AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 8.

Microbiology diagnosis of *Tuberculosis, Lepra, Actinomycosis* and *Nocardiosis*

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. General characteristics, classification of bacteria from the genus *Mycobacterium*.

- Tuberculosis agents, morpho-biological characteristics, pathogenicity factors. Drug resistance. Multidrug-resistant (MDR), extensively drug-resistant (XDR), pandrug-resistant (PDR). Pathogenesis of the disease. Microbiological diagnosis (microscopic, bacteriological, biological, serological, molecular-genetic methods and skin-allergic test). Application of automated cultivation systems in the microbiological diagnosis of tuberculosis. Specific prevention and treatment of tuberculosis. BCG vaccine.

- The causative agent of *leprosy*. Morpho-biological features, distinguishing features from other mycobacteria, pathogenicity factors, ways of infection, pathogenesis, clinical forms. Microbiological diagnosis of leprosy (microscopic, skin-allergic, molecular-genetic methods), principles of specific treatment and prevention

2. *Actinomycetes*, classification, morpho-biological characteristics, pathogenicity factors. Pathogenesis, clinical forms and microbiological diagnosis of actinomycosis.

3. *Nocardia*, their role in human pathology

Purpose of the lesson:

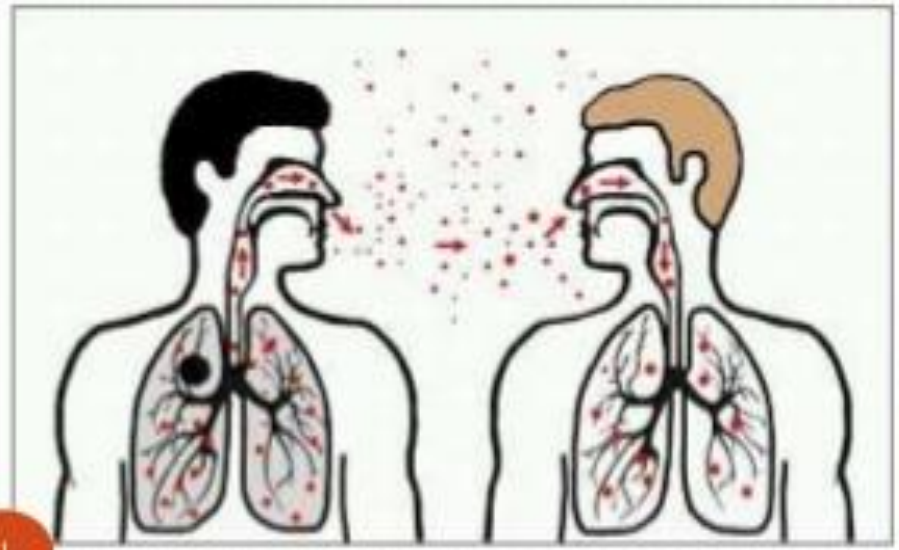
- Students will learn the morpho-biological characteristics of bacteria from the Mycobacterium (multidrug-resistant (MDR), extensively drug-resistant (XDR), pandrug-resistant (PDR)) and Actinomyces and Nocardia genusespathogenicity factors, pathogenesis, clinical signs, microbiological diagnosis of diseases caused by these bacteria, to provide information on specific treatment and prevention principles.

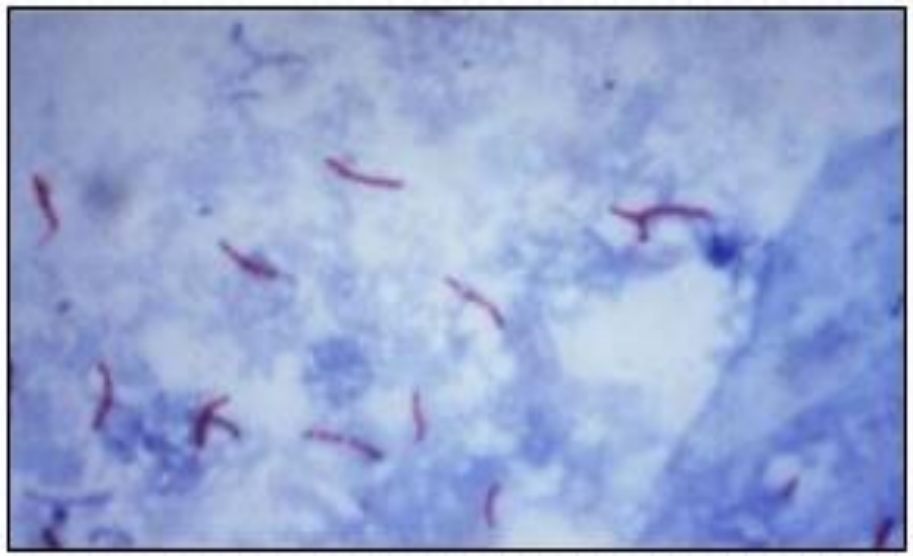
INTRODUCTION

- Tuberculosis is a worldwide public health problem

PULMONARY TUBERCULOSIS

MYCOBACTERIUM TUBERCULOSIS





Classification of Mycobacteria

1. **Tubercle bacilli**
   a) **Human – MTB**
   b) Bovine – *M. bovis*
   c) Murine – *M. microti*
   d) Avian – *M. avium*
   e) Cold blooded – *M. marinum*

   MTB Complex (*M. africanum* also included)

2. **Lepra bacilli**
   a) Human – *M. leprae*
   b) Rat – *M. leprae murium*
3. **Mycobacteria causing skin ulcers**
   a) *M. ulcerans*
   b) *M. belnei*
4. **Atypical Mycobacteria (Runyon Groups)**
   a) Photochromogens
   b) Scotochromogens
   c) Nonphotochromogens
   d) Rapid growers
5. **Johne's bacillus**

   M. paratuberculosis
6. **Saprophytic mycobacteria**
   a) *M. butyricum*
   b) *M. phlei*
   c) *M. stercoralis*
   d) *M. smegmatis*
   e) Others

What are Mycobacteria?

- Obligate **aerobes** growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative **intracellular pathogens** usually infecting **mononuclear phagocytes** (e.g. macrophages).

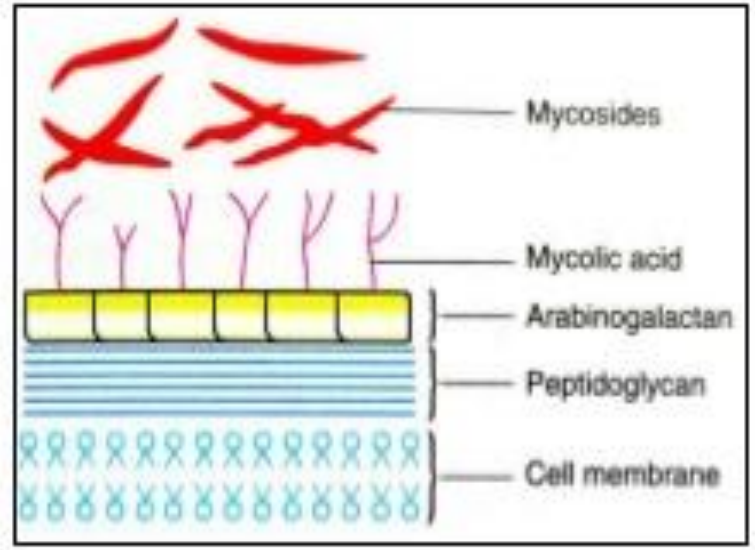
Mycobacterium differ from other routinely isolated Bacteria

- **Slow-growing** with a generation time of 14 to 15 hours (20-30 minutes for *Escherichia coli*).
- **Hydrophobic** with a high lipid content in the cell wall. As they are hydrophobic and tend to clump together, they are impermeable to the usual stains,

  e.g. **Gram's stain**

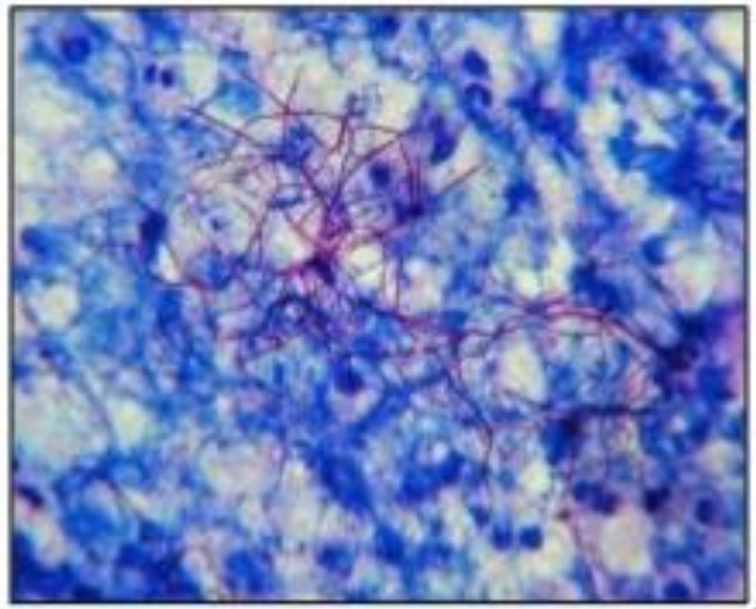
Acid fast bacilli

- Known as **"Acid-fast bacilli"** because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol.



How they are Acid fast

- Once stained, the cells resist decolourization with acidified organic solvents and are therefore called "acid-fast". (Other bacteria which also contain mycolic acids, such as *Nocardia*, can also exhibit this feature.)



Mycobacterium tuberculosis

MORPHOLOGY:-

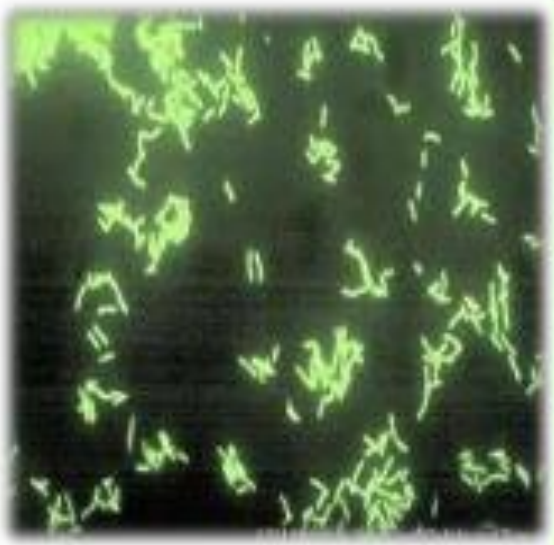
- Slender, straight or slightly curved bacilli with rounded ends, occurring singly or in pairs or in clumps.
- Non-sporing, non-capsulated and non-motile.

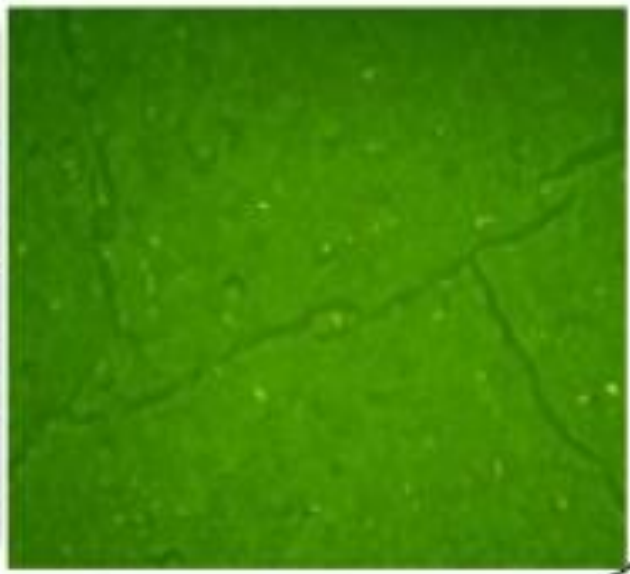
1. **Ziehl Neelsen stain** – stained by carbol fuschin; heat melts wax; resist decolourisation by 20% sulphuric acid . Resist decolourization by absolute alcohol.

   (Acid fast and alcohol fast)

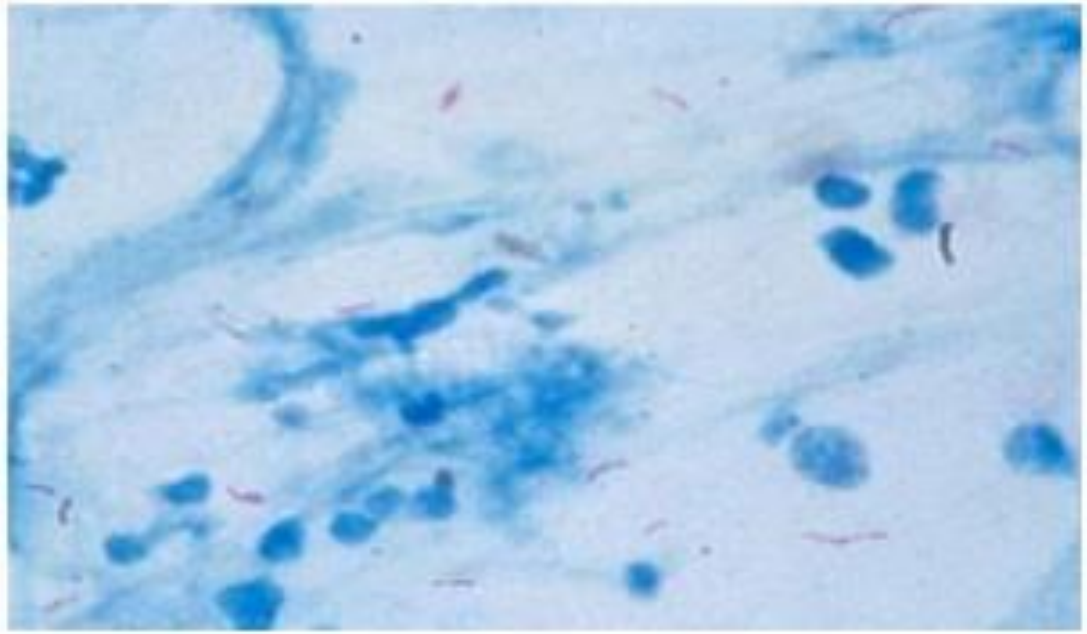
2. **Auramine rhodamine stain**

   (fluorescent stain)



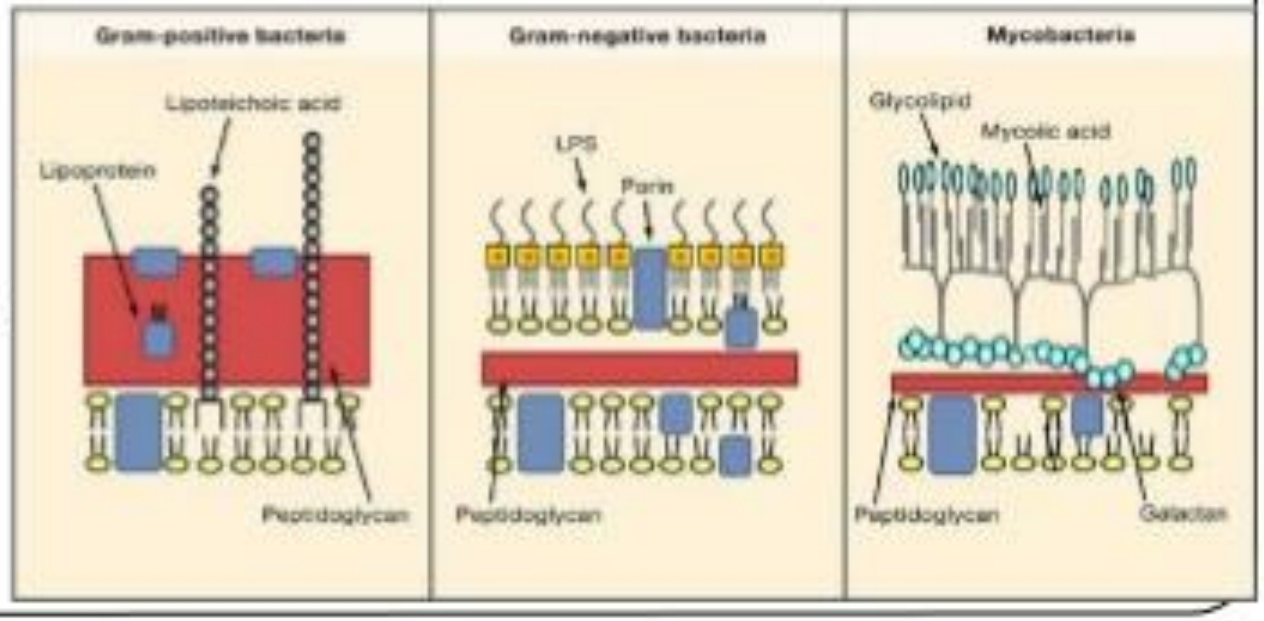


- **Acid fast bacilli**
- Straight or slightly curved.
- 1- 4 x 0.2-0.8 µm.
- Single, small clumps, pairs, long filamentous forms may be seen.
- Other (bacteria, cells – stained blue by)
- Counter stain (methylene blue)



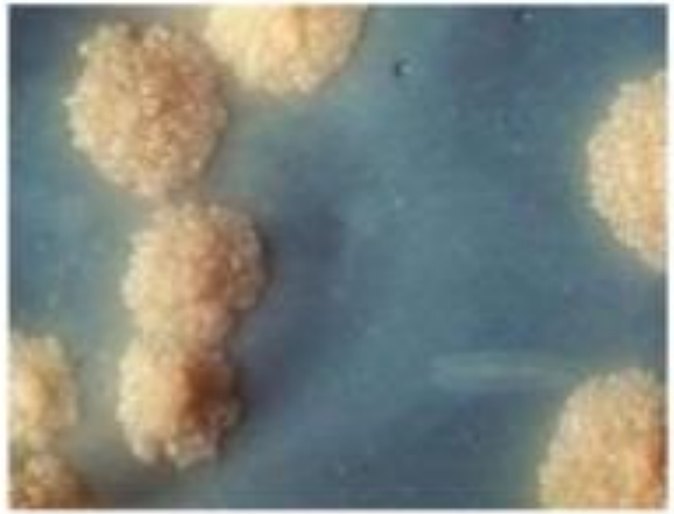
COUNTER STAINS USED:-

- Methylene blue – Blue background
- Malachite green – Green ”



CULTURAL CHARACTERS:-

- Aerobe.
- Growth stimulation by 5-10% CO_2
- Bacilli grow slowly, generation time 14- 15 hrs.
- Colonies appear in about two weeks or delayed upto 6-8 weeks.
- Optimum temp. 37°c
- Optimum pH 6.4-7.0
- Colonies - rough, tough and buff
- *M. tuberculosis* – obligate aerobe
- *M. bovis* – Microaerophilic



1. Solid media:-

i. Containing egg – **Lowenstein Jensen**, Petragnin, Dorset's egg.
ii. Containing blood – Tarshis medium.
iii. Containing potato – Pawlowsky's medium.

