



Mycobacteria & Tuberculosis

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Objectives

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

Objectives

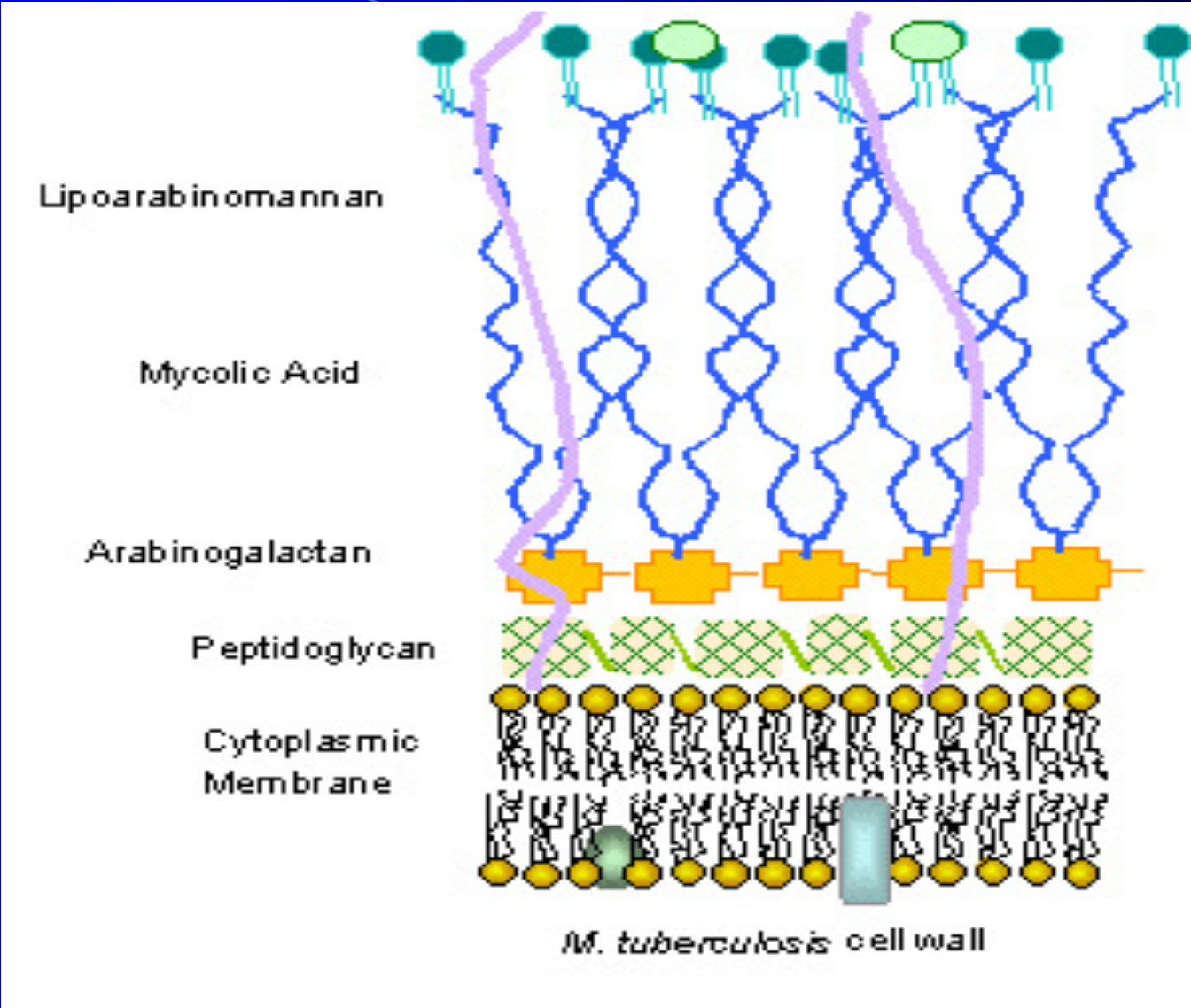
- Recall the causative agents , their characteristic and staining methods .
- Describe the pathogenesis of tuberculosis.
- Differentiate between primary and secondary tuberculosis and the clinical features of each.

Objectives

- Recall the laboratory diagnostic methods.
- Recall the chemotherapeutic agents and other methods of management .
- Describe the methods of prevention and control of tuberculosis

Characteristics of the Genus Mycobacteria

- Slim, rod shaped, non-motile, do not form spores.
- Do not stain by Gram stain because cell wall contains high lipid concentration (**Mycolic acid**) which resist staining by Gram stain .
- **Acid- Alcohol Fast Bacilli (AFB)** resist decolorization with up to 3% HCL, 5% ethanol or both.



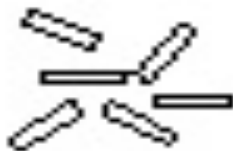
Acid-Fast Bacilli (AFB)

- Stains : **Ziehl-Neelsen (ZN)** and **Auramine Rhodamine**.
- Strict aerobes, slowly growing (2-8 weeks)
- Intracellular multiplication
- Immune response : delayed hypersensitivity reaction

Ziehl-Neelson Stain Kinyoun Modification

Acid Fast
Organisms

Not 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.



