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## Members of the Genus Mycobacterium

- 1. Mycobacterium tuberculosis Complex (MTC):
  - \* *M. tuberculosis*, Human is the only reservoir
  - \* *M. bovis,* Both cows and humans extrapulmonary TB (hunched backs)
- 2. Non-tuberculous mycobacteria (NTM): e.g. M. avium. TB-like disease especially in AIDS patients
- 3. Mycobacterium leprae: causes leprosy.

## **Mycobacterium Tuberculosis**

### **General Characteristics**

- Strictly aerobic,
- Non motile
- Non spore forming
- Facultative intracellular bacilli. macrophage
- Slow generation time (15-20) hours
- They are resistant to decolorization by acid alcohol, hence called 'acid-fast bacilli'.

### **Mycobacterium Tuberculosis**

### Morphology:

- Slender
- Straight or curved
- Arranged singly or in small groups
  Chains of cells in smears made from colonies called serpentine cords
- Ziehl-Neelsen (Z-N) pink in a blue background

## Lipid-Rich Cell Wall

Mycobacterium tuberculosis is not classified as either Gram-positive or Gram-negative because it does not have the chemical characteristics of either, although the bacteria do contain peptidoglycan (murein) in their cell wall.

Over 60% of the mycobacterial cell wall is lipid, consists of Mycolic acid, Cord factor, and Wax-D



# **Laboratory Diagnosis**

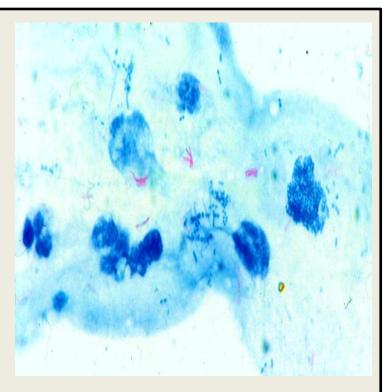
- Specimens: include sputum,
- Early morning sputum specimens collected on 3 consecutive days, from a deep productive cough, give the best results
- an excess of 10,000 bacteria
  per ml of sputum are needed
  to visualize the bacilli with a
  100X



# **Laboratory Diagnosis**

#### • Direct Detection:

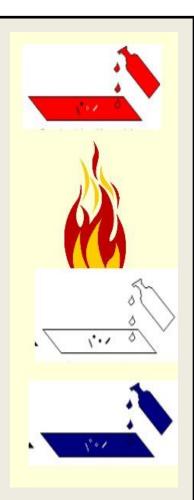
1. Smears: Ziehl-Neelsen (Z-N). Under the ordinary light microscope, AFB appear pink in a blue background



2. Molecular tests: • For rapid detection.

# Ziehl-Neelsen (Z-N) Stain

- Cover with carbol-fuchsin dye
- Heat gently for 5 minutes over direct flame
- Acid-alcohol (decolourization)
- Wash with water
- Counterstain for 10-30 seconds with Methylene blue
- Wash with water and let dry



# **Laboratory Diagnosis**

#### • Cultivation:

- Media include: Lowenstein-Jensen (L-J) medium – Middlebrook's medium
- Incubation is at 35-37 °C in 5-10% CO2 for up to 8 weeks.
- If culture negative and acid-fast Positive, a set of inoculated media should be incubated at 24-33°C for 12 weeks.

### **Cultural Characters**

#### They grow as brown pigmented "rough, tough and buff " colonies





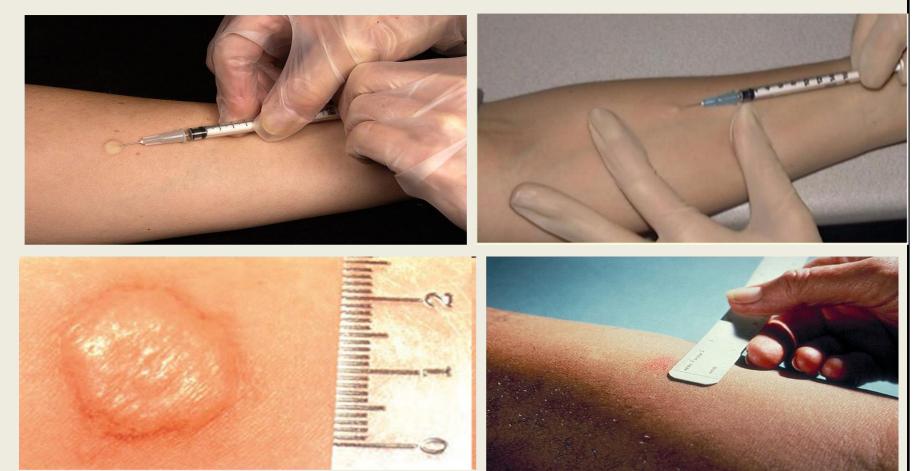
Colony of Mycobaterium On Lowenstien – jensen Agar Photochromogenic Middlebrook

### **The Mantoux test**

- Also known as the Tuberculin Sensitivity Test, is a diagnostic tool for tuberculosis.
- Tuberculin is a glycerol extract of the tubercle bacillus.
- A standard dose of 5 Tuberculin units (0.1mL) is injected intradermally and read 48 to 72 hours later.
- The reaction is read by measuring the diameter of induration across the forearm in millimeters.

### **The Mantoux test**

• It is considered positive if the induration 10mm or more in diameter.



### The Mantoux test

- A positive test indicates that an individual has been infected in the past.
- It does not imply that active disease or immunity to disease is present.
- Tuberculin positive person are of risk of developing disease from reactivation of the primary infection.

#### **Mycobacterium avium**

Also known as M avium intracellulare MAI. These organisms grow optimally at 41°C and produce smooth, soft, nonpigmented colonies. Cultured from water, soil, food, and animals, including birds.

Cause disease in immunocomptent human.

#### **Mycobacterium leprae**

- Typical acid-fast bacilli, singly, in parallel bundles, or in globular masses,
- ➢ It causes leprosy.
- Diagnosis: scraping from skin or nasal mucosa are smeared a slide and stained by the Ziel-Neelsen technique.
- Biopsy of skin for histological study.



# Thank You For Listening

#### **MCQ Questions**

- *1.M. tuberculosis are characterized by being:* 
  - a. Strict anaerobes
  - b. Acid fast bacilli
  - c. Capsulated
  - d. Motile

e. Able to grow on ordinary media

#### **MCQ Questions**

2.One of the following media is used for growing M. tuberculosis:

- a. MacConkey's agar
- b. Blood agar
- c. Nutrient agar

d. Lowenstein Jensen medium

e. Loeffler's serum

#### **MCQ Questions**

- 3.The following methods are used in the diagnosis of pulmonary tuberculosis except:
  - a. Examination of ZN stained direct sputum smear
  - b. PCR of sputum
  - c. Culture on selective medium
  - d. INVC Tests
  - e. Detection of specific antibodies