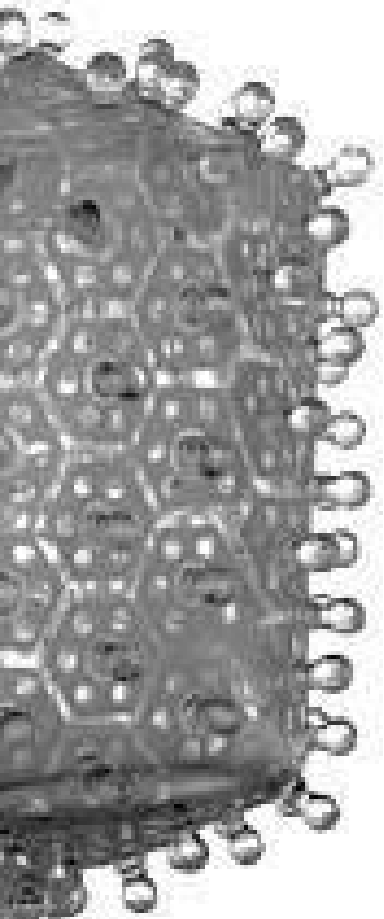

# Microbiology

Lecture No:....26.....

Dr Name:...Dr. Asem....

Done by:....Hasan Hammo.....

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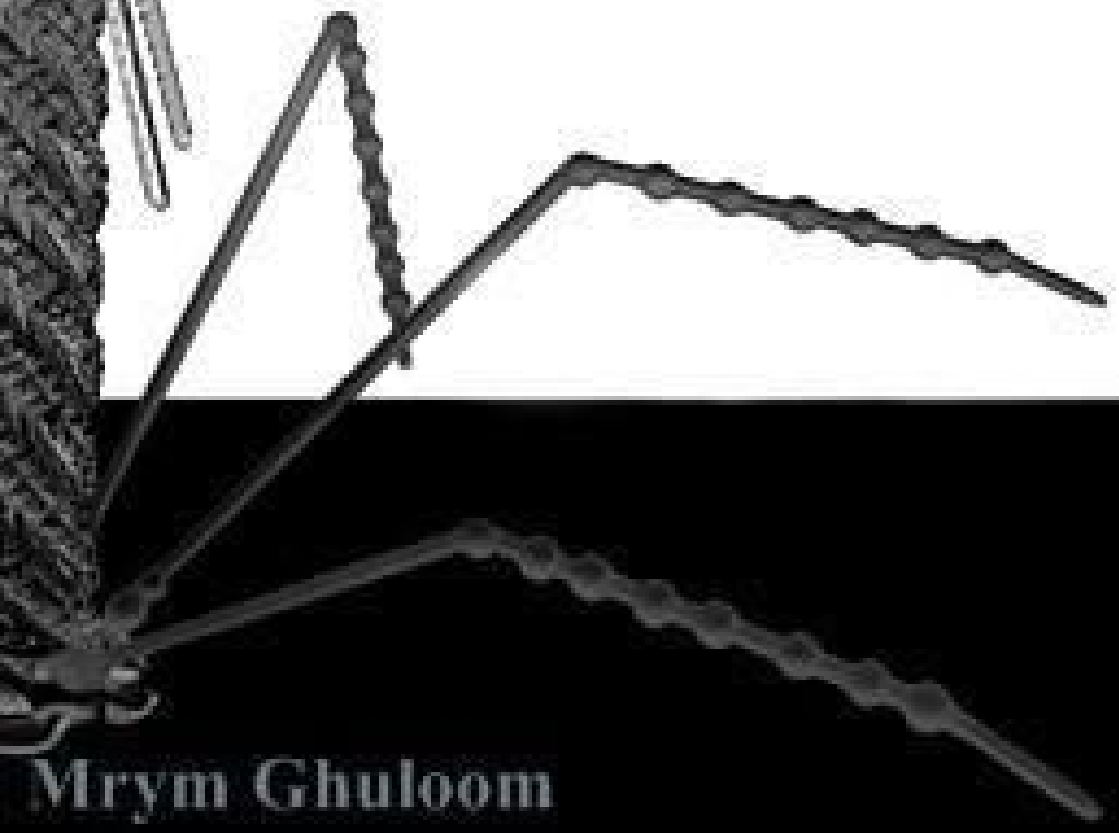


Mrym Ghuloom

# Mycobacteria

We are coming to the end of the introductory course of bacteriology, then after this we will have 4 lectures in parasitology by Dr. Hassan, and an introduction to mycology by Dr. Asem.