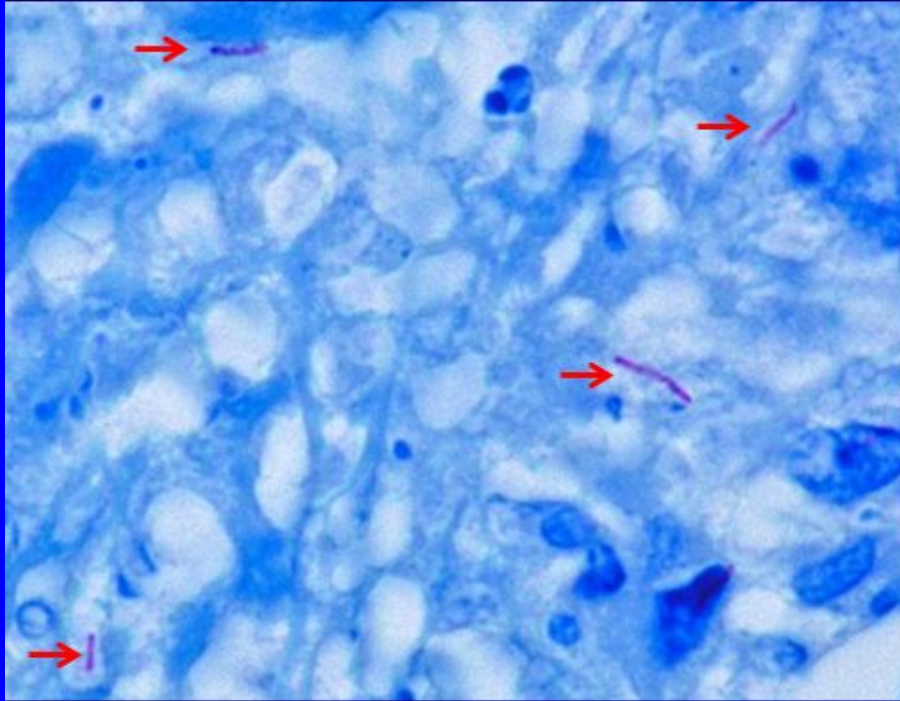
Slide is decolorized with 3% HCl in 70% alcohol until color appears to be removed (approx. 2 mins), and rinsed with water.



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



ZN Stain vs. Auramine



Species of Mycobacteria

Mycobacteria

- *Mycobacterium tuberculosis* complex
- *Mycobacterium leprae*
- Atypical mycobacteria (Mycobacterium other than tuberculosis (MOTT))

Disease

- Tuberculosis
- Leprosy
- Infections in immunocompromised patients

Mycobacterium tuberculosis ***complex Include***

1- *M. tuberculosis* (Human type)

2- *M. bovis* (Bovine type)

3- *M. Africanum*

4- *M. microti*

5- *BCG strains*

Cause tuberculosis in human

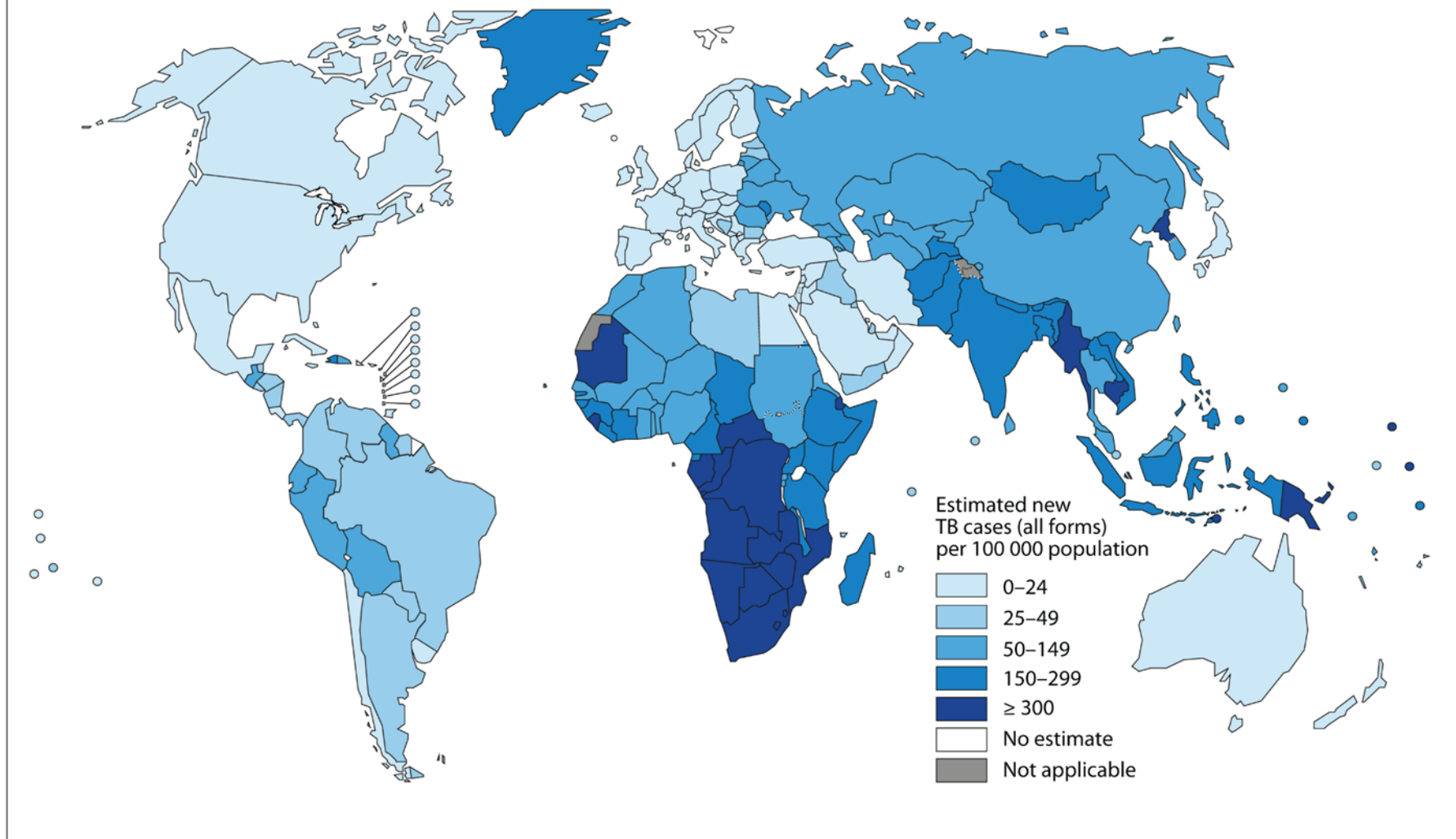
Tuberculosis (TB)

