



# Mycobacteria & Tuberculosis

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COLLEGE OF MEDICINE**

# Objectives

- Recognize that tuberculosis as a chronic disease mainly affecting the respiratory system.
- Know the epidemiology of tuberculosis world wide and in the kingdom of Saudi Arabia.
- Understand the methods of transmission of tuberculosis and people at risk.

# Objectives

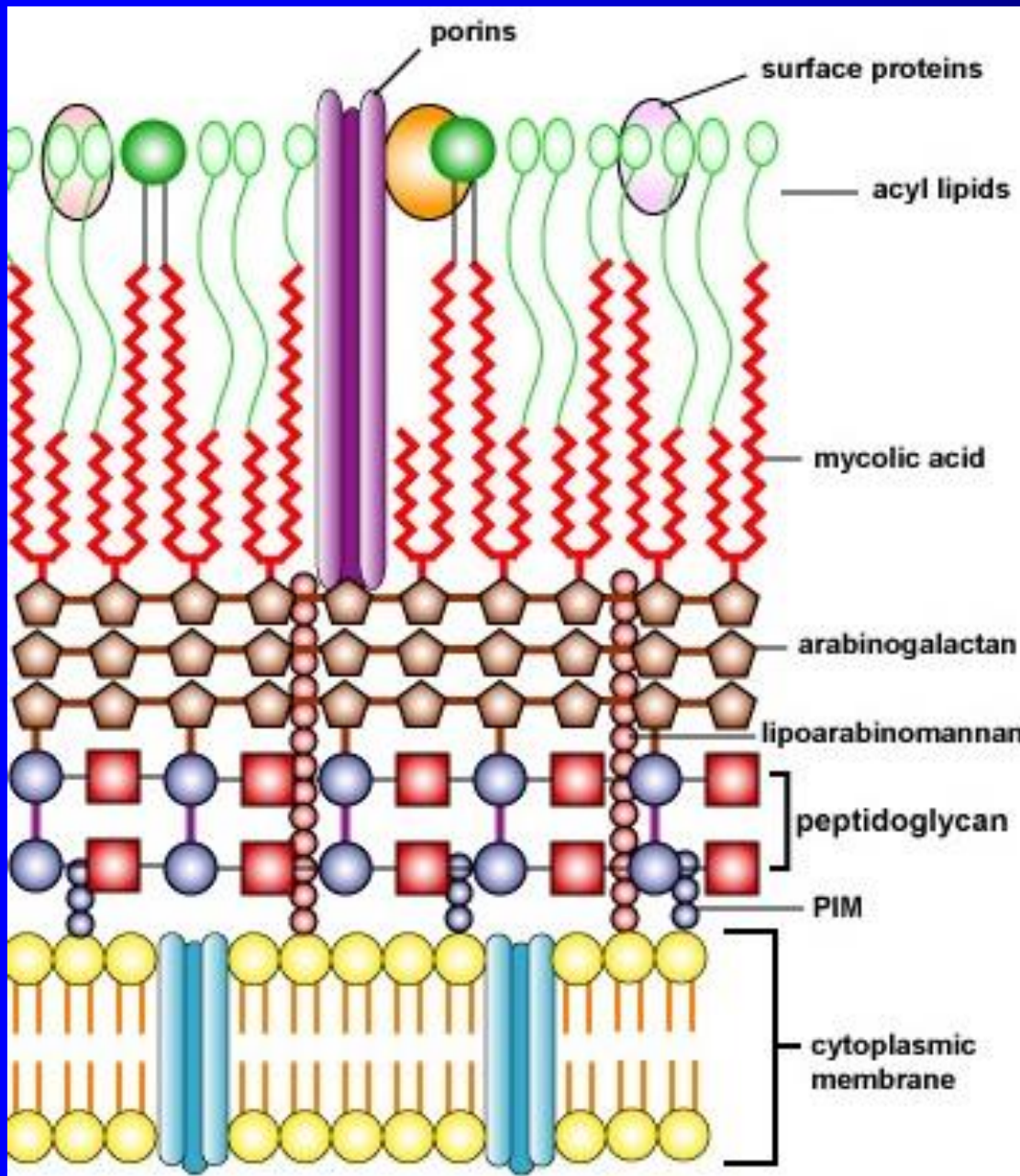
- Know the causative agents , their characteristic and staining and culturing methods .
- Understand the pathogenesis of tuberculosis.
- Differentiate between primary and secondary tuberculosis and the clinical features of each.

# Objectives

- Understand the method of tuberculin skin test and result interpretation .
- Know the laboratory diagnostic methods.
- Know the chemotherapeutic agents and other methods of management .
- Describe the methods of prevention and control of tuberculosis

# Characteristics of the Genus Mycobacteria

- Non-spore forming, slim, non-motile bacilli.
- Do not stain by Gram stain .
- Cell wall contains high lipid concentration (**Mycolic acid** ) which resists staining by Gram stain .
- **Acid- Alcohol Fast Bacilli (AFB)** : resist decolorization with up to 3% HCL, 5% ethanol or both.



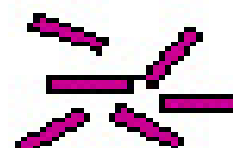
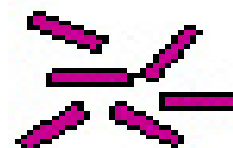
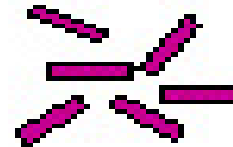
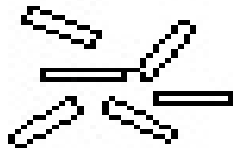
Glycolipid  
 PIM=Phosphatidylinositol  
 mannosides and  
 lipoarabinomannan

# Acid-Fast Bacilli (AFB)

- Stains used : **Ziehl-Neelsen** stain (ZN stain) and **Auramine Rhodamine** stain.
- Strict aerobes
- Multiply intracellularly
- Cause delayed hypersensitivity reaction type of immune response
- Slowly growing ( between 2 - 8 wks.)