- Medium most commonly used is Lowenstein Jensen medium contain:-

i. Coagulated hen's eggs (neutralise fatty acid)
ii. Glycerol (C source)
iii. Mineral salt solution
iv. Asparagines (nitrogen source)
v. Malachite green (inhibits growth of other bacteria)

2. Liquid media:-

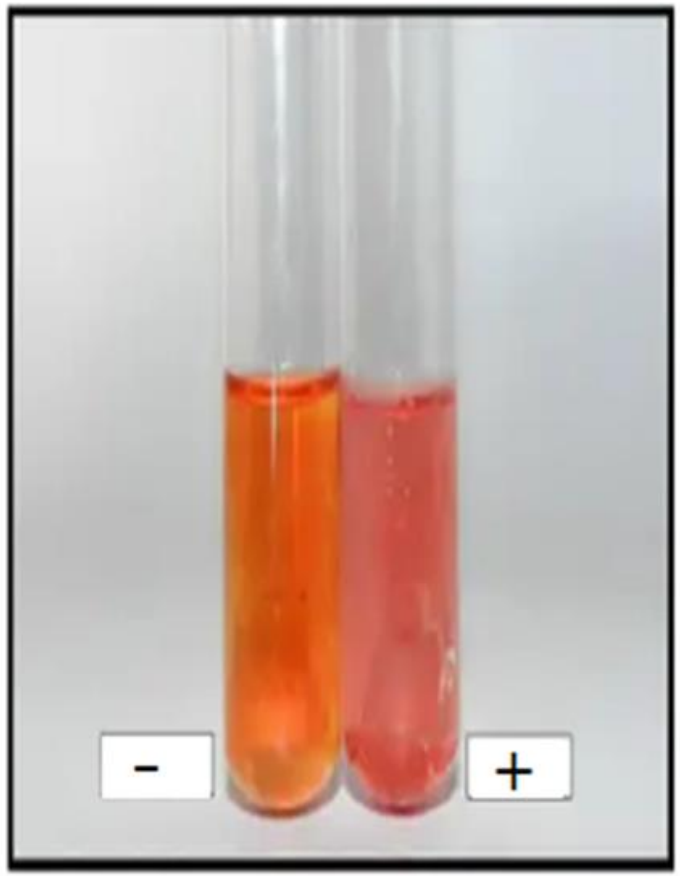
- Dubo's, Middlebrooke's, Prouskeur & Beck's, Sula's & Sauton's.

- Liquid media useful for – sensitivity tests, for extraction of Ag & vaccines.
  i. Growth in liquid media- pellicle at surface.
  ii. Dubo's medium with tween 80 – diffuse growth

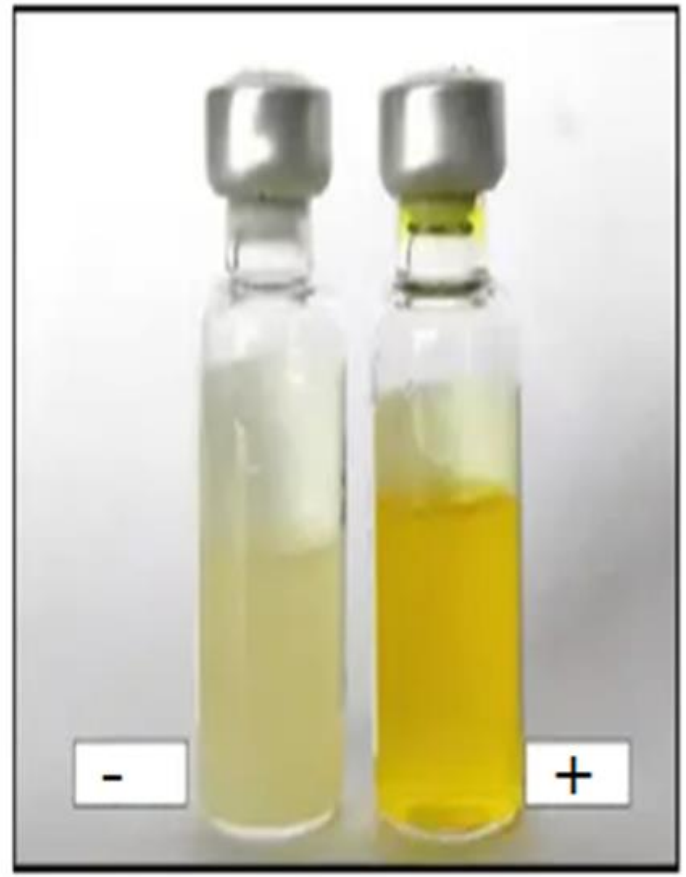
- **Virulent strain – Serpentine cords**
- **Avirulent strain – Dispersed growth.**

- Tubercle bacilli also grow in chick embryo & tissue culture.

	M. tuberculosis	M. bovis
Morphology	Long, slender and usually curved	Short, stout and straight
Staining	Barred or beaded appearance	Uniform staining
Growth on LJ medium	Eugonic	Dysgonic
Presence of glycerol in medium	Enhances the growth	Inhibits the growth
Colony	Dry, rough, tough, raised & wrinkled, difficult to emulsify	Moist, smooth, flat, white and friable
Biochemical reactions		
Niacin test	+	-
Nitrate reduction	-	-
Animal pathogenicity		
In guinea pig	+ (progressive & fatal)	+ (similar)
In rabbit	- Or mild lesion	+ generalised lesion



Tween-80 hydrolysis test



Niacin test

Nitrate reduction test

RESISTANCE :

- Not heat resistant
- Resistant to chemical disinfectants like phenol
- Destroyed by tincture iodine -5 min
- 80% ethanol – 2-10 minutes
- Sensitive to formaldehyde and glutaraldehyde

VIABILITY :

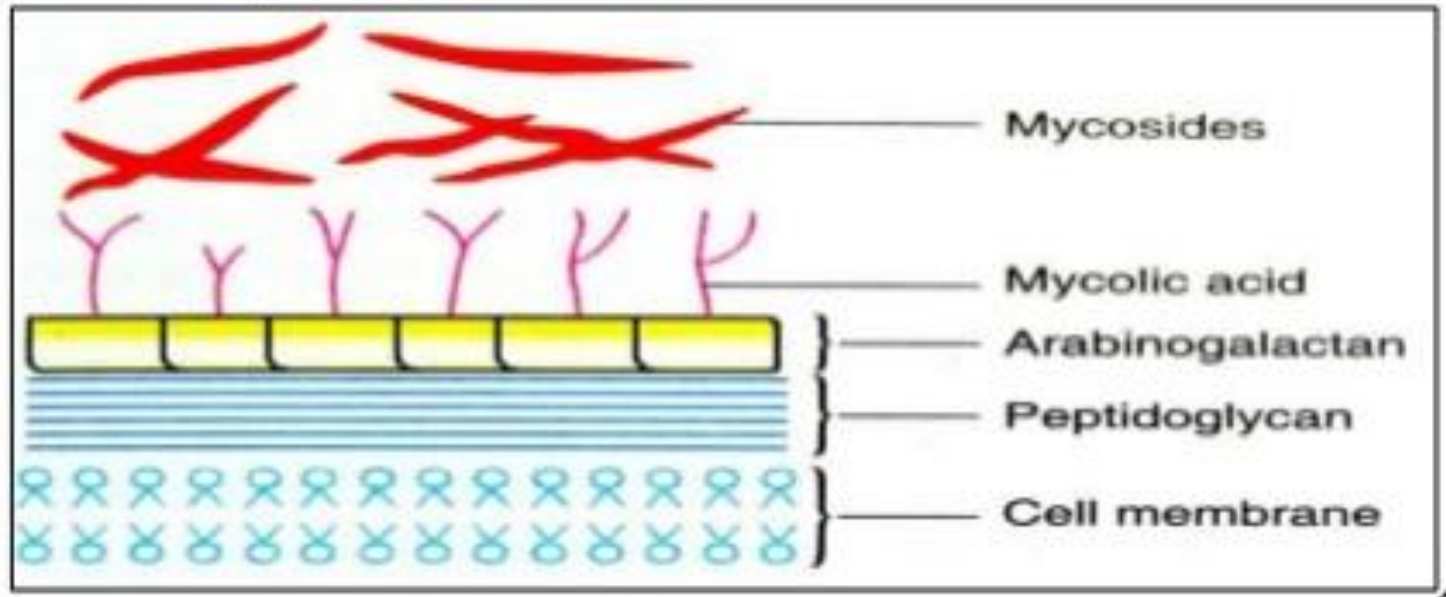
- Sputum – 20-30 hrs
- Droplets - 8-10 days
- Cultures- 6-8 months

Antigenic Structure

- **Cell Wall Antigens:**
  - Peptidoglycan layer
  - Arabinogalactan layer
  - Mycolic acid layer
  - Mycosides

- **Mycolic Acid**
  - Difficult to stain.
  - Difficult to phagocytose.
  - Intracellular survival.
  - Hypersensitivity.
  - Slow growth.
  - Resistant to heat and chemical disinfectants.

- **Cytoplasmic Antigens (Protein antigens)**



Virulence Factor:

- **Cord factor- Trehalose 6-6 dimycolate**, is a glycolipid molecule found in the cell wall of *Mycobacterium tuberculosis* and similar species. It is the primary lipid found on the exterior of *M. tuberculosis* cells.
  - **Serpentine growth** (filaments, cords) grows in close parallel arrangement.
  - Toxic to leukocytes
  - Role in development of granulomatous lesions

- **Sulfolipids-** Sulfated glycolipid (sulfatide) prevent phagosome- lysosome fusion which is important for intracellular survival.

IMMUNITY :

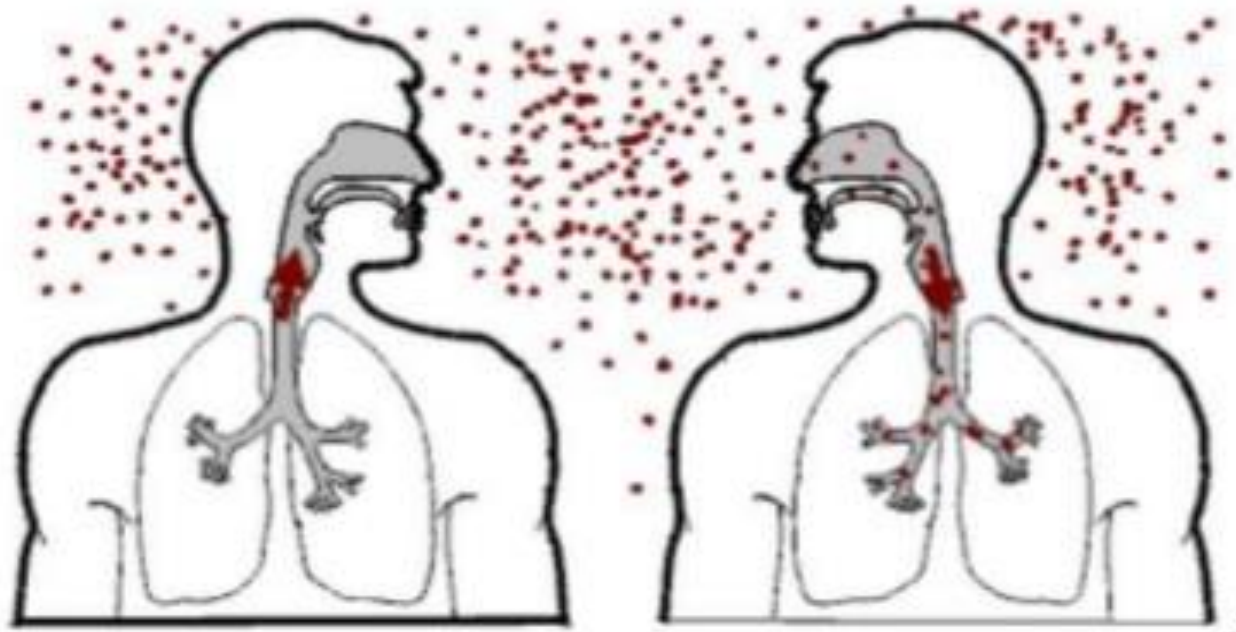
- Following injection by tubercle bacilli, delayed hypersensitivity develops against tuberculoprotein. Antibodies also develop but they don't have any diagnostic value and not relevant in immunity. Immunity in tuberculosis is mainly cell mediated by sensitized T-lymphocytes and macrophages.

- Tubercle Bacilli do not produce any toxin. Various bacterial components have biological effects.

  - **Cell wall** – Causes **Delayed Hypersensitivity**.
  - **Tuberculoprotein** – Induces D.H. Formation of cellular reaction of lymphocytes, monocytes, macrophages, epitheloid cells & giants cells.
  - **Lipids**- Accumulations of macrophages and neutrophils.

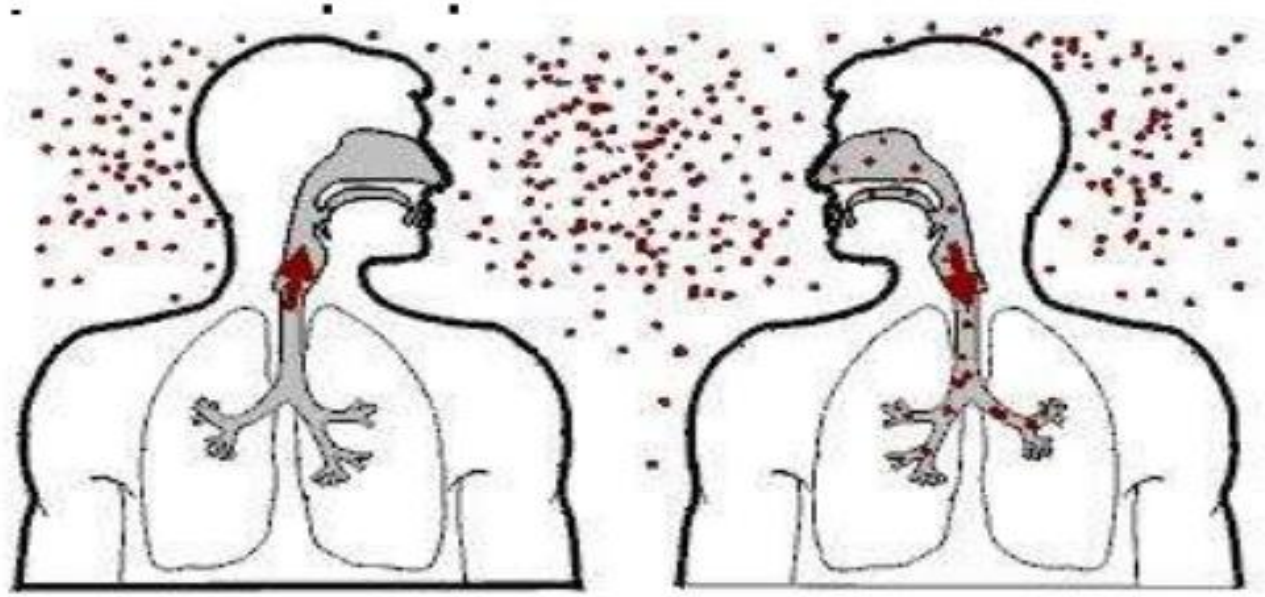
How tuberculosis spreads

- Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel bacilli into the air. A person needs only to inhale a small number of these to be infected.



