

CHAPTER IX

MYCOBACTERIA

Upon completion of this unit of instruction, the student will be able to:

- Discuss the basic characteristics of Mycobacteria
- Describe the virulent factors of Mycobacteria
- Discuss pathogenicity, clinical manifestations, laboratory diagnosis, prevention & control of members of the Mycobacteria.

- Mycobacteria are slender rods with lipid-rich cell walls that are resistant to penetration by chemical dyes, such as those used in the Gram stain. They stain poorly but, once stained, cannot be easily decolorized by treatment with acidified organic solvents. Hence, they are termed **acid-fast**.
- Mycobacteria are long, slender rods that are non motile and do not form spores.
- Mycobacterial cell walls contain unique class of very long-chain fatty acids (**mycolic acids**).
- Mycobacteria are strictly aerobic.
- Most species grow slowly.

Table: Lineage of the agents of TB

Kingdom	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Subclass	Actinobacteridae
Order	Actinomycetales
Suborder	Corynebacterineae
Family	Mycobacteriaceae
Genus	<i>Mycobacterium</i> unique genus
Species	<i>M. tuberculosis</i> <i>M. bovis</i> <i>M. africanum</i> <i>M. microti</i> <i>"M. canettii"</i> <i>M. caprae</i> <i>M. pinnipedii</i>

Mycobacterium tuberculosis

- It is currently estimated that about one third of the world's population is infected with *M. tuberculosis* (tubercle bacillus), with thirty million people having active disease.
- In some of Asian and sub Saharan africa nations, nearly fifty percent of the HIV- infected population is co-infected with *M. tuberculosis*.
- Patients with active pulmonary tuberculosis shed large numbers of organisms by coughing, creating aerosol droplet nuclei. Because of resistance to dessication, the organisms can remain viable in the environment for a long time.
- The principal mode of transmission is person-to-person transmission by inhalation of the aerosol.

- Humans are the only reservoir
- Obligate aerobe & non - sporulated
- Slow generation time (**15-20** hours)
- Withstand week disinfectants and drying
- Does not have the chemical characteristics of either Gram-positive or Gram-negative bacteria
 - **Acid-fast bacteria** due to their impermeability to certain dyes and stains
 - Once stained, acid-fast bacteria will retain primary dye when heated and treated with decolorizer (**3% acid alcohol**)

- High concentration of **lipids/mycolic fatty acids** in the cell wall of *Mycobacterium tuberculosis* has been associated with:
 - 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
 - Prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule

Virulence Mechanisms and Virulence Factors of *M.tb*

- ***M.tb*** does not possess the classic bacterial virulence factors such as **toxins, capsules** and **fimbriae**
- However, a number of structural and physiological properties of the bacterium are contributed to bacterial virulence and the pathology tuberculosis
- Cell wall of *M.tb* is thick consisting of plasma membrane surrounded by a complex wall structure harboring virulence factors such as:
 - Peptidoglycan
 - Arabinogalactans
 - Mycolic fatty acid (long chain fatty acids)
 - Glycolipids
 - Down regulate the oxidative cytotoxic mechanism
 - Interact with PMNLs and macrophage lysosomal membrane and prevent their fusion with **phagosomes**
 - Lipoarabinomannans (down regulate the oxidative cytotoxic mechanism)

■ **Risk factors**

- Low socio - economic status
- Genetic disposition
- Chronic infections (HIV/AIDS, Diabetes mellitus, lung damage)
 - **More than 10%** of all HIV +ve individuals harbor *M.tb*
 - This is **400-times** the rate associated with the general public!!
- Previous exposure to mycobacterial infection
- Malnutrition

Epidemiology

WHO, 2007 report:

- **1/3** of world population infected with TB
- **8 - 10** million new active TB cases
- **1.97** million deaths / yr
- Sub - Saharan African countries: Account for **6.9 million** new TB case
- Incidence rate ranging b/n **305 & 525/100, 000**

Pathogenesis

Transmission

- Inhalation of air borne droplet nuclei that contain *M.tb* bacilli (Particle of 1 - 5 μm in diameter)
 - One droplet nuclei contains not more than **3 bacilli**
 - Coughing generates about **3000** droplet nuclei
 - Talking for 5 minutes generates **3000** droplet nuclei
 - Singing generates **3000** droplet nuclei in one minute
 - Sneezing generates the most droplet nuclei by far, which can spread to individuals up to 10 feet away

