

• **MICROBIOLOGICAL
PULMONARY**

TUBERCULOSIS

- Causative agent
- Tuberculosis is caused by a genetically closely related group of *bacteria* together called mycobacterium tuberculosis complex
- Majority of the cases(90%) caused by *Mycobacterium tuberculosis*

- **How TB Spreads?**
- *Mycobacterium tuberculosis* spread via airborne particles called droplet nuclei
- Expelled when person with infectious TB coughs, sneezes,
- Transmission occurs when droplet nuclei inhaled and reach the lungs

- Risk factors
- Weakened immunity
 - Malnutrition
 - Advanced age
 - HIV/AIDS
 - Diabetes
 - Cancer treatment,
- Poverty

- Overcrowding
- Pathogenesis
- Pathogenesis
- Latent TB Infection (LTBI)
- The immune system is usually able to stop the multiplication of bacilli
- Persons with LTBI are not infectious and do not spread organisms to others
- Active TB Disease
- In some, the granulomas break down, bacilli escape and multiply, resulting in TB disease
- Can occur soon after infection, or years later

- Persons with TB disease are usually infectious and can spread bacteria to others
- Positive *M. tb* culture confirms TB diagnosis
- Primary Vs. secondary tuberculosis
- Primary tuberculosis
 - Primary pulmonary tuberculosis occurs soon after the initial infection with tubercle bacilli(first time). Most people with good immunity control it and patient enter latent phase
- Secondary tuberculosis
 - results from endogenous reactivation of latent infection
- Risk of Developing Disease

- Untreated, 5% of infected persons develop TB in first 1–2 years post infection, another 5% later in life
- Thus, about 10% of infected persons will develop TB at some point in life if not treated

- Lab diagnosis of tuberculosis
- Diagnosis of active disease
 - Microscopy
 - Culture
 - Nucleic Acid Amplification(PCR)
 - Drug Susceptibility Testing
- Diagnosis of latent tuberculosis(to check person is exposed to MTB or not)
 - Montoux skin test

- Specimens for pulmonary tuberculosis
- Specimens from the respiratory tract
 - Sputum
- Microscopy
- Ziehl neelson staining
- Rhodamine auramine staining
- Ziehl neelsen stain
- The waxy mycolic acid of cell wall of *Mycobacterium tuberculosis* allows it to retain the carbol fuschin even after exposure to strong acid solutions, thus called acid-fast.
 - AFB ---pink
 - Background ---blue

- Auramine rhodamine staining
- Fluorochrome dyes Auramine-Rhodamine have affinity with mycolic acid in the cell walls of Mycobacteria, which appear bright yellow against a dark background.
- Advantage ----easy to visualize AFB especially in labs with high workload
- Disadvantage----needs fluorescent microscope
- Culture
- Culture on solid media
 - Egg based
 - Agar based
- Semiautomated/Automated culture methods
 - Bactec 460

- MGIT 960
 - ESP culture system
 - MB bacT alert 3D
 - Bactec 9000 MB
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- Culture on solid media
 - Egg based
 - Lowenstein Jensen(LJ) medium
 - Selective for mycobacterium
 - Culture Characteristics of MTB on LJ medium
 - incubated aerobically at 35⁰ C with 5-10% CO₂ at high humidity
 - MTB is slow growing and colonies take 4-6 weeks to grow
 - Colony appearance is dry, rough, raised with wrinkled surface and creamy white in color
 - Conatmination and liquifaction of egg based medium is a disadvantage

- Culture on solid media
- Agar based media
 - Middlebrook 7H10 agar
and Middlebrook 7H11 agar
 - Less affected by contamination
 - Growth in two weeks
- BACTEC MGIT 960
- The MGIT tube contains a fluorochrome that is suppressed by oxygen in tube. During bacterial growth within the tube, the free oxygen is utilized by bacteria. With depletion of free oxygen, the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube .
- This fluorescence is measured by the machine . The intensity of

fluorescence is directly proportional to the bacterial growth.

- BACTEC MGIT 960(cont.)
- Fully automated
- Growth in less than 9 days
- Antibiotic susceptibility testing
- Nucleic Acid detection
- PCR
- Drug resistance genes detected
- Rapid
- PCR is highly sensitive(1-10 bacilli/ml of sample)
- Expensive
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Diagnosis of latent tuberculosis

- Mantoux skin test

- is the standard method of determining whether a person is exposed to *Mycobacterium tuberculosis*
 - The TST is performed by injecting 0.1 ml of tuberculin purified protein derivative (PPD) into the inner surface of the forearm. The TST is an intradermal injection. When placed correctly, the injection should produce a pale elevation of the skin (a wheal)
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- Montoux test (cond.)
 - The skin test reaction should be read between 48 and 72 hours after administration.
 - The reaction should be measured in millimeters of the induration (palpable, raised, hardened area or

swelling). The reader should not measure erythema (redness).

- Interpretation of montoux skin test
- Infection control in hospitals
- **Airborne precautions that includes:**
 - **Personal respiratory protection**
 - N95 respirator
 - **Airborne Infection Isolation Room (AIIR)**
 - Provide negative pressure room
- BCG vaccination
- BCG was derived from an attenuated strain of *M. bovis* .
- Vary in efficacy, ranging from 80% to nil in clinical trials

- WHO recommends routine use at birth in countries with high tuberculosis prevalence
- Studies have found higher rates of protection in infants and young children from serious forms of tuberculosis, such as TB meningitis and miliary TB.