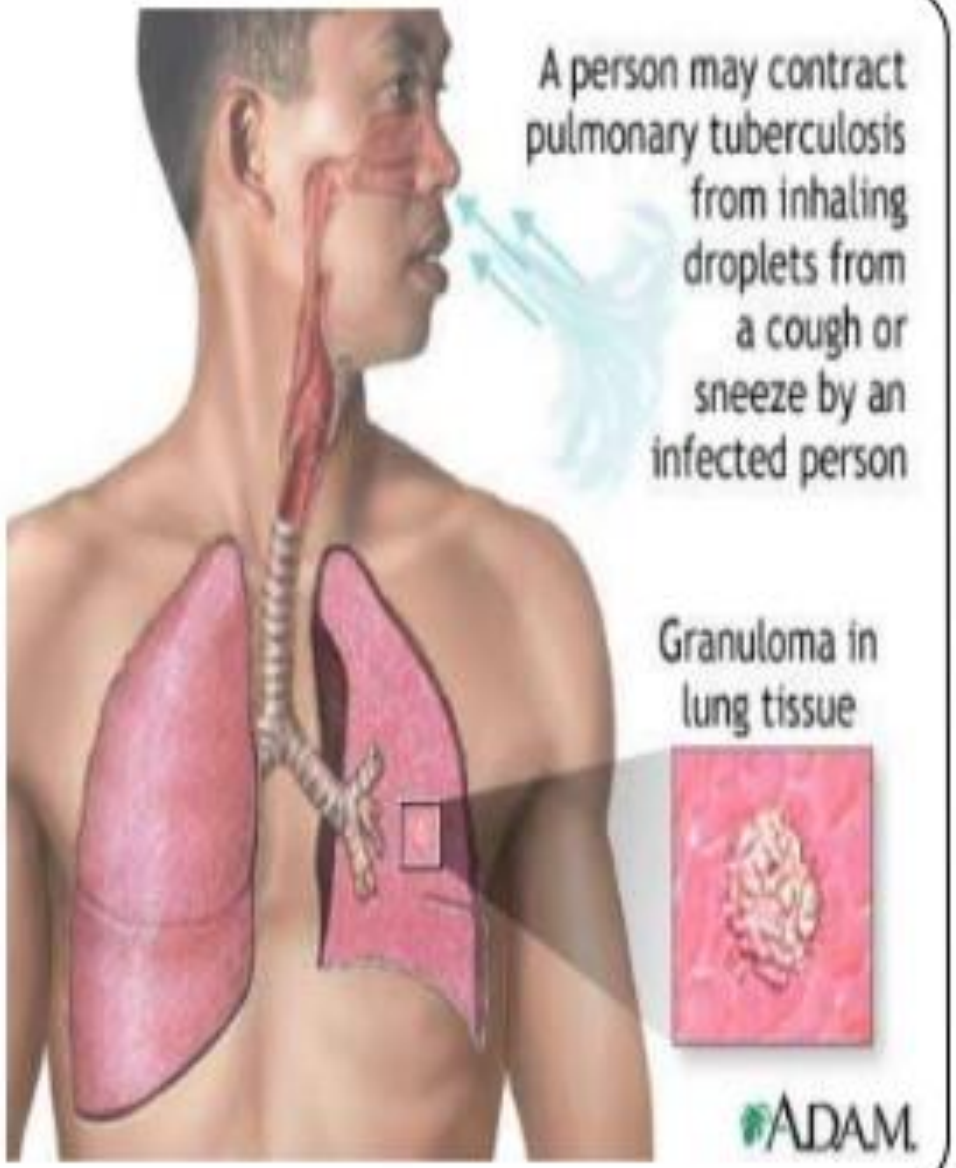
TRANSMISSION

- TB spreads from person to person by airborne



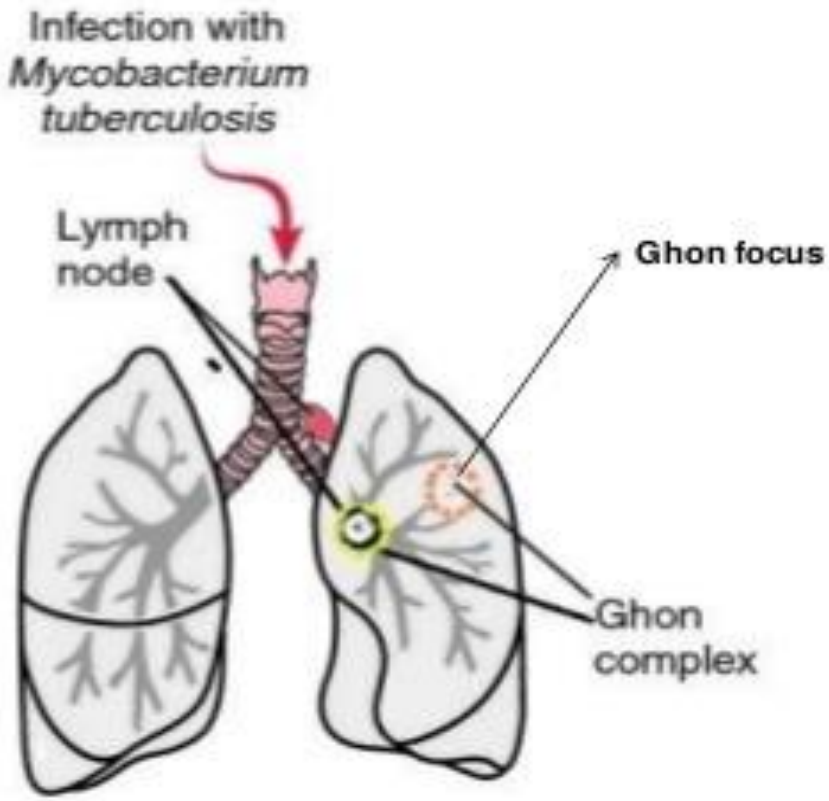
PATHOGENICITY:-

- *M. tuberculosis* can infect any organ or tissue but most commonly lungs are infected; intestines, kidneys, bones, soft tissues, brain etc.
- Infection acquired by inhalation of infected droplets.
- Engulfed by macrophages but survive and multiply.
- Lyses host cell and infect other macrophages.



Primary Tuberculosis:

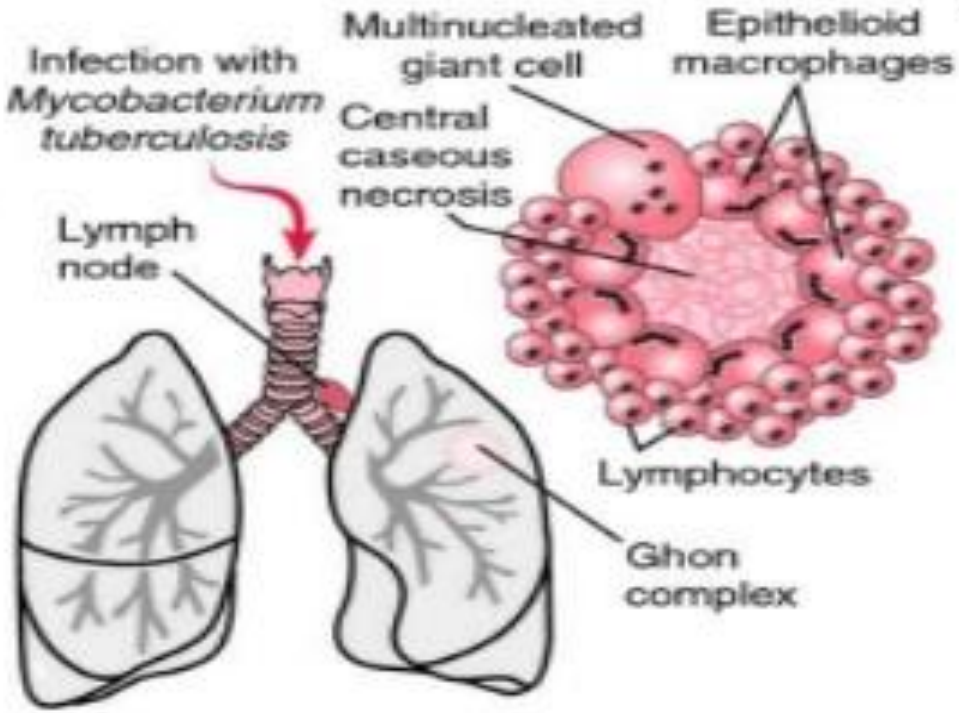
- Mostly asymptomatic.
- Some may have flu like symptoms; chest pain, mild fever and lack of appetite.
- Within 3 weeks, cell mediated immunity checks the bacilli.
- Engulfed bacilli in alveoli forms a lesion called **Ghon focus** in lower lobe. (Anton Ghon, Austrian pathologist)

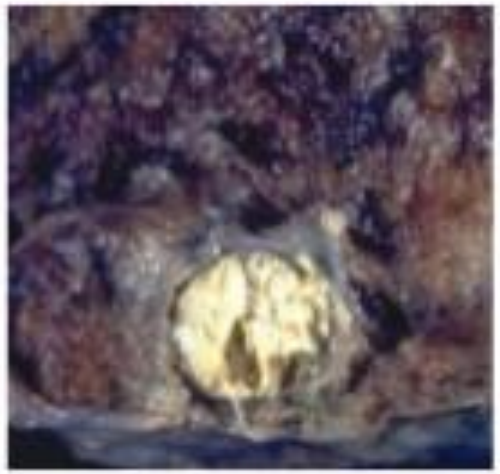


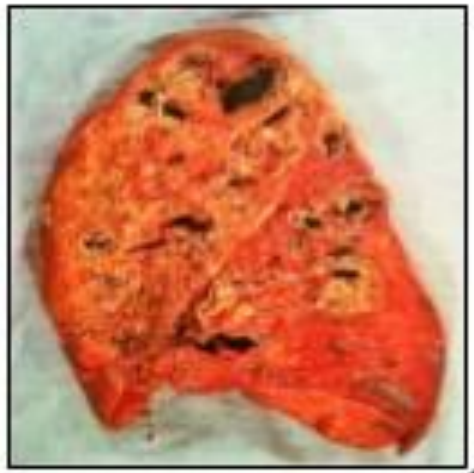
- Some bacilli are transported **to hilar lymph nodes**.
- Ghon focus together with the enlarged hilar lymph nodes is called **Primary Complex (Ghon Complex)**. (Karl Ernst Ranke, German pulmonologist)

Secondary Tuberculosis:

- Caused by reactivation (**immunosuppression**) of the primary lesion.
- Spreads to upper lobes.
- **Granuloma** occurs in apex of lungs.
- Memory T cells releases cytokines.
- Causes tissue destruction and necrosis called **tuberculomas (caeseous necrosis)**.
- Cavities may rupture into blood vessels, spreading bacilli throughout body and in sputum. Causing systemic **Miliary tuberculosis**.

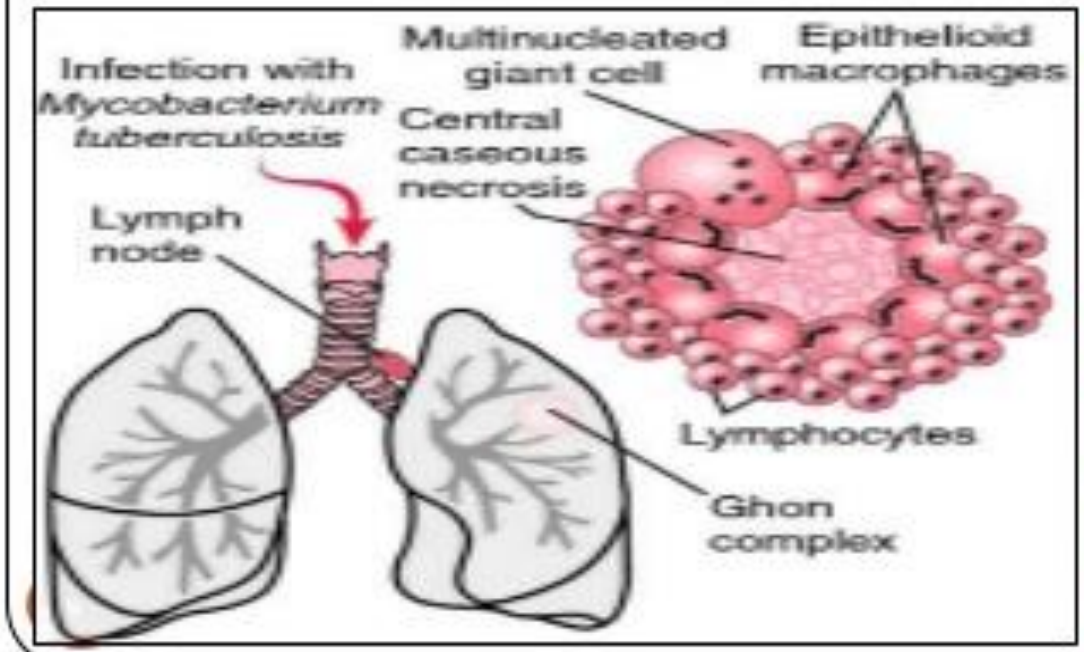


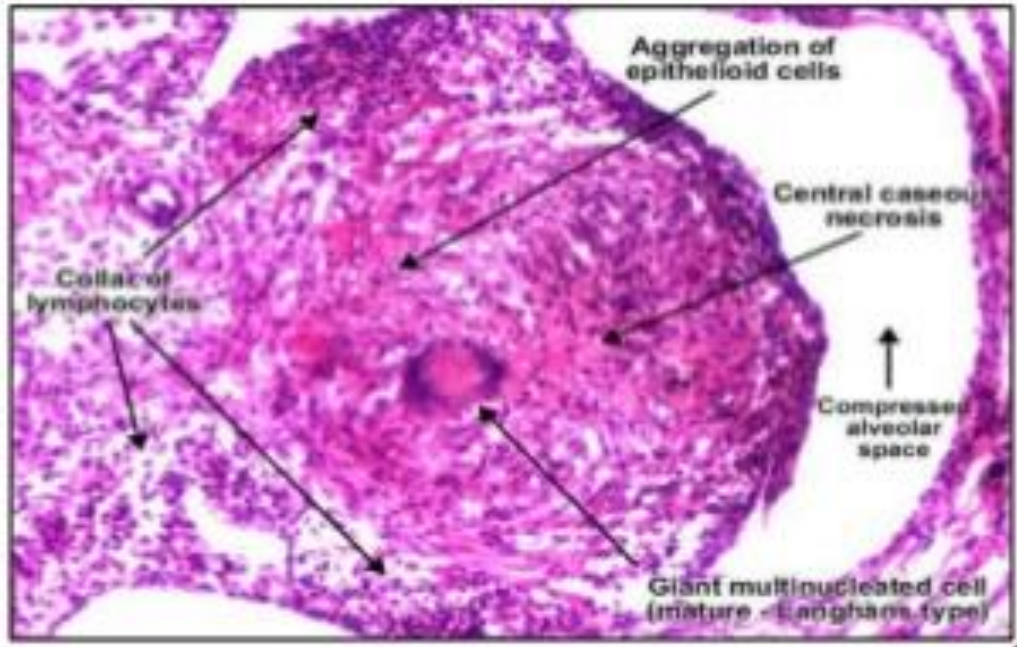




Secondary Tuberculosis: (in 10% cases caused by)

- HIV infection
- Alcoholism and liver cirrhosis
- Malnutrition
- Diabetes
- Steroid and immunosuppressive therapy
- Old age

