Chapter 15. Mycobacteria Infections

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- *Mycobacterium tuberculosis*
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Mycobacteria are acid-fast obligate aerobes. They can be classified into:

- *Mycobacterium tuberculosis* complex: It is responsible for tuberculosis in man
- *Mycobacterium leprae* (Hansen's bacillus): causes leprosy
- Nontuberculous mycobacteria (NTM): causes cutaneous, and pulmonary infections.

MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis complex causes tuberculosis, which is one of the oldest disease of mankind and is a major cause of death worldwide. It usually affects the lungs, although other organs are also involved. India accounts for the highest burden of tuberculosis (20% of total TB cases) worldwide.

Pathogenesis

- **Transmission:** *M. tuberculosis* is mainly transmitted by inhalation of aerosols (<5µm), generated while coughing or sneezing by infected patients
- **Bacillary load:** At least 10^4 bacilli/mL in sputum is required for an effective transmission
- A fraction of small droplet nuclei containing bacilli reaches the lungs, where the bacilli are phagocytosed by the alveolar macrophages
- *M. tuberculosis* is an obligate intracellular pathogen, that survives inside the macrophage by inhibition of phagolysosome fusion

- **CMI:** Host's cell-mediated immune response to *M. tuberculosis* is critical to contain the infection
- **Macrophage-activating response:** If the host mounts a good immune response, the activated macrophages kill the tubercle bacilli and form characteristic granuloma called tubercles
- **Tissue-damaging response:** In case bacilli are more virulent and the host mounts a delayed hypersensitivity reaction (DTH) to contain the infection, which leads to lung tissue destruction.

Clinical Forms

Tuberculosis occurs both in pulmonary and extrapulmonary forms.

Pulmonary Tuberculosis (PTB)

It is the most common type, presents either as primary PTB in children or as post-primary PTB in adults.

- **Primary PTB:** It is characterized by fibrotic nodular lesions (Ghon focus) in the lungs and associated hilar lymphadenopathy—together referred to as primary complex. The middle and lower lobes of the lungs are commonly affected
- **Post-primary PTB:** It usually occurs in adults, where the apical lobe of the lungs gets involved. Common features observed are hematogenous spread, cavitation, and caseating granuloma formation
- **Clinical features:** Patients usually present with fever, productive cough (±hemoptysis) and occasionally pleuritic chest pain, night sweating, weight loss, etc.

Extrapulmonary Tuberculosis (EPTB)

EPTB results from hematogenous dissemination of tubercle bacilli to various organs. In HIV patients, the occurrence of EPTB is much higher. The common types of EPTB include—tuberculous lymphadenitis (35%), pleural tuberculosis (20%), genitourinary tuberculosis, skeletal tuberculosis, tuberculous meningitis, gastrointestinal tuberculosis, and tuberculous skin lesions.

Epidemiology

About a quarter of the current world population is infected asymptomatically with *M. tuberculosis*, of which 5–10% develop the clinical disease during their lifetime.

- **World:** The WHO has estimated 10 million new cases of TB occurred in 2018 with a global incidence of 130 new cases per one lakh population per year
- Countries with high TB burden are *India*, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and South Africa
- **India:** In 2018, about 27 lakh cases occurred India; with highest burden from Uttar Pradesh (20% of total TB cases) followed by Maharashtra
- TB is one of the top 10 causes of death worldwide and the leading cause among infectious diseases.

Laboratory Diagnosis

The specimens collected for the diagnosis of tuberculosis depend upon the clinical forms.

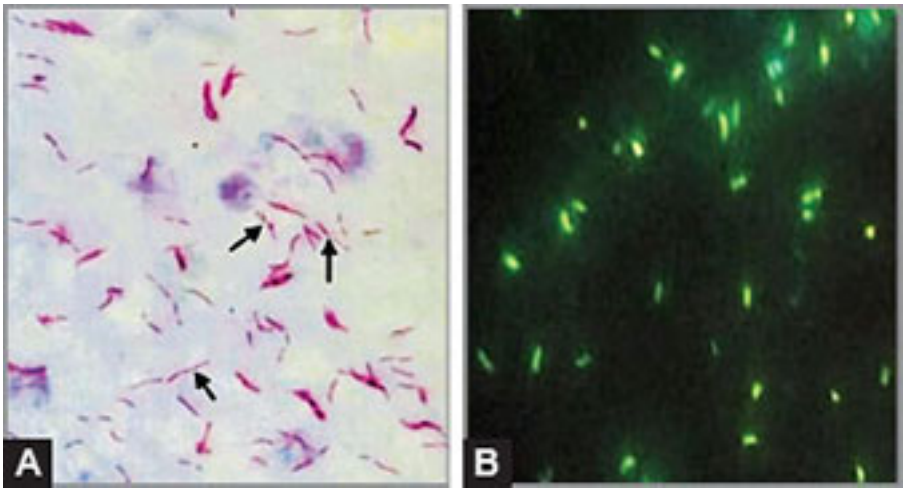
- In PTB, a minimum of *two sputum specimens* are examined—early morning and spot
- Whereas in EPTB, the specimens vary depending on the site involved such as pleural fluid, CSF, urine, etc.

