



دانشگاه اصفهان

دانشکده علوم و فناوری های زیستی، گروه زیست شناسی سلولی مولکولی و میکروبیولوژی،
آزمایشگاه میکروبیولوژی



آزمایشگاه باکتری شناسی ۱

تست های جداسازی و شناسایی میکوباکتریوم

1

Kingdom:

Bacteria

Phylum:

Actinobacteria

Order:

Actinomycetales

Suborder:

Corynebacterineae

Family:

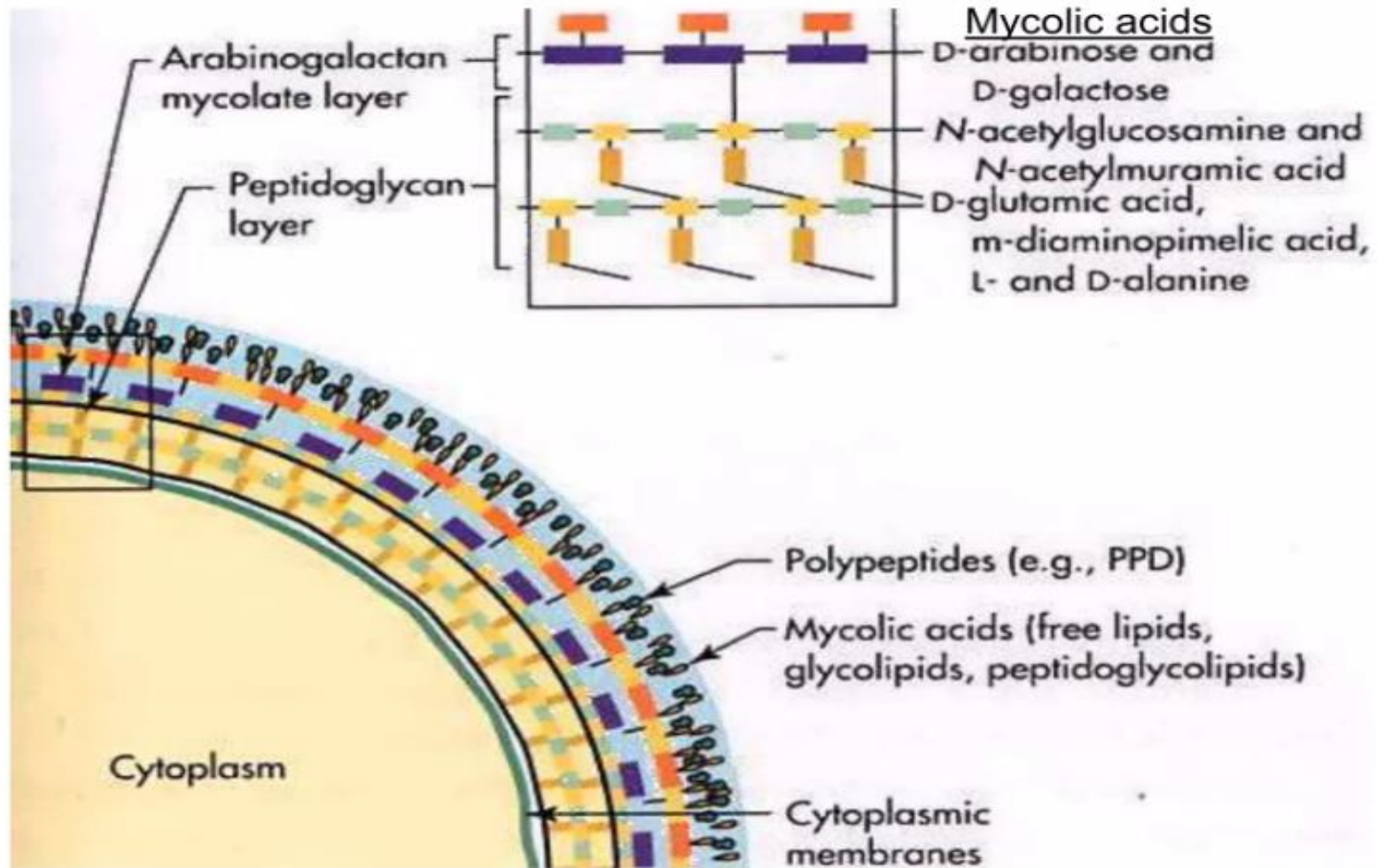
Mycobacteriaceae

Genus:

Mycobacterium

- **Mycobacterium Tuberculosis**
- **Mycobacterium Leprae**
(uncommon)

Lipid Rich Cell Wall



Acid-Fast (Kinyoun) Stain



- Cord growth (Serpentine arrangement) of virulent strains.
- Kinyoun similar to Ziehl-Neelsen Stain

Ziehl-Neelsen Stain Procedure

- 1. Cover with tissue paper or if not then without paper its possible.
- 2. Flood slide with carbolfuchsin, the primary stain, for 3-5 minutes while heating with steam or heating on hot plate.