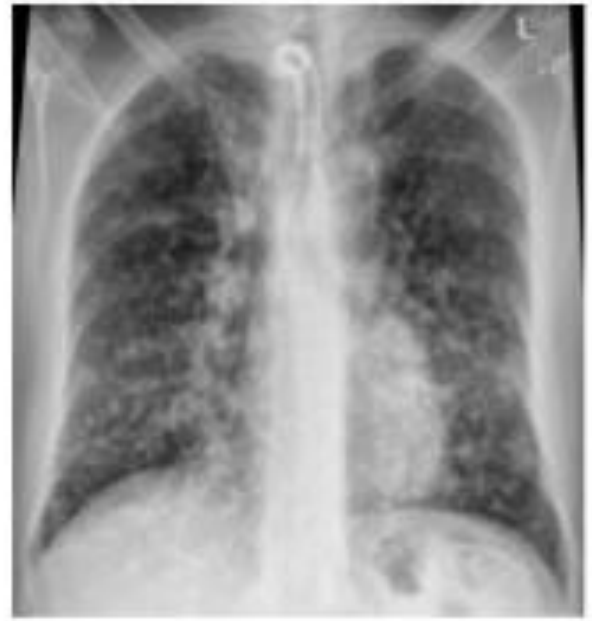


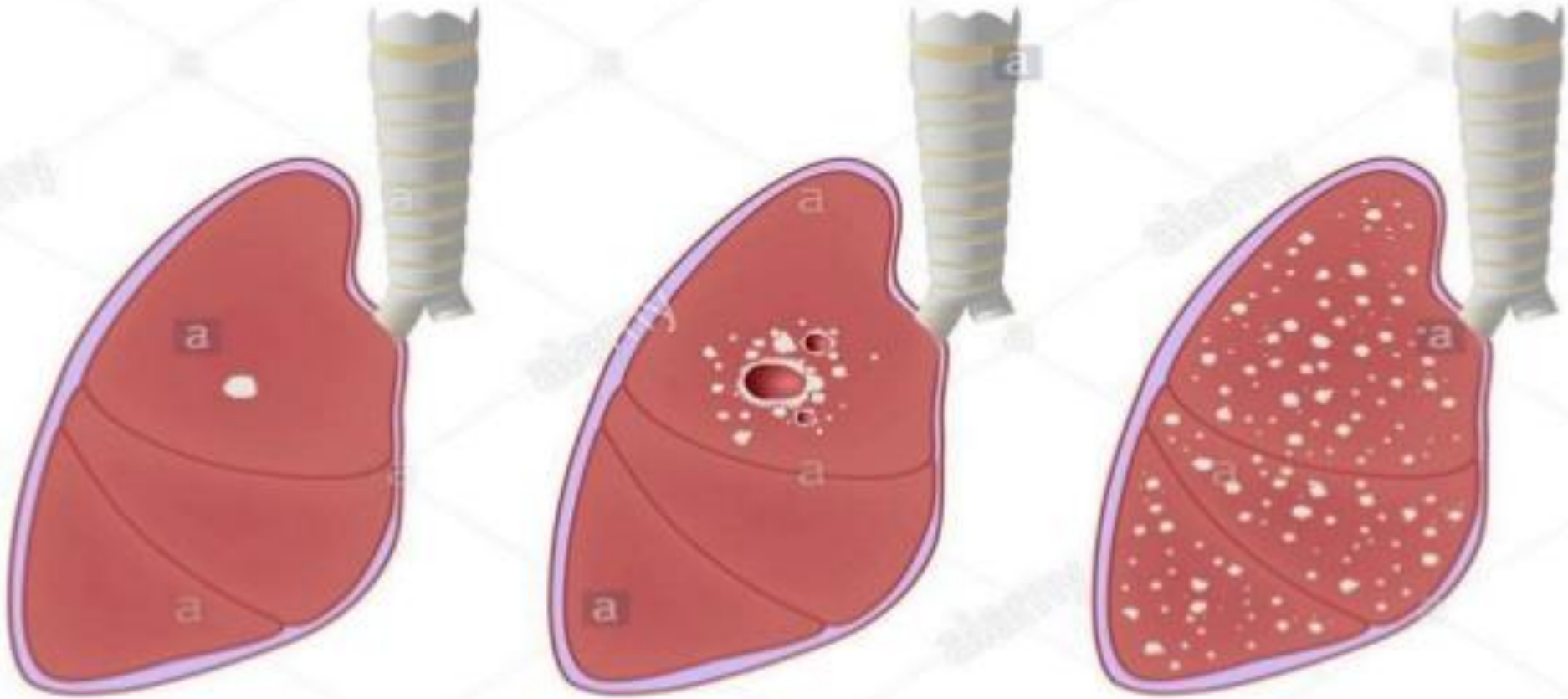


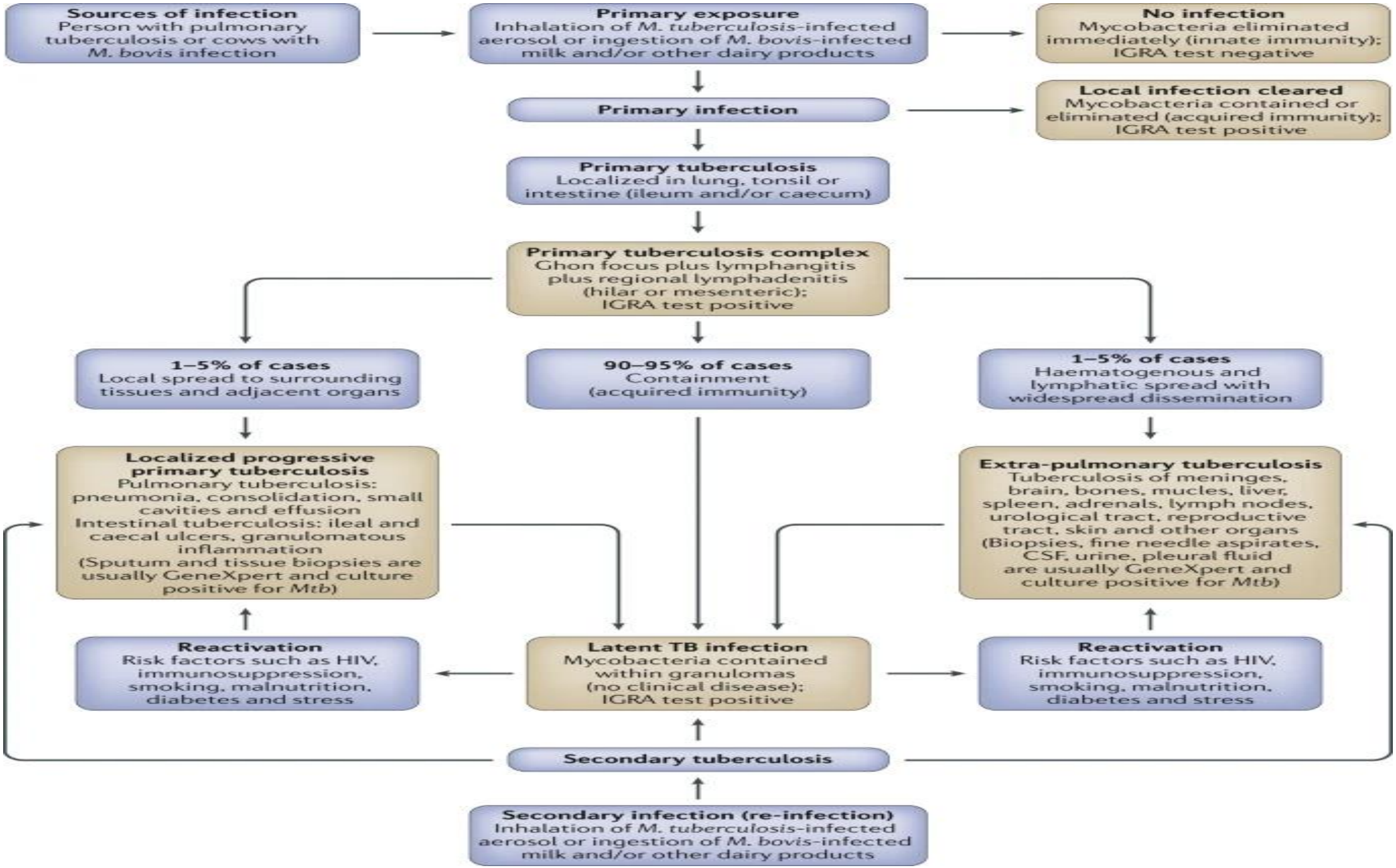
Secondary Tuberculosis:

- Miliary tuberculosis may develop in any organ of the body.
- Certain tissues like heart, striated muscles, thyroid and pancreas are resistant.
- Localization sites are the bone marrow, eye, lymph nodes, liver, spleen, kidneys, adrenal, prostate, seminal vesicles, fallopian tubes, endometrium and meninges.

- **Clinical signs:**
  - Temperature elevation usually in mid-afternoon, night sweats, weakness, fatigability, loss of appetite and weight.
  - Productive cough, blood streaked sputum (hemoptysis)



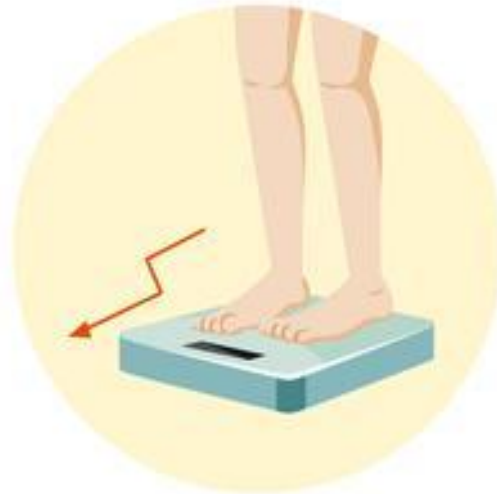

Latent infection
Cavitary tuberculosis
Miliary tuberculosis


Sources of infection
Person with pulmonary tuberculosis or cows with M. bovis infection
Primary exposure
Inhalation of M. tuberculosis-infected aerosol or ingestion of M. bovis-infected milk and/or other dairy products
No infection
Mycobacteria eliminated immediately (innate immunity); IGRA test negative
Primary infection
Local infection cleared
Mycobacteria contained or eliminated (acquired immunity); IGRA test positive
Primary tuberculosis
Localized in lung, tonsil or intestine (ileum and/or caecum)
Primary tuberculosis complex
Ghon focus plus lymphangitis plus regional lymphadenitis (hilar or mesenteric); IGRA test positive
1–5% of cases
Local spread to surrounding tissues and adjacent organs
90–95% of cases
Containment (acquired immunity)
1–5% of cases
Haematogenous and lymphatic spread with widespread dissemination
Localized progressive primary tuberculosis
Pulmonary tuberculosis: pneumonia, consolidation, small cavities and effusion
Intestinal tuberculosis: ileal and caecal ulcers, granulomatous inflammation
(Sputum and tissue biopsies are usually GeneXpert and culture positive for Mtb)
Extra-pulmonary tuberculosis
Tuberculosis of meninges, brain, bones, mucles, liver, spleen, adrenals, lymph nodes, urological tract, reproductive tract, skin and other organs
(Biopsies, fine needle aspirates, CSF, urine, pleural fluid are usually GeneXpert and culture positive for Mtb)
Reactivation
Risk factors such as HIV, immunosuppression, smoking, malnutrition, diabetes and stress
Latent TB infection
Mycobacteria contained within granulomas (no clinical disease); IGRA test positive
Reactivation
Risk factors such as HIV, immunosuppression, smoking, malnutrition, diabetes and stress
Secondary tuberculosis
Secondary infection (re-infection)
Inhalation of M. tuberculosis-infected aerosol or ingestion of M. bovis-infected milk and/or other dairy products

Tuberculosis Symptoms

fever

fatigue



weight loss

persistent cough

blood in cough

night sweats

LAB DIAGNOSIS:-

- **Specimen** depending on clinical presentation –Sputum, Pus, Urine, CSF, Pleural/ Ascitic fluid.
- **Pulmonary tuberculosis** - Early morning sputum sample on 3 consecutive days. (Bacillary shedding is intermittent).
- Sputum is collected in wide mouth containers.

PROCESSING

Physical exam

Quantity

Colour

Consistency

Presence of blood

Direct smear stained by Z.N. method

Conc. Petroff's method

Smear

Culture

Interpretation of sputum stained by Z - N Stain (WHO)

More than 10 bacilli / field ---------- +++

From 1 – 10 bacilli / field ---------- ++

From 10 – 99 bacilli / 100 fields ---------- +

From 1 -9 bacilli/100 fields ---------- write the exact no.

No bacilli seen ---------- NEGATIVE

***(10,000 bacilli / ml of sputum): shows positive**

TEST PRINCIPLES :-

1. **Niacin test-** suspension of tubercle bacilli +10% cyanogen bromide 4% aniline in ethanol- positive gives yellow colour. (+MTB)

2. **Arylsulphatase test-** Bacteria grown in solution of disulphate + 2N NaOH – pink (-MTB)

3. **Neutral Red Test** – colonies of Tub. Bacilli in neutral red solution in alk buffer –colonies pick up red colour (+MTB)

4. **Catalase Peroxidase test-** 5ml culture suspension + H_2O_2 and 2% catechol – effervescence - Catalase Peroxidase positive. Point mutation is a catalase gene, makes the strains resistant to isoniazide. (weakly + MTB)

5. **Amidase test-** Acetamide, benzamide, carbamide, nictonamide, pyrizinamide. (Split)
   - 0.00164M solutions of amide + tub. bacterial suspension incubate at 37°c.
   - Add solution of phenol, $MnSo_4$, hypochlorite.
   - Boil tube for 20 mins.
   - Blue colour indicates – Positive reaction

Drug sensitivity tests: -

1. **Absolute conc. Method**:-

   L.J. media containing serial conc. Of drug are inoculated & minimum inhibitory conc. Noted.

2. **Resistance ratio**:-

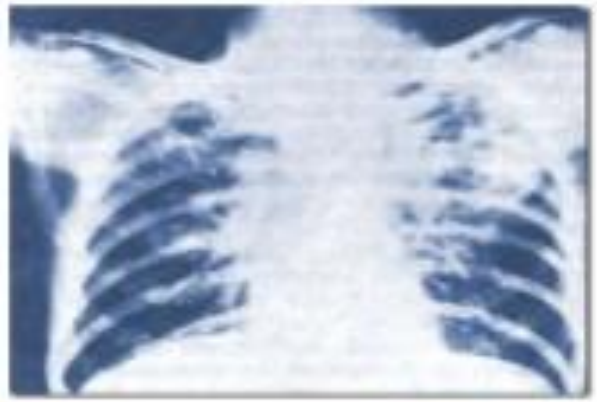
   Two sets of media containing serial conc. Of drugs are inoculated.

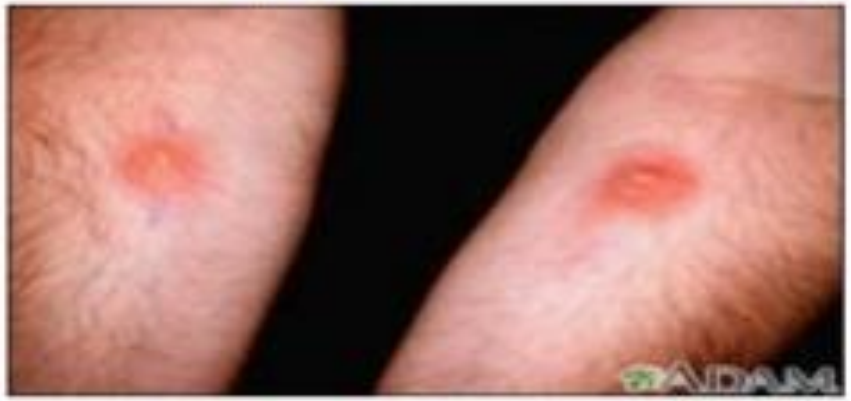
- 1st set – test strain
- 2nd set – standard strain

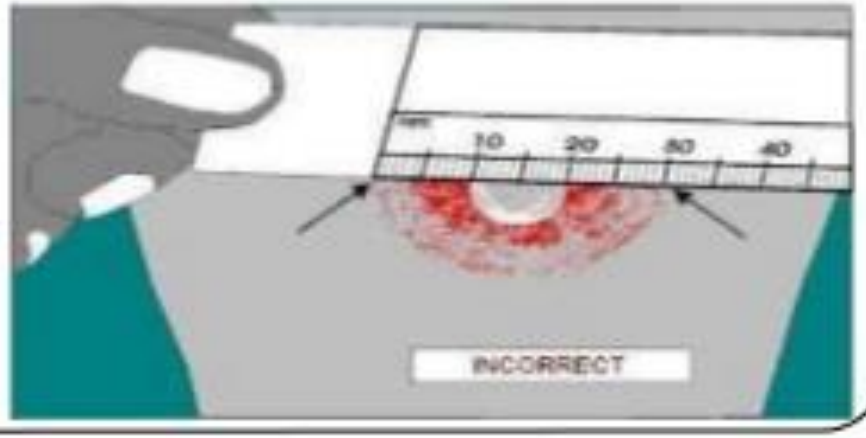
OTHER METHODS OF DIAGNOSIS OF TUBERCULOSIS:-

1. **X-ray chest**
2. **Blood exam** – lymphocytosis, increased ESR
3. **Mantoux test** – Tuberculin test.

- Routinely 5TU is used. 0.1 ml of PPD is injected intradermally in forearm. The area is marked by pen do not press or wash.
- Readings taken after 48-72 hrs.
- Erythema & indurations > 10mm – positive
  < 5mm – negative (+ in HIV)
  6-9mm – equivocal







1

Result Interpretation

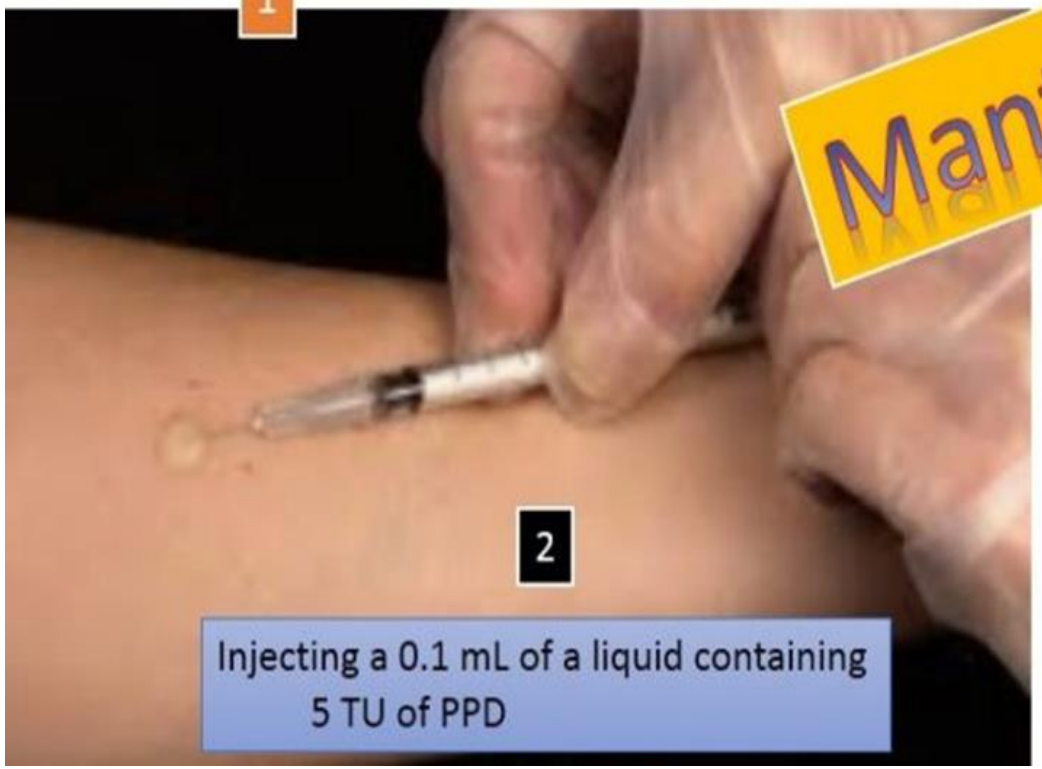
Negative: Induration < 5 mm (always)

Positive: Induration ≥ 5 mm not always but conditional e.g. in Immunosuppressed persons

10 mm induration to be positive e.g. IV drug abusers, children under 4 years old, people in high risk areas

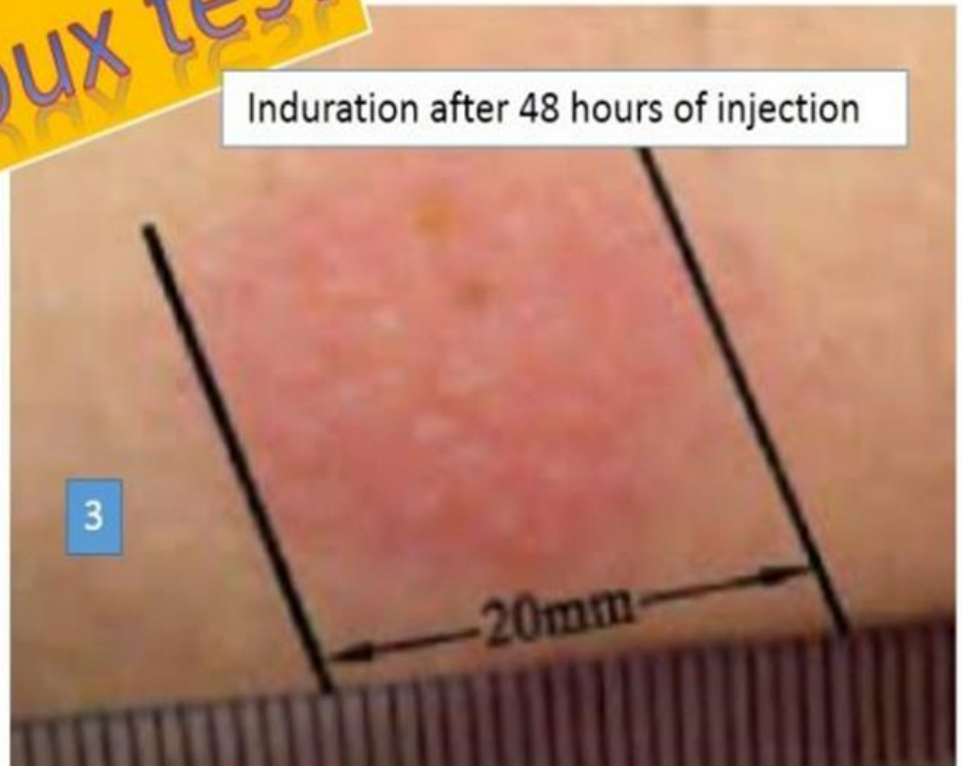
≥ 15 mm induration to be positive in a healthy person whose immune system is normal

Mantoux test



2

Injecting a 0.1 mL of a liquid containing 5 TU of PPD



3

NEWER METHODS FOR LAB DIAGNOSIS OF TUBERCULOSIS:-

1. **Radiometric methods** –

Advantage:- rapid growth

- Specific identification,
- Result within 7 days

Instrument:-

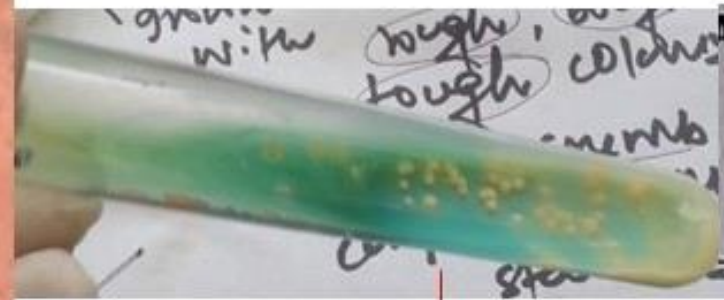
- **BACTEC**
- Fully automated

2. **PCR** – high sensitivity.
   - DNA amplified.
   - Cannot differentiate living and dead bacteria; both reported positive.