Microscopy

Acid-fast staining is the microscopic method performed for the detection of *M. tuberculosis*.

- **Digestion and decontamination:** The sputum specimens are prior subjected to digestion (to liquefy the thick pus) and decontamination by treatment with sodium hydroxide (Petroff's method) or N-acetyl L-cysteine (NALC)
- **Methods:** The various acid-fast staining methods available are:
  - Ziehl–Neelsen (ZN) staining (hot method) using 25% sulfuric acid as the decolorizer—*M. tuberculosis* appears as long slender, beaded, red colored acid-fast bacilli *(Fig. 15.1A)*
  - Kinyoun's cold acid-fast staining
  - Fluorescent (auramine phenol) staining—is more sensitive and smears can be screened more rapidly than ZN staining. Tubercle bacilli appear bright brilliant green against the dark background *(Fig. 15.1B)*.
- **Grading** of the sputum smear is done (0 to 3+) which helps to determine the severity of disease, and infectiousness and also to monitor the response to treatment
- **Detection limit:** At least 10^4 bacilli/mL in sputum is required for the bacilli to be detected in acid-fast stained smear.

Figs. 15.1A and B. *A.* ZN staining of sputum smear showing long, slender and beaded red colored acid–fast bacilli; *B.* Auramine phenol staining of sputum smear—Tubercle bacilli appear bright brilliant green against the dark background.



Source: ***A and B.*** Department of Microbiology, JIPMER, Puducherry *(with permission)*.

Culture

Culture is more sensitive than microscopy, with a detection limit of 10-100 bacilli/mL. Various culture methods/media available are:

- **Conventional media** such as Lowenstein-Jensen (LJ) medium: *M. tuberculosis* produces rough, tough, and buff-colored colonies after an incubation of 6-8 weeks *(Fig. 15.2A)*
- **Automated culture systems,** e.g. MGIT (Mycobacteria growth indicator tube): Takes less time than LJ culture (3 weeks). It can also be used for drug susceptibility testing *(Fig. 15.2B)*

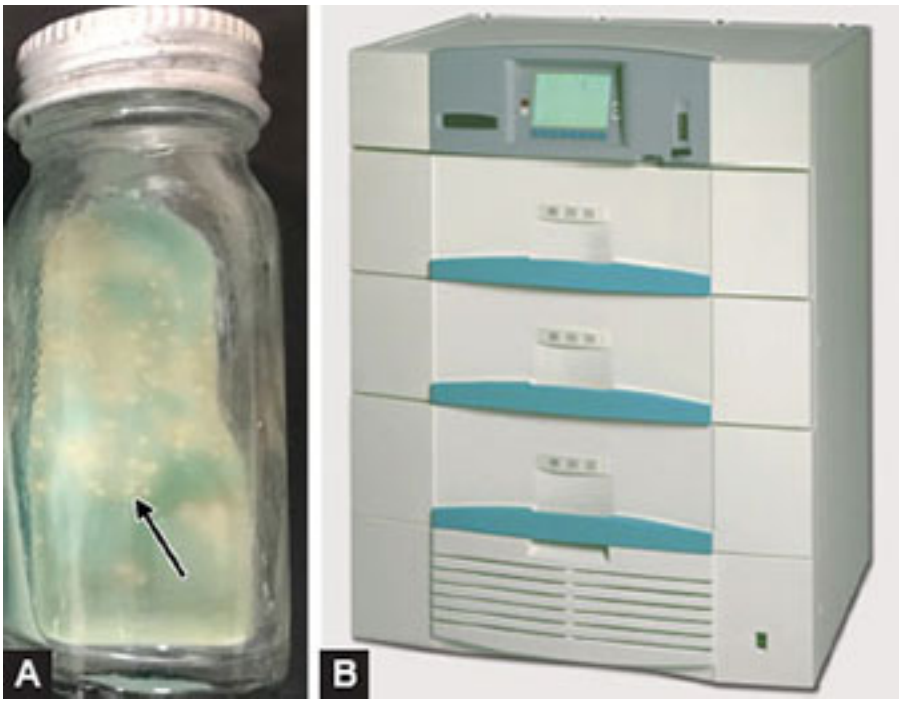
- **Identification** of *M. tuberculosis* in culture is made by:
  - Automated identification—by MALDI-TOF
  - Antigen detection by ICT—detecting MPT 64 antigen.

