Mycobacterium sp

General characters:

1- They are slender acid fast bacilli (Gram positive but cannot stain), strict aerobic, non-spore forming and non capsulated.

2- Usually can not grow in ordinary culture media and require special media.

3- Slow growing bacteria 3-8 weeks.

4- Important species to human are
A- M. tuberculosis (T.B)
B- M leprae (Leprosy venearal disease, STD (syphilis)
C- M.bovis (zoonotic and form BCG vaccine)
D- Atypical mycobacteria or non-tuberculous Mycobacteria like M.avium-intracellular complex (common in AIDS patients).

MYCOBACTERIUM TUBERCULOSIS

Morphology and Identification

A. Typical Organisms

- **Are slender, straight or slightly curved bacilli occurring in single, pairs or bundles (sputum) as shown in figure 1.**
- **They are acid fast bacilli in which** 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) or 20% sulfuric acid in alcohol quickly decolorizes all bacteria except the mycobacteria (**Ziehl-Neelsen stain)**. Acid fastness depends on the integrity of the cell wall.
- Can be stained by fluorochrome stains(auramin and rhodamin) and glow yellow-orange by fluorescent microscope as shown in figure 2)

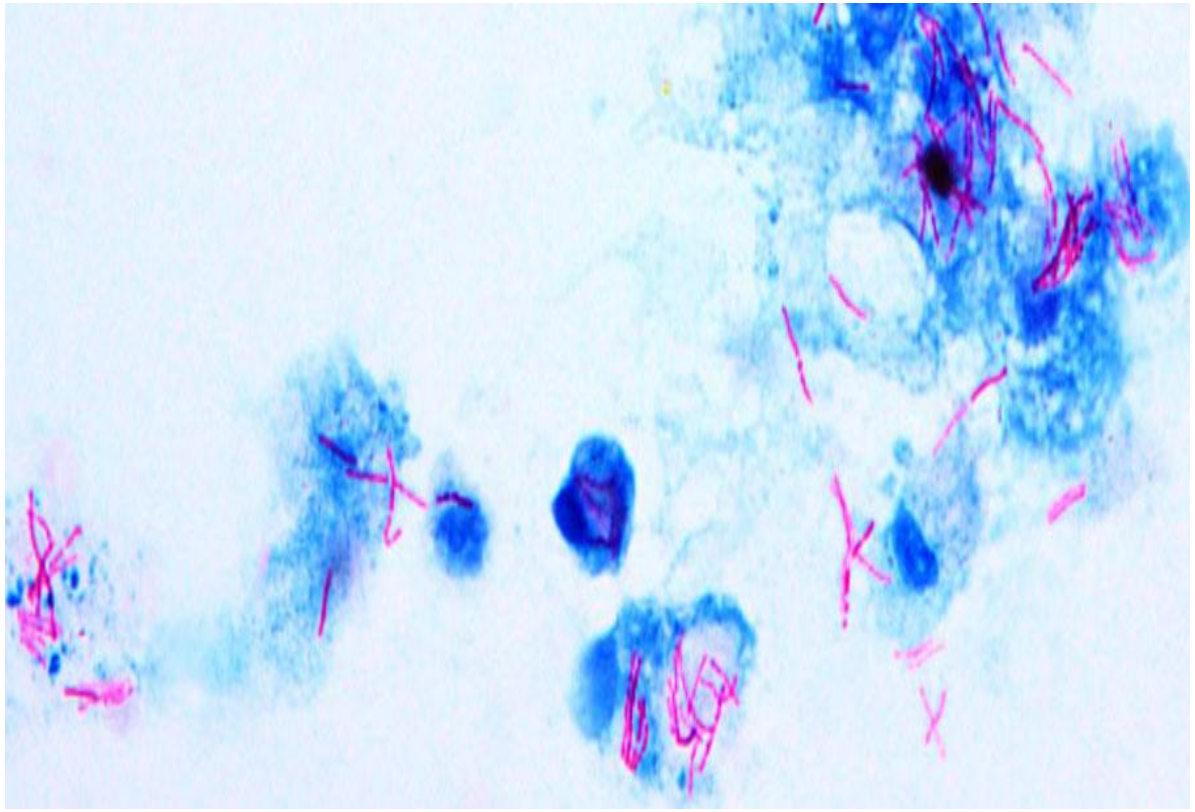

Fig.1. Acid fast bacilli from sputum

Fig.2. Yellow-orange glowing *M. tuberculosis* by fluorescent microscope.