3. **Serology** – antibodies against *M.tubercule* antigen by ELISA.

Stain

Culture



PCR

FOR POSITIVE SAMPLE

Sputum smear minimum contain 10000 bacilli /ml

Sputum culture 10-100 bacilli/ml

PCR-load should be 1-10 bacilli/ml

Mycobacterium tuberculosis

TREATMENT

FIRST LINE DRUG:-

- Rifampicin(R) & Pyrizinamide (Z) – kill bacilli in lesions
- Isoniazid (H) – kills replicating bacilli
- Streptomycin (S) – kills extracellular bacilli
- Ethambutol (E) – bacteristatic

- **Intensive phase** – 3 times a week, 2 months – H, E, R, Z
- **Continuing phase** – 3 times a week, 4-5 months – H, R

SECOND LINE DRUG:-

- Quinolones, Aminoglycosides, Macrolides, Thiacetazone, Cycloserine, Capneomycin.

- **MDR-TB** – Resistance to Rifampicin & Isoniazid ; **DOTS** (directly observed therapy under supervision) important.

TYPES OF DRUG RESISTANCE

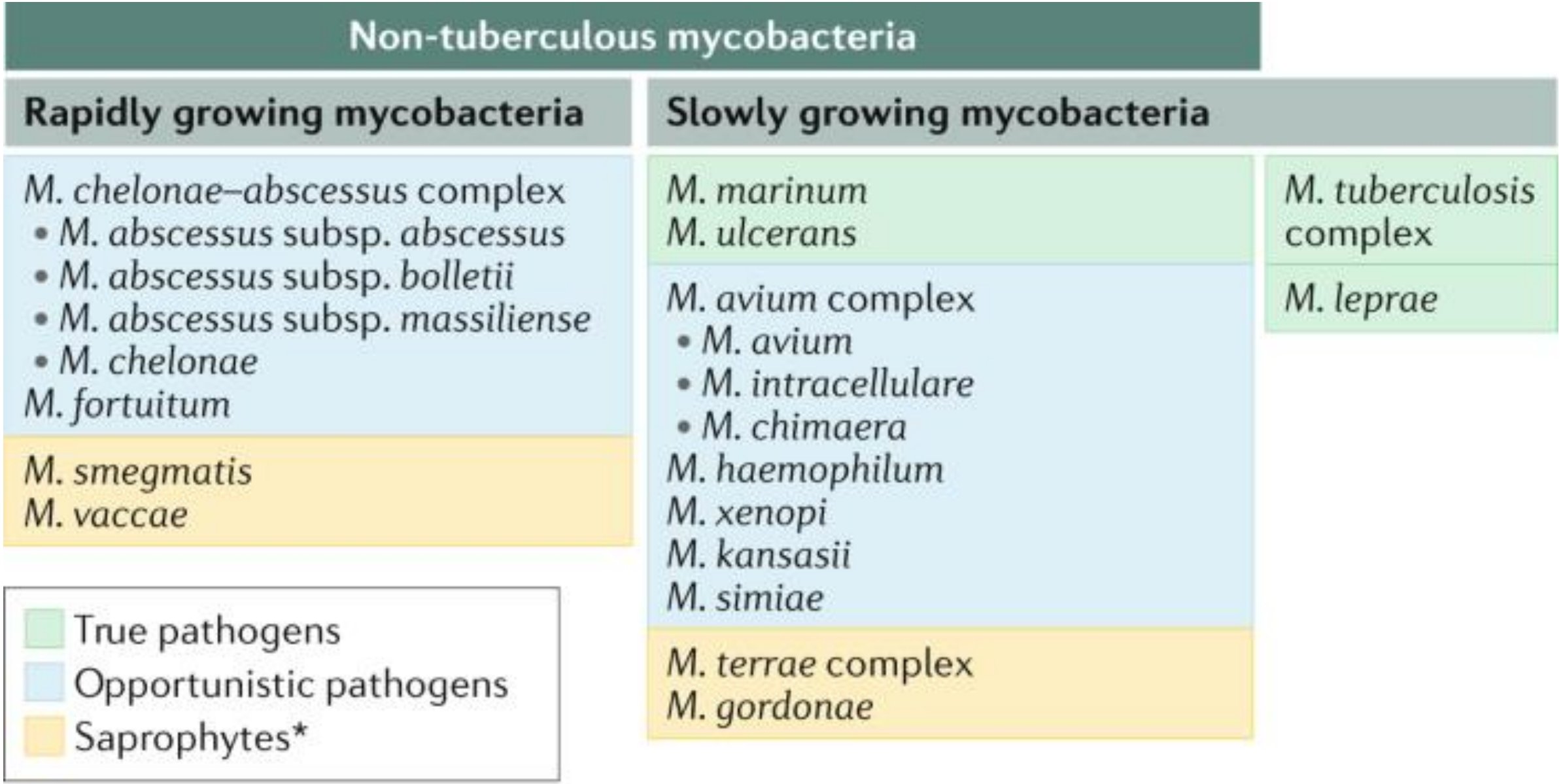
- Mono resistance: resistant to one drug
- Poly resistance: resistant to 2 or more drugs
- Multidrug-resistant tuberculosis (MDR-TB)- resistant to at least isoniazid and rifampin
- Pre- XDR TB – MDR TB + resistance to a fluoroquinolone or a 2nd line injectable drug
- Extensively Drug Resistant TB (XDR TB) – MDR-TB + resistance to a fluoroquinolone and a second line injectable (amikacin/ kanamycin/capreomycin)
- Totally Drug Resistant TB (TDR-TB) ???

BACILLUS CALMETTE GUERIN (BCG) :–

- Live attenuated vaccine. Strain of ***M. bovis*** attenuated by serial sub cultures in glycerine bile potato medium over 13 years.
- 0.1ml injected intradermally on deltoid muscle soon after birth.
- Immunity last for about 15 years.

BCG not to be given –

- Infants & children with active HIV disease.
- Babies born to sputum AFB positive mother.

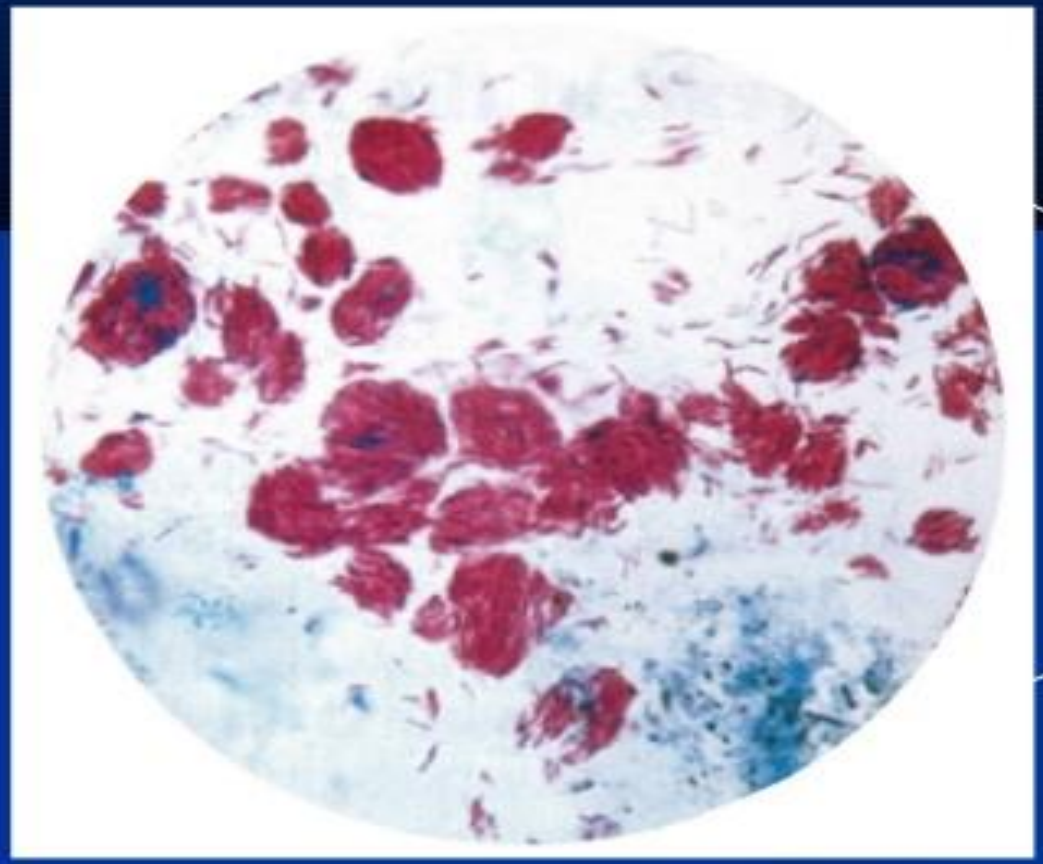

Non-tuberculous mycobacteria
Rapidly growing mycobacteria
Slowly growing mycobacteria
M. chelonae–abscessus complex
• M. abscessus subsp. abscessus
• M. abscessus subsp. bolletii
• M. abscessus subsp. massiliense
• M. chelonae
M. fortuitum
M. smegmatis
M. vaccae
M. marinum
M. ulcerans
M. avium complex
• M. avium
• M. intracellulare
• M. chimaera
M. haemophilum
M. xenopi
M. kansasii
M. simiae
M. terrae complex
M. gordonae
M. tuberculosis complex
M. leprae
True pathogens
Opportunistic pathogens
Saprophytes*
*can be detected in clinical samples and need retesting to confirm infection

Leprosy

Lepra bacilli

- Gram positive Obligate intracellular bacillus - due to its large pool of non functional genes.
- Acid fast stained with modified Fite stain or ZN stain
- Short, thick, pink stained rods of Size: (5μ X 0.5 μ)
- Occurs characteristically in clumps or bundles("globi")
- Affinity for Schwann cells & cells of R-E system .
- *M. leprae* grows best in cooler tissues (the skin, peripheral nerves, anterior chamber of the eye, upper respiratory tract, and testes), sparing warmer areas of the skin (the axilla, groin, scalp, and midline of the back).
- Optimal temp. for growth is 30-33 centigrade

The Leprosy Bacteria



Cultivation

- *M. leprae* is found only in cases of human infection.
- They have not yet been grown ion artificial media or tissue culture.
- The generation time of leprae bacillus is found to be 12-13 days on an average.
- When ground tissue or nasal scrapping from lepromatous leprosy containing lepra bacilli are inoculated intradermally into foot pad of mouse and kept at low temperature (20°C), a granulomatous lesion develops at the site of injection in 1-6 months.
- When nine band armadillo is inoculated with lepra bacilli, generalized infection develops with extensive multiplication of the bacilli.

Reservoir of infection

- **Main reservoir :** Human being
  - Lepromatous case> Non lepromatous cases

- **Animal reservoirs**
  - 9-banded armadillos
  - Chimpanzees
  - Mangabey monkeys
  - Sphagnum moss

Mode of transmission

- **Transmission by inhalation**
  - Droplet infection(most common)
- **Transmission by contact**
  - Skin to skin contact with infectious cases
  - Contact with soil or fomites
- **Other Routes**
  - Insect Vectors e.g.. Mosquito, Bedbugs
  - Tattooing needles

NB : Breast feeding and Transplacental infection do not occur.

Incubation period

- **Long incubation period**
  - Ranged: 2 to 40 years or more
  - Average: 3-5 years
- **Generation time** : 12 days.
- **Infectivity** : Leprosy is a highly infectious disease with low pathogenicity. Among household contacts of lepromatous cases about 5 to 12 percent is expected to show signs of leprosy within 5 yrs.

VIRULENCE FACTOR

The bacterium's complex cell wall contains large amounts of an *M. leprae*–specific **phenolic glycolipid (PGL-1),** which is detected in serologic tests. The unique trisaccharide of *M. leprae* binds to the basal lamina of Schwann cells; this interaction is probably relevant to the fact that *M. leprae* is the only bacterium to invade peripheral nerves.

Bacilli discharged from nose

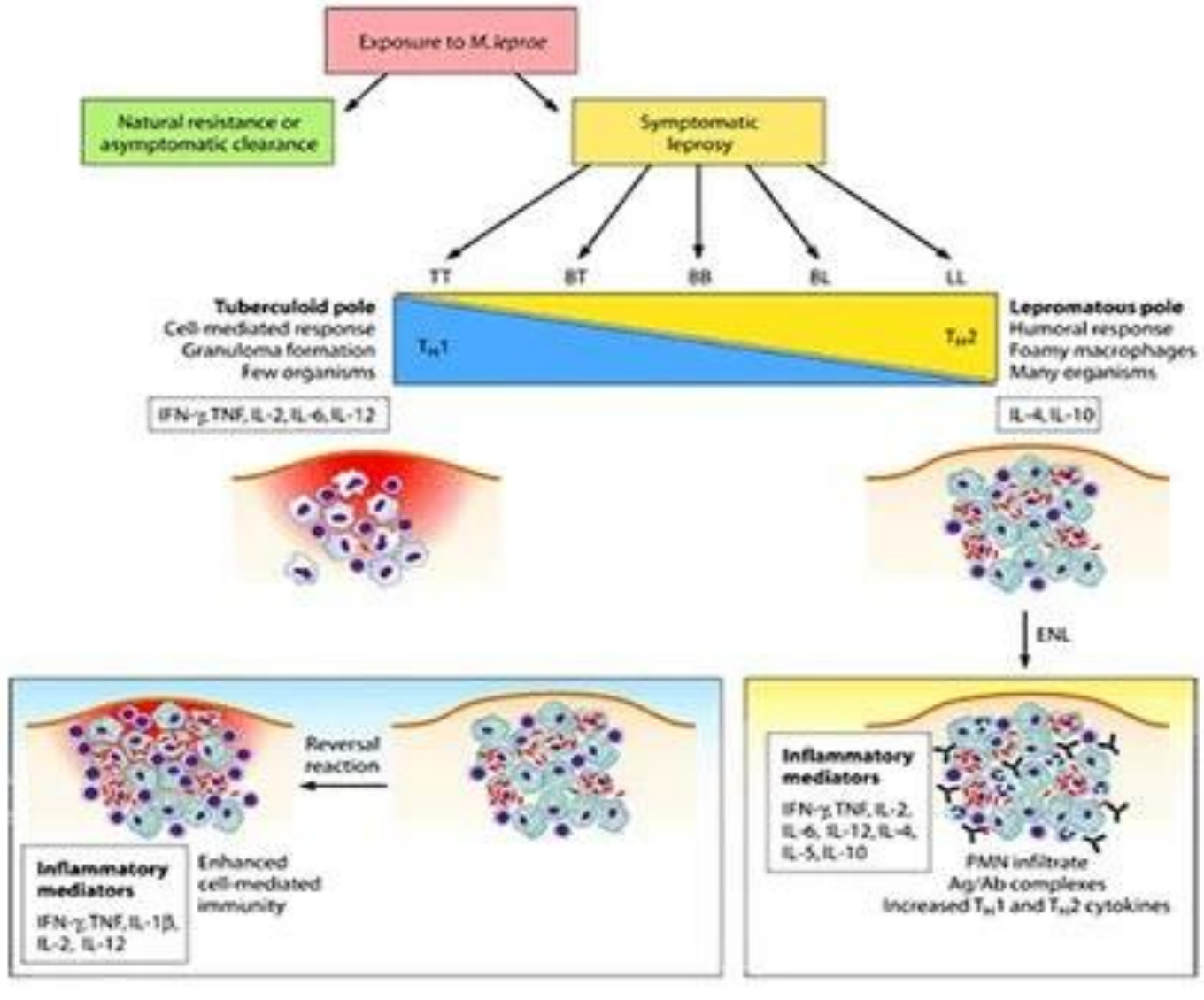
Inhaled by susceptible person

Taken up by alveolar macrophages

Disseminated through blood

Spreads to nerve and skin

Bacilli proliferate especially in Schwann cells



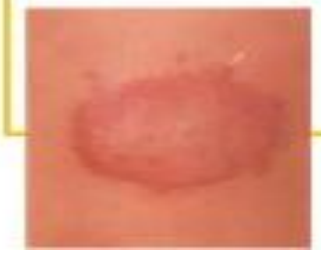
Ridley- Jopling 1966 (Research purposes)