Molecular Methods

As culture is time-consuming, and microcopy is less sensitive, the diagnosis of TB greatly relies on molecular methods. Various molecular methods available are:

- **Cartridge-based nucleic acid amplification test (e.g. GeneXpert):** It is an automated real-time PCR system that has completely revolutionized the diagnosis of TB
  - *Uses:* It serves two purposes—(i) detection of *M. tuberculosis* complex in the specimen, and (ii) detection of rifampicin resistance
  - *Advantages:* It is rapid, takes <2h of time, and is highly sensitive (detection limit 131 bacilli/mL of the specimen).
- **Chip-based NAAT: Truenat** is a chip-based real-time PCR system, developed in India, that works in a similar principle as GeneXpert
- **Line probe assay (LPA):** It involves probe-based detection of amplified DNA in the specimen
  - *Uses:* (i) Identification of MTB complex, (ii) detection of resistance to first-line and second-line antitubercular drugs
  - *Disadvantages:* It takes 2-3 days. It is less sensitive, and can be performed only on smear-positive specimens.

Figs. 15.2. Culture media/culture systems for *M. tuberculosis*: *A.* Lowenstein-Jensen medium (arrow showing rough, tough and buff-colored colonies); *B.* BACTEC MGIT.



Source: Department of Microbiology, JIPMER, Puducherry *(with permission).*

Drug Susceptibility Test (DST)

Universal-DST refers to performing DST for all TB patients—first performing CBNAAT to determine rifampicin susceptibility; followed by line probe assay (LPA) or MGIT to detect susceptibility to other anti-tubercular drugs.

Diagnosis of Latent Tuberculosis

Latent tuberculosis is diagnosed by demonstration of delayed or type IV hypersensitivity reaction against the tubercle bacilli antigens.

- **Two methods** are available: (1) tuberculin skin test (or Monteux test), (2) IFN-$_{\gamma}$ release assay
- **A positive test** indicates prior exposure to *M. tuberculosis*, but cannot differentiate between past exposure and active infection. However, in infants, it can suggest an active infection.

TREATMENT

Tuberculosis

Treatment of tuberculosis involves a multidrug regimen of first-line agents, given for a longer duration (6 months) and under the direct supervision:

- Intensive phase (2 months) with four drugs (HRZE): Isoniazid, rifampicin, pyrazinamide, and ethambutol
- Continuation phase (4 months) with three drugs (HRE): Isoniazid, rifampicin, and ethambutol
- FDC: All drugs must be given in fixed-dose combination (FDC) tablets as per body weight
- Daily-oral regimen: The FDC tablets should be taken orally, once a day.

Drug Resistance

Failure to adhere to the multidrug regimen is a common practice in patients, which often leads to the emergence of drug resistance in *M. tuberculosis*. The common pattern of drug resistance are:

- **MDR-TB:** Defined as resistance to both isoniazid and rifampicin with or without resistance to other first-line drugs
- **XDR-TB:** Defined as MDR-TB plus resistance to second-line agents such as at least one injectable aminoglycoside and one fluoroquinolone.

National TB Elimination Programme

The National Tuberculosis Elimination Programme (NTEP) is the national program implemented in India for the control of tuberculosis. It was earlier called as Revised National Tuberculosis Control Programme (RNTCP).

Nikshay: It is a web portal for surveillance of TB, where all health care facilities need to notify all new TB cases through this web.

Infection Control Measures

Airborne precautions (e.g. negative pressure isolation room, N95 respirator, etc.) must be followed (Chapter 38).

Vaccine

Bacillus Calmette-Guérin (BCG) is a live attenuated vaccine for tuberculosis.

- **Strain:** In India, WHO recommended Danish 1331 strain of BCG is used. It is prepared in Central BCG laboratory, Guindy, Chennai
- **Indication:** It is given to newborn, at birth
- **Administered** by intradermal route above the insertion of left deltoid. If properly given, a permanent tiny round scar is developed in 6-12 weeks of time
- **Protection:** BCG has a variable efficacy of 0–80% and only up to 15–20 years. However, it surely gives protection against the development of complications such as tuberculous meningitis and disseminated tuberculosis
- **Contraindications to BCG include:** Child born to a TB-positive mother or child with low immunity or HIV-positive.