# Ziehl-Neelson Stain Kinyoun Modification

Acid Fast  
Organisms



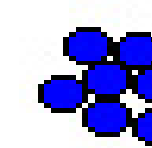
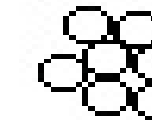
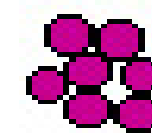
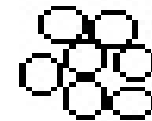
A small amount of organism suspended in saline solution is fixed on a slide.

Slide is flooded with Carbol Fuchsin and phenol for 3 minutes, and gently rinsed with water.

Slide is decolorized with 3% HCl in 70% alcohol until color appears to be removed (approx. 2 mins), and rinsed with water.

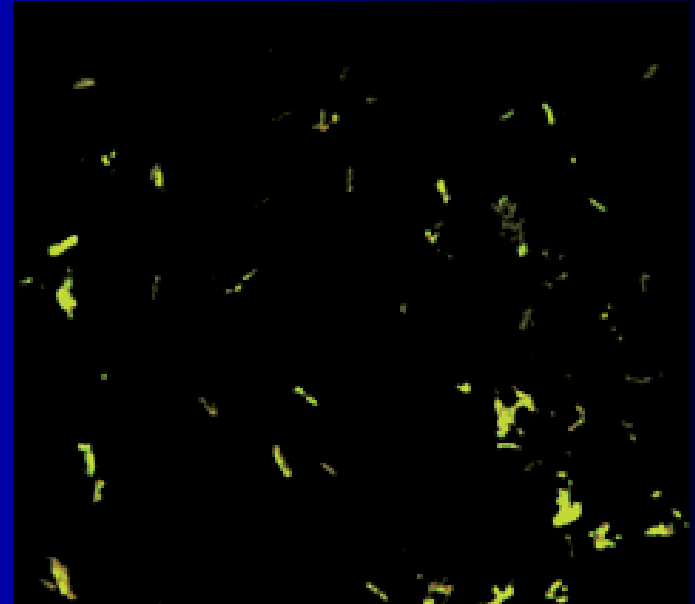
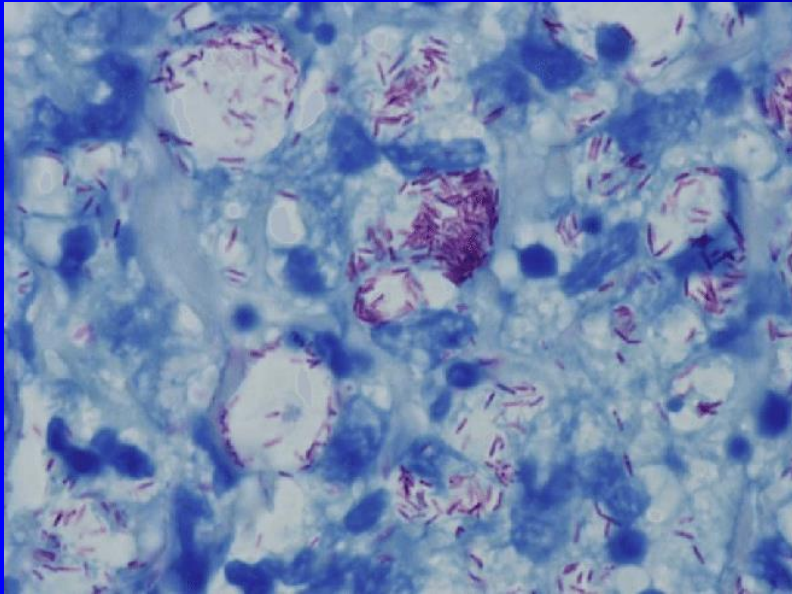
Slide is flooded with methylene blue counterstain for 30 secs, rinsed with water and air-dried.

Not Acid Fast  
Organisms





# ZN-Stain vs Auramine



# Species of Mycobacteria

- *Mycobacterium tuberculosis complex* cause tuberculosis.
- *Mycobacterium leprae* causes leprosy.
- *Atypical Mycobacteria* / Mycobacteria other than tuberculosis ( MOTT) cause infections in immunosuppressed patients.

# *Mycobacterium tuberculosis* *complex*

1- *M.tuberculosis* (Human type)

2- *M. bovis* (Bovine type)

3- *M. Africanum*

4- *BCG strains*

All are called *Mycobacterium tuberculosis*  
*complex* and cause tuberculosis in human

