Assist. Prof. Dr. Ameer Mezher Hadi
Dr.Zaid Abdel Hadi Abd
Lecture. 14
Second Stage

College of Health & Medical Techniques
Department of Medical Laboratories Techniques

MYCOBACTERIUM

Mycobacterium

Table-1. Classification of mycobacteria

Tubercle bacilli
1. Human—*M. tuberculosis*
2. Bovine—*M. bovis*
3. Murine—*M. microti*
4. Aviam—*M. avium*
5. Cold blooded—*M. marinum*

Lepra bacilli
Human—*M. leprae*
Murine—*M. lepraemurium*

Mycobacteria causing skin ulcers
1. *M. ulcerans*
2. *M. balnei*

Atypical mycobacteria
1. Photochromogens
2. Scotochromogens
3. Nonphotochromogens
4. Rapid growers

Johne's bacillus
M. paratuberculosis

Saprophytic mycobacteria
M. butyricum, M. phlei, M. stercoris.

Morphology

M. tuberculosis is a slender, straight or slightly curved rod with rounded ends, about 3 μm × 0.3 μm, in pairs or as small clumps. The bacilli are non-motile, non-sporing, non-capsulated and acid-fast. They are gram-positive but are difficult to stain.

When stained with carbol fuchsin by the Ziehl-Neelsen method, they resist de-colorization by 20 percent sulfuric acid and absolute alcohol for 10 minutes **(acid and**

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alcohol fast). With this stain, the *Tubercle bacilli* stain **bright red,** while the tissue cells and other organisms are stained blue (Fig. 1). Organisms in **tissue** and **sputum smears** often stain irregularly and have a beaded or barred appearance, presumably because of their **vacuoles** and **polyphosphate** content.

Acid fastness has been ascribed to the presence in the bacillus of **mycoloic acid.** It is related to the **integrity of the cell** and appears to be a property of the lipid-rich waxy cell wall. Staining may be uniform or granular. In *M. tuberculosis* beaded or barred forms are frequently seen, but *M. bovis* stains more uniformly. *M. bovis* appear straighter, bolder and shorter with uniform staining.

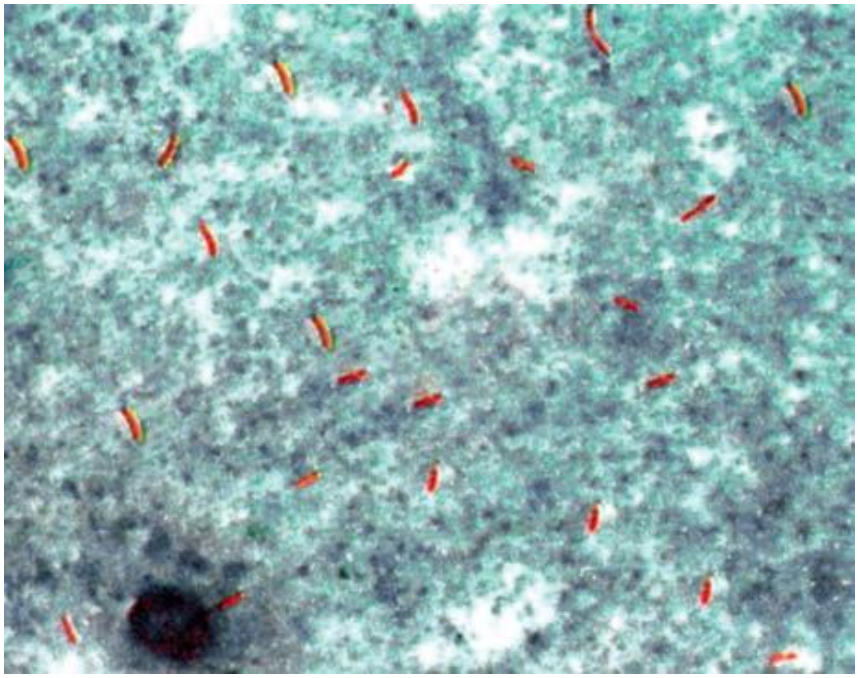

Figure-1: *Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear

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Cultural Characteristics

M. tuberculosis is an **obligate aerobe** while *M. bovis* is **microaerophilic** on primary isolation, becoming aerobic on subculture. The optimal growth temperature of tubercle bacilli is 35 to 37°C but they fail to grow at 25°C or 41°C. Most other mycobacteria grow at one or other, or both, of these temperatures. Optimum pH is 6.4 to 7.0. The bacilli grow slowly, the **generation time** *in vitro* being **14** to **15** hours. Colonies appear in about **two weeks** and may sometimes take up to **eight weeks**.

The solid medium most widely employed for routine culture is **Lowenstein-Jensen (LJ) medium** without starch.

Human tubercle bacilli produce visible growth on LJ medium in about 2 weeks, although on primary isolation from clinical material colonies may take up to 8 weeks to appear. On solid media, *M. tuberculosis* forms **dry**, **rough**, **raised**, **irregular** colonies with a **wrinkled** surface. They are **creamy white**, **becoming yellowish or buff colored on further incubation**. They are tenacious and not easily emulsified. *Mycobacterium tuberculosis* has a luxuriant growth **(eugenic growth***)* as compared to *Mycobacterium bovis* which grows poorly on LJ glycerol medium **(dysgenic growth)** and colonies, in comparison are **flat** , **smooth**, **moist**, **white** and **break up** easily when touched. The growth of *M. bovis* is much better on LJ pyruvate medium (media containing sodium pyruvate in place of glycerol).