MYCOBACTERIUM LEPRAE

Mycobacterium leprae (Hansen's bacillus) causes leprosy; characterized by anesthetic skin lesions, bony deformities, and disfigurement.

- Due to fear, ignorance, superstitious beliefs, and characteristic disfigurement produced in the patients, leprosy remained as a social stigma over many years and patients have been socially outcasted
- However today, with early diagnosis and effective treatment, patients can lead a productive life in the community and the deformities can largely be prevented.

Clinical Manifestations

Based on the number of skin lesions, presence of nerve involvement and identification of bacilli in the slit-skin smear, leprosy can be classified into two categories. This classification is used by the leprosy control program for the treatment of patients (described later).

- **Paucibacillary (PB) leprosy:** A case of leprosy that fulfills all the criteria—(i) 1 to 5 skin lesions, (ii) no nerve involvement, and (iii) slit-skin smear-negative for lepra bacilli
- **Multibacillary (MB) leprosy:** A case of leprosy that fulfills any one of the criteria—(i) >5 skin lesions; or (ii) nerve involvement (neuritis); or (iii) slit-skin smear-positive for lepra bacilli.

Table 15.1. Differences between lepromatous leprosy and tuberculoid leprosy.

Characters	***Lepromatous leprosy (LL)***	***Tuberculoid leprosy (TT)***
Bacillary load	Multibacillary	Paucibacillary
Skin lesions	• Many, symmetrical • Margin is irregular	• One or few, asymmetrical • Margin is sharp
Nerve lesion	Appear late	• Early anesthetic skin lesion • Enlarged thickened nerves

Characters	*Lepromatous leprosy (LL)*	*Tuberculoid leprosy (TT)*
CMI	Low	Normal
Lepromin test	Negative	Positive
Humoral immunity	Exaggerated	Normal
Abbreviation: CMI, cell-mediated immunity.		

Leprosy is a bipolar disease. Depending upon the host cell-mediated immune response (CMI), the patient can develop various clinical forms ranging from lepromatous, borderline, or tuberculoid forms

- There are various *classification schemes* described in the literature such as Ridley-Jopling, Madrid, and Indian classification
- The *differences* between lepromatous leprosy (LL) and tuberculoid leprosy (TT) are depicted in **Table 15.1**.

Epidemiology

Unlike its superstitious beliefs, leprosy is not a highly communicable disease. Intimate and prolonged contact is necessary for transmission. Only about 5% of spouses living with leprosy patients develop the disease.

- **Transmission:** *M. leprae* is transmitted mainly through:
  - Nasal droplet inhalation (common mode)
  - Contact transmission (skin).
- **Leprosy elimination:** As per WHO, leprosy is said to be eliminated if the caseload is <1 case per 10,000 population. Many countries have achieved this target including India
- **The situation in World**: Once leprosy was worldwide in distribution, but now, it is almost exclusively confined to the developing nations of Asia, and Africa
- **The situation in India**: Although India has achieved leprosy elimination in 2005, cases still occur in various pockets of India such as Bihar, Chhattisgarh, etc. India still accounts for the highest-burden of leprosy in the world.

Laboratory Diagnosis

Smear Microscopy

Smear microscopy is done to demonstrate the acid-fast bacilli in the lesions.

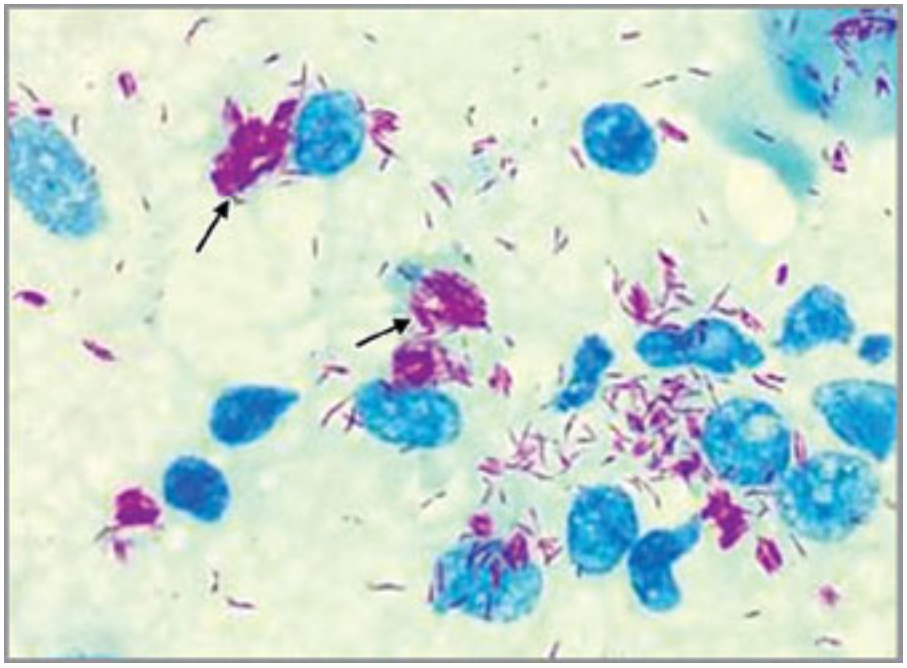
- **Specimens:** A total of six samples are collected; four from the skin (forehead, cheek, chin, and buttock), one from the ear lobe and nasal mucosa by nasal blow/scraping. Skin specimens are collected by a technique called slit-skin smear
- **Acid-fast stain** using 5% sulfuric acid: *M. leprae* appears red acid-fast bacilli arranged in cigar-like bundles to form globi, found inside the foamy macrophages *(Fig. 15.3)*.