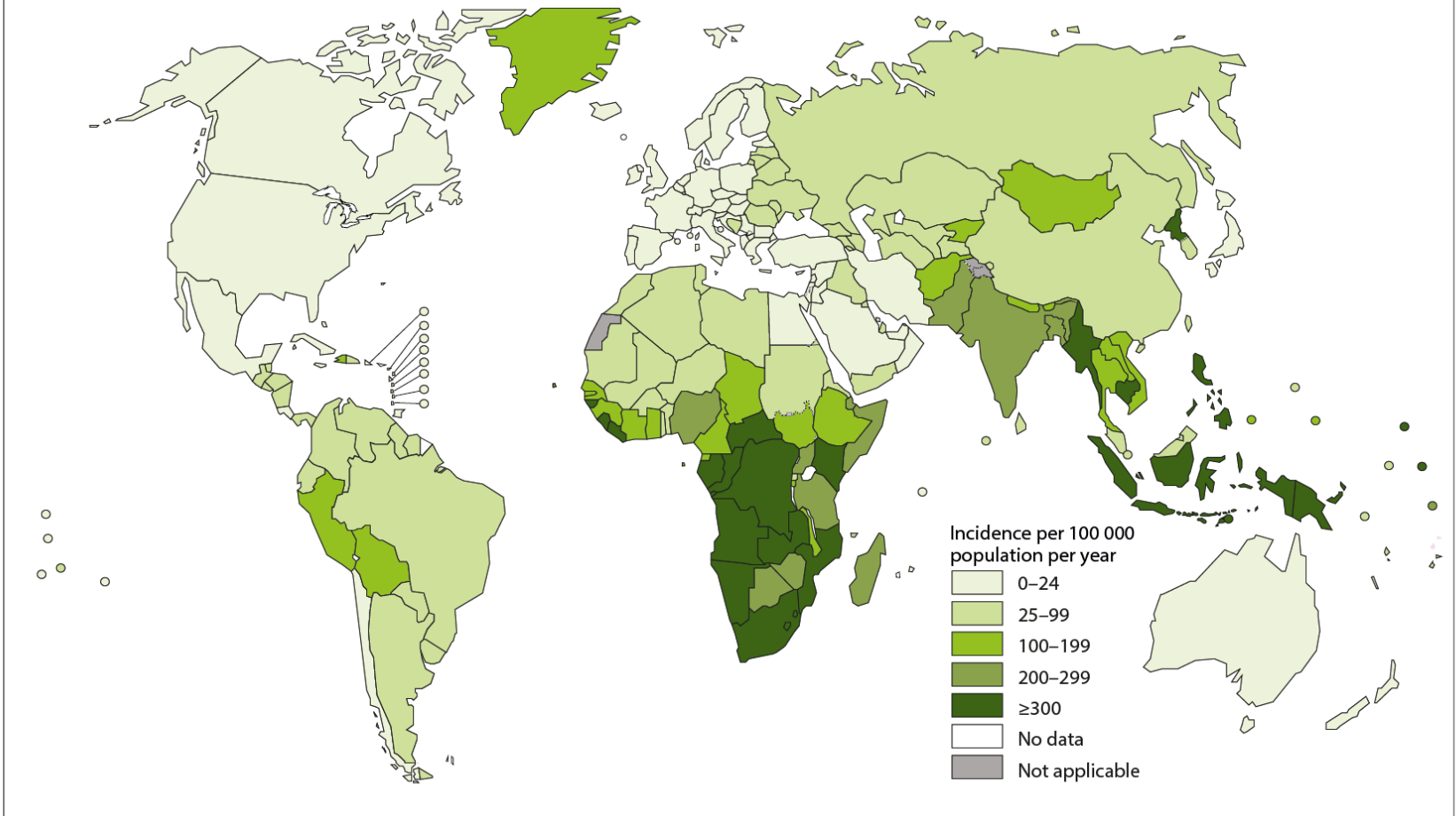
# Tuberculosis

- Tuberculosis (TB) is an ancient chronic disease affects humans , caused by ***Mycobacterium tuberculosis complex***. (Robert Koch 1882) , >15,000 year
- A major cause of death worldwide.
- Usually affects the lungs, other organs can be affected in one third of cases.
- If properly treated is curable, but fatal if untreated in most cases.

# Epidemiology

- TB affects 1/3 of human race as a latent dormant tuberculosis.
- Globally in 2017, there were an estimated 10.0 million incident cases of TB, equivalent to 133 cases per 100 000 population.
- **Incidence:** a world wide disease , more common in developing countries ( India , China , Indonesia , the Philippines , Pakistan , Nigeria , Bangladesh and South Africa ). The incidence among HIV 20 times.
- 1.3 million deaths from TB among HIV-negative people in 2017 and an additional 300 000 deaths from TB among HIV-positive people

## Estimated TB incidence rates, 2017

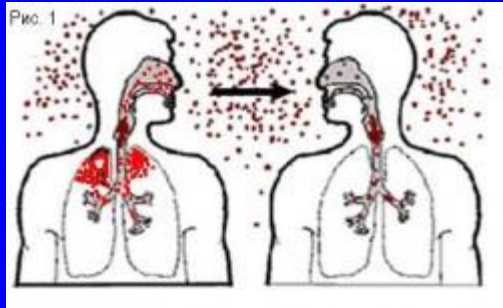


The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: *Global Tuberculosis Report 2018*. WHO, 2018.