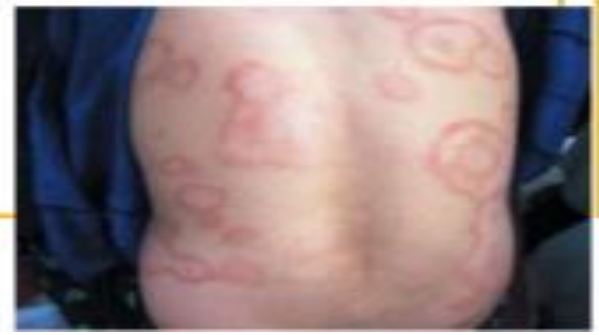
- **Most widely accepted**
  - Divides Leprosy cases into five groups according to their position on an immunohistological scale.
  - It can be used only when full research facilities are available :
    - Tuberculoid (TT)
    - Borderline Tuberculoid (BT)
    - Borderline Borderline (BB)
    - Borderline Lepromatous (BL)
    - Lepromatous (LL)

Differences

Tuberculoid Leprosy (TT)

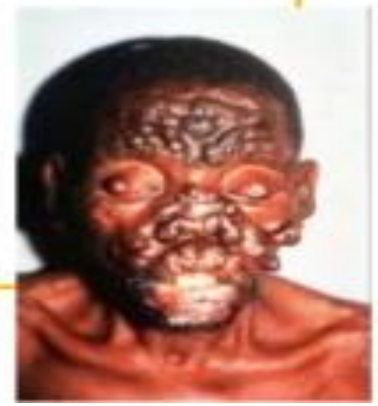
- Well demarcated, dry patch
- Minimal disfigurement
  - No leonine facies
  - No claw-shaped hands
  - No pendulous ear lobes
- Good immune response (high resistance)



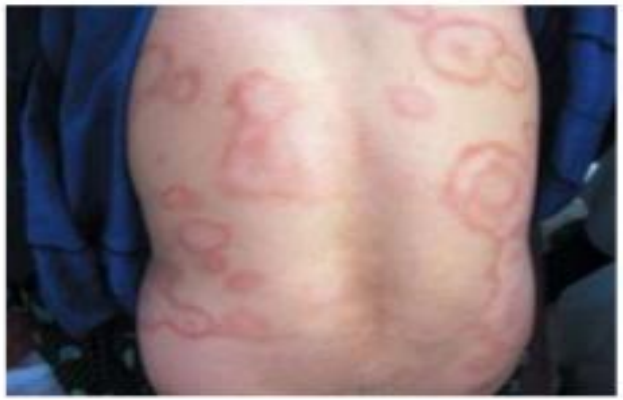


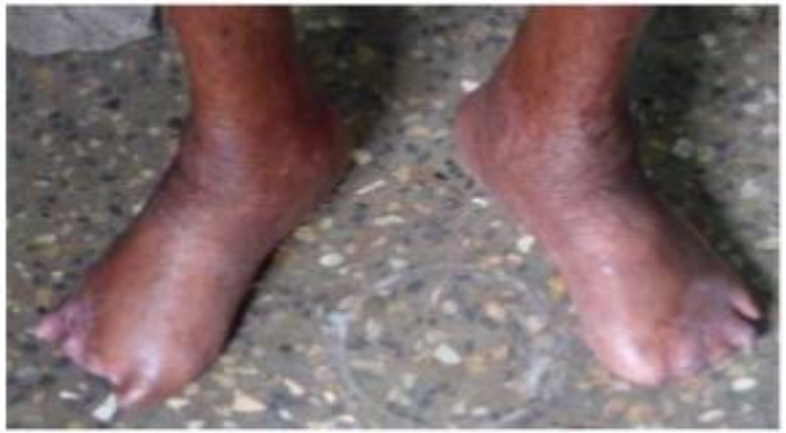
Lepromatous Leprosy (LL)

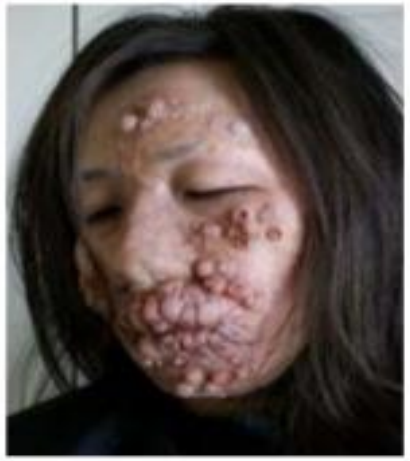
- Disfigurement is there
  - Leonine facies
  - Claw-shaped hands
  - Pendulous ear lobes
  - Saddle nose
- Suppressed (low resistance)

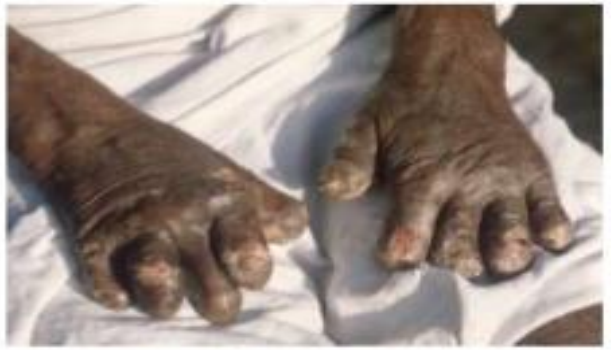


Leprosy









DIAGNOSIS

BACTERIOLOGICAL EXAMINATION

This includes :

- Skin Smears :
- Nasal Smears or blows :
- Nasal Scrapings :

DIAGNOSIS

BIOPSY

- Usually resorted to when there is high clinical suspicion but the other test are unyielding. It also gives information about the bacterial content of skin.

DIAGNOSIS

IMMUNOLOGICAL TESTS

- **Tests for cell mediated immunity(CMI)**
  - **LEPROMIN TEST**

- **Tests for humoral antibodies(serological tests)**
  - **FLA-ABS test** : used for detecting subclinical infections. **92.3** percent sensitive and **100** percent specific in detecting specific antibodies in all types leprosy irrespective of type and duration of disease.
  - Monoclonal antibodies
  - Others : RIA, ELISA.

DIAGNOSIS

LEPROMIN TEST

Method : it is performed by injecting 0.1ml of lepromin into inner aspect of the forearm. The reaction is read at 48 hours and 21 days. Two types of reaction have been described :

EARLY REACTION(FERNANDEZ REACTION) :

an inflammatory reaction develops within 24 to 48 hours and this tends to disappear in 3 to 4 days. If the diameter of the red area is more than 10mm the test is considered positive. It is a delayed type hypersensitivity reaction to soluble constituents of lepra bacilli and indicates whether or not a person has been sensitized by exposure to and infection by lepra bacilli.

DIAGNOSIS
LEPROMIN TEST

LATE REACTION(MITSUDA REACTION) : It is characterized by the appearance of a nodule which becomes apparent in 7 to 10 days and reaches its maximum in 3 to 4 weeks. The test is read at 21 days. If the nodule is more than 5 mm it is considered positive. It is induced by the bacillary component and indicates cell mediated immunity.

- In the first six months of life most children are lepromin negative
- BCG vaccination is capable of converting lepra reaction from negative to positive.

DIAGNOSIS
LEPROMIN TEST

VALUE OF LEPROMIN TEST :

- Useful tool for evaluating the immune status of leprosy patients.
- Aid to classify the type of disease.
- Estimating the prognosis
- Strongly positive in a typical tuberculoid case and getting weaker towards the lepromatous end, the typical lepromatous case being lepromin negative indicating failure of CMI.

The greatest drawback being high false positive and false negative cases hence not used as a diagnostic test.

OTHER TESTS FOR CMI :

- Lymphocyte transformation test(LTT)
- Leucocyte migration inhibition test(LMIT)

Treatment

- **Multiple drug therapy** for 12 – months is key to treatment, this is carried out by WHO guideline using.
  1- Rifampicin
  2- Dapsone
  3- Clofazimine

- During treatment, patient may develop acute manifestation, which controlled by **steroids**

- **Surgical treatment** is indicated in advance stage of disease for functional disability of limbs, cosmetic disfigurement of face and visual problems.

- Surgical reconstruction requires the expertise of hand surgeon, orthopedic surgeon and plastic surgeon.

Actynomyces - Taxonomy

- (Domain): Bakteriyalar
- (Kingdom): Actinomycetota
- (Class): Actinomycetia
- (Order): Actinomycetales
- (Family): Actinomycetaceae
- (Genus): Actinomyces
- (Species): ***A.israelli***

Actinomycetes

- Fungus-like characteristics
  - Branching filaments in tissues / culture
  - looks like mycelia
- Filaments frequently segmented
  - Pleomorphic forms (Diphtheroid & club shaped)
- Cell wall and the internal structures are typical of bacteria.
- Aaerobic OR Anaerobic.
- Slow growers

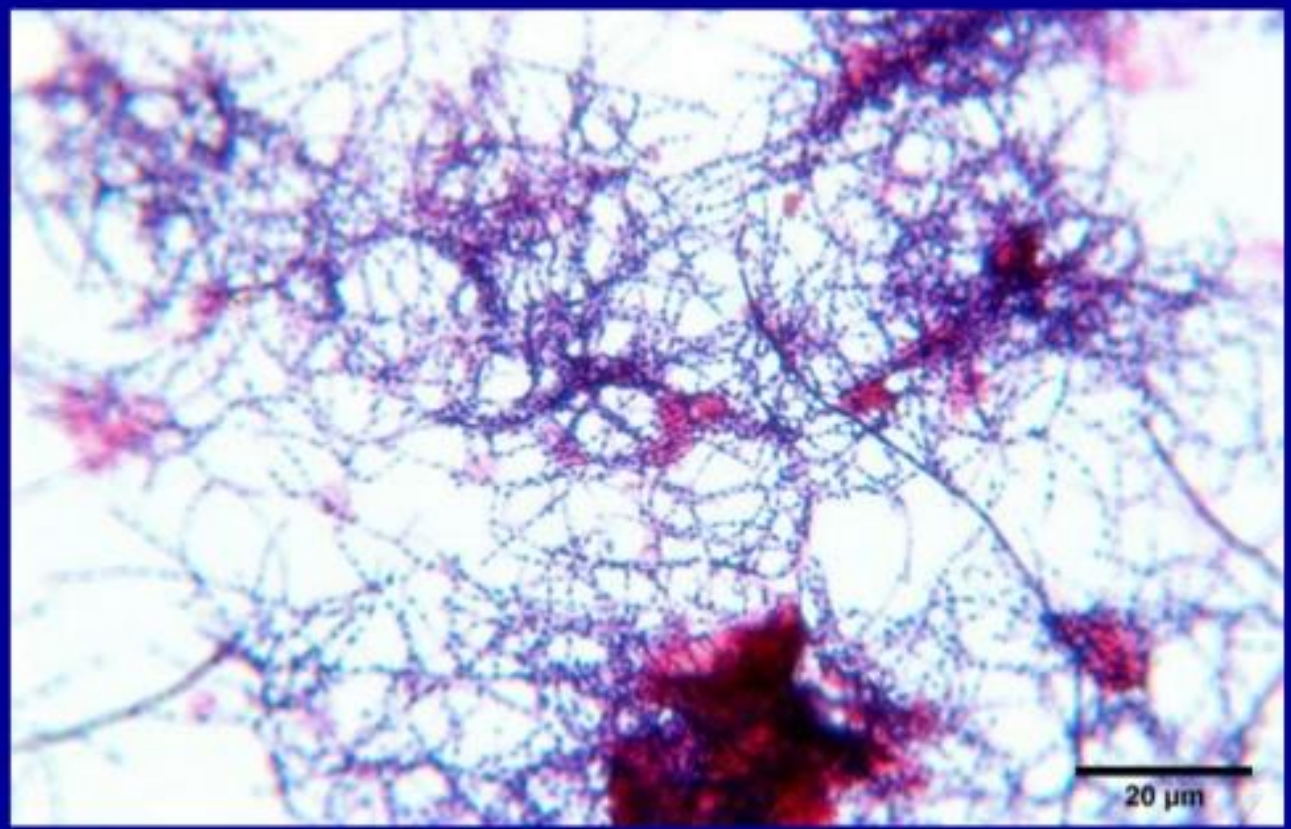
Actinomycetes

- Classification
  - Anaerobic
    - Actinomyces spp
  - Aerobic
    - Nocardia spp
    - Actinomadura spp
    - Streptomyces spp

Actinomyces

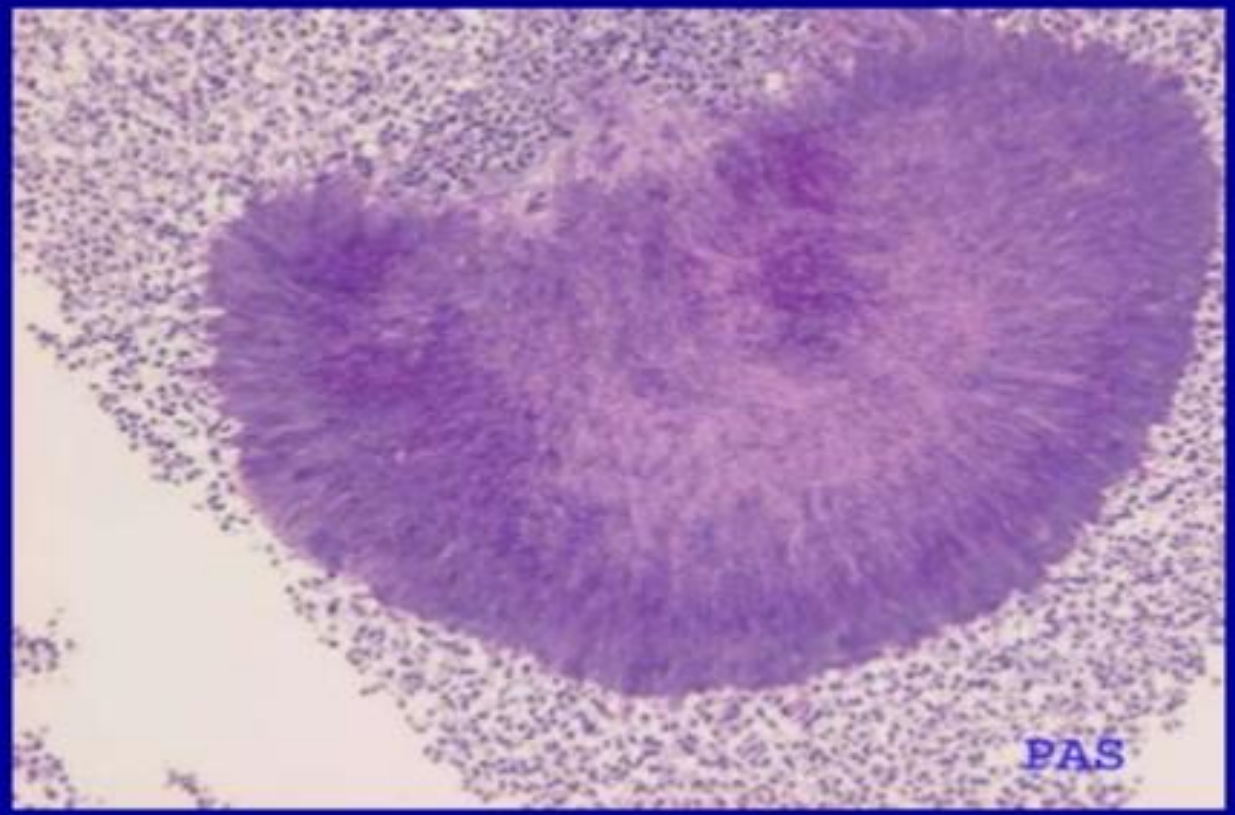
- Anaerobic Actinomycetes
  - Morphology and cultural characteristics
    - Gram positive branching, or diphtheroid-like bacilli
    - Anaerobic and require CO_2 for growth
    - Non-sporing
    - Grows well on Blood Agar.