- TB is an ancient chronic disease affects humans , caused by *Mycobacterium tuberculosis complex*.
- 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.
- WHO estimated 8.9 million new cases and 2-4 million death in 2014
- **Incidence:** a worldwide disease , more common in developing countries (see diagram).
- Affects all age groups who are subject to get the infection.

Estimated tuberculosis (TB) incidence rates, 2011



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.

Source: *Global Tuberculosis Report 2012*. WHO, 2012.



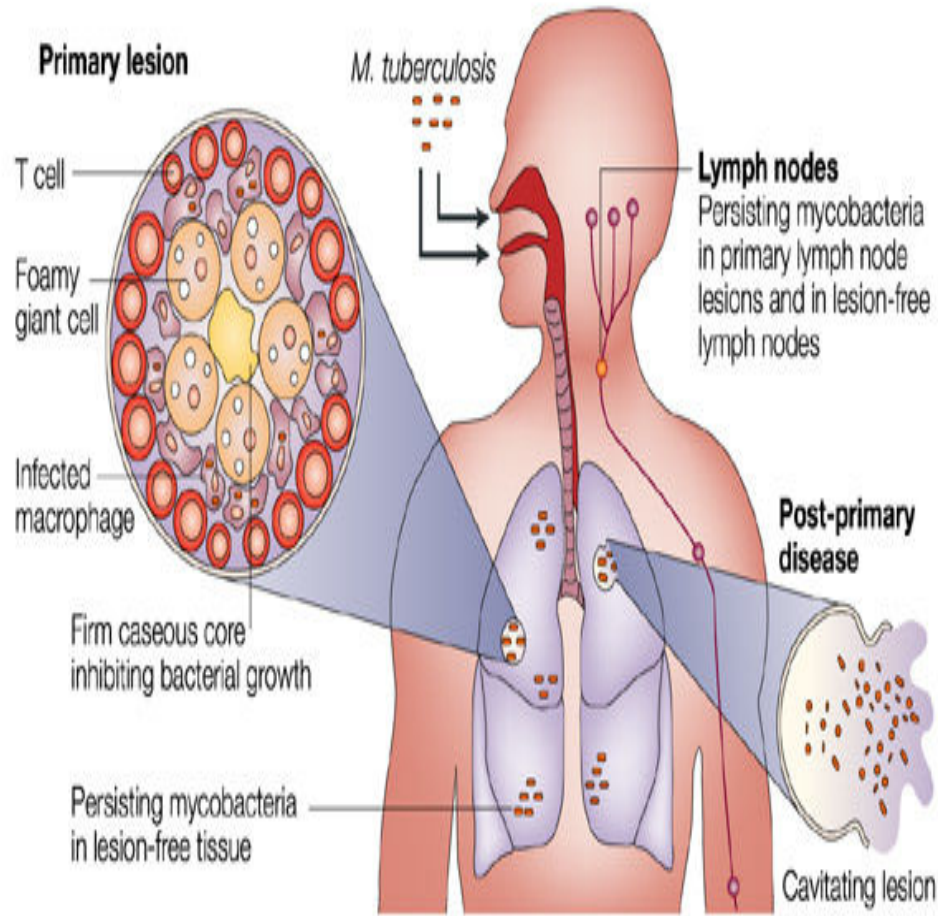


Epidemiology

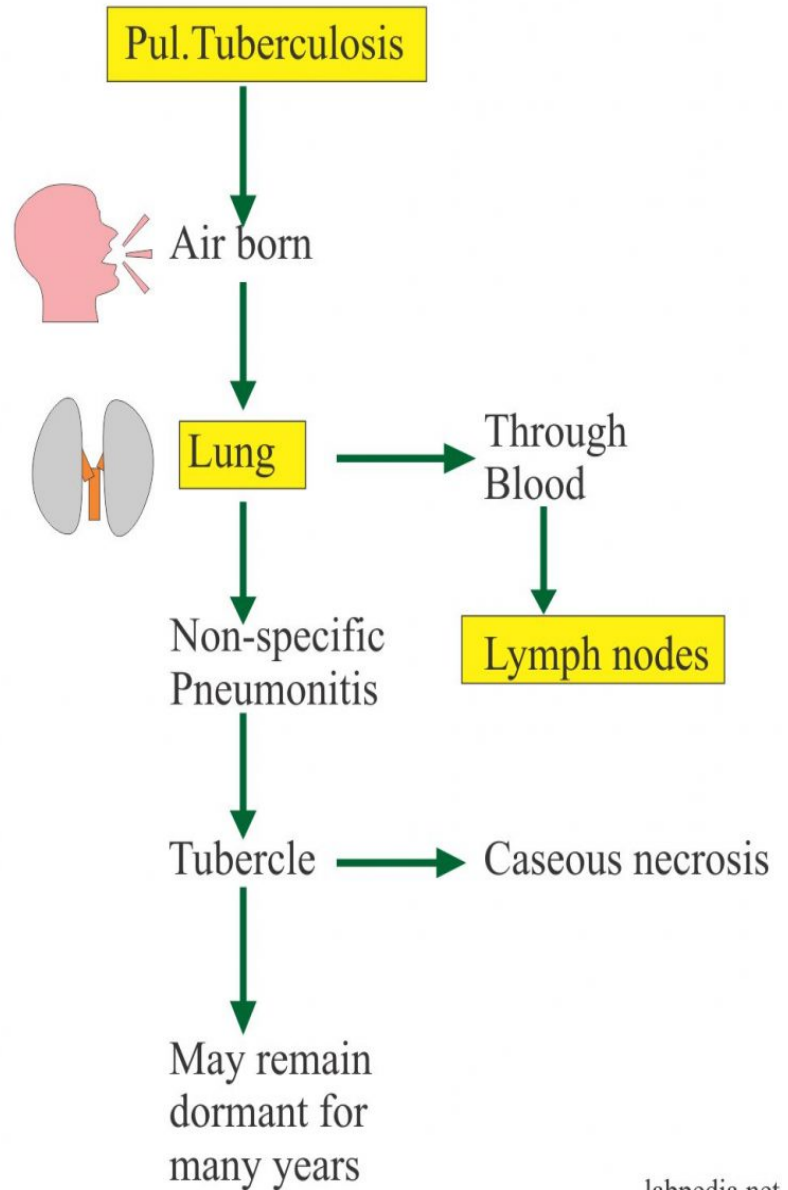
- Transmission :inhalation of airborne droplet nuclei (1-5 μm) for pulmonary disease, rarely through GIT & skin
- **Reservoir**: patients with open TB.
- **Age**: young children & adults
- **People at risk** : include lab technicians, immunosuppressed patients ,workers in mines and contacts with index case.

Pathogenesis of Tuberculosis

- Mycobacteria acquired by airborne droplet which reach and survive in the **alveolar macrophages** .
- Simulates CMI which controls multiplication of the organism but does not kill it.
- Disease due to destructive effect of CMI.
- **Granuloma** formed, organism lives in dormant state (**latent tuberculosis infection**)



Nature Reviews | Microbiology



TUBERCULOSIS

TREATMENT

- Medication
- Vaccination

SYMPTOMS

- Fatigue
- Appetite Loss
- Chest Pain
- Weakness
- Night Sweats
- Fever
- Cough, Coughing up blood