B. Culture

1- Agar based media such as Middlebrook 7H10 and 11 with added antibiotics to prevent contamination. It is more transparent than egg based media so small colonies are can easily be detected

2- Egg based solid media (egg yolk) like Lowenstein-Jensen media with added antibiotics and malachite green.

3- Broth media like Middlebrook 7H9. Growth is often more rapid than on complex media. There are several commercial sources of these media that are used in many clinical and reference laboratories.

C. Growth Characteristics

1- Aerobic but 5-10% enhances growth

2-Slow growing bacteria with doubling time 18 hrs and growth take 2-8 weeks. Growth faster in broth (4-25 days than agar

3- The optimum temperature for growth is 37 C (No growth below 25C and more than 40 C)

4- Saprophytic forms tend to grow more rapidly, to proliferate well at 22–33°C

D. Reaction to Physical and Chemical Agents

Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth. Dyes (eg, malachite green) or antibacterial agents (eg, penicillin) that are bacteriostatic to other bacteria can be incorporated into media without inhibiting the growth of tubercle bacilli. Acids and alkalies permit the survival of some exposed tubercle bacilli and are used to help eliminate contaminating organisms and for "concentration" of clinical specimens like sputum to eliminated normal flora. Tubercle bacilli are resistant to drying and survive for long periods in dried sputum.

Temperature 60°C for 20 minutes can kill it (pasteurization). Moist heat at 100°C kill it readily. In sunlight the culture may be killed in 2 hours (TB occurs mainly among poor people who living in dark and damp houses).

Main components of Mycobacterium cell wall

A. Lipids

Mycobacteria are rich in lipids. These include mycolic acids ,waxes, and phosphatides.

In the cell, the lipids are largely bound to proteins and polysaccharides (glycolipids).

Functions:

A- Glycolipids responsible for granuloma formation (no pus) and serpentine cords (bacilli arranges in parallel chains)

B- Glycolipids inhibit migration of PMN cells.

C- responsible for acid fastness

D- Sulfatids and glycolipids inhibit phagolysosomal formation allowing the bacteria to survive inside the macrophages after being engulfed or prevent lowering of pH inside the phagolysosomes, therefore hydrolytic enzymes no longer discharged and worked

Analysis of lipids by gas chromatography reveals patterns that aid in classification of different species.

B. Proteins

Purified protein derivate of M.tuberculosis is used antigen in tuberculin skin test which elicit delayed type hypersensitivity reaction in sensitized persons (vaccinated and exposed).

Types of T.B
1- Localized T.B most common is pulmonary T.B (Fatigue, weakness, weight loss, fever, and night sweats with chronic cough tinged with blood=hemoptysis) or Tubercle meningitis or renal TB or Bone TB
2- Severe systemic generalized TB called military TB in which small tubercle lesions occurs in most body organs.

The lesions are called tubercles which granulomatous lesions with giant cells containing T.B bacilli in the center surrounded by epitheloids, lymphocytes and monocytes. The whole lesion may surrounded by a fibrous capsule and

In Pulmonary T.B infection there is no bacteremia and septicemia and no toxin production just hypersensitivity reaction of body cells like macrophages and lymphocytes that release inflammatory cytokines.

Routes of infection

M.Tuberculosis by droplet infection (inhalation)

M.bovis by ingestion

Atypical Mycobacterium sp. by skin trauma

Diagnostic Laboratory Tests

A. Specimens

Specimens consist of fresh sputum, gastric washings, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy material and blood (HIV patients) so depends on the type of clinical presentation.

The following criteria should be taken into consideration

- Volume of the sample: Large volumes are preferred because body fluids may contain few bacilli/ml so large volume increase the chance of recovery of the bacteria.
- The number of specimens also important. E.g samples of urine , gastric aspiration and urine should be submitted in 3-5 days.
- Timing: early morning samples are preferred because the organisms had been accumulated overnight.

In renal T.B three early morning urine samples are required in order to give the final decision

B. Decontamination and Concentration of Specimens

Specimens from sputum and other nonsterile sites should be liquefied with *N*-acetyl-L-cysteine decontaminated with NaOH (kills many other bacteria and fungi), neutralized with buffer, and concentrated by centrifugation. Specimens processed in this way can be used for acid-fast stains and for culture. Specimens from sterile sites, such ascerebrospinal fluid, do not need the decontamination procedure but can be directly centrifuged, examined, and cultured.

C. Smears

Sputum, exudate, or other material is examined for acid-fast bacilli by staining. Stains of gastric washings and urine generally are not recommended because saprophytic mycobacteria may be present and yield a positive stain. A negative report should not be given till at least 100 fields are examined taking about 10 minutes time. Fluorescence microscopy with auramine-rhodamine stain is more sensitive than traditional acid-fast stains, such as Ziehl-Neelsen, and is the preferred method for clinical material.