Continued...

- 3. Remove paper cover, decolorize slide with a mixture of hydrochloric acid and ethanol.
- 4. Counterstain with methylene blue or Malachite green.

Pathogenic Mycobacterium

M. tuberculosis Complex

- M. tuberculosis - Common
 - M. leprae - Uncommon
 - M. africanum
 - M. bovis
 - M. ulcerans
- Rare

All are Strictly Pathogenic

Continued...

Runyon Group I (Slow growing photochromogens)

- *M. kanasii* - Common
 - *M. marinum*
 - *M. simae*
- } Uncommon

All are usually pathogenic **not strictly**

Continued...

Runyon Group II (Slow growing scotochromogens)

- *M. szulgai*
- *M. scrofulaceum*
- *M. xenopi*

Uncommon

Usually pathogenic

Sometimes pathogenic

Continued...

Runyon Group III (Slow growing nonchromogens)

- *M. avium* complex – common
 - *M. genavense*
 - *M. hemophilum*
 - *M. malmoense*
- } uncommon

Strictly pathogenic

Usually pathogenic

Continued...

Runyon Group IV (Rapid growers)

- M. fortuitum
 - M. chelonae
 - M. abscessus
 - M. mucogenicum
- Common
- Uncommon

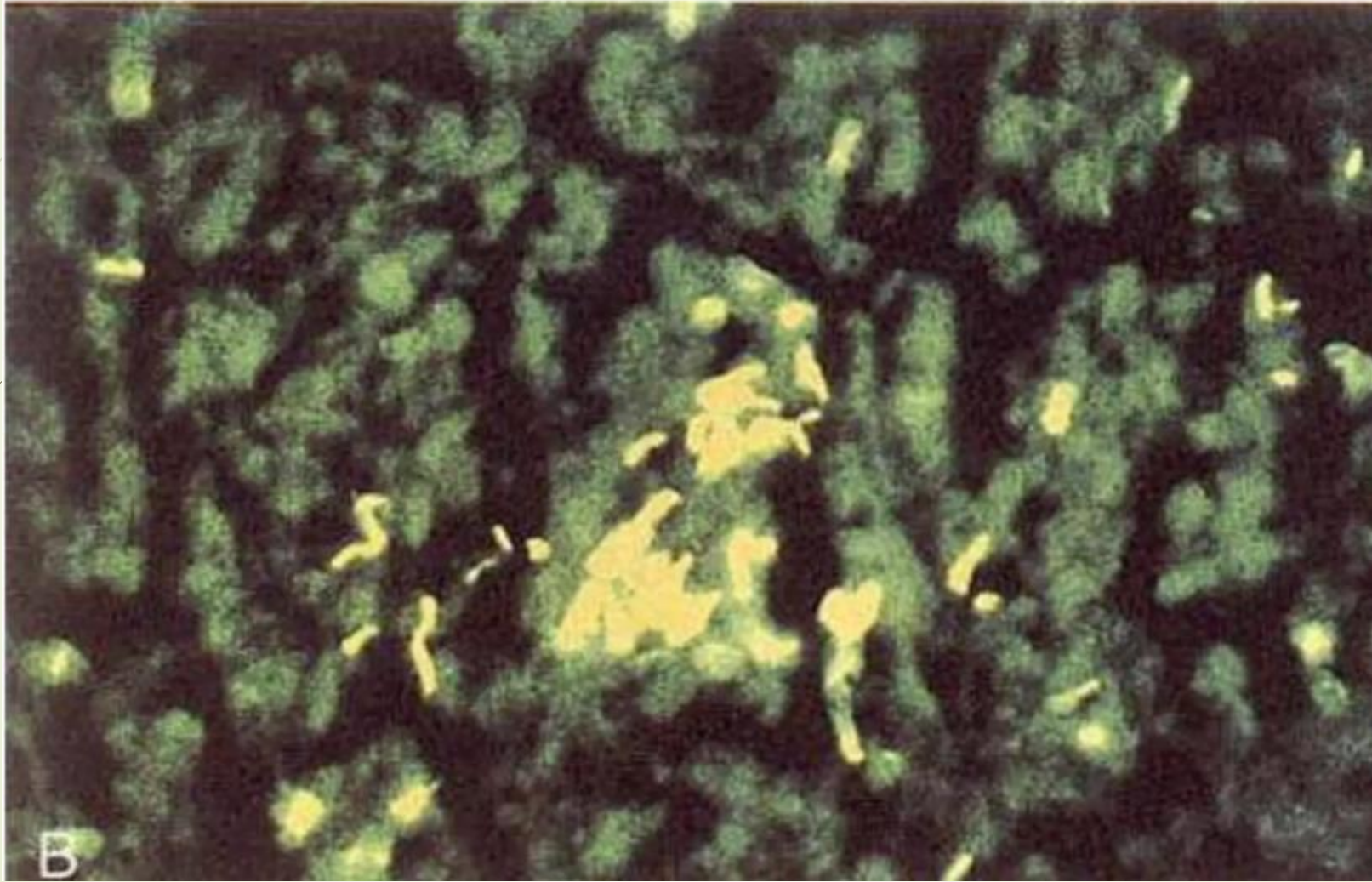
Sometimes pathogenic

MYCOBACTERIUM TUBERCULOSIS

Structure and Physiology

- Weakly gram positive
- Strongly acid fast
- Aerobic bacilli

Stained with Fluorescent Dye



تهیه کننده: سهیلا عباسی

Lipid rich cell wall, makes organism resistant to

- Disinfectants
- Detergents
- Common antibacterial antibiotic

Virulence

- Capable of intracellular growth in unactivated alveolar macrophages
- Disease primarily host response to infection

Epidemiology

- Worldwide , one third of the population is infected
- 16 million existing cases and 8 million new cases
- Most common in Southeast Asia, Sub- Saharan Africa and Eastern Europe

Continued...

Patients at the greatest risk are the

- Immunocompromised patients (HIV)
- Drug and alcohol abusers
- Homeless
- Individuals exposed to infected patients

Humans are only the reservoir

Person to person infection by aerosols

Diseases

- Primary infection is lungs
- Dissemination to any other site occurs mostly in the immunocompromised and untreated persons