Actinomyces – Gram stain

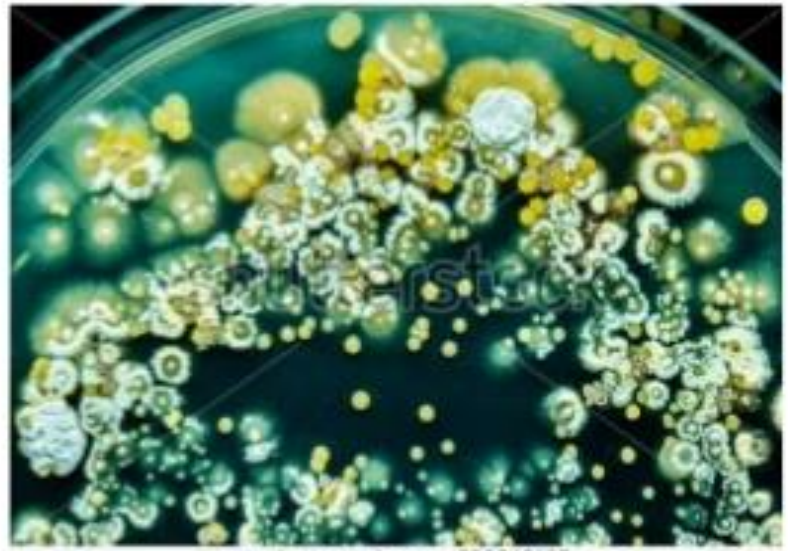


Actinomycetes - Gram staining

Ray fungus



Acrtinomycetes - culture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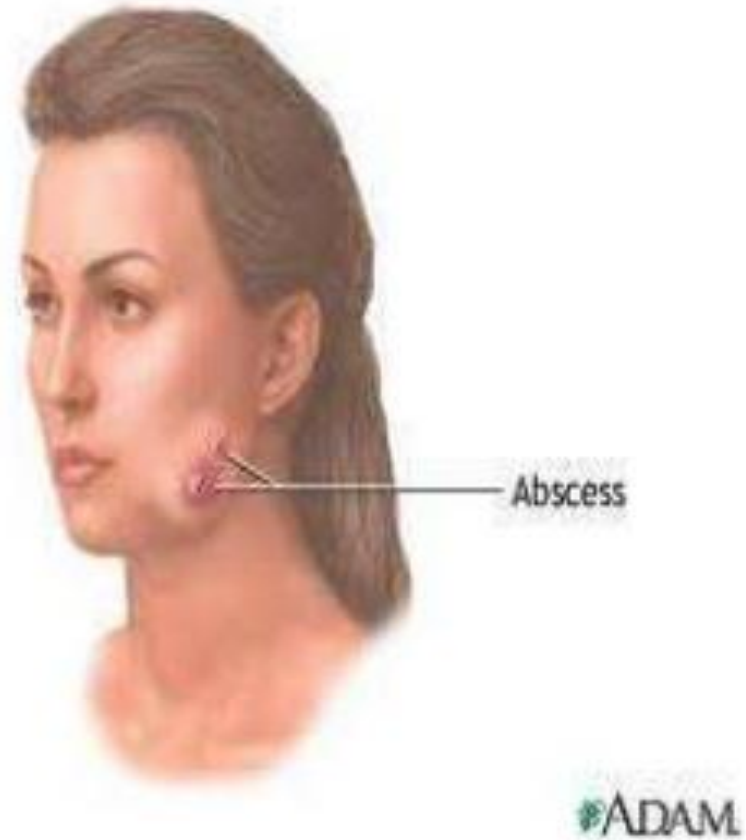
Actinomyces

- Clinical significance
  - Part of the normal oral bacterial flora in humans and animals.
  - Three clinical types
  - Cervico facial actinomycosis or "lumpy jaw"
    - » occur following tooth extractions or dental surgery
    - » rare today because of prophylactic antibiotic therapy
  - Thoraco Lumbar actinomycosis
  - Abdominal actinomycosis
  - Meningitis, endocarditis, or genital infections

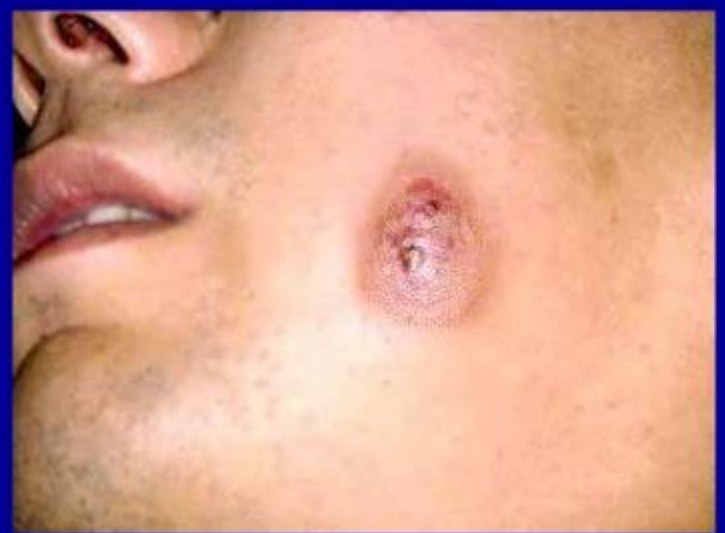
Actinomycosis

- Characterized by draining sinuses,
- containing characteristic granules
  - which are micro colonies of bacteria
  - look like dense rosettes of club-shaped filaments in radial arrangement
- Ray fungus

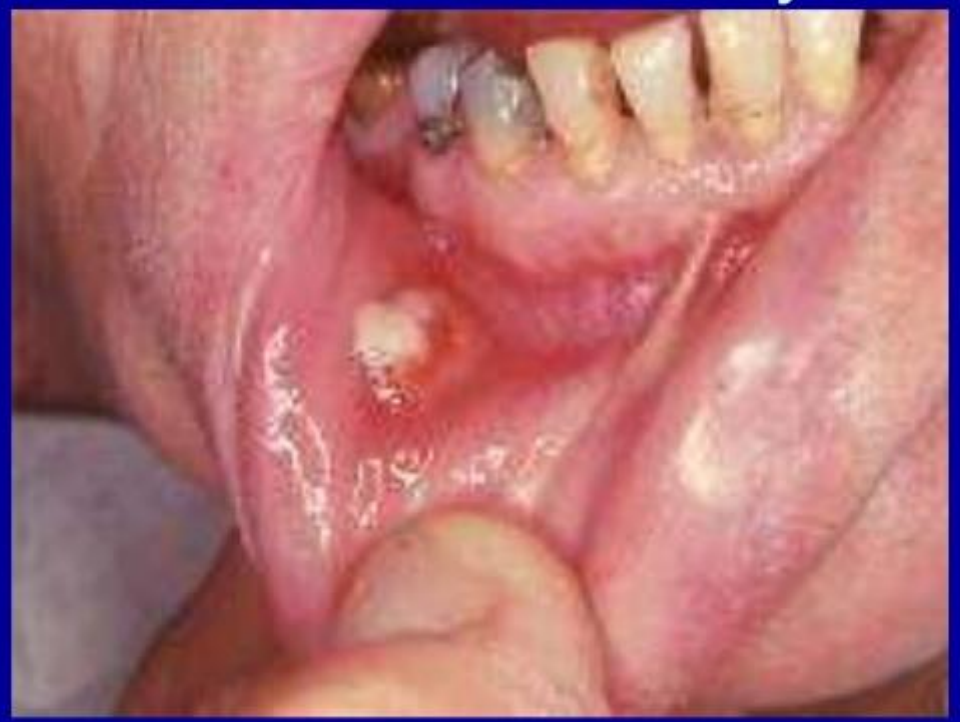
Clinical presentation



Cervicofacial Actinomycosis



Cervicofacial Actinomycetes



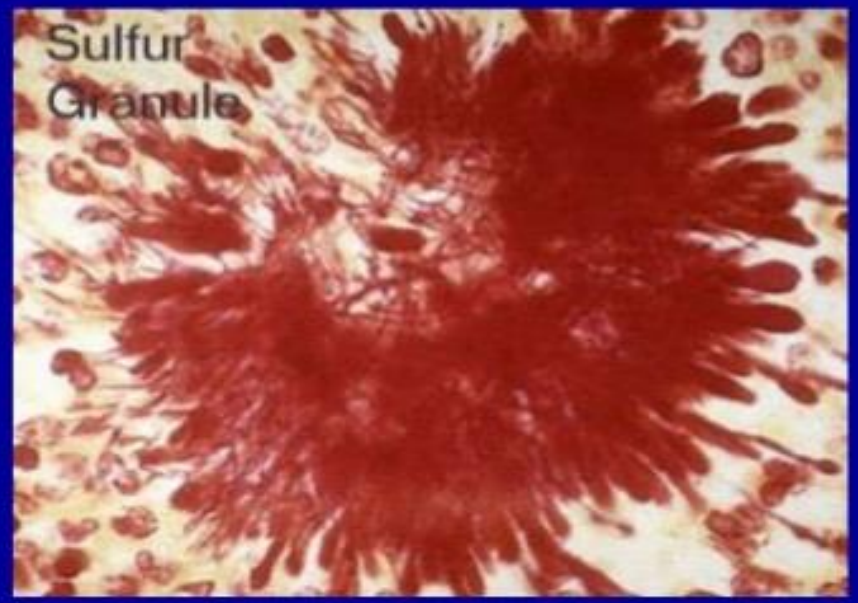
Actinomycosis – Lab Diagnosis

- Macroscopic examination of Granules
- Microscopy
  - Gram stain
- Isolation / Anaerobic Culture
- Serology – Not useful
- Molecular diagnostic tests - PCR

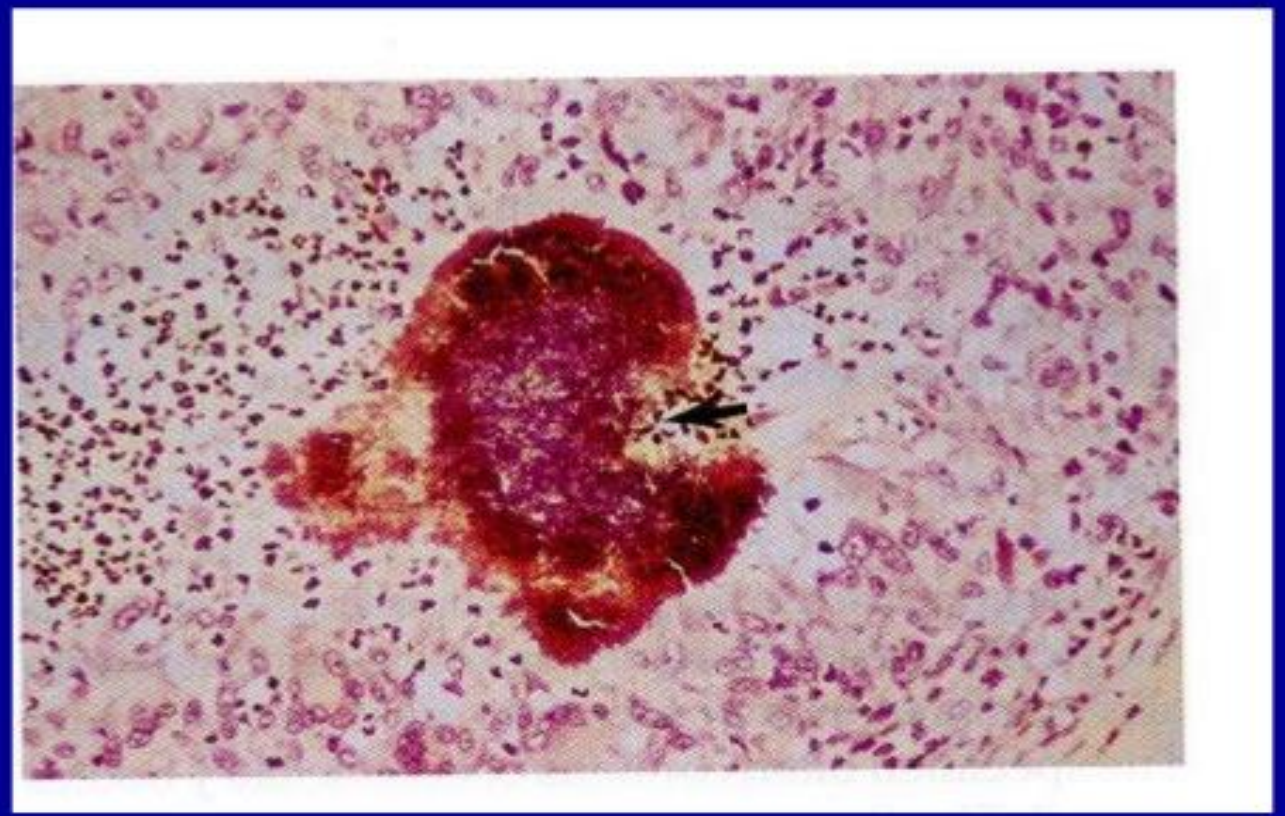
Macroscopic examination of Granules

- Yellow in colour (Hence the name Sulur granules)
- But may be white / brown
- Firm and round
- Size : 0.5 - 5mm in diameter

Sulfur granule



Granules



Treatment - Actinomycosis

- Penicillin

Aerobic Actinomycetes

Nocardia
Actinomadura
Streptomyces

Nocardia - Taxonomy

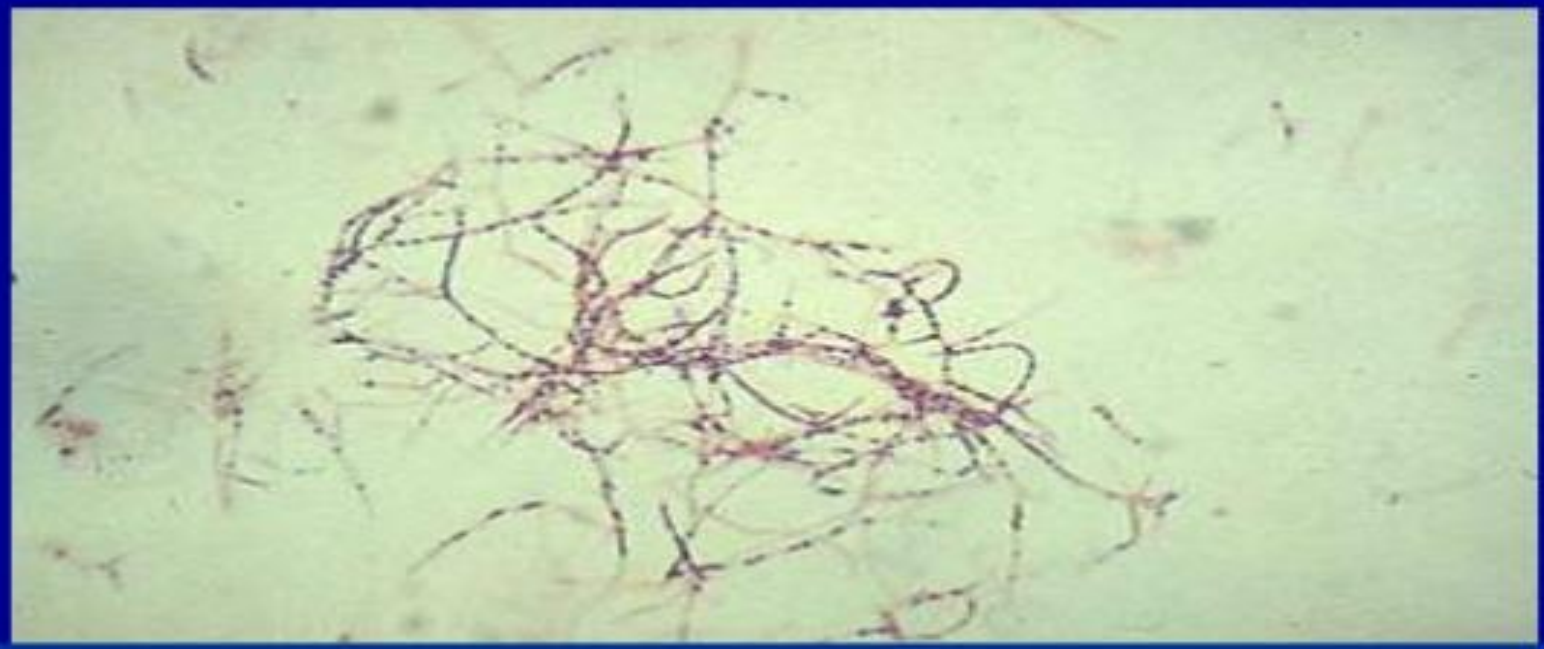
- (Domain): Bacteria
- (Kingdom): Actinomycetota
- (Class): Actinomycetia
- (Order): Mycobacteriales
- (Family): Nocardiaceae
- (Genus): Nocardia
- (Species): ***N.asteroides, N.brasiliensis etc.***

Nocardia spp.

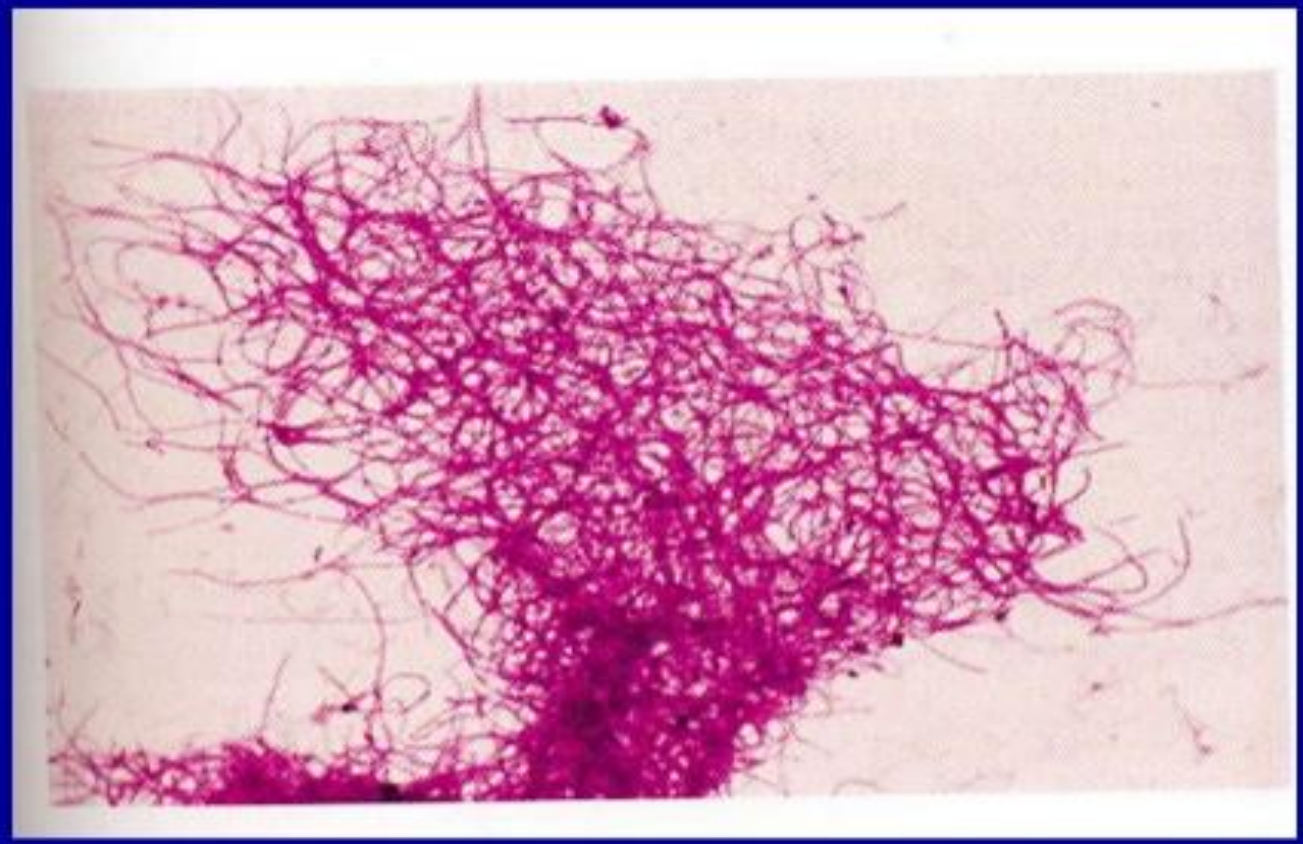
Three clinically important species

- *N. asteroides,*
- *N. brasilensis*
- *N. caviae*
  - Morphology and cultural characteristics
    - Gram positive branching filamentous bacteria
    - May fragment to bacillary or coccoid forms
    - Aerobic
    - The organisms are weakly acid fast or non acid fast
    - Ubiquitous in soil

Nocardia – Gram stain



Nocardia acid fast stain



Culture

- Nocardiae grow on blood agar, although growth is better on enriched media including Löwenstein-Jensen medium, brainheart infusion agar and Sabouraud's dextrose agar containing chloramphenicol as a selective agent.