D. Culture, Identification, and Susceptibility Testing

A selective agar media (eg, Löwenstein-Jensen or Middlebrook 7H10/7H11 biplate with antibiotics) should be inoculated in parallel with broth media cultures. Incubation is at 35–37°C in 5–10% CO2 for up to 8 weeks. However, rapid growing mycobacteria may appear in 4 days' time.

Special broth media by automated systems like BACTEC 460 can rapidly detect M.tuberculosis (Figure 3)

Conventional methods for identification of mycobacteria include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles.

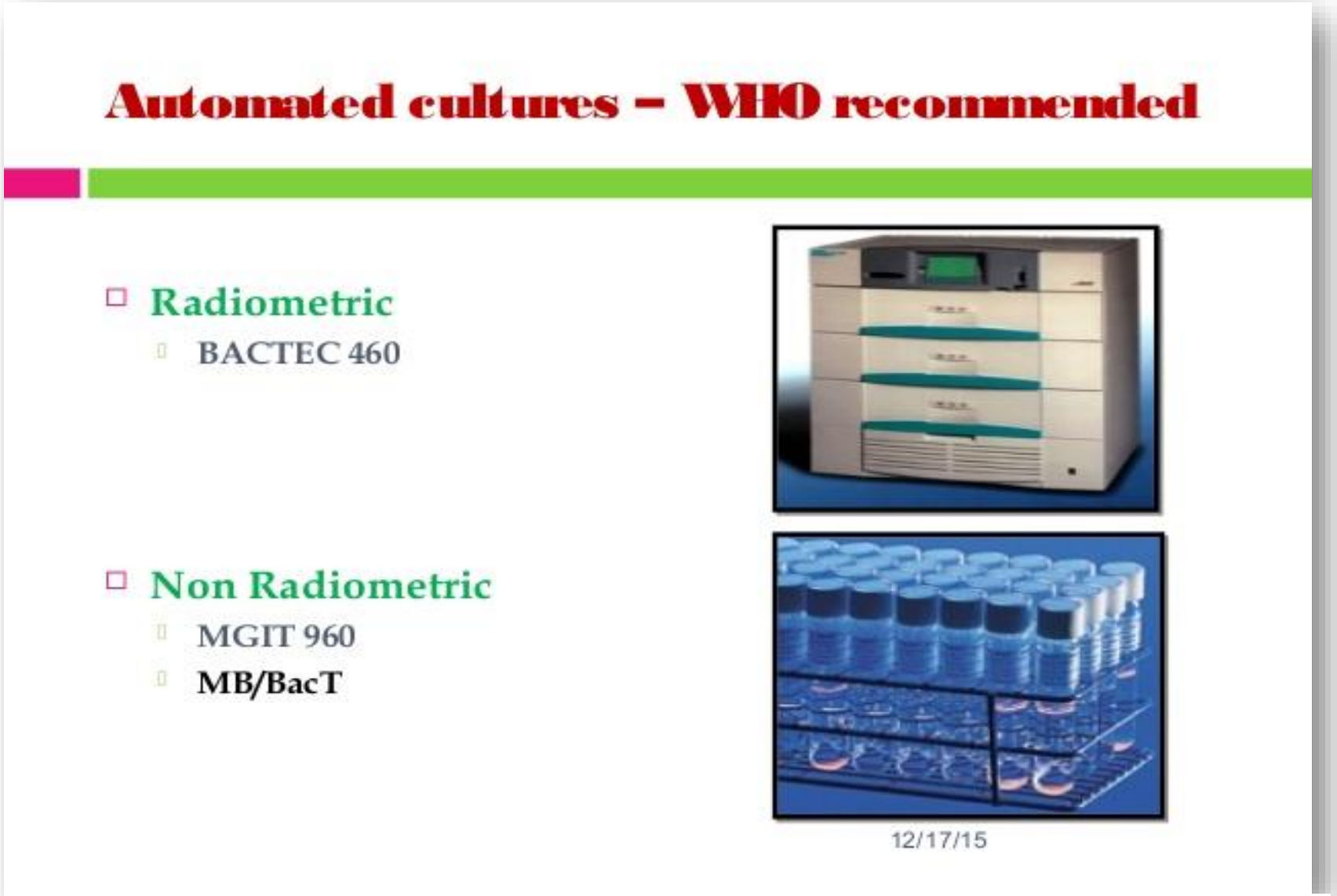

Fig.3. Automated radiometric culture systems for identification of *M.tuberculosis*