## **Mycobacteria**,

The name originated form the Greek language. "Myco" is used to describe any type of roots, whether in relation to plants or filamentous fungi. Scientists have used the term for Mycobacteria in the 19th century following the discovery of these organisms by Robert Koch, a famous children's scientist with a lot of studies relating to many types of organisms, like cholera and mycobacteria. At that time they used the term mycobacteria because they thought mycobacteria wasn't a typical bacteria, but they considered mycobacteria as part of the filamentous fungi. However, after many investigations of their cell walls and morphology and replication style, they have separated mycobacteria from fungus and have been included in the kingdom of prokaryotes, in relation to bacteria.

Mycobacteria is composed of 30 specific species. These mycobacteria can be found as obligate pathogens in humans and only produce infections in humans. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis. We have many other types of bacteria related to animals and birds which only produce infection there. Only one species can produce infection in both, animals and humans, the *Mycobacterium bovis*. This bacteria infects mainly cattle and can infect sheep etc. It can contaminate dairy products, and in the case of infection in animals, it may produce lesions in the lung, liver, and spleen.



Written by Hasan Hammo

Here, in the Arab countries and many other parts of the world, we eat the lungs and liver of animals, especially sheep and goats (mu3lag). This might result in intestinal infection in humans, granuloma and damage to intestines and intestinal tuberculosis. Mycobacterium bovis is rarely associated with lung tuberculosis, but there are some cases.

## Biological features of mycobacteria

### Cell Wall

Mycobacteria is a special type of organism due to its special composition of its cell wall. Its cell wall is composed mainly of special specific proteins and polysaccharides with long chain Fatty Acid known as **mycolic acid** (in relation to mycobacteria). Together these features, due to the high amounts of phospholipid and long fatty acids with phosphate groups, will make the cell wall hydrophobic and not easily dissolved in water or other reagents. Therefore, mycobacteria cannot be demonstrated by using the gram stain method. We cannot stain it gram positive or negative.

### Classification

So how do we classify mycobacteria? We use the acid fast stain AFS. Mycobacteria are resistant to low concentrations of acidity, and often the cell wall is not easily stained with dyes. They need a special stain, like methylene blue etc.

Tubercle bacilli (mycobacterial bacilli) can be differentiated form many other types of bacteria (except spore forming bacteria) in the fact that they can survive for many years in the environment. They are not easily killed by environmental factors like dryness, even despite the fact that they don't form spores. Their cell wall resists damage by difference in osmotic pressure, presence of other types of organisms, and presence of biochemical reactions etc.

*Mycobacteria tuberculosis* is highly susceptible to UV radiation (in sunlight). Therefore, in the 19$^{th}$ and beginning of the 20$^{th}$ century, if doctors had

a patient infected with mycobacteria TB, they would let him sit under the sun to cure his infection. Sunlight cannot cure TB, but it can reduce the transmission of mycobacteria from the lung of an infected person to anybody in close contact, which is a useful means of preventing the dissemination (spread) of mycobacteria to the community.

## Infectivity

Mycobacteria has a special importance as infectious cells. Few cells (like brucella) can produce infection. Infection can be easily transmitted by close contact, especially if the infected person carries the organism in their lungs (as in the case of pulmonary tuberculosis). In this way, the organism can be easily transmitted by coughing and spitting of sputum drops, which allows the organism to easily spread in close-contact communities, like classrooms. One positive case can infect 20 people by just one cough. Imagine how easily infection with TB can spread, especially to people who haven't had asymptomatic infections previously (aren't immune).

In the case of tuberculosis, we have to distinguish between two important features. Features which are related to inhaled few cells of tubercle bacilli especially during childhood, where these few bacilli might produce primary infection with tubercle bacilli. These infections are often lung infections with tubercle bacilli, and these few bacilli can produce mild lesions due to the multiplication of tubercle bacilli in macrophages. The presence of bacilli in a few macrophages can produce inflammatory reactions with the surrounding tissue, resulting in a granuloma. Simply, you have necrotizing tissue and an immune response due to the development of cytokines and alpha interferons, etc.
We will end up with small lesions in the lung. However, these lesions do not indicate the presence of disease. It means that the person only came in contact with the tubercle bacilli. The development of hypersensitive cell mediated immunity might contribute to resistance against re-infection with mycobacteria bacilli.

We have to distinguish between asymptomatic infection and clinical infection associated with more severe forms of tuberculosis.