Tuberculosis Transmitted from Person to Person

Latent Infection, Cavitary Tuberculosis, Miliary Tuberculosis

Respiratory Droplets, Aerosols

Tuberculosis Bacteria

Bronchiole, Bronchi, Right Lung, Left Lung, Infection

SIGNS AND SYMPTOMS OF ACTIVE TB

COUGHING THAT LASTS THREE OR MORE WEEKS

CHEST PAIN, OR PAIN WITH BREATHING OR COUGHING

COUGHING UP BLOOD

LOSS OF APPETITE

FEVER

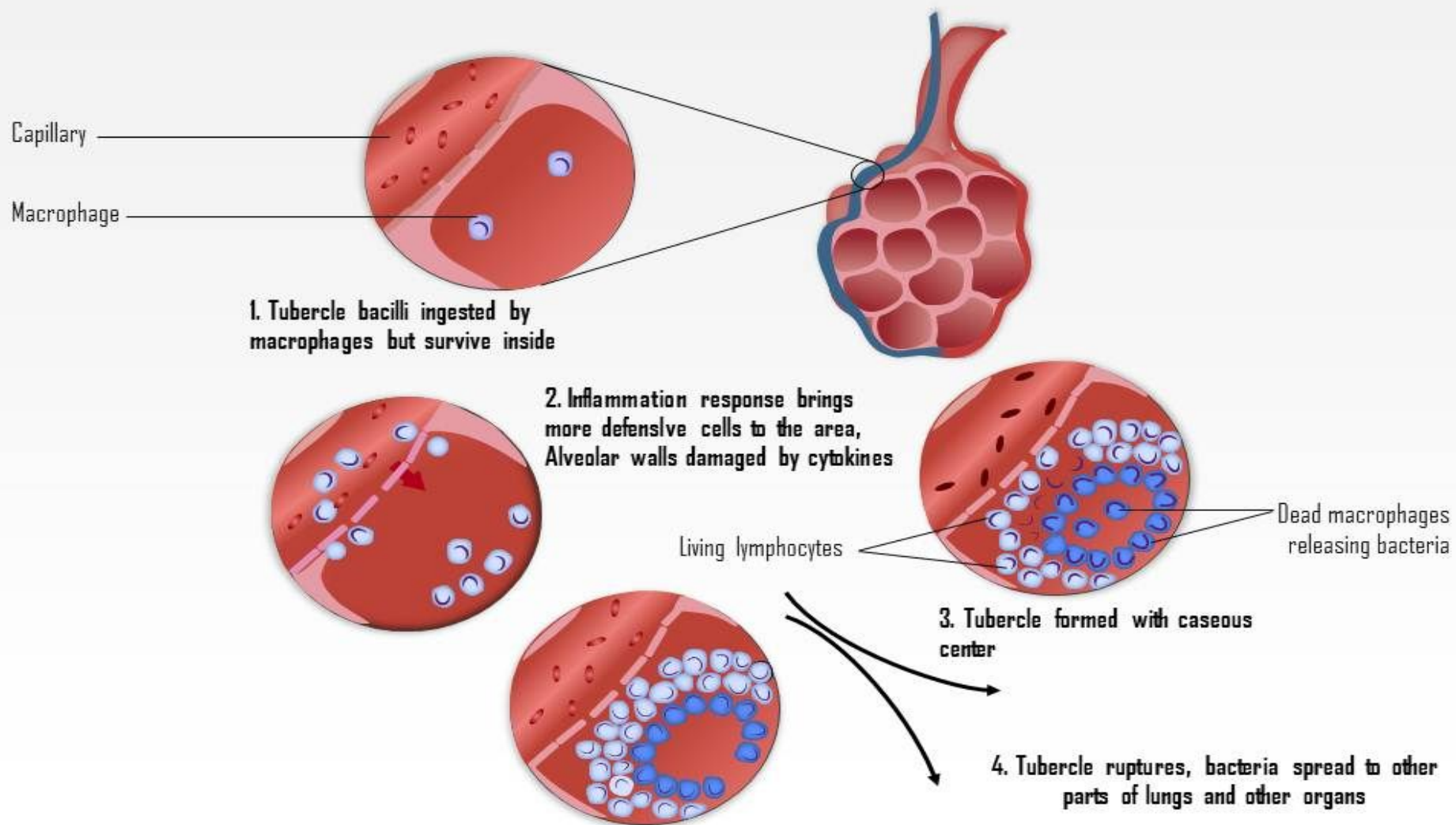