Clinical significance:

- **Primary tuberculosis** occurs in a person who has had no previous contact with the organism.
 - For the majority of cases (about 95 percent), the infection becomes arrested, and most people are unaware of this initial encounter. Approximately ten percent of those with an arrested primary infection develop clinical tuberculosis at some later time in their lives.
 - Primary disease initial phase: Primary tuberculosis is usually acquired via the respiratory tract; therefore, the initial lesion occurs in a small bronchiole or alveolus in the mid lung periphery.

□ Primary tuberculosis follows one of two courses:

- If the lesion arrests, the tubercle undergo fibrosis and calcification,
- alternatively, if the lesion breaks down, the caseous material is discharged, and a cavity is created that can facilitate spread of the infection. The organisms are dispersed by the lymph and the bloodstream, and can be seeded in the lungs, regional lymph nodes, or various distant tissues, such as liver, spleen, kidneys, bone, or meninges.

- In progressive disease, the resulting tubercles may expand, leading to destruction of tissue and clinical illness; for example,
 - **chronic pneumonitis,**
 - **tuberculous osteomyelitis, or**
 - **tuberculous meningitis**
 - In the extreme instance, active tubercles develop throughout the body, and a serious condition known as **miliary (disseminated) tuberculosis** results.

N.B: The disease can undergo haematogenous & lymphatic dissimulation

- Reactivation of tuberculosis: Reactivation is apparently caused by an impairment in immune status, often associated with malnutrition, alcoholism, advanced age, or severe stress.
 - Immunosuppressive medication or diseases such as diabetes and, particularly, AIDS, are common preconditions.

Clinical Presentation

- ☐ Weight loss
- ☐ Productive cough (For more than a month)
- ☐ Chest pain
- ☐ High fever
- ☐ Night sweating
- ☐ Haemoptysis

Laboratory identification:

Specimens: Sputum, CSF, Biopsy or FNA

1. Ziehl Neelson staining / AFS

- ☐ Specific & rapid
- ☐ Cheap
- ☐ Low sensitivity (65 - 70% with repeated smear) -----
HIV??

2. Cytology → using FNA / biopsy samples

- ☐

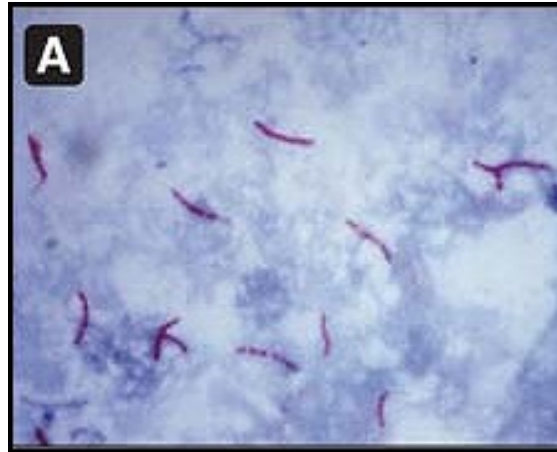


Figure: *Mycobacterium tuberculosis*. A. Acid-fast stain of sputum from a patient with tuberculosis.

3. Mycobacterial culture

Three general formulations:

1. Semi - solid agar media

- ☐ Include Middle brook 7H10 & 7H11 media
- ☐ Contain important ingredients that enhance the growth of the
- ☐ organism
- ☐ The media are used for:
 - Observing colony morphology (3-8 weeks)
 - Susceptibility testing
 - Selective media

2. Lowenstein - Jensen egg based media

- Is a solid media used as a selective media with added ABCs
- Requires about **3 - 8 weeks** for growth to occur

3. Broth media: E.g. Middlebrook 7 H9 & 7H12

- Support growth of small inocula
- Growth is more rapid than solid media

■ Variation

- Can occur in:
- Colony appearance
- Pigmentation
- Virulence/strain
- Optimal growth T0



Figure: *Mycobacterium tuberculosis* colonies grown on Lowenstein-Jensen medium.