Diagnosis

- Positive PPD
- Chest X-Ray
- Microscopy and culture – it is sensitive

Treatment, Prevention and Control

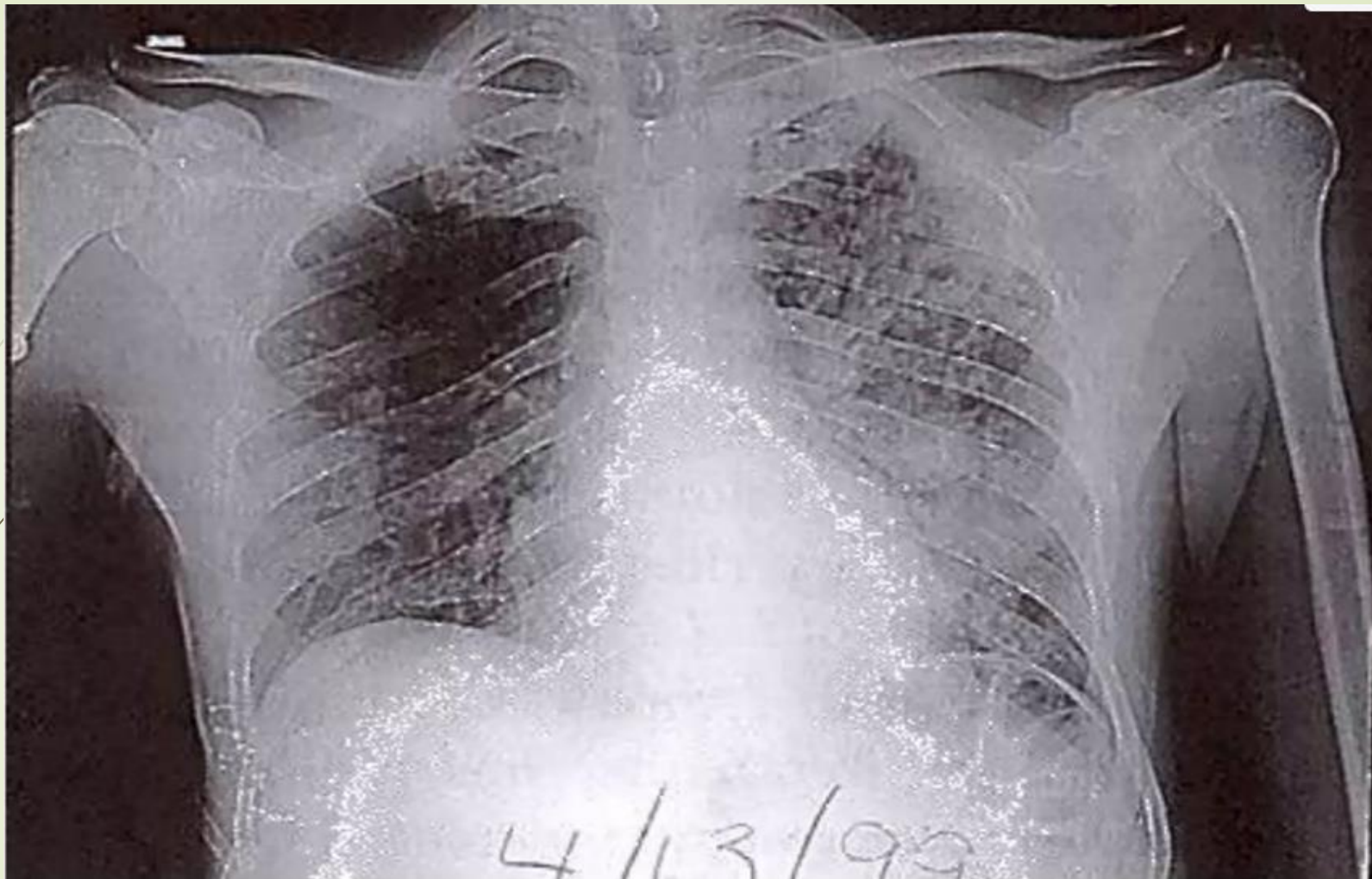
- Multiple drugs regimens and prolonged treatment are required to prevent development of drug resistance strains
- **MDR-TB is global health threat**

Continued...

- Treatment include **isoniazid** and **rifampin** for 9 months + **pyrazinamide** + **ethambutol** or **streptomycin**
- Immunoprophylaxis with BCG in endemic countries
- **Control-** **active surveillance** , prophylactic and therapeutic interventions and monitoring of the case

Progression of Pulmonary TB

- Pneumonia
- Granuloma formation with fibrosis
- Caseous necrosis
 - Tissue becomes dry & amorphous (resembling cheese)
 - Mixture of protein & fat (assimilated very slowly)
- Calcification
 - Ca^{++} salts deposited
- Cavity formation
 - Center liquefies & empties into bronchi



تهیه کننده: سهیلا عباسی

MYCOBACTERIUM LEPRAE

Structure and Physiology

- Weakly gram positive
- Strongly acid fast bacilli
- Lipid rich cell wall
- Unable to culture on artificial medium

Virulence

- Capable of intracellular growth
- Disease primarily from host response to infection

Epidemiology

- Common in Africa and Asia
- Armadillos are naturally infected and are reservoir
- Lepromatous form of disease is highly infectious
- Spreads by inhalation of aerosols
- Individual in direct contact with patients are at greater risk

Forms of Diseases

- Tuberculoid form of leprosy
- Lepromatous form of leprosy
- Intermediate form of leprosy

Diagnosis

- Microscopy is sensitive for the lepromatous but not for tuberculoid form
- Skin testing is required for tuberculoid leprosy
- **Culture cannot be used**

Treatment, Prevention and Control

- Dapsone with or without rifampin is used to treat tuberculoid form
- Clofazimine is added for the lepromatous form
- Therapy is usually prolonged
- For prophylaxis –DAPSONE
- Control – by prompt recognition and treatment of infected patients

Lepromatous form

33



تهیه کننده: سهیلا عباسی

Tuberculoid form



تهیه کننده: سهیلا عباسی



تهیه کننده: سهیلا عباسی

Pre and Post Treatment