CHILLS

NIGHT SWEATS

UNINTENTIONAL WEIGHT LOSS

FATIGUE

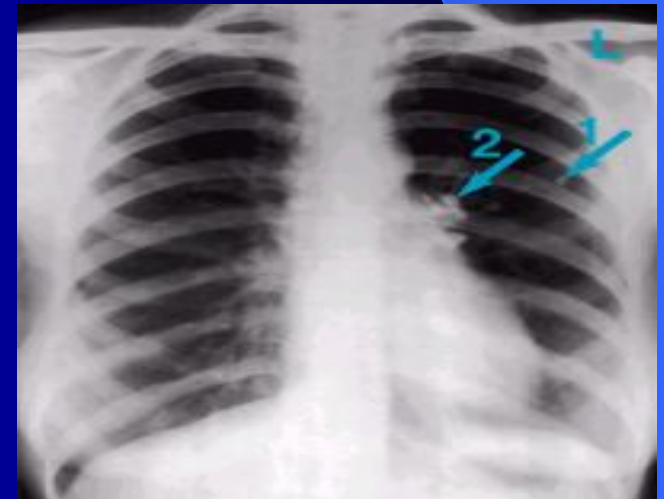
Progression of pulmonary tuberculosis



Clinical Presentation Primary Tuberculosis

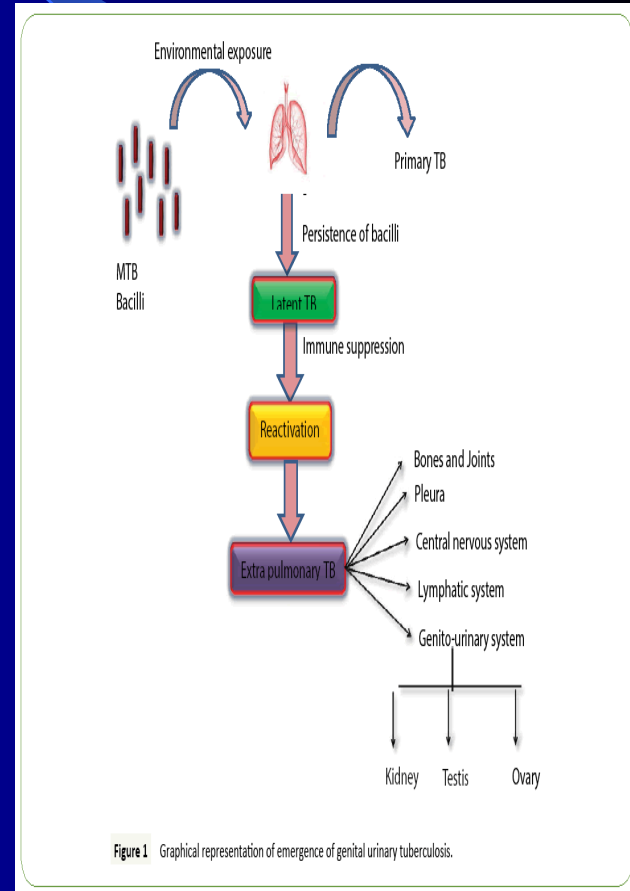
➤ Pulmonary tuberculosis

- . Patient not previously infected.
- . Asymptomatic or mild illness
- . Chest X-ray: **Ghon Focus (Primary Complex)** at the periphery of mid zone of lung.