On solid media M.tuberculosis give rise to discrete, raised, irregular, dry and wrinkled colonies which creamy white in color (Figure 4) but those of bovine type are flat, white, smooth, moist colonies which break up more readily when touched (Figure 4).Table 1 shows the differences between human type and bovine type

	Test	*M. tuberculosis*	*M. bovis*
1.	Morphology	Long, thin and curved	Shorter, stout and straighter
2.	Staining	Barred and beaded	Uniform
3.	Growth	Eugonic	Dysgonic
4.	Action of glycerol on growth	Enhanced	Inhibited
5.	Colony	Dry, rough, raised and wrinkled	Moist, smooth and flat
6.	Progressive disease in rabbit	—	+
7.	Niacin production	+	—
8.	Nitrate reduction	+	—
9.	Growth in semisolid medium	Grows at surface (aerobic)	Grows 10 to 20 mm below surface (microaerophilic)

Acid fast stain smear from colonies: AFB

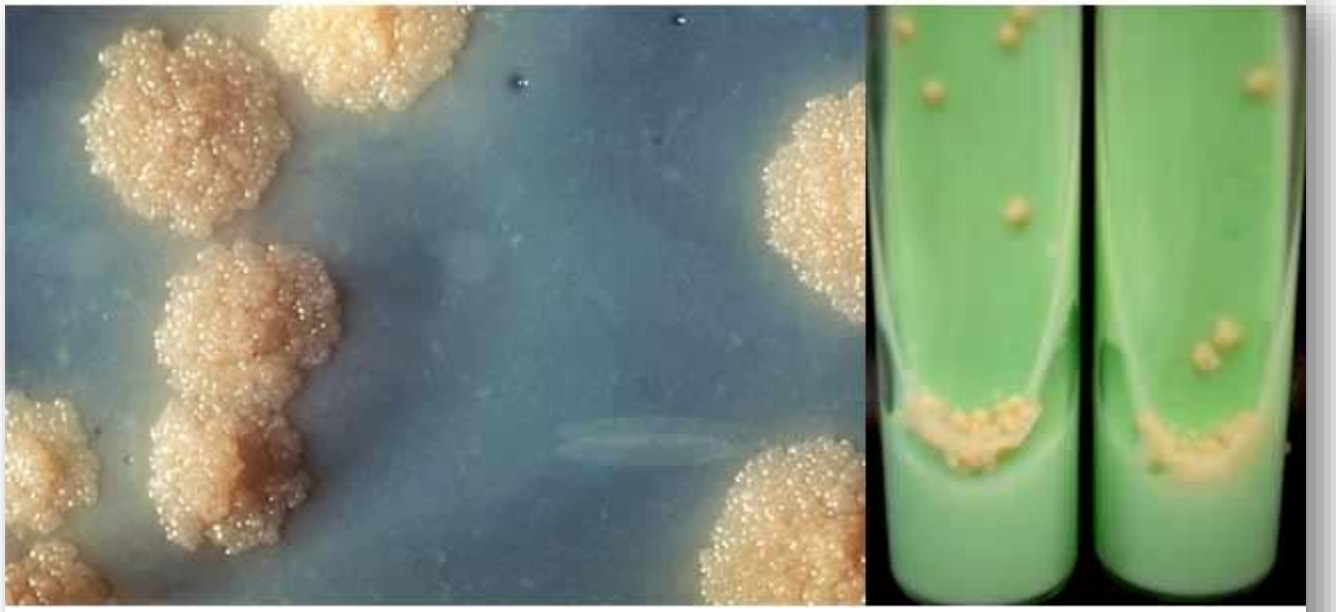

Fig.4. Cultural characteristics of *M.tuberculosis*

Biochemical tests

Niacin test is positive (Figure 5)
Aryl phosphatase is negative
Nitrate reduction is positive

Tuberculin test (intradermal PPD)
To detect persons exposed to TB

Molecular methods like DNA probe or by PCR technique

Atypical mycobacteria

Mycobacteria other than human and bovine tubercle bacilli that cause human disease resembling tuberculosis are called atypical mycobacteria or mycobacteria other than tubercle bacilli (MOTT). The important characters of this group are:

1- Low virulence (no cord factor)
2- Non pathogenic for guinea pigs
3- Environmental saprophytes
4- opportunistic pathogens
5- Not readily transmissible from man to man
6- Resistant to anti-tuberculous drugs
7- Some produce pigment in culture
8- Capable to grow at 22C
9- Negative for Aryl phosphatase
10- Niacin test are negative