Other Methods

The other methods for diagnosis of leprosy include:

- **Mouse footpad cultivation:** *M. leprae* is not cultivable in culture media or in tissue culture, but can grow in mouse foot pads

Fig. 15.3. Acid-fast stained slit skin smear showing numerous *Mycobacterium leprae* singly or in globi (arrows).



Source: Dr Isabella Princess, Apollo Hospital, Chennai *(with permission)*.

- *Antibody detection* by FLA-ABS (fluorescent leprosy antibody absorption test)
- **Lepromin test:** It is a skin test, similar to the tuberculin test. A positive test indicates CMI is intact and good prognosis, while a negative test indicates low CMI and poor prognosis.

TREATMENT

Leprosy

Multidrug therapy (MDT) is recommended for the treatment of leprosy, because of the risk of development of drug resistance to a single drug.

- **3-drug regimen:** WHO recommends a 3-drug regimen of rifampicin, dapsone and clofazimine for all leprosy patients
- **Duration of treatment**-6 months for paucibacillary leprosy and 12 months for multibacillary leprosy
- **Follow-up** is conducted annually for 2 years for paucibacillary leprosy and 5 years for multibacillary leprosy cases.

NONTUBERCULOUS MYCOBACTERIA (NTM)

They are a diverse group of mycobacteria, exist either as saprophytes or commensals, but can occasionally cause opportunistic infection in man. The various NTMs can be classified, as follows:

- **Photochromogens:** Produce pigments only in light, e.g.
  - *M. marinum:* It causes skin ulcers known as *swimming pool granuloma* or fish tank granuloma
  - *M. kansasii:* It causes chronic pulmonary disease resembling tuberculosis.
- **Scotochromogens:** Produce pigments both in light and dark, e.g.
  - *M. scrofulaceum:* It causes scrofula (cervical lymphadenitis) in children
  - *M. gordonae:* It is often found as commensal in tap water.
- **Non-chromogens:** Do not produce pigments, e.g. *M. avium-intracellulare* complex (MAC). It causes opportunistic infection, especially in HIV-infected people such as lymphadenitis, respiratory infection, and disseminated disease
- **Rapid growers:** Grow within one week, e.g. *M. chelonae, M. fortuitum,* they cause post-trauma injection abscess and catheter-related infections.

EXPECTED QUESTIONS

1. **I. Write an essay on:**
   1. Discuss the pathogenesis, clinical manifestations and laboratory diagnosis of pulmonary tuberculosis.
2. **II. Write short notes on:**
   1. Clinical manifestations of leprosy.
   2. Nontuberculous mycobacteria (NTM) infections.
   3. BCG vaccine.
   4. Drug resistance in TB.
3. **III. Multiple Choice Questions (MCQs):**
   1. **How much bacillary load in sputum is required for effective transmission of** *M. tuberculosis?*
      a. 10 bacilli/mL
      b. 100 bacilli/mL
      c. 1,000 bacilli/mL
      d. 10,000 bacilli/mL
   2. **Survival of** *M. tuberculosis inside the macrophages is due to:*
      a. Inhibition of entry into the host cell
      b. Inhibition of entry into the phagosome
      c. Inhibition of phagosome-lysosome fusion
      d. Inhibits degradation by lysosomal enzymes
   3. **GeneXpert can detect resistance to:**
      a. Isoniazid

b. Rifampicin

c. Pyrazinamide

d. Ethambutol

4. **Which of the following is a rapid grower mycobacteria?**

a. *M. marinum*

b. *M. kansasii*

c. *M. scrofulaceum*

d. *M. chelonae*

Answers

1. d	**2.** c	**3.** b	**4.** d