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# Epidemiology

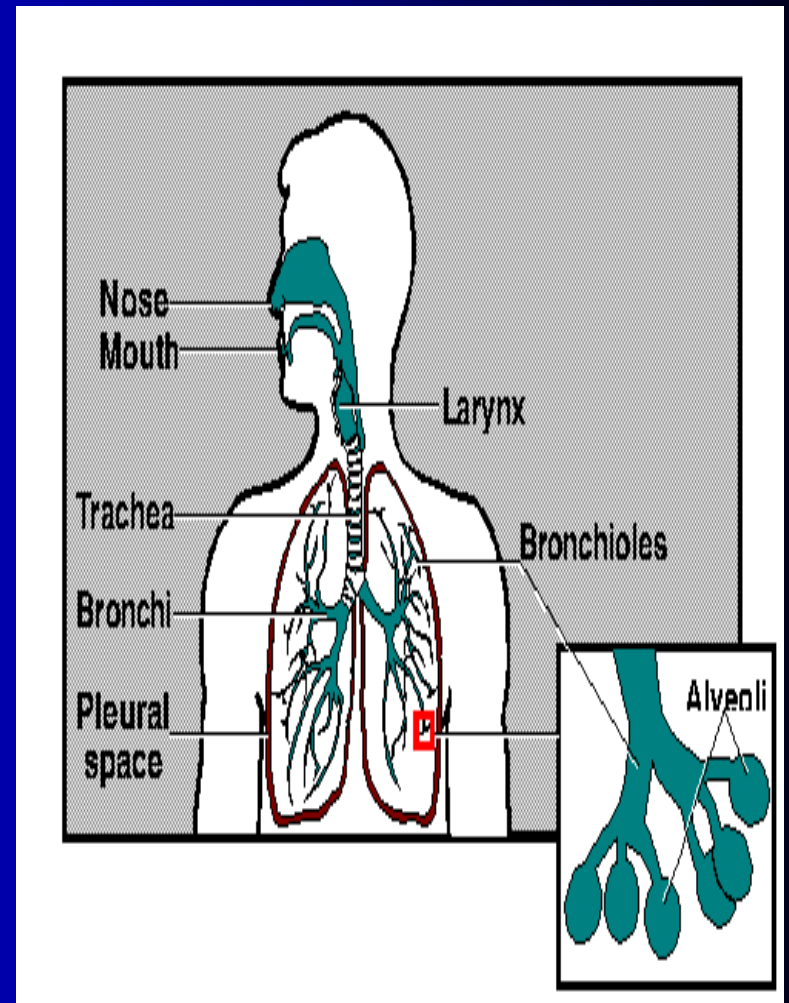
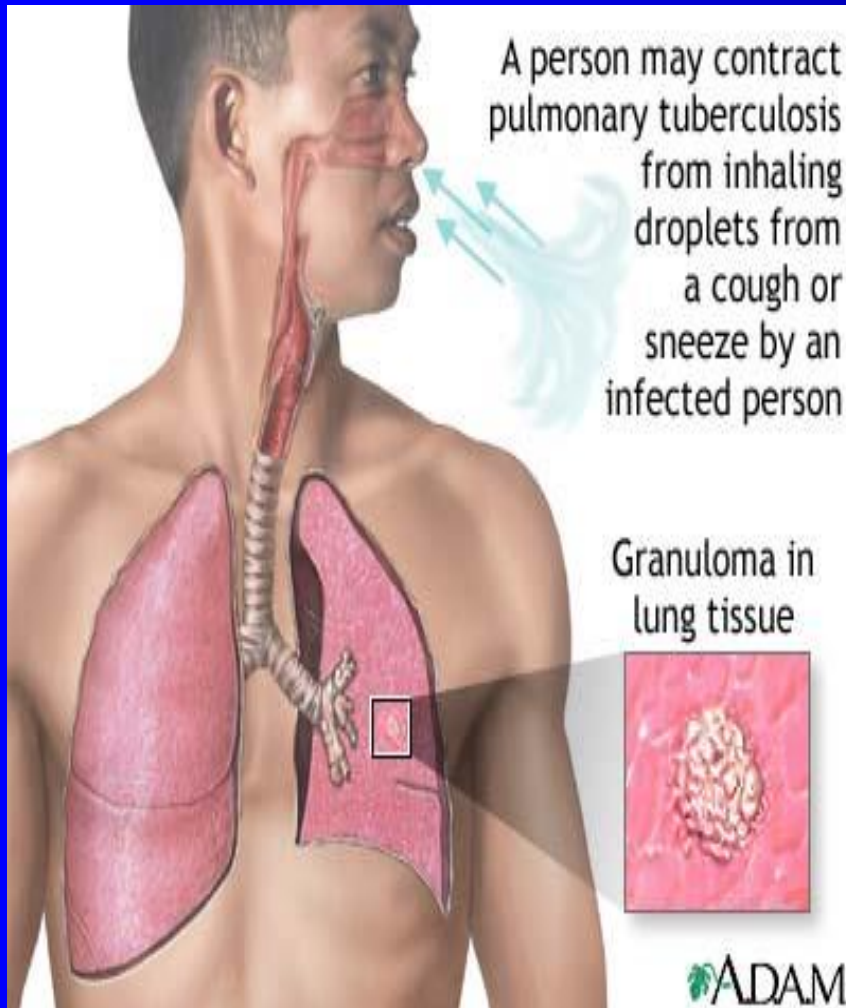
- Transmission mainly through inhalation of airborne droplet nuclei ( $< 5 \mu\text{m}$ ) in pulmonary diseases cases , rarely through GIT & skin
- **Reservoir:** patients with open TB.
- **Age:** young children & adults
- **People at risk :** include ;lab technicians, immunosuppressed patients ,workers in mines and contacts with index case.

# Pathogenesis of Tuberculosis

- Mycobacteria is acquired by airborne droplet which reach the **alveolar macrophages** and are able to survive their (**main virulence factor**).
- Simulates cell mediated immune response which controls the multiplication of the organism but does not kill it.
- Granuloma formed and organism lives in dormant state ( **latent tuberculosis infection**)



# Pathogenesis of TB





# Pathogenesis of Tuberculosis

- Patient show evidence of delayed cell mediated immunity ( **CMI** ).
- Disease results due to destructive effect of **CMI** .
- Clinically the disease is divided into **primary** or **secondary** .

# Pathogenesis of Tuberculosis

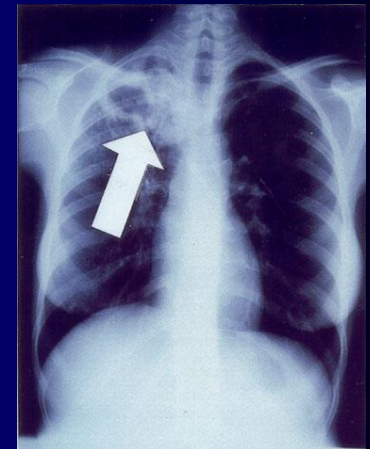
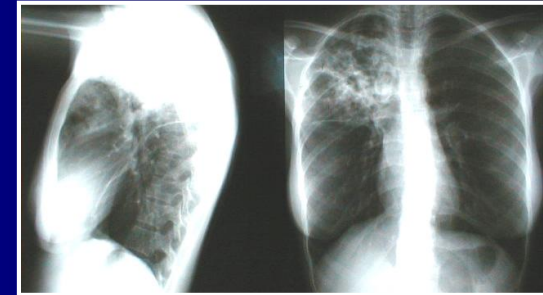
## ➤ Primary Tuberculosis

Occurs in patients not previously infected.