Primary Tuberculosis

- Granuloma at lesion site
- Extra pulmonary TB:
 - TB meningitis
 - TB lymph nodes (cervical ,mesenteric)
 - TB bone & joint
 - Genitourinary TB
 - Miliary TB (blood)

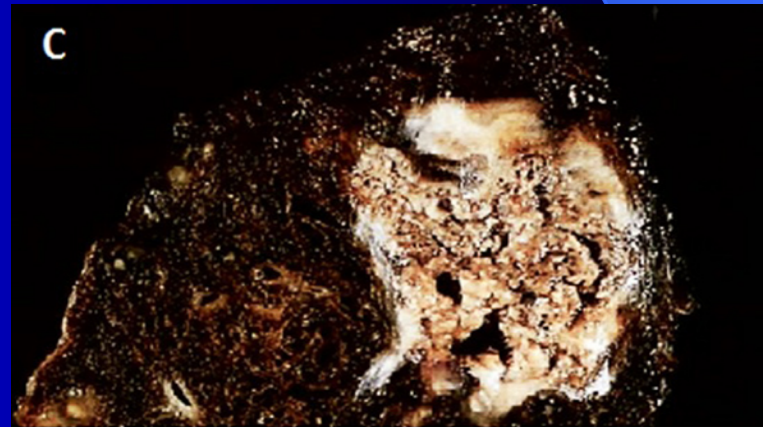
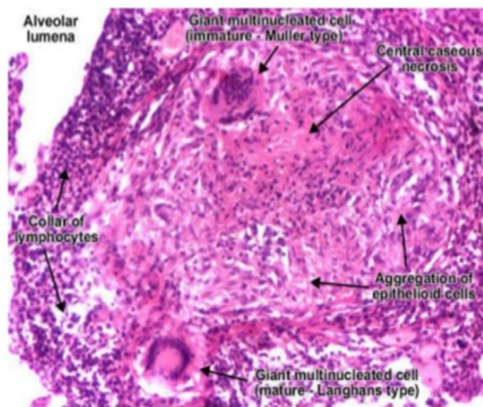


Primary Tuberculosis

- Soft tissue (**cold abscess**) with caseation.

Caseation: due to delayed hypersensitivity reaction. Contains many bacilli, enzymes, O_2 , N_2 intermediates, necrotic center of granuloma (cheesy material).

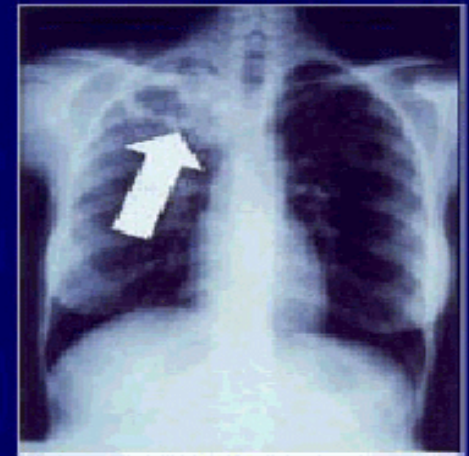
HISTOLOGY



Caseous necrosis in lung tissue

Secondary TB (reactivation)

- Occurs later in life
- Lung more common site
- Immunocompromised patients.
- Lesion localized in **apices**
- **Infectious & symptomatic**
- Many bacilli at lesion, 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.
- *To be discussed further in immunology lecture.*

Laboratory Diagnosis of TB

specimens depend on affected organ:

- **Pulmonary TB:** **Three** sputum samples (at least one early morning), or bronchial lavage, or gastric washing (infants) .
 - **TB meningitis:** cerebrospinal fluid (CSF)
 - **Urinary tract tuberculosis :** **three early morning urine**
 - Bone , joint aspirate
 - Lymph nodes, pus or tissues **NOT** swab.
- Repeat sample as required.

Laboratory Diagnosis of TB

- **Staining & direct microscopy of specimen :**
Z-N or (Auramine) stain.
- **Culture is the gold standard** . Important for identification and sensitivity.
 - **Culture Media :Lowenstein-Jensen media (L J).**
Contains: eggs, asparagine, glycerol, pyruvate / malachite green.
Two LJ media used ,one with glycerol (*MTB*),one with pyruvate (*M. bovis*)

Laboratory Diagnosis of TB

- Colonies appear in LJ media after 2-8 weeks as eugenic, raised, buff, adherent growth .
- Other culture media and methods **plus** LJ media used :
 - Automated methods : Bactec MGIT (Mycobacterium Growth Indicator Test).
 - Molecular methods :
 - ProbTech** detects nucleic acid directly from respiratory samples.
 - Xpert MTB/RIF** detects nucleic acid and resistance to rifampicin

