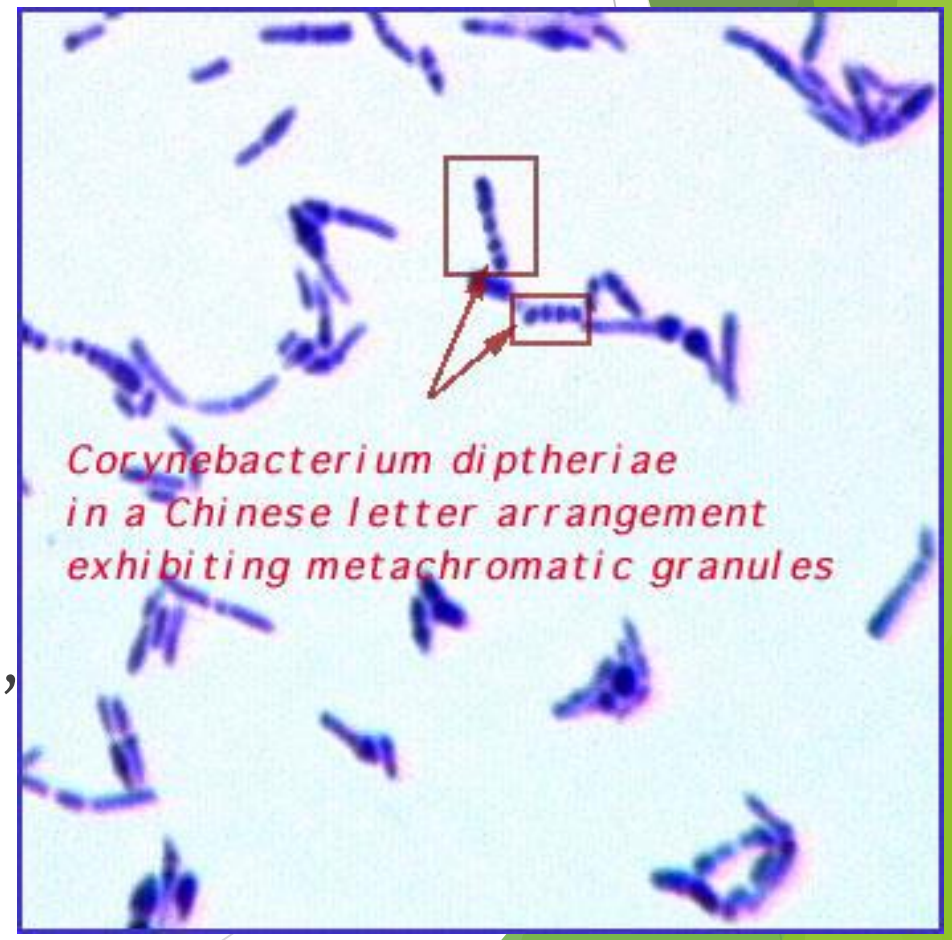


Diagnosis

Specimens: swabs from nose, throat, lessions

Bacterioscopic method:

- **Staining:** alkaline blue, methylene blue, Gram's, Albert's and Neisser's.
- **Characteristic:** dark purple volutin granules
- **Arrangement:** angled pairs, parallel rods (palisades), chinese letering