4. Molecular Techniques

- ☐ Routine PCR: Gene amplification
- ☐ Real Time PCR: Gene amplification plus quantification
- ☐ Sensitivity: 80 - 85%, specificity ~ 99%

Table: Colony morphological and biochemical characteristics of species in the *M. tuberculosis* complex

Test	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. bovis</i> BCG	<i>M. africanum</i>	<i>M. microti</i>	" <i>M. canettii</i> "
Morphology	rough	rough	rough	rough	rough	smooth
Pyruvate rather than glycerol as carbon source	-	+	+	-	-	-
Pyrazinimidease	+	-	-	+	+	+
Niacin	+	-	-	+/-	+	-
Nitratase	+	-	-	+/-	-	+
Urease	+/-	-	+	+/-	+/-	+
Susceptibility to TCH	R	S	S	S	S	R
O ₂ requirement	aerobic	Micro-aerophilic	aerobic	Micro-aerophilic	Micro-aerophilic	Unknown

R= resistant, S= susceptible, TCH= Thiochrome-2-carboxylic acid hydrazide

- The following are among the tuberculosis diagnostic technologies which are currently (2005) under development
- *FASTPlaque TB*, a bacteriophage-based assay to detect within 48 h, *M. tuberculosis* in sputum and *FASTplaque TB-RIF* to identify rifampicin resistance in *M. tuberculosis* strains. The tests are produced by Biotec Laboratories.
- *TK Medium*, a mycobacterial solid culture medium which contains colour dye indicators to detect mycobacterial growth at an earlier stage (average 10–18 days) than other culture media. When there is mycobacterial metabolic activity, the colour of the medium changes from red to yellow. Bacterial contamination is indicated by the development of a green colour. The medium has a shelf-life of 4 months.

- *MTB ICT Strip*, an immunochromatographic urinary antigen test based on the detection of lipoarabinomannan in urine.
- *LAMP (loop-mediated isothermal amplification) test*, a sensitive molecular amplification technique to diagnose tuberculosis by detecting *M. tuberculosis* DNA in clinical samples.
- *Proteome Systems TB test*, a rapid technique to detect antigens produced in active tuberculosis and to measure severity of infection. The test is being developed by Proteome Systems

Table: Current methods of tuberculosis diagnosis

Method	Advantages	Disadvantages
Clinical signs	Rapid diagnosis	Not specific, not conclusive Not always present
X-Ray	Readily available	Not specific or conclusive
Microscopy (smear for acid-fast bacilli)	Low cost Rapid diagnosis	Low sensitivity (up to 2/3 of pulmonary TB cases are negative) Difficult sample collection
Culture	Specific	Time consuming (up to 4-8 weeks) Not always possible
PCR	Relatively quick Very specific	Relatively expensive High level of training required Expensive instrumentation Can detect latent disease
Method	Advantages	Disadvantages
BACTEC	Specific	Slow, 2-3 weeks Expensive Not possible in all cases
Tuberculosis ELISA test kits	Quick (procedure time: 110 min) Reproducible Minimal training	Some equipment required
Rapid tests	Very quick (procedure time: only 15 min) Minimal training No special equipment required	Lower sensitivity compared to ELISA test kits No quantitative results

Treatment

- **First line anti-TB drugs:** Isoniazid (INH), Rifampin, Ethambutol, Pyrazinamide, Streptomycin
 - **N.B:** 1 in 106 & 1 in 108 tubercle bacilli are spontaneous mutants resistant to first line drugs ----
MDR-TB????
- **Second line anti-TB drugs:** Kanamycin, Capreomycin, Cycloserine, Ethionamide, Ciprofloxacin

- Because strains of the organism resistant to a particular agent emerge during treatment, multiple drug therapy is employed to delay or prevent emergence.
- Drug resistance: Mutants resistant to each of these agents have been isolated even prior to drug treatment.
- Therefore, the standard procedure is to begin treatment with two or more drugs to prevent outgrowth of resistant strains. The higher incidence of multiple-drug-resistant strains (**MDR-TB**) is present in some locations.

