

# Mycobacterium tuberculosis

Kusum Shrestha

Lecturer

School of Health and Allied Sciences

Pokhara University

# Introduction

- Robert Koch identified *Mycobacterium tuberculosis* (MTB) as the causative agent of tuberculosis
- MTB complex responsible for the disease
- MTB complex includes many species and important ones include: *M. tuberculosis*, *M. bovis*
- *Tuberculosis* is one of the oldest disease of mankind
- Major cause of death worldwide
- Usually affects the lungs, although can affect other organs

# Characteristics of causative agent

- Belongs to the family Mycobacteriaceae
- Non-motile, non-sporing, noncapsulated
- Weakly gram-positive, straight or slightly curved rod-shaped bacteria
- Obligate aerobes
- Acid fast in nature
- Sometimes show branching filamentous forms resembling fungal mycelium
- Mould-like pellicle formation on liquid media

# contd

## ❖ Virulence factors

### ➤ Lipoarabinomannan (LAM)

- binds to complement receptors and mannose receptors present on surface of macrophage
  - Survival inside macrophage
- ### ➤ Mycolic acid: reduces entry of many antibiotics

# Epidemiology

- About one-third of world population is infected asymptotically and of which 5-10% develop clinical disease during their lifetime
- Patient with infectious pulmonary TB can infect 10- 15 people in a year
- In 2022, 202 countries and territories with more than 99% of the world's population and TB cases reported data.
- One of the top 10 causes of death worldwide
- Leading cause of death from a single infectious agent (ranking above HIV/AIDS)
- During the COVID-19 pandemic, there have been reductions in the reported numbers of people newly diagnosed with TB
- An estimated 10.6 million people fell ill with TB worldwide in 2021, an increase of 4.5% from 10.1 million in 2020, reversing many years of slow decline
- The global number of deaths officially classified as caused by TB in 2021 (1.4 million) was more than double the number caused by HIV/AIDS (0.65 million)

# contd

❖ In Nepal:

- Based on the National TB prevalence survey report 2018-19, the revised burden estimated shows higher incidence 245 per 100K (1.6 times), higher prevalence 416 per 100K (1.8 times), and higher mortality rates 9000-26000 (3.1 times) than previously estimated
- Burden significantly higher among men and in elderly population
- In terms of ecoterrain distribution, the Terai belt reports more than half of the cases
- Childhood TB is around 6.0%

- Multidrug resistant TB(MDR): resistant to rifampicin and isoniazid
- Pre-XDR TB : MDR TB plus which are also resistant to any fluoroquinolone.
- Extensively drug resistant(XDR) TB: Pre-XDR plus resistance to either bedaquiline or linezolid

# Risk Factors

- Low cell-mediated immunity: HIV infected people
- Comorbid conditions such as-post silicosis, post-transplantation (renal, cardiac), jejunioileal bypass, gastrectomy, chronic renal failure/hemodialysis, diabetes, IV drug abuse, smoking
- Age: Late adolescence and early adulthood period more prone
- Sex: Risk is higher in women at 25-34 years of age while at older ages, men have greater risk

# Mode of transmission

- Transmitted mainly by droplet infection and droplet nuclei
- In case of *M.bovis*, it can be transmitted via ingestion of unpasteurized milk of infected cow

# Pathogenesis

- Droplet nuclei containing tubercle bacilli from infectious patients are inhaled and only a fraction (usually <10%) of small droplets reach alveoli
- Adhesion to macrophages by lipoarabinomannan (LAM) leads *to* internalization of bacilli.
- Phagocytosis by macrophages is enhanced by complement mediated opsonization of bacilli.
- LAM **impairs phagosome-lysosome fusion** which allows bacteria to multiply intracellularly
- The macrophage eventually ruptures and releases its bacillary contents which infect other phagocytes and the cycle continues.

# Clinical features

- Incubation period is about 4 to 12 weeks
- Disease develops slowly
- Causes pulmonary and extrapulmonary tuberculosis
- Symptoms of pulmonary tuberculosis are
  - ✓ Fever and fatigue
  - ✓ weight loss and night sweating
  - ✓ Loss of appetite
- cough, which is characteristic of pulmonary involvement, may result in expectoration of bloody sputum
- Symptoms of extrapulmonary tb depends on site of infection

# Laboratory diagnosis

- ❖ Specimen: based on site of infection

- Sputum samples(two spot sample), urine, CSF, lymphnode aspirate, pus

- ❖ Microscopy

- Fluorescent microscopy: uses auramine phenol as primary stain, acid alcohol as decolorizer and potassium permanganate as counter stain. The bacilli appear bright brilliant green against dark background

- ZN staining

- ❖ Culture: solid culture performed on Lowenstein Jensen media. Should be kept for 8 weeks before giving negative result. Produces Rough, tough, buff colony

- ❖ Liquid culture: Middlebrook 7H9 media

- ❖ Antigen detection:

- ELISA, dip stick and latex agglutination tests are available

# ZN staining

- **Procedure:** (1) Smear is covered with primary stain, strong carbol fuchsin (basic fuchsin and phenol mixture) for 5 minutes. Intermittent heating of the slide is done till the fumes appear. Heating helps in better penetration of the stain; (2) Decolorization is done with 25% sulphuric acid for 3 minutes; (3) Counter staining is done with methylene blue for 1 min. Slide is examined under oil immersion objective.
- **Negative result:** At least 100 oil immersion fields should be examined before giving a negative report.
- **Positive result:** *M. tuberculosis* appears as long slender, beaded, less uniformly stained, red colored acid fast

# contd

- ❖ Mycobacterium growth indicator tube(MGIT): liquid media is used. Automated and continuous monitoring of the growth, faster and can report negative within 6 weeks
- ❖ Molecular methods
  - Probe based identification (Line probe assay): can simultaneously detect presence of MTB and resistance to rifampicin and isoniazid. Using second line LPA, resistance to aminoglycoside and fluoroquinolones can also be detected
  - Fully automated system(GeneXpert): simultaneously detect Presence of MTB and multidrug resistance. Detect resistance to rifampicin which acts as surrogate marker for isoniazid resistance as well

# contd

- ❖ Drug susceptibility testing
- ❖ Diagnosis of latent tb
  - Mantoux test
  - Interferon gamma assay:
    - ✓ uses highly specific *M. tuberculosis* antigens such as CFP10 and ESAT6 (early secreted antigenic target-6)
    - ✓ both coded by RD1 genes

# Prevention and control

- Rational and proper case management of TB cases
- Intensified Case Finding
- Contact tracing and investigation
- Preventive therapy (PT) - treatment of TB infection. -(Nepal is transitioning to 3HR Regimen for preventive therapy in children under five years) except for those children living with HIV who are on Nevirapine
- BCG vaccination
- Community based DOTS
- Early diagnosis and treatment

# Treatment

- First line drugs: Isoniazid(H), Rifampicin(R), Pyrazinamide(Z), ethambutol(E)
- Second line drugs: Levofloxacin, Moxifloxacin, Linezolid, Bedaquiline, clofazimine, amikacin
- Now only one category of treatment for TB patients needing first-line treatment
- All TB patients whether bacteriologically confirmed or clinically diagnosed will receive Treatment Regimen (2HRZE/4HR)
- EPTB patients will receive the same treatment as pulmonary TB patients
- For drug resistant TB second line drugs are used according to national guidelines

Thank You!