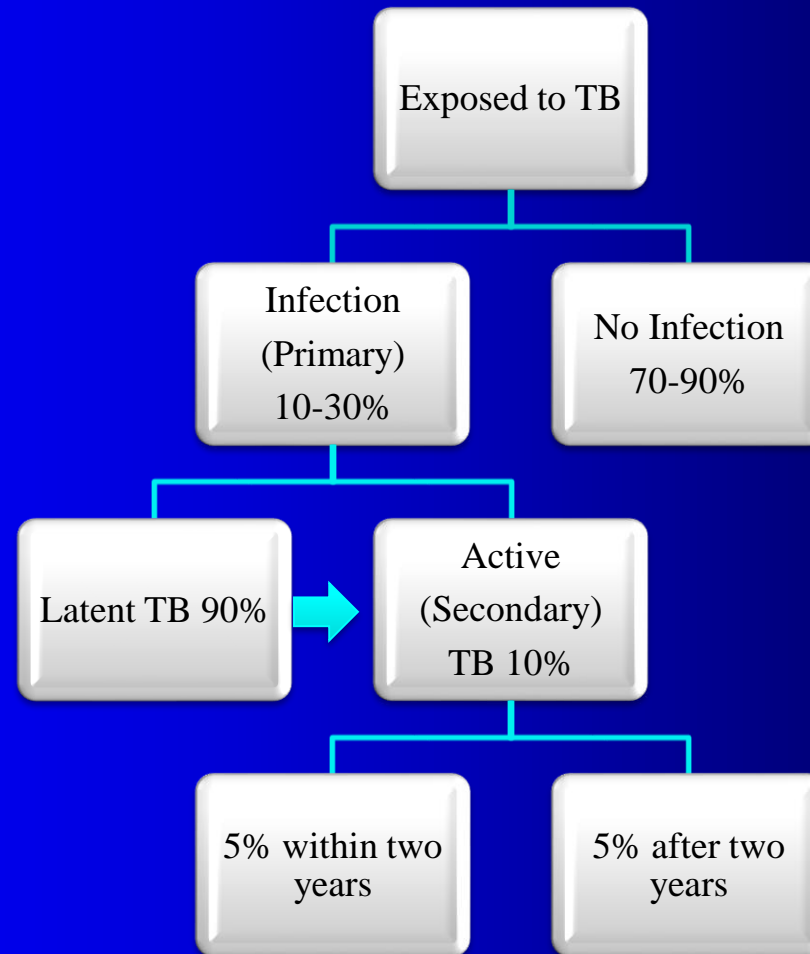
Inhalation of bacilli  Phagocytosis   
lymph nodes calcify to produce **GHON Focus**  
(or **Primary Complex**) at the periphery of mid  
zone of lung.

# Chest radiography

- No chest X-ray pattern is absolutely typical of TB
- 10-15% of culture-positive TB patients not diagnosed by X-ray
- 40% of patients diagnosed as having TB on the basis of x-ray alone do not have active TB
- **Classical radiograph appearance**
  - Infiltration
  - Cavitation
  - Fibrosis with traction
  - Enlargement of hilar and mediastinal lymph node
  - Pleural thickening
- **In reactivation TB**
  - Classically fibrocavitary apical disease
- **Primary TB**
  - Middle or lower lobe consolidation