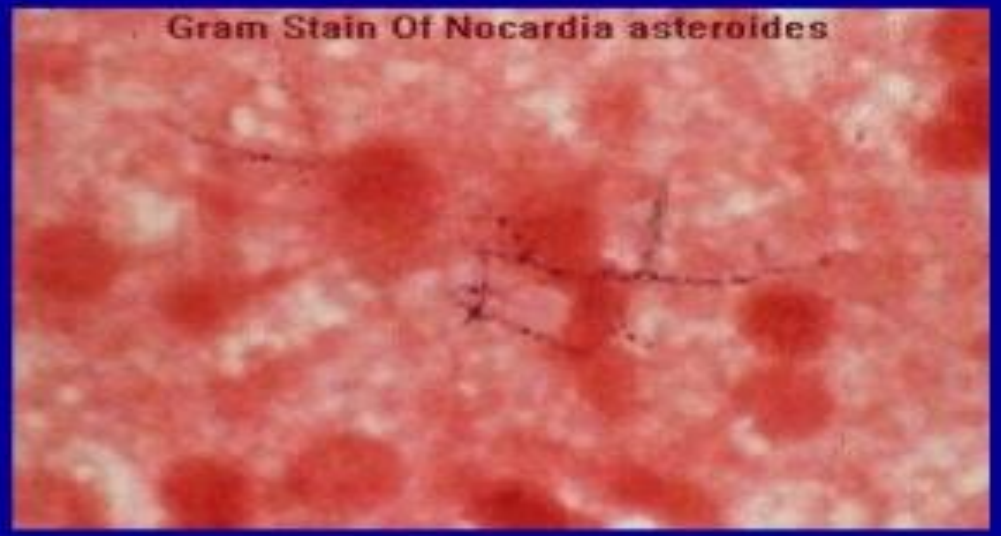
- Growth is visible after incubation for between 2 days and 1 month; selective growth is favoured by incubation at 45°C. Colonies are cream, orange or pink coloured; their surfaces may develop a dry, chalky appearance, and they adhere firmly to the medium

- On tap-water agar, *Nocardia* species have recursively branching hyphae with aerial hyphae.

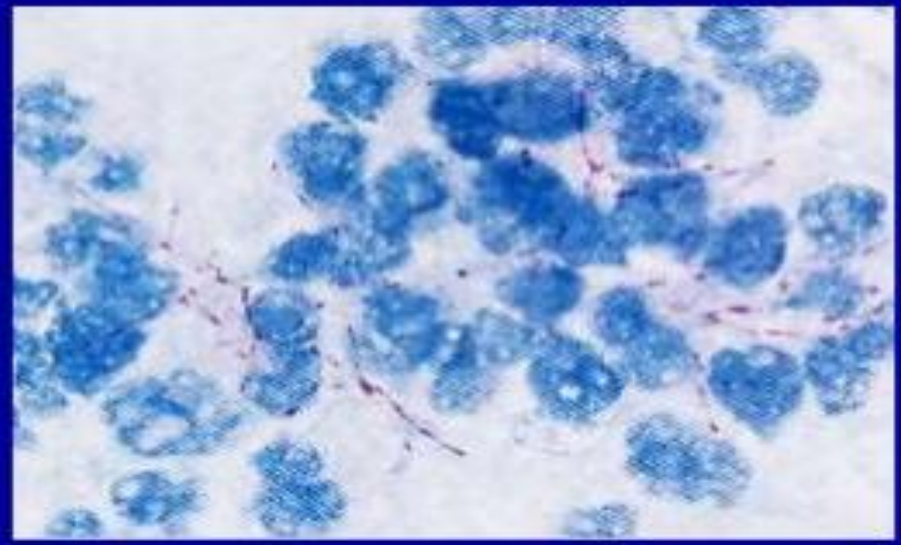
Culture character

- Plate culture of the bacteria Nocardia asteroides grown on 7H10 agar plates at 37° C.
- Media: Nutrient agar, sabouraur agar, brain heart in fusion agar, yeast extract malt extract agar.
- Specimens with mixed flora can over grow the Nocardia colonies
- Selective media may increase yield:
  - **Thayer-Martin agar with antibiotics**
  - **paraffin agar.**
  - Buffered charcoal-yeast extract (BCYE) medium

Nocardia spp.



Gram staining



Modified Acid Fast Staining

Nocardia culture on Blood Agar

Biochemical test

- hydrolysis of casein, tyrosine, and/or xanthine, (2) presence of urease, (3) utilization of rhamnose, and (4) positive resistance to lysozyme.

Table 1: Hydrolysis Tests for differentiating *Nocardia* strain

	Casein hydrolysis	L-tyrosine hydrolysis	Xanthine hydrolysis
N. asteroides complex, N. farcinica, or N. nova	-	-	-
N. brasiliensis	+	+	-
N. otitidis	-	+	-
N. caviae	-	-	+
Streptomyces or Nocardiopsis	+	+	+

Spectrum of illness

- Skin / Soft tissue infections most common presentation
- Can spread hematogenously in rare cases
  - CNS & pulmonary System
- Persons with impaired host defense are more likely to develop systemic disease

Nocardiosis – Clinical manifestations

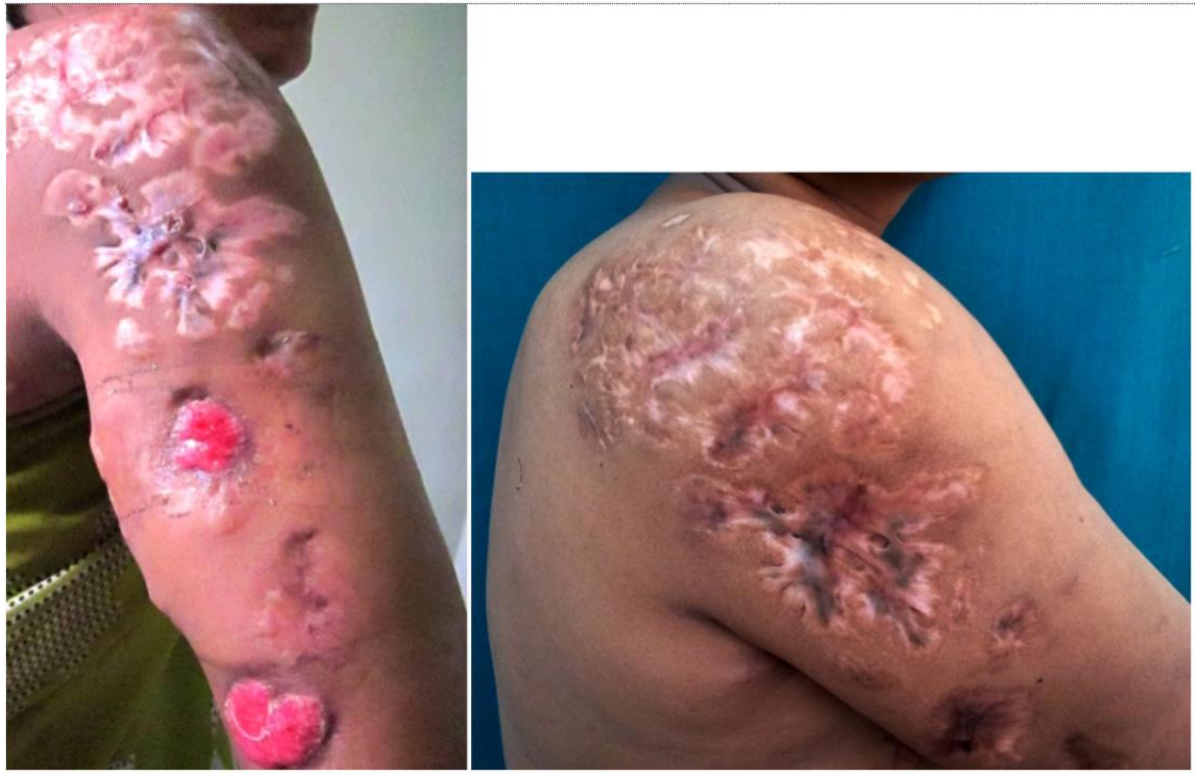
- Three broad types
  - Mycetoma
    - Most common cutaneous manifestation of *N. brasiliensis* worldwide
    - Chronic, indurated, granulomatous masses, mostly found on the Lower Extremities
    - Draining nodules & sinuses that contain sulfur granules
    - tend to invade underlying connective tissue, muscle, bone

Nocardiosis – Clinical manifestations

- Localized cutaneous Nocardiosis
  - Cellulitis, subcutaneous abscesses, pustules, pyoderma, ulcerations
- Pulmonary Nocardiosis
- Lymphocutaneous Nocardiosis
  - Also called the "sporotrichoid" form of nocardiosis
  - Rare

Nocardia asteroides

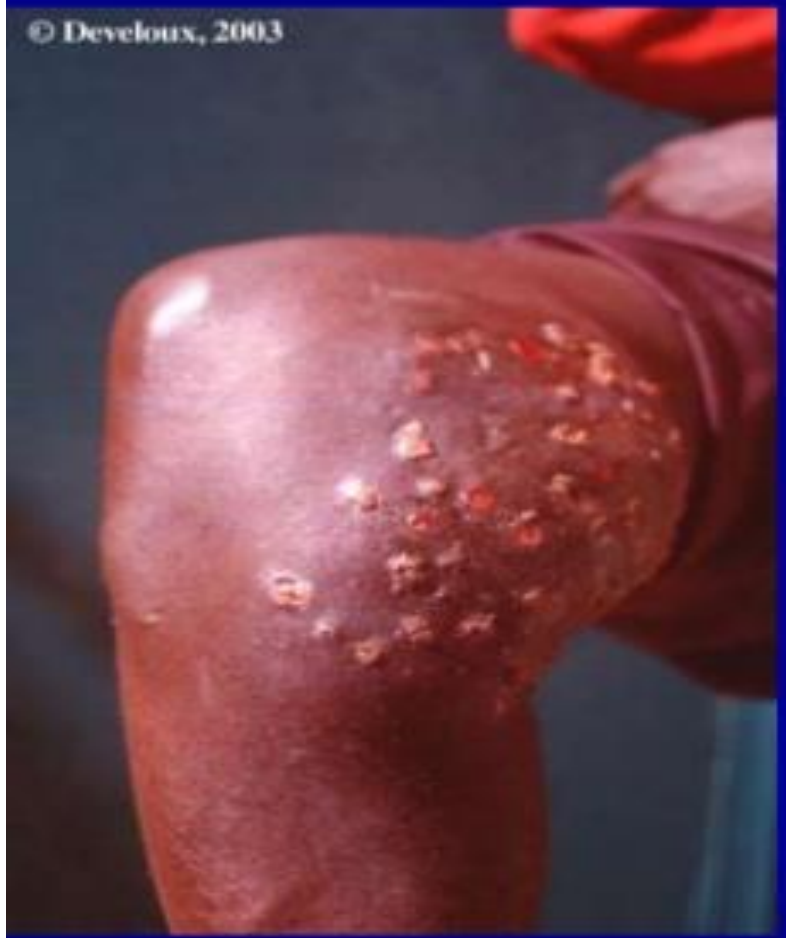
Nocardia brasiliensis

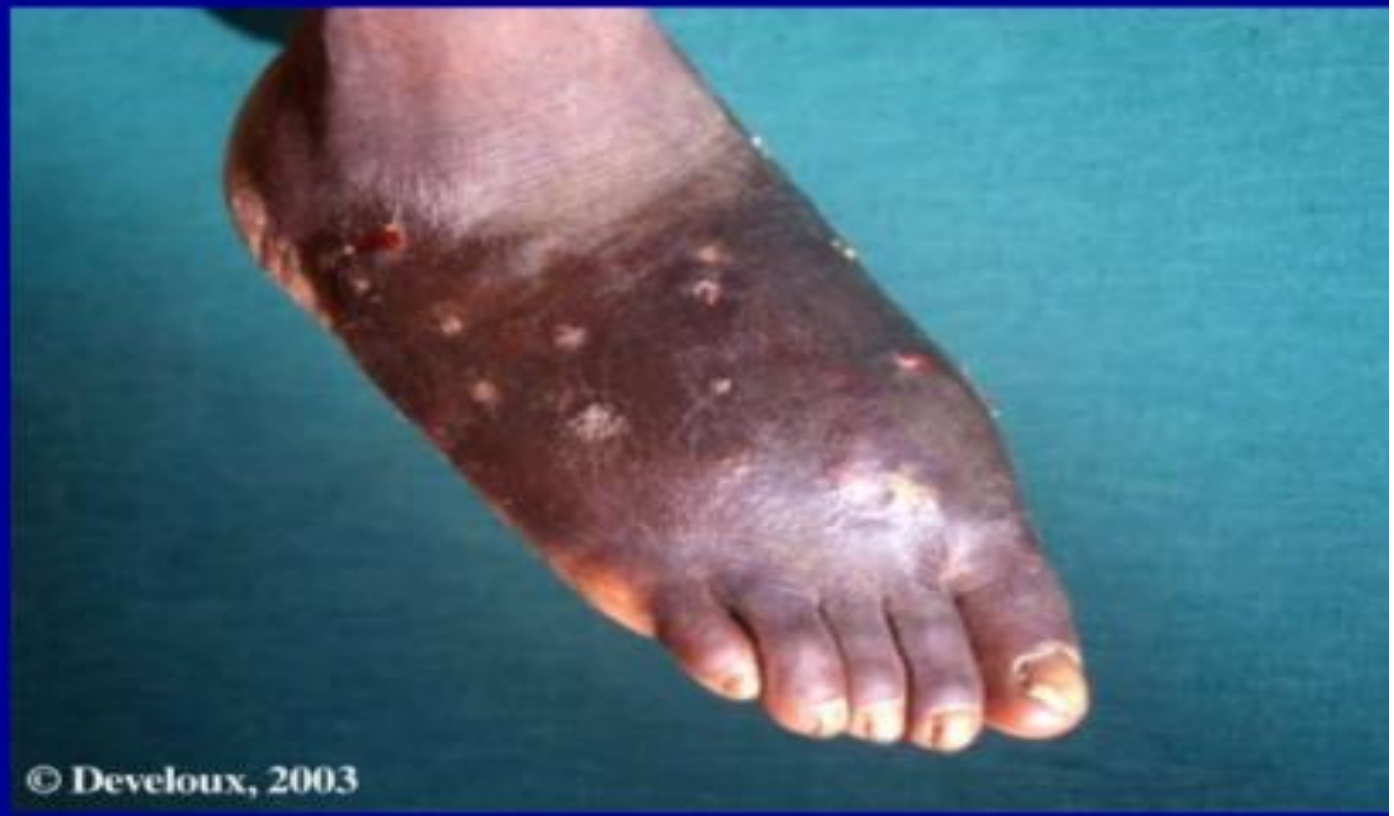


Mycetoma

- Organism enters the body through breaks in the skin
- Causes a localized infection involving skin, cutaneous, and subcutaneous tissue.
- The three most characteristic features seen in Mycetoma
  - » **swelling (Tumifaction)**
  - » **draining sinuses**
  - » **granules**
  - – This disease can also be caused by fungi

Mycetoma –Nocardia spp.





Nocardiosis

- Pulmonary nocardiosis
  - Localized or disseminated disease
  - Occurring after inhalation of organisms.
  - Pulmonary infections resemble tuberculosis
  - May disseminate to brain and meninges
  - Usually a disease of compromised hosts.

Laboratory Diagnosis

- Macroscopic examination of granules
- Direct Microscopy
  - Gram Staining
  - Ziehl Neelsen's staining
- Culture
  - Specimens should be inoculated onto
    - **7H10 agar**
    - **Lowenstein-Jensen agar**
    - **Brain heart infusion agar**
  - Orange, dry, crumbly, and adherent colonies
- Serology – Not useful
- Molecular Diagnosis - PCR

Nocardiosis - Treatment

- Mycetoma – aminoglycosides
- Pulmonary Nocardiosis – sulfonamides

Nocardiosis - Treatment

- Optimal duration of tretment not known
- Clinical outcome related to the duration of antibiotic therapy
- Tendency of Nocardia to recur
  - Treatment best continued for 3-12 months.
    - **depending on severity of disease**
    - **immune status of pt.**
  - Immunocompromised hosts, consider indefinite low-dose prophylaxis after full-dose therapy is completed

Other causes of actinomycotic mycetoma

- Actinomadhura madhurae
- Actinomadhura palletieri
- Streptomyces somaliensis