Antigenic Structure

Mycobactria contain many unique immune-reactive substances, most of which are components of the cell wall. Mycobacteria possess two types of antigens, **cell wall** (insoluble) and **cytoplasmic** (soluble) antigens.

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1. Cell wall antigens

The basic structure of the cell wall is typical of gram-positive bacteria: an inner cytoplasmic membrane overlaid with a thick peptidoglycan layer and no outer membrane. The cell wall consists of **lipids**, **proteins** and **polysaccharides**. These lipids constitute **60%** of the cell wall weight and contributes to several biological properties. Lipids of the cell wall particularly **mycolic acid** fraction جزء are responsible for acid-fastness of bacteria and the cellular reaction of the body. The cell wall is made up of four distinct layers (Fig. 2).

(i) Peptidoglycan (murein) layer

(ii) Arabinogalactan layer

(iii) Mycolic acid layer

(iv) Mycosides (peptidoglycolipids or phenolic glycolipids)

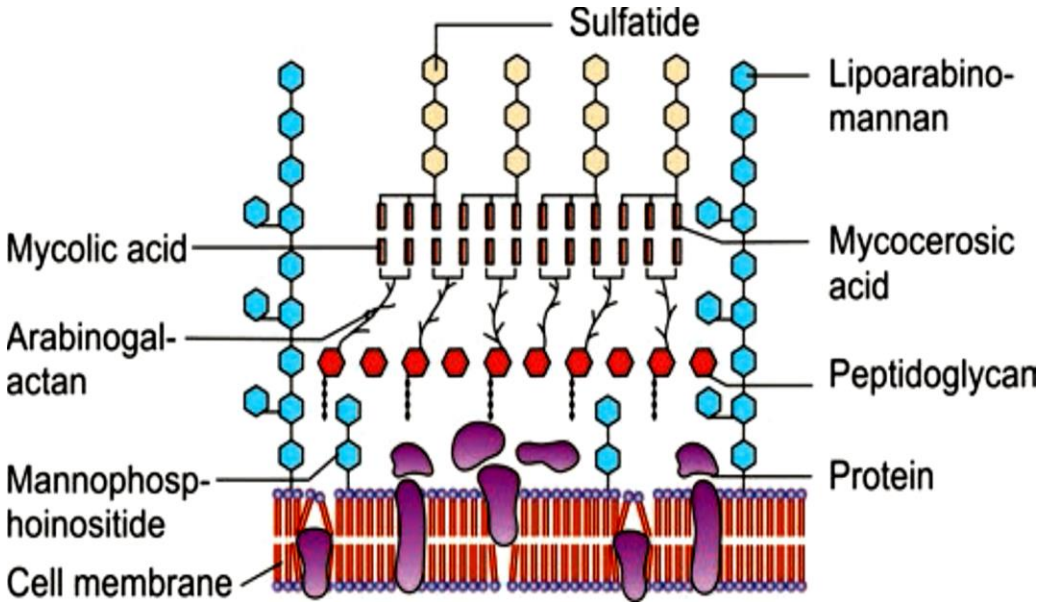

Figure – 2: Cell wall of *Mycobacterium tuberculosis*

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Tabl-1: Biochemical characteristics of Mycobacterium species

Test	*M. tuberculosis*	*M. bovis*	*Atypical mycobacteria*
Production of niacin	+	–	–
Binding of neutral red	+	+	+/–
Hydrolysis of Tween 80	–	–	+
Production of enzymes:			
• Nitrate reduction	+	–	+/–
• Arylsulphatase	–	–	–/+
• Catalase at room temp	–	–	+
at 68°C	–	–	+
• Catalase-Peroxidase	Weak +	Weak +	Strong +
Nicotinamidase	+	–	–
• Pyrazinamidase	+	–	+/–
Susceptibility to:			
• Pyrazinamide	+	–	–
Uptake of iron	–	–	–/+

Specimen Collection

Persons suspected of having pulmonary or laryngeal TB should have at least three sputum specimens examined by smear and culture. It is best to obtain a series سلسلة of early-morning specimens collected on 3 consecutive متعاقب days. Specimens should be obtained in an isolated, well-ventilated area or a sputum collection booth.

For patients unable to cough up sputum, deep coughing may be induced by inhalation of an aerosol of warm, hypertonic (5%-15%) saline. Patients should be given time — 15 minutes is usually sufficient — to produce sputum, which is usually brought up by a deep

cough. Because induced sputum is very watery and resembles saliva, it should be labeled "induced" to ensure that the laboratory staff do not discard it.

Bronchoscopy can be done if there is suspicion of TB and the patient cannot cough up sputum.

Gastric aspiration can also be used to obtain specimens of swallowed sputum.

During specimen collection, patients produce an aerosol that may be hazardous to health care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures.

Because TB can occur in almost any anatomical site, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid, pleural fluid, pus, or biopsy specimens) may be submitted for examination when extra-pulmonary TB disease is suspected. Tissue specimens for the culture of *M. tuberculosis* should be placed in a transport medium (e.g., Dubos) or a normal saline solution. Formalin or other preservatives should not be used because these solutions kill or inhibit the growth of *M. tuberculosis*.