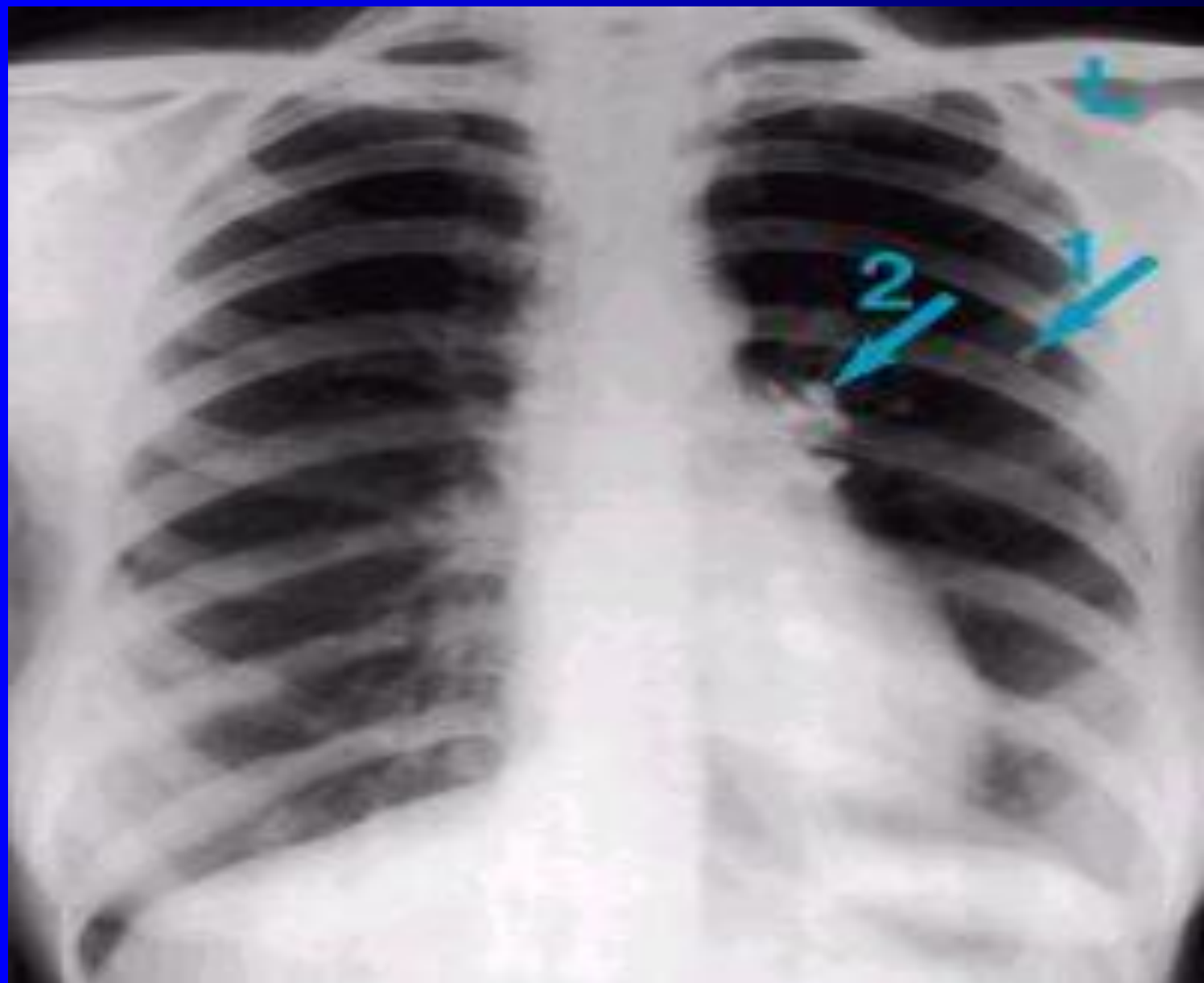


# Natural History of TB Infection




# Primary Tuberculosis

- Microscopy of lesion shows **Granuloma**.
- **Clinically**: primary TB usually asymptomatic or / minor illness.
- **Non-pulmonary TB**: may spread from pulmonary infections to other organs eg.:
  - TB of lymph nodes (cervical, mesenteric).
  - TB meningitis
  - TB bone & joint



# Primary Tuberculosis

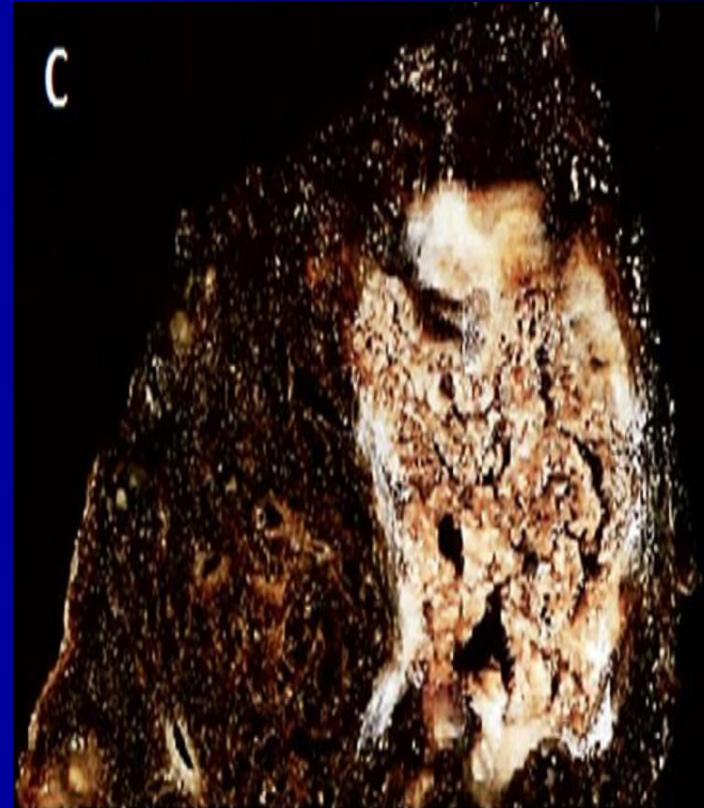
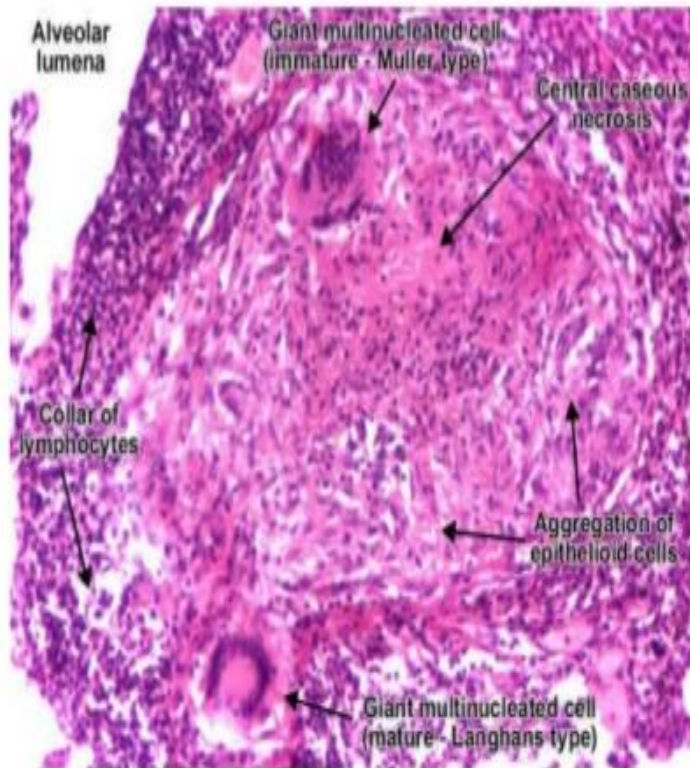
- Genitourinary TB
- Miliary TB  (blood)
- Soft tissue (**cold abscess**): lack of inflammation with caseation.

**Caseation:** due to delayed hypersensitivity reaction. Contains many bacilli, enzymes, O<sub>2</sub>, N<sub>2</sub> intermediates,  necrotic center of granuloma (cheezy material).




# CASEATION

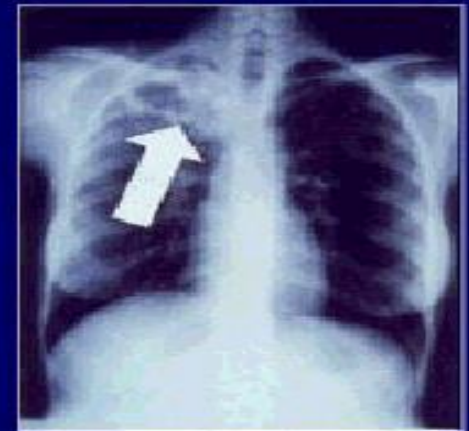
## HISTOLOGY



Caseous necrosis in lung tissue

# Secondary TB (reactivation)

- Occurs later in life
- Lung more common site
- Immunocompromised patients.
- Lesion localized in **apices**
- **Infectious & symptomatic**
- **Microscopy: many bacilli, large area of caseous necrosis**  **cavity (open TB) with granuloma and caseation.**



Arrow points to cavity in patient's right upper lobe.