- Directly observed therapy (**DOT**): Patient compliance is often low when multiple drug schedules last for six months or longer. One successful strategy for achieving better treatment completion rates is directly observed therapy in which patients take their medication while being supervised and observed.
- If the drugs are effective in the pulmonary form of tuberculosis, sputum acid-fast bacteria smears become negative, and the patient becomes non infectious in two to three weeks.

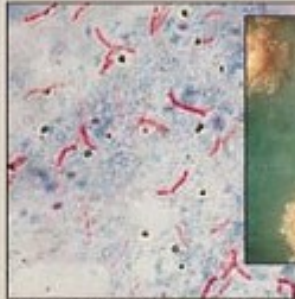
Prevention & control

- Early case detection & treatment
- Decreasing of over crowding
- Pasteurization of milk --- ↓ *M. bovis* infection
- Immunization (BCG)
- Health education

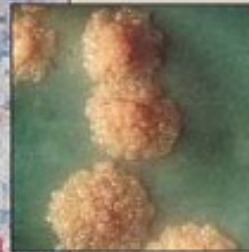
Other



Mycobacterium species



Mycobacterium tuberculosis (acid-fast stain)



Mycobacterium tuberculosis (colonies on Lowenstein-Jensen media)

- Acid-fast rods
- Not colored by Gram stain due to lipid-rich cell walls
- Long, slender, non-motile rods
- Aerobic
- Resistant to drying
- Culture *M. tuberculosis* on specialized medium such as Lowenstein-Jensen agar; *M. leprae* does not grow in culture

Mycobacterium tuberculosis

- Tuberculosis:



Mycobacterium leprae

- Hansen disease (leprosy)



Primary tuberculosis



Early leprosy

Mycobacterium leprae

- Leprosy (Hansen disease) is a worldwide much larger problem, with an estimated ten to twelve million cases.
- *M. leprae* is transmitted from human to human through:
 - prolonged contact; for example, between exudates of a leprosy patient's skin lesions and the abraded skin of another individual and
 - more commonly through nasal droplets from a patient with lepromatous disease.
- Leprosy is a chronic granulomatous condition of peripheral nerves and mucocutaneous tissues, particularly the nasal mucosa.

- It occurs as a continuum between two clinical extremes: tuberculoid and lepromatous leprosy.
 - **In tuberculoid leprosy**, the lesions occur as large maculae (spots) in cooler body tissues such as skin (especially the nose, outer ears, and testicles), and in superficial nerve endings. Neuritis leads to patches of anesthesia in the skin.
 - **In lepromatous leprosy**, nodular dermal and mucosal lesions develop. Nerve inflammation and neuroparalysis follow, eventually resulting in mutilations. Large numbers of organisms are present in the lesions

Tuberculoid Leprosy



Fig. 4.14 Tuberculoid leprosy is the benign, nonprogressive form of the disease, characterized by spotty dermal depigmentations.

Lepromatous Leprosy



Fig.4.15 In lepromatous leprosy, nodular dermal and mucosal lesions develop. Nerve inflammation and neuroparalysis follow, eventually resulting in mutilations.

- **Clinical findings**

- Lesions involving:
 - Skin (causing disfigurement)
 - Superficial nerves
 - Nose, pharynx, eyes and testicles
 - Lymphadenopathy

- **Incubation period- 2 - 10 years**

Laboratory identification:

Specimen:

- Scrapings from skin/nasal mucosa
- Biopsy from earlobe

■ Ziehl Neelson staining technique

- Look for bacilli (at least examine **200** fields)
- Laboratory diagnosis of lepromatous leprosy, where organisms are numerous, involves **acid-fast stains** of specimens from nasal mucosa or other infected areas. In tuberculoid leprosy, organisms are extremely rare.

- **Culture**

- M. has not been successfully maintained in artificial culture, but can be grown in the footpads of **mice** and in the **armadillo**.

- **Histology**

- **Molecular techniques**

Treatment

- Dapsone & rifampin
- Suppress the growth of *M. leprae*

N.B: Without prophylaxis, about **10%** of exposed children acquire the disease

■ **Prevention and control**

- Identification & treatment of cases
- Provision of chemoprophylaxis----- risk groups
- BCG vaccination -----endemic areas
- Health education