## Growth

We don't only have obligate pathogenic (*Mycobacterium tuberculosis*) and *Mycobacterium bovis*, we have other types of mycobacterium. These obligate pathogens can be easily differentiated form other saprophytic mycobacterium (which are less important as causative agent for any type of disease) by their slow growth in vitro and in vivo. For example, if we have a clinical specimen from an infected person with mycobacterium TB, we need at least 2-6 weeks to recognize any growth of mycobacterium TB or bovis under optimum temp, this shows that they are slow growing.

Whereas other types of saprophytic bacteria (widely found in the environment associated with animals and sometimes in our body) need only 3-5 days for us to recognize their growth. Rapidly growing mycobacterium are usually not as important as causative agents of TB or serious diseases in humans. They may however, produce skin lesions. For example, we have a type of mycobacterium called *Mycobacterium smegmatis* from the skin. This can be found as a normal part of our flora, especially around the genital area, and can be recovered from urine (but not significantly). This can confuse some physicians, because this would represent inadequate information from the lab.

If we recover these rapidly growing *Mycobacterium smegmatis* or other species (mycobacterial cells) from urine within 3-5 days, then this is considered unimportant and non pathogenic, and should not be reported in the physician's treatment.

## Pathogenesis and Infectivity (Again)

In children, infection with mycobacterium TB (an obligate pathogen) can spread via the lymphatic channels and the hematogenous route to the kidneys and produce kidney TB (infection to the kidney). Later on, the organism will be excreted in the urine. It might even reach the meninges and produce meningitis in children.

1% of infected people with mycobacterium TB can end up with TB meningitis or kidney TB, and both can be fatal in children.

## Atypical Mycobacteria

Last item in relation to other types of mycobacteria, atypical mycobacteria may be rapid growers. They may produce pigmentation during growth, and often produce non serious infections in healthy persons.
Often, atypical mycobacterium may produce skin lesions in immune-suppressed patients. They may also be rarely associated with pulmonary TB. 99% of the time, Pulmonary TB is caused by mycobacterium TB of the obligate pathogenic type (mainly related to humans).

Mycobacterium TB, which is adapted to infect humans, cannot produce infections in animals of any type. Whereas mycobacterium bovis, which originates from animals, can produce infection in humans. Other mycobacteria of animal origin rarely produce infection in human.

Last item in relation to biological characteristics of this important organism, is that it can survive in the environment. It is resistant to dryness yet susceptible to UV light. It can produce infection with only a few number of cells. It isn't necessary to have many cells for infection (one or more may be enough to induce lung infection). That has two main signs to recognize it with:

Primary pulmonary TB is often recognized in children and is often acquired in childhood. A few bacilli will be lodged in the lung tissue. This results in the development of mild lesions, but these mild lesions are not developed into cavities. They do not result in severe damage necrosis. They don't develop into cavities in the lungs.

### The tuberculin test:

In addition, there are no signs whatsoever that indicate a child is infected with mycobacterium TB. No fever, no malaise, no night sweating, weight loss etc. These unrecognized mild infections result in the development of a partial immune response, and can be tested later on in life by use of the **tuberculin test**, which is composed of an amount of the cell wall and the cell membrane of these bacteria. It is prepared by a special procedure called PPT, (purified

protein derivative of mycobacterium TB or simply, tuberculin test).

This tuberculin test/tuberculin antigen is used to detect only contact with tubercle bacilli. We can find out if someone has already been in contact with tubercle bacilli (whether asymptomatic or symptomatic) by using the tuberculin test.

It is a skin test; usually a small amount ex. 5 micro grams of this antigen is introduced subcutaneously in the skin. We then wait 48 hours to recognize if there are any skin reactions. These can be recognized if there had already been contact with tubercle bacilli. The skin will react by the development of induration and swelling as well as irritation and redness of the skin. This reaction can be measured, i.e. it can be mild or intensive, and can produce large zones or small zones.

Generally if there is a mild reaction, this indicates a positive tuberculin reaction, which means only you have been in contact with the tubercle bacilli. Keep in mind that this does not mean you have a clinical infection of mycobacterium TB. The tuberculin test can be used to evaluate patients with infection, if the induration and erythema is large and intense, this means we have active TB. Active TB is considered as active productive TB.

What does active productive TB mean in relation to the lung? It means that you have a person infected with mycobacterium. He has one or more lesions in his lungs and these lesions are open. This means that if you cough or spit sputum, then this cough may carry a few bacilli. So the patient is highly infectious and he is now a source of infection.