Mycobacterium

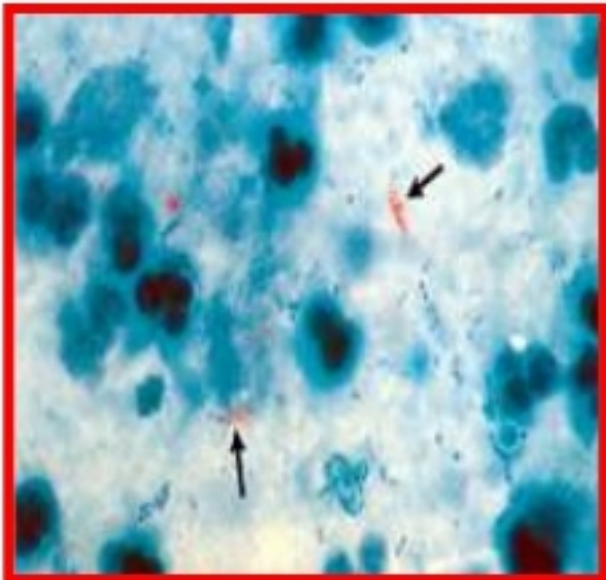


Fig. 5.3: **Myc. TB in Sputum, Z.N stain**
(few thin pink bacilli with blue background)



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

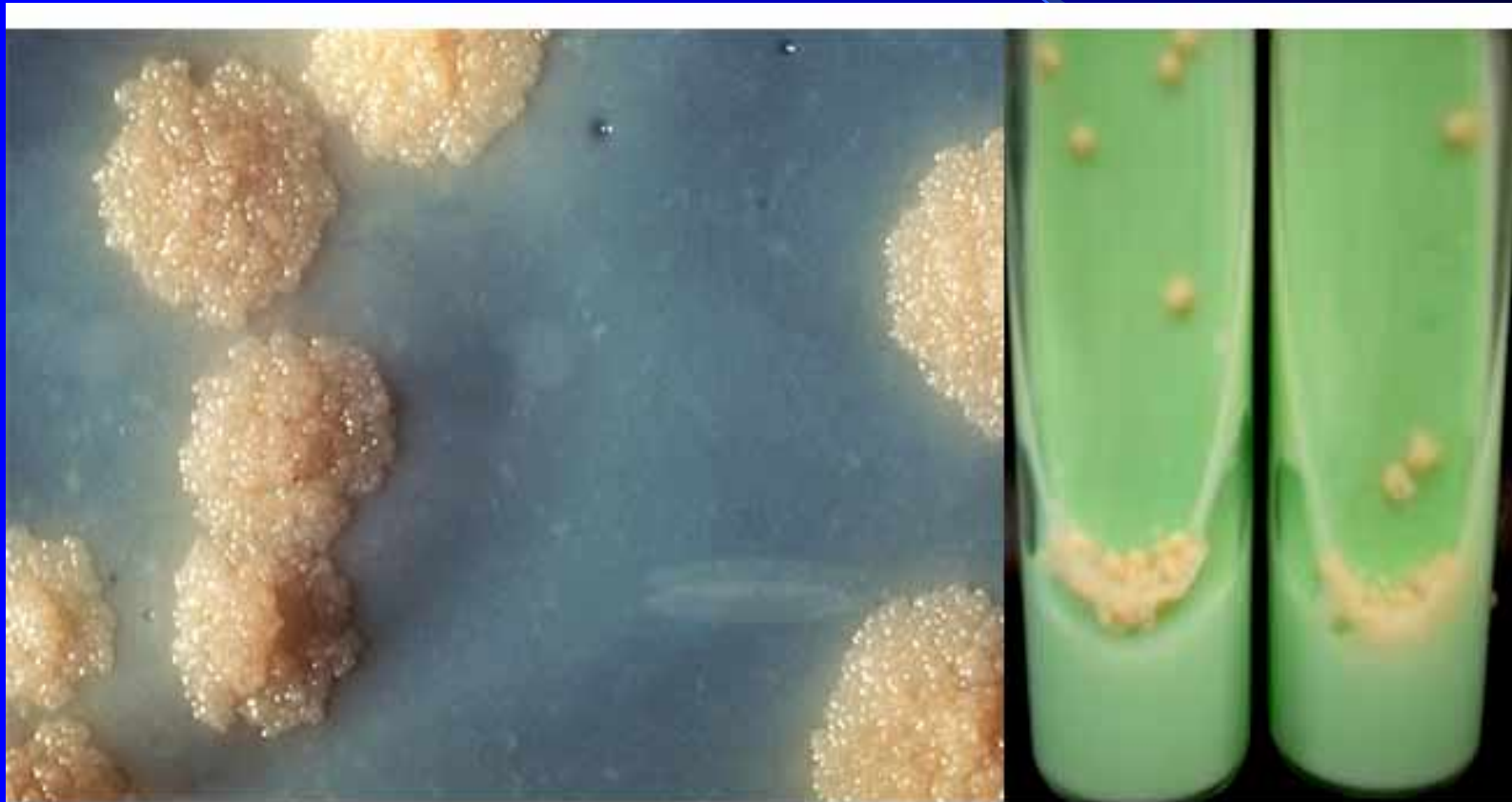


Fig: Cultural Characteristics of *Mycobacterium tuberculosis*

Identification & antimicrobial susceptibility testing

- Morphology, growth at 37C + 5 -10 % CO₂
- Biochemical tests : Niacin production & Nitrate test.
- Antimicrobial susceptibility testing: to detect resistance to anti-tuberculosis agents.