CDC

# Secondary TB

- Clinically: fever, cough, hemoptysis ,weight loss & weakness.

## Sources of secondary TB :

- **Endogenous** (reactivation of an old TB) or
- **Exogenous** (re-infection with new strain)

# Immunity to Tuberculosis

- **CMI** associated with delayed hypersensitivity reaction.
- Detected by **tuberculin skin test**.
- Tuberculin test takes 2-10 weeks to react to tuberculin and becomes positive.

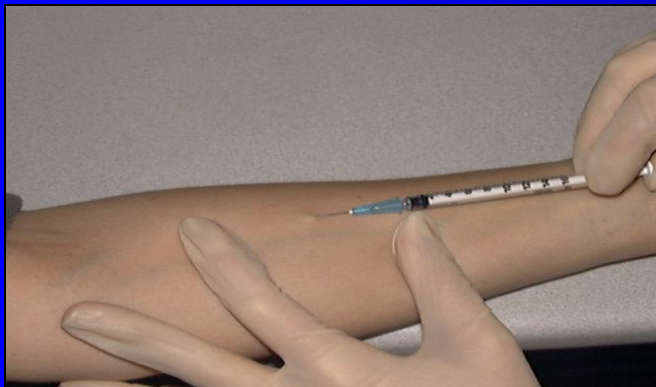
# Tuberculin Test

- Uses purified protein derivative (**PPD**).
- Activity expressed by Tuberculin unit .
- **Activates synthesized lymphocytes to produce CMI which appear as skin induration.**
- May not distinguish between active and past infection except in an individual with recent contact with infected case.
- **Low level activity induced by environmental mycobacteria.**

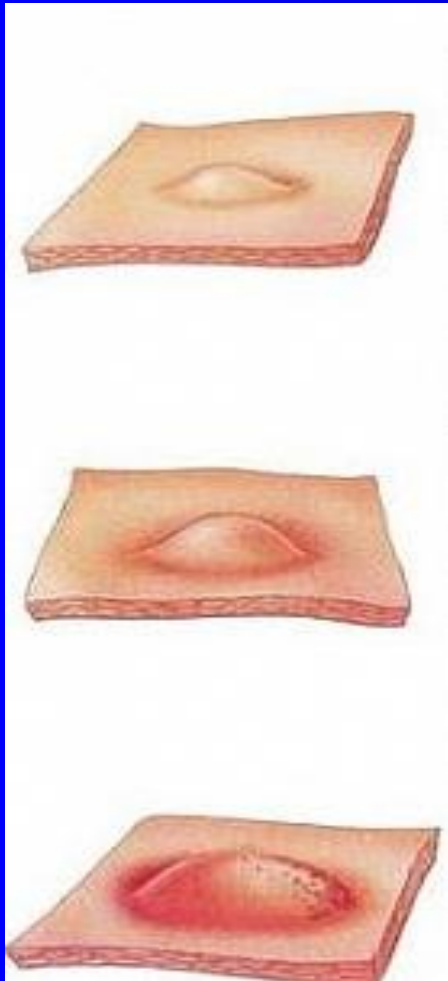
# Method of Tuberculin Skin Test

## *Mantoux test:*

- Intradermal inoculation of 0.1 ml of PPD (purified protein derivative), 5 tuberculin units.
- Read after 48-72hrs.



# Positive Tuberculin Skin Test



>5mm induration :

1. Recent contact with active TB.
2. HIV or high risk for HIV
3. Chest X-ray consistent with healed TB.
4. Organ transplant

> 10mm induration:

1. IV drugs user, HIV seronegative patient.
2. Medical conditions eg. *diabetes* , *malignancy*.
3. Residents & employee at high risk
4. Patients from country with high incidence.
5. Children < 4yrs or exposed to adult high risk group.
6. Mycobacteriology lab. personnel.

>15 mm induration:

1. Considered positive in any persons including those with no risk factors for TB.

# Negative Tuberculin Skin Test

- No induration , either due to:
  - No previous infection
  - Pre-hypersensitivity stage
  - Lost TB sensitivity with loss of antigen.
- AIDS patients are anergic and susceptible to the infection.



# Laboratory Diagnosis of TB specimens:

- Pulmonary TB: **Three** early morning sputum samples ,or bronchial lavage, or gastric washing (infants) .
- Cerebrospinal fluid ( CSF) ( TB meningitis)
- **Three** early morning urine
- Bone , joint aspirate
- Lymph nodes, pus or tissues **NOT** swab.

Repeat sample as required.



# Laboratory Diagnosis of TB

**Direct microscopy of specimen :**

**Z-N** or (Auramine ) stain.

**Culture** is the **gold standard** . Important for identification and sensitivity.

- **Media used:** Lowenstein-Jensen media (L J).

Contains: eggs, asparagine, glycerol, pyruvate / malachite green.