Biochemical reactions

Niacin test

- *M. tuberculosis* lacks the enzyme that converts Niacin to Niacin ribonucleotide due to this large amount of Niacin accumulates in the culture medium

- Niacin is detected by addition of 10% cyanogen bromide and 4% aniline in 96% ethanol

- Positive reaction – canary yellow

- *M. tuberculosis* – Positive

- *M. bovis* - Negative

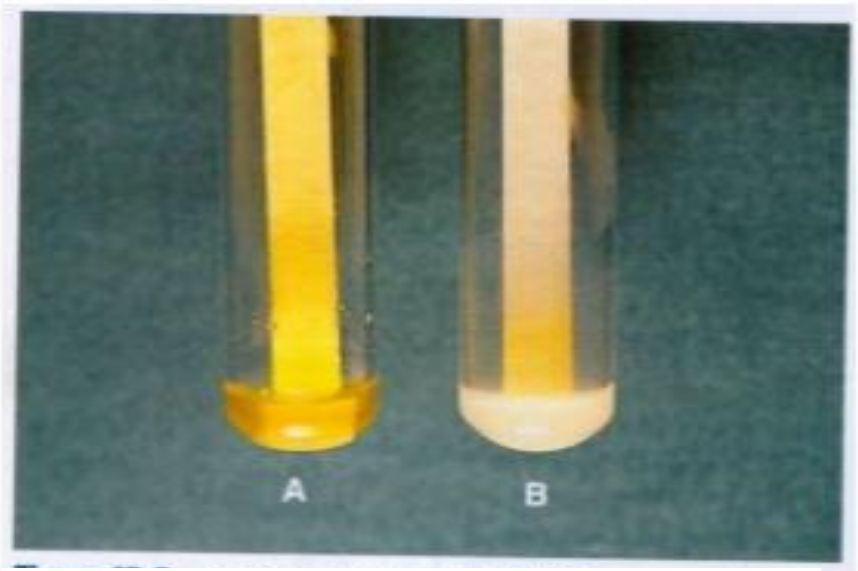

Fig.5. Niacin test

Atypical Mycobacteria are classified into 4 groups based on pigment production and rate of growth:

Group I: Photochrmogens produce pigment in light.

Group II: Scotochromogens produce pigment in the dark.

Group III: Nonchromogenes no pigment production neither in light nor in the dark.

Group IV: Rapid growers : Grow less than 7 days

Treatment

Multiple drugs used in order to prevent drug resistant which due to the mutation in bacterial drug receptors.
The first lines drugs are isoniazid, rifampin, ethambutol, pyrazinamide
Preferred regimen is the aforementioned drugs for 8 weeks
Afterward, maintenance therapy includes daily isoniazid and rifampin for 18 weeks

All children during 1st week of age receive BCG (Bacille, Calmette and Guarin) vaccine (attenuated M.bovis)

MYCOBACTERIUM LEPRAE or Hansen's Bacilli

Cause leprosy disease

It has not been cultivated on nonliving bacteriologic media.

Only grow when inoculated the footpad of mice or armadillo

Typical acid-fast bacilli—singly, in parallel bundles, or in globular masses—are regularly found in scrapings from skin or mucous membranes (particularly the nasal septum) in patients with lepromatous leprosy.

It is weakly acid fast (5% H_2SO_4 is used for decolorization).

Clinical Findings

Lepromatous type, the course is progressive and malignant, with nodular skin lesions; slow, symmetric nerve involvement; abundant acid-fast bacilli in the skin lesions; continuous bacteremia; and a negative lepromin (extract of lepromatous tissue) skin test result.In lepromatous leprosy, cell-mediated immunity is markedly deficient.

In the tuberculoid type, the course is benign and nonprogressive, with macular skin lesions, severe asymmetric nerve involvement of sudden onset with few bacilli present in the lesions, and a positive lepromin skin test result. cell-mediated immunity is intact.

Transmission of leprosy is most likely to occur when small children are exposed for prolonged periods to heavy shedders of bacilli. Nasal secretions are the most likely infectious material for family contacts. The incubation period is probably 2–10 years.

Diagnosis

Scrapings with a scalpel blade from skin or nasal mucosa or from a biopsy of earlobe skin are smeared on a slide and stained by the Ziehl-Neelsen technique. No serologic tests are of value.

Treatment

Dapsone is the mainstay of antibacterial treatment of leprosy and is administered for at least 3 to 4 years.