**Signs and symptoms of TB + diagnosis:**

The presence of active TB can associated with specific and nonspecific clinical signs. In general, it is easy to recognize the following in any active lung TB (or TB in other parts of body but mainly in the lung),

1- There is a **continuous productive cough**, associated with the accumulation of sputum and spitting of sputum. The sputum contains large amounts of epithelial cells and damaged cells as well as the microorganism.

2-It can be bloody sputum. The presence of blood is 100% associated with pulmonary TB. *Note that other types of organisms which cause pneumonia like streptococcus pneumonia and pseudomonas can often induce the production of a cough but without damage to the blood vessels and thus without the presence of blood. So, bloody sputum is a good indication of TB.
3-Bloody coughing will also be associated with non-continuous fever. A fever will increase and decrease according to the release of a number of tubercle bacilli, along with night sweats, weight loss, and general weakness.

All of these can be considered together in the differential diagnosis of a case of TB.

To confirm the presence of TB, you have to take an X-ray of the lung (or ultrasound for other parts of body like the kidney or intestine) to see if there are any lesions or granulomas.

However all of these indications are not enough to confirm that this is a positive case of TB without isolating the organism from a clinical specimen like the sputum. To get a good culture you have to collect the sputum in the early morning and then send it to the lab. Sputum in the lab will be firstly be prepared for direct smearing by use of a special staining method called Ziehl-Neelsen stain with the Acid Fast bacilli Stain. You must demonstrate in this direct smear the presence of the tubercle bacilli.

You might think this is very easy, but it is not for two reasons:
1- Sometimes the number of tubercle bacilli in the sputum at the beginning is very small. You have to concentrate the sputum by treating it with NaOH, and then neutralize it with low percent of acid solution etc. It's a long process.
2- To stain the smear and examine it in order to recognize the presence of 1 or 2 cells, you need at least 1 hour of examining the smear in order to confirm this as a positive or negative case. This confirmation is very important. You cannot misdiagnose the case of TB because it has many consequences, especially in the prevention of dissemination (spread) of disease.

We have to culture the bacteria on a special culture medium. Tubercle

bacilli is a slow growing organism. It is aerobic, and grows at an optimum temp of 37. We use a special culture medium called **Lowenstein-Jensen Medium**. This medium, there are other media but this is the best used worldwide, but in order to recognize the presence of tubercle bacilli (especially obligate pathogens), we have to incubate for at least 6 weeks. However we may recognize the first colonies after 2 weeks. From these colonies we have to prepare an Acid Fast Stain and then do biochemical tests to confirm a case of TB.

Recently they began to use molecular techniques like PCR, but it is still not accurate in confirming cases of TB, because there is cross reactivity between mycobacterium TB and other atypical mycobacterium which may be found in the respiratory tract of certain patients. Therefore, PCR still isn't approved as a confirmation test for detection of TB. The only absolute confirmation test is the culturing of the bacteria.

It is more difficult to confirm the presence of TB by using CSF (cerebrospinal fluid) because in CSF, we have very few bacilli. You must enrich these bacilli before demonstrating the organism and this requires many other fluids and this is not easy.

**Infectivity (AGAIN)**

The active productive type of TB, in 95% of this type of TB is not developed through direct contact with tubercle bacilli. Non-acute infection is acquired from direct contact with infected people. You will be surprised, that the majority is due to the reactivation of old lesions of childhood. According to a study done 10 yrs ago, in our country, 80% of children who reach 10 years old are considered tuberculin test positive. This means that we have huge reservoir of this bacteria in the environment. We acquired the tubercle bacilli in early childhood, but our body responded with an immune response against this, resulting in asymptomatic infection. We have only the positive tuberculin test without presence of clinical disease. And this might help us to reduce the severity of infection in other people but reactivation of old TB might end with active TB, or it can be acquired from infected cases or from the environment, etc. This results in localized infection in the lung and in some cases, the

organism might spread (like in children) in adults from lungs to meninges, kidneys, etc. TB can produce granuloma in any part of the body, it is not restricted to the lungs, but the majority is related to the lungs as the majority originates from the lungs. In short, TB is a very important infectious agent and the health community in any country should do everything to prevent the occurrence and spread of TB, and the only way to do that is by detection of positive cases. Once we identify the positive cases, they should be isolated for 2-4 weeks, and at the same time must be treated with specific antibiotics to reduce the spread of the organism in the community.

One single case of lung TB can infect 100s of people in a community. In Sweden, where they don't have endemic TB cases, and they control their hygiene well (nobody spits on the floor/streets), they have less than 1% of their population positive for TB, and this means that there are no infections in the community. There are no positive cases where the organism spreads and they don't have problems.