# Laboratory Diagnosis of TB

- Colonies appear in LJ media after 3-6 weeks as eugenic, raised, buff, adherent growth enhanced by glycerol (*MTB*) or by pyruvate (*M.bovis*).
- Other media and methods **plus** LJ media used ,eg:
  - Automated methods : using Bactec MGIT (Mycobacterium Growth Indicator Test).
  - Liquid media
  - Faster growth of mycobacterium 4-14 days
- **Molecular methods** : eg. **ProbTech** detects nucleic acid directly from respiratory samples.
  - **Xpert MTB/RIF** detects nucleic acid and resistance to rifampicin
- **Measurement of interferon -gamma ( IF- $\gamma$  )** :
  - Positive in latent TB. More specific than TST



# Mycobacterium

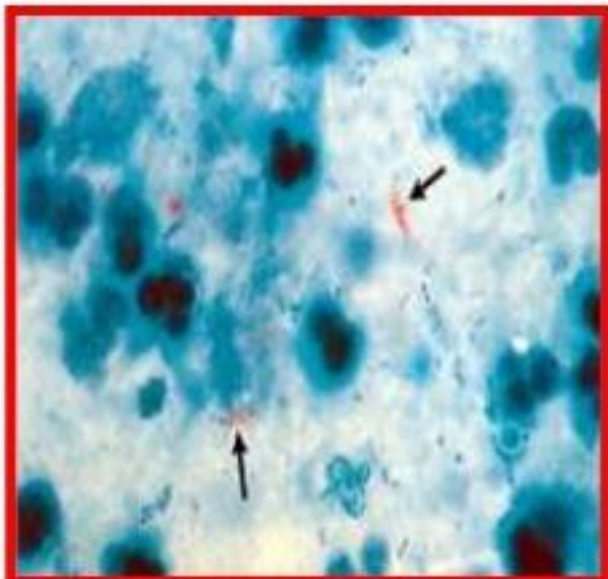


Fig. 5.3: **Myc. TB in Sputum, Z.N stain**

(few thin pink bacilli with blue background)



Fig. 5.1: **Selective media**

for Myc. T.B.



Fig. 5.2: **Culture of Myc. TB on L.J. media**

- Grow after 6-8 week

# Growth of MTB on LJ media



**Fig: Cultural Characteristics of *Mycobacterium tuberculosis***

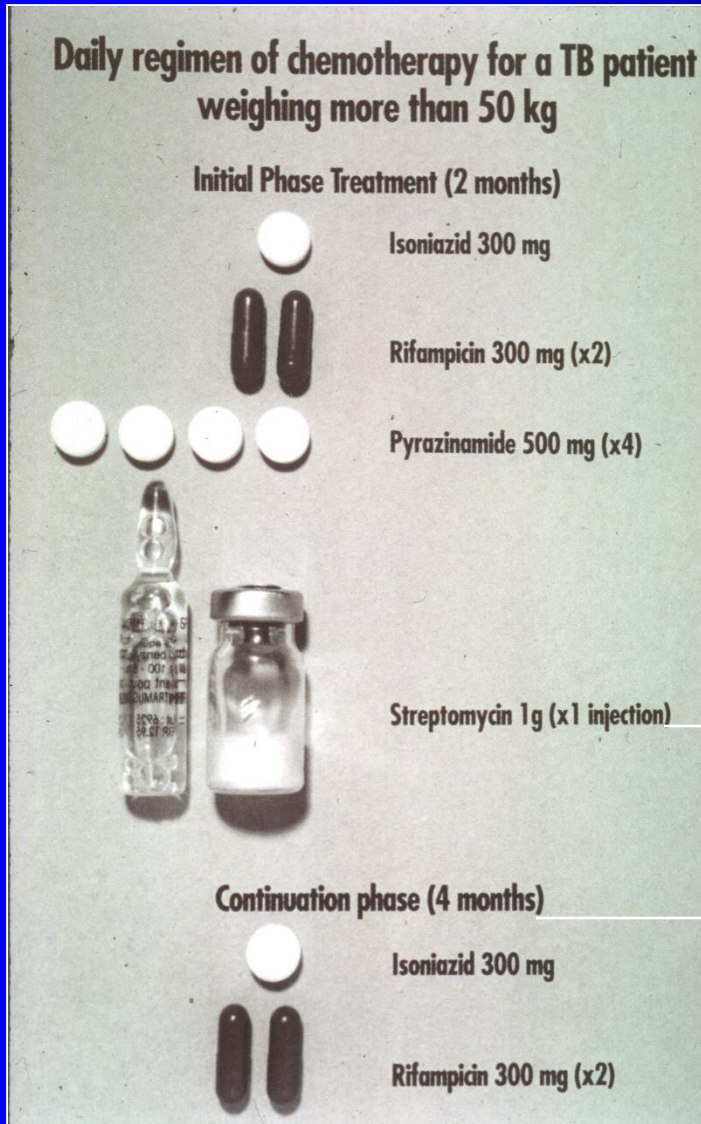
# Identification & Susceptibility Testing

- Morphological characteristics , growth at 37°C and 5 -10 % CO<sub>2</sub>
- **Biochemical tests** : Niacin production & Nitrate test.
- **Susceptibility testing**: to detect resistance to anti-tuberculosis agents.

# Management of a TB case

- **Isolation** of the patient for 10-14 days (smear positive cases contain  $> 1000$  organisms / ml of sputum and considered infectious).
- **Triple regimen of therapy** .Why ?
  - To prevent resistant mutants
  - To prevent relapse
- Treatment must be guided by sensitivity testing.

# Standard Treatment of TB(1940-1960)



2monthsHRZEor S  
+  
4months H<sub>2</sub>R<sub>2</sub>

Or

Ethambutal  
As effective  
twice or trice  
weekly

➤ Directly Observed Therapy (DOT).



# Second Line

Used for the bacteria resistant to first line drugs (MDR, XDR).

More toxic than the first line drugs.

- PAS ( Para-Amino Salicylic acid)
- Ethionamide
- Cycloserine,
- Kanamycin,
- Fluroquiolones

# Prevention of TB

- Tuberculin testing of herds.
- Slaughter of infected animals.
- Pasteurization of milk to prevent bovine TB
- Recognition of new cases.
- Prophylaxis with INH of contacts.
- Follow up cases .
- Immunization with Bacillus Calmette Guerin(**BCG**) ( live attenuated ) to all new borne.



# Reference book

Ryan, Kenneth J.. Sherris Medical  
Microbiology, Latest Edition.  
McGraw-Hill Education.