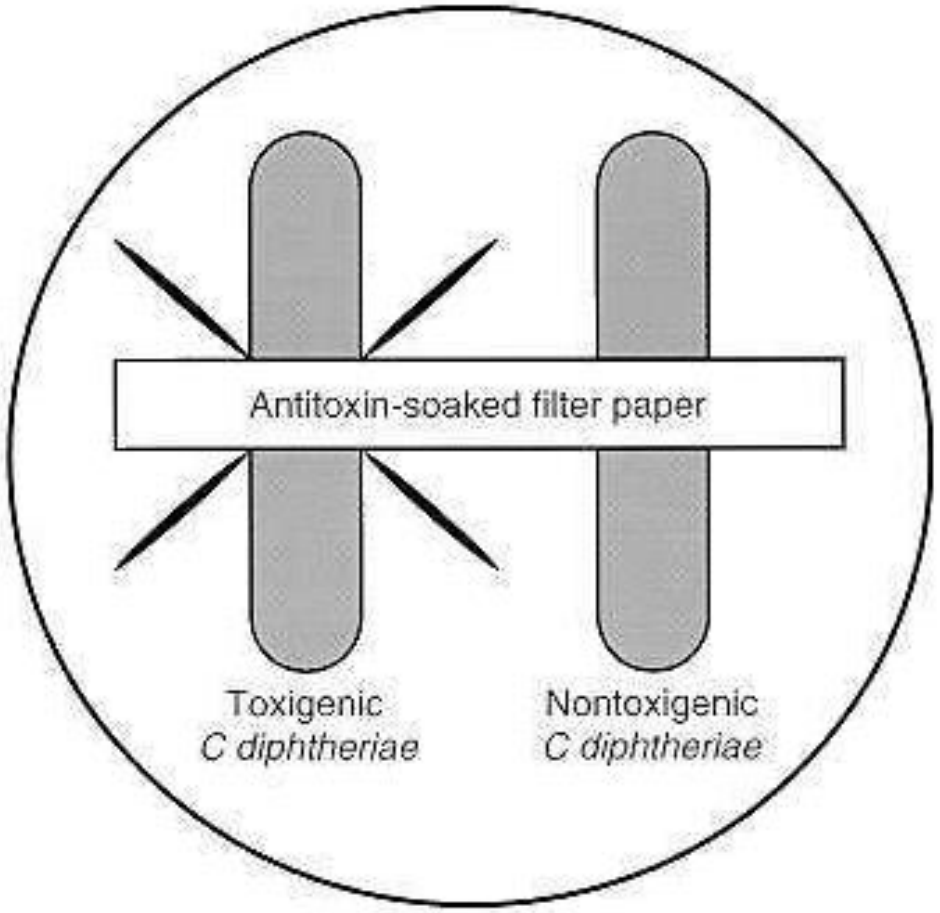


Bacteriological method

- For primary isolation, a variety of media may be used: Loeffler agar, Mueller-Miller tellurite agar, or Tinsdale tellurite agar.
- Following initial isolation, *C diphtheriae* may be identified as mitis, intermedius, or *gravis* biotype on the basis of carbohydrate fermentation patterns and hemolysis on sheep blood agar plates.
- The toxigenicity of *C diphtheriae* strains is determined by a variety of *in vitro* and *in vivo* tests. Some isolates of C.diphtheriae, especially mitis strains, are not toxigenic and are therefore non-virulent.

The most common *in vitro* assay for toxigenicity is the Elek immunodiffusion test. This test is based on the double diffusion of diphtheria toxin and antitoxin in an agar medium.

A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper. The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.



Alternatively, many eukaryotic cell lines (e.g., African green monkey kidney, Chinese hamster ovary) are sensitive to diphtheria toxin, enabling *in vitro* tissue culture tests to be used for detection of toxin.

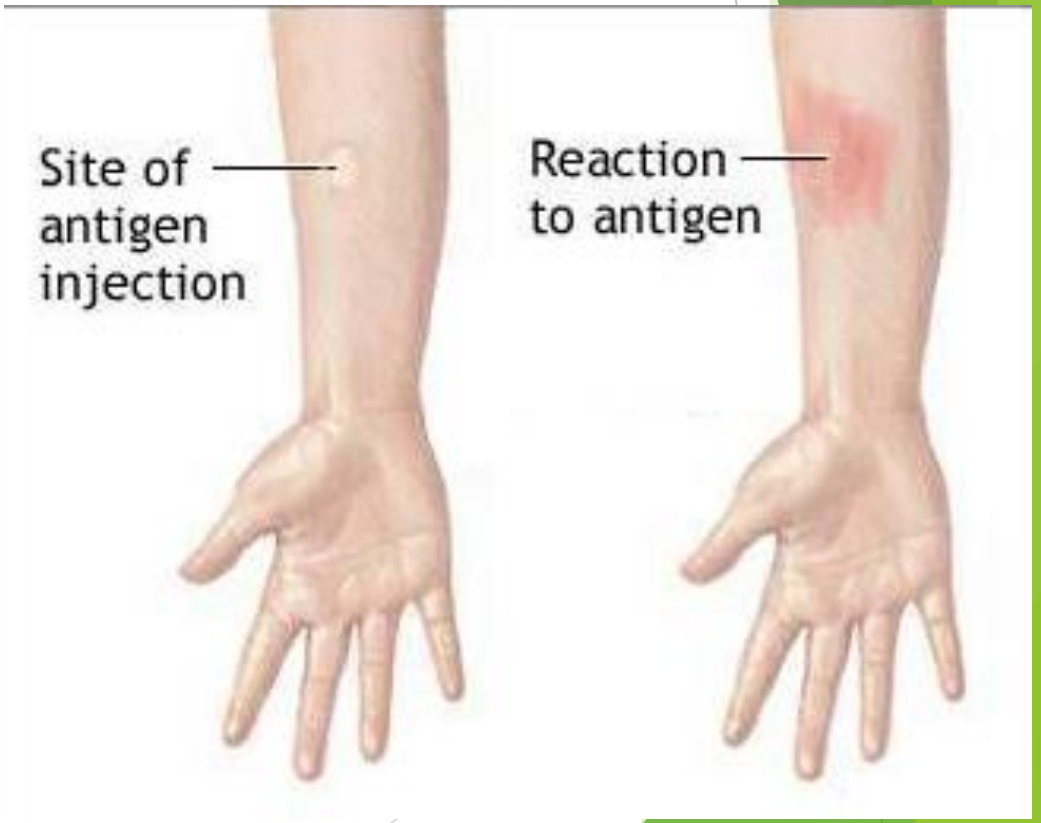
There are several sensitive *in vivo* tests for diphtheria toxin (e.g., guinea pig challenge test, rabbit skin test).