Management of a TB case

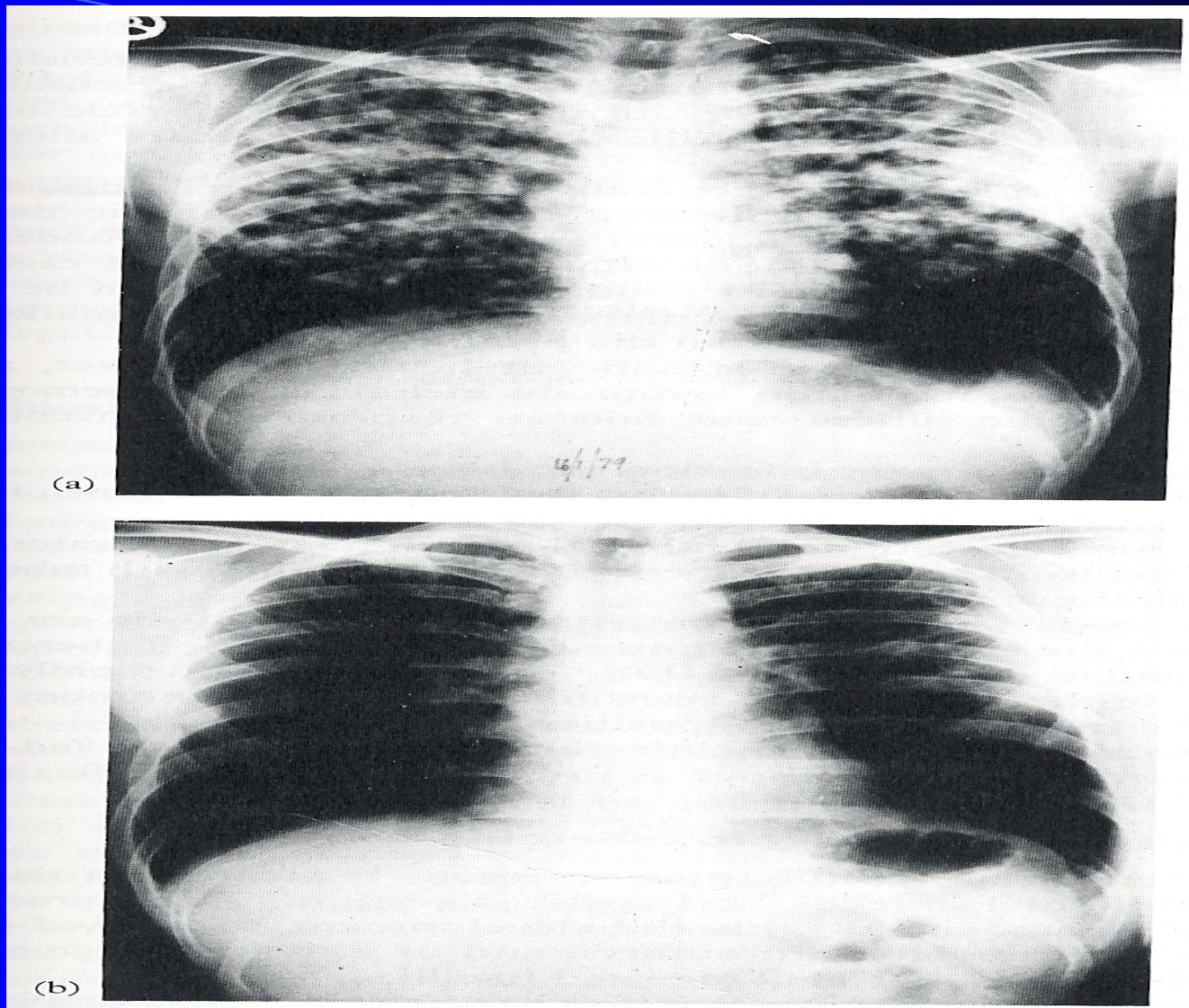
- **Isolation of the patient** for 10-14 days (*smear positive cases contain > 1000 organisms / ml of sputum and considered infectious*).
- *Negative smear does not exclude TB (may be low number of AFB)*
- **Triple regimen of therapy** .Why ?
 - To prevent resistant mutants
 - To prevent relapse
- Treatment must be guided by sensitivity testing.

First Line Treatment

- Isoniazide (INH)
- Rifampicin (RIF)
- Ethambutol (E)
- Pyrazinamide (P)

Combination therapy : All 4 first line or INH+ RIF +P for 2 months then continue with INH+RIF for **4-6 months.**

- Directly Observed Therapy (DOT).



Tuberculosis: (a) Chest X-ray of a patient with tuberculosis bronchopneumonia. (b) Chest X-ray of the same patient 10 months after antituberculous therapy. (Courtesy of Dr. R.S.Kennedy)

Second Line

Used for the bacteria resistant to first line drugs. More toxic than the first line drugs.

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

Diagnosis of Latent TB

- Measurement of interferon -gamma release (IGRA) :
- Tuberculin Skin testing
 - See *immunology lecture*.

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 infected contacts.
- Follow up cases .
- Immunization with **BCG** (live attenuated) after the first year of life.

Reference book

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