

### The MYCOBACTERIA

<u>Scientific classification</u>	
<b>Domain:</b>	<u><b>Bacteria</b></u>
<b>Phylum:</b>	<u><b>Actinobacteria</b></u>
<b>Order:</b>	<u><b>Actinomycetales</b></u>
<b>Family:</b>	<b>Mycobacteriaceae</b>
<b>Genus:</b>	<b>Mycobacterium</b>

*Mycobacterium tuberculosis* is an obligate pathogenic bacterial species in the family *Mycobacteriaceae* and the causative agent of most cases of tuberculosis. First discovered in 1882 by Robert Koch, *M. tuberculosis* has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cells impervious to Gram staining; *M. tuberculosis* can appear Gram negative and Gram positive in clinical settings. The Ziehl-Neelsen stain, or acid-fast stain, is used instead.

#### - **Characteristics:**

- Non-spore forming, non-motile, aerobic, Acid-fast bacilli.
- Acid-fastness depends on the waxy envelope-mycolic acid of cell wall.
- More resistant to chemical agents than other bacteria.
- Once stained with primary stain, they resist decolorization by acid-alcohol.
- All bacteria are decolorized by acid-alcohol except *Mycobacteria*.

#### - **Mycobacteria of medical importance:**

*M. tuberculosis*

*M. leprae*

*Mycobacterium tuberculosis* Characteristics:

- . Strictly aerobic acid-fast bacilli.
- . The main reservoir is an infected human.

Antigenic structure:

1. Lipids: Mycolic acid, waxes, phosphatides .responsible for acid-fastness, granuloma formation and caseation necrosis.
2. Proteins. Elicits the tuberculin reaction and antibody production.

3. Polysaccharides . Induce the immediate type of hypersensitivity.

### Cell Wall Structure:

Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of (M.TB's) cell wall consists of three major components. Mycolic acids are unique alpha-branched lipids found in cell walls of *Mycobacterium* and *Corynebacterium*. Mycolic Acids are thought to be a significant determinant of virulence in (M.TB). Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.

**Cord Factor** is responsible for the serpentine cording (chains of cells form distinctive serpentine cord). Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of (M.TB) . When smear is prepared from culture, the bacilli appear short, thick and tend to arrange in a sort of bundles a phenomenon referred to as a cord formation due to the cord factor.

**In summary**, the high concentration of lipids in the cell wall of *Mycobacterium tuberculosis* has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- Resistance to lethal oxidations and survival inside of macrophages

### Clinical manifestation:

Incubation period: 4-6 weeks.

### Route of infection:

Respiratory- Inhalation of droplet nuclei, Ingestion of infected milk. Source of infection, Tuberculous patients.

### Disease:

Pulmonary and extra pulmonary tuberculosis. The disease generally manifests with low-grade persistent fever, night sweating, significant weight loss, fatigue and generalized weakness.

The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system, it infects the lungs. The most

frequently used diagnostic methods for tuberculosis are the tuberculin skin test, acid-fast stain, and chest radiographs. It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall, rich in lipids (e.g., mycolic acid), is likely responsible for this resistance and is a key virulence factor.

Humans are the only known reservoirs of *M. tuberculosis*. When in the lungs, *M. tuberculosis* is taken up by alveolar macrophages, but they are unable to digest and eradicate the bacterium. Its cell wall prevents the fusion of the phagosome with the lysosome, which contains a host of antimycobacterial factors.

person to person spread by direct contact or inhalation of infectious aerosol. Microscopy  
Organisms are identified by their red color on acid-fast staining. Cells are often seen wrapped together, due to the presence of fatty acids in the cell wall that stick together. This appearance is referred to as **chording**, like strands of chord that make up a **rope**.



### Morphology and identification:

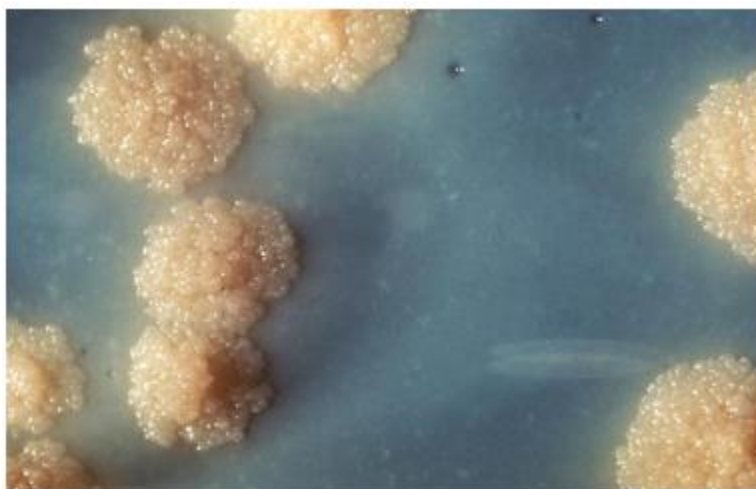
Typical organisms, In tissue, tubercle bacilli are thin straight rods with variable morphology from one species to another. All bacteria except the mycobacteria, acid-fastness depends on the integrity of the waxy envelope. The Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria.

In order to detect *Mycobacterium tuberculosis* in a sputum sample, in excess of 10,000 organisms per ml of sputum are needed to visualize the bacilli with a 100X microscope objective. One acid-fast bacillus/slide is regarded as "suspicious" of an M.TB. infection.



***Mycobacterium tuberculosis*. Acid-fast stain.**

**Culture:** Egg media (eg, Lowenstein-Jensen) contain salts, glycerol, and complex organic substances (eg, fresh eggs, egg yolks, potato flour, and other ingredients). Malachite green is included to inhibit other bacteria. Small inoculate in specimens from patients will grow on these media in 3-6 weeks. These media with added antibiotics are used as selective media. Glycerol broth used as liquid media for this bacteria, the bacteria appear as white pellicle on the surface of media, Dubose broth used for rapid culturing and for saving the bacteria for many years.



***Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar The growth characterized as rough-tough-puff***

### Treatment:

Anti-tuberculosis drugs:

1. First-line drugs . Isoniazid . Ethambutol . Rifampin . Pyrazinamide . Streptomycin
2. Second-line drugs . Kanamycin . Cycloserine . Capreomycin . Ofloxacin . Ethionamide . Para-aminosalicylic acid (PAS).

### Prevention and control:

Prompt and effective treatment of patients with active tuberculosis and careful follow up of their contacts with tuberculin test and CXR .

Immunization with BCG (Bacillus-Calmette-Guerin) vaccine .

Pasteurization of milk and milk products.

#### - *Mycobacterium leprae*

Leprosy: Is caused by the organism *Mycobacterium leprae*. Leprosy has two common forms, tuberculoid and Lepromatous. Both forms produce lesions on the skin, but the Lepromatous form is most sever, producing large disfiguring nodules. person to person spread by direct contact or inhalation of infectious erosome.

Clinical features: Incubation period is months to years. Route of infection is through nasal mucus secretion.

All forms of the disease eventually cause peripheral neurological damage (nerve damage in the extremities by sensory loss in the skin and muscle weakness). People with long-term leprosy may lose the use their hands or feet due to repeated injury resulting from lack of sensation.

#### Lepromin skin test :

The lepromin skin test is used to determine what type of leprosy a person has. It involves the injection of a standardized extract of the inactivated "leprosy bacillus",(*Mycobacterium leprae* or "Hansen's Bacillus") under the skin. The reaction continue for 3-5 weeks and it is called Mitsuda Reaction. This test is positive in tuberculoid form and negative in lepromatous form (which detect by staining).