- Guinea pig inoculation: inject subcutaneously a suspension of the isolated strain of C.diphtheriae into two guinea pigs, one protected with diphtheria antitoxin.

If the strain is toxigenic, the upprotected animals dies in 2-3 days but the protected animal survives.

- PCR assays (test presence of specific bacteriophate gene (tox)

Schick test: a skin test formerly used to demonstrate immunity, i.e. circulating diphtheria antitoxin. Toxin was injected into the skin of the forearm, and this caused an erythematous reaction in those who were susceptible. Immunity checks in individuals at special risk are now carried out by the measurement of antitoxin levels in serum.



Treatment

Antibiotic sensitivity: C.diphtheriae is sensitive to penicillin, erythromycin and other antibiotics.

Although antibiotics (e.g., penicillin and erythromycin) are used as part of the treatment of patients who present with diphtheria, prompt passive immunization with ***diphtherial antitoxin*** is most effective in reducing the fatality rate.

Specific prophylaxis

In non immune person (Schick positive) immunity can be produced by active or passive immunization.

- For active immunization formal toxoid adjuvant such as alum precipitated toxoid (ATP), purified alum precipitated toxoid (PAPT) and toxoid antitoxin floccules (TAF); DTP (DPT)-tripple vaccine containing diphtheria toxoid, tetanus toxoid and pertussis vaccine, Td-contains absorbed tetanus and diphtheria toxoid are available. In endemic areas, active immunization is given at the age of 6 month. A booster dose is given at 18 months and another at school entry (6 years). This gives lasting protection.
- Passive immunity: this is an emergency measure when the susceptible are exposed to infection. 500 or 1000 units of antitoxin (anti diphtheria serum) is given subcutaneously. Being a horse serum one should take precaution against hypersensitivity.

DPT (Diphtheria, Pertussis, and Tetanus) "3-in-1" vaccine

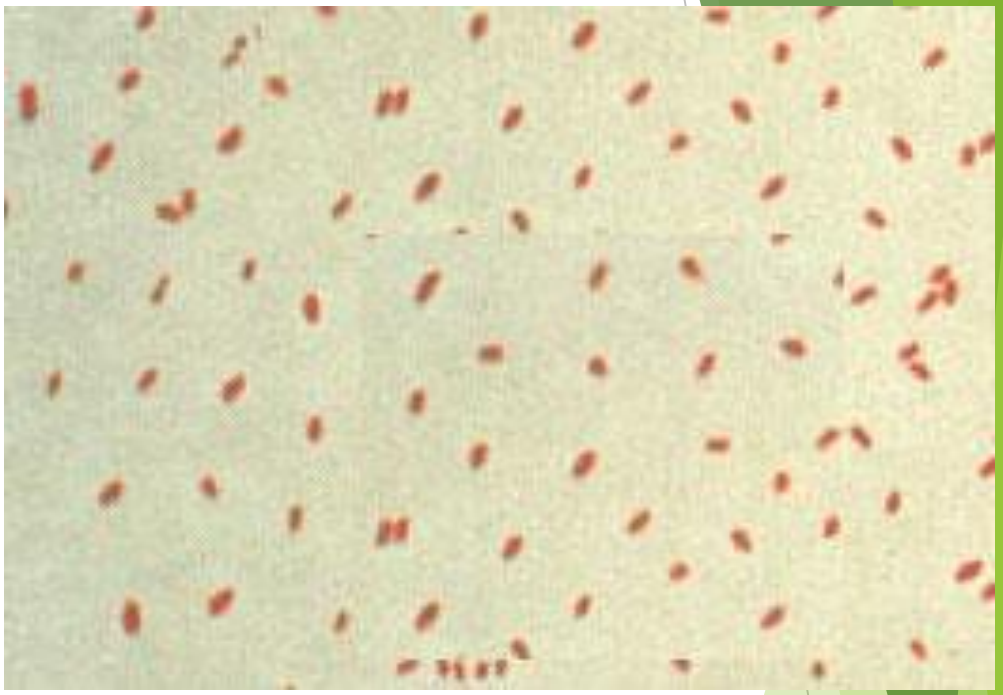
DT (Diphtheria and Tetanus) "2-in-1" vaccine; no pertussis

Td (Tetanus and Diphtheria) "2-in-1" vaccine for adults

Bordetella pertussis

Genera Bordetella

Species B. pertussis



Morphology and staining: *B. pertussis* is a very small, Gram-negative coccobacillus that appears singly or in pairs.

It is non-motile, non-sporing, have a capsule.

Cultural characteristic

B. pertussis is aerobic. The bacteria are nutritionally fastidious and are usually cultivated on rich media supplemented with blood. They can be grown in synthetic medium, however, which contains buffer,salts, an amino acid energy source, and growth factors such as nicotinamide (for which there is a strict requirement). Even on **blood agar** the organism grows slowly and requires 3-6 days to form pinpoint colonies.

- Culture: special enriched medium is required for primary isolation: the most widely used medium is charcoal blood agar. This has largely replaced the traditional Bordet-Gengou medium, which contain 30% blood, potato extract, glycerol and agar. The media are usually made selective by the addition of penicillin or cephalexin.
- Colonial morphology: colonies like “split pearls” or “mercury drops” appear after 3 or more days of incubation in a moist aerobic atmosphere at 35^{o}C.

Colonies of Bordetella pertussis growing on Bordet-Gengou media.

Biochemical activity

Bordetella pertussis is not active biochemically. Don't ferment of proteins, carbohydrates, urea.
Catalase is positive.

Antigenic structure

Bordetella pertussis have general heat stable somatic (O) antigen and various specific agglutinogens. There are 14 antigenic components in Bordetella.

Resistance

Bordetella pertussis is a unstable organism, susceptible to heat (destroyed in 10-15 mins at 56°C), chemical disinfectants (3% solution of phenol), sun light.

Virulence Factors

Adherence mechanisms of B. pertussis involve a **"filamentous hemagglutinin" (FHA),** which is a fimbrial-like structure on the bacterial surface, and cell-bound **pertussis toxin (PTx).** Short range effects of soluble toxins play a role as well in invasion during the colonization stage.

Colonization of tracheal epithelial cells by *Bordetella pertussis*

Toxins Produced by B. pertussis

B. pertussis produces a variety of substances with toxic activity in the class of **exotoxins** and **endotoxins (LPS)**.

- **invasive adenylate cyclase**. This toxin acts locally to reduce phagocytic activity and probably helps the organism initiate infection;
- **lethal toxin** (formerly called dermonecrotic toxin) which causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located;
- **tracheal cytotoxin** which is toxic for ciliated respiratory epithelium. The tracheal cytotoxin is a peptidoglycan fragment, which appears in the extracellular fluid where the bacteria are actively growing. The toxin kills ciliated cells and causes their extrusion from the mucosa. It also stimulates release of cytokine IL-1, and so causes fever;
- **pertussis toxin, PTx**, a protein that mediates both the colonization and toxemic stages of the disease.

Pathogenesis

The disease pertussis has two stages. The first stage, **colonization**, is an upper respiratory disease with fever, malaise and coughing, which increases in intensity over about a 10-day period.

The second or **toxemic** stage of pertussis follows relatively nonspecific symptoms of the colonizaton stage.

It begins gradually with prolonged and paroxysmal coughing that often ends in a characteristic inspiratory gasp (whoop). This stage is mediated by a variety of soluble toxins.

Epidemiology and Clinical features

Pertussis, also known as 'whooping cough', is a highly infectious bacterial disease of the respiratory tract and is spread by breathing in droplets expelled by an infected person when they talk, cough or sneeze.
Bordetella pertussis has an incubation period of 7 – 10 days. Pertussis is divided into three stages. (1) The catarrhal stage, so named because of the mucous membrane inflammation, which is insidious and resembles the common cold. (2) Prolonged coughing sieges characterize the paroxysmal stage. During this stage the infected person tries to cough up the mucous secretions by making 5 to 15 rapidly consecutive coughs followed by the characteristic whoop—a hurried deep inspiration. The catarrhal and paroxysmal stages last about 6 weeks. (3) Final recovery may take several months (the convalescent stage).

Diagnosis of Pertussis

Laboratory diagnosis of pertussis is by culture of the Bacterium (Bacteriological method), fluorescent antibody staining of smears from nasopharyngeal swabs, and serological tests (agglutination reaction, complement fixation test, direct hemagglutination reaction).

Treatment and Prevention

Treatment is with erythromycin, tetracycline, or chloramphenicol. Treatment ameliorates clinical illness when begun during the catarrhal phase and may also reduce the severity of the disease when begun within 2 weeks of the onset of the paroxysmal cough.

Prevention is with the DPT vaccine; vaccination of children is recommended when they are 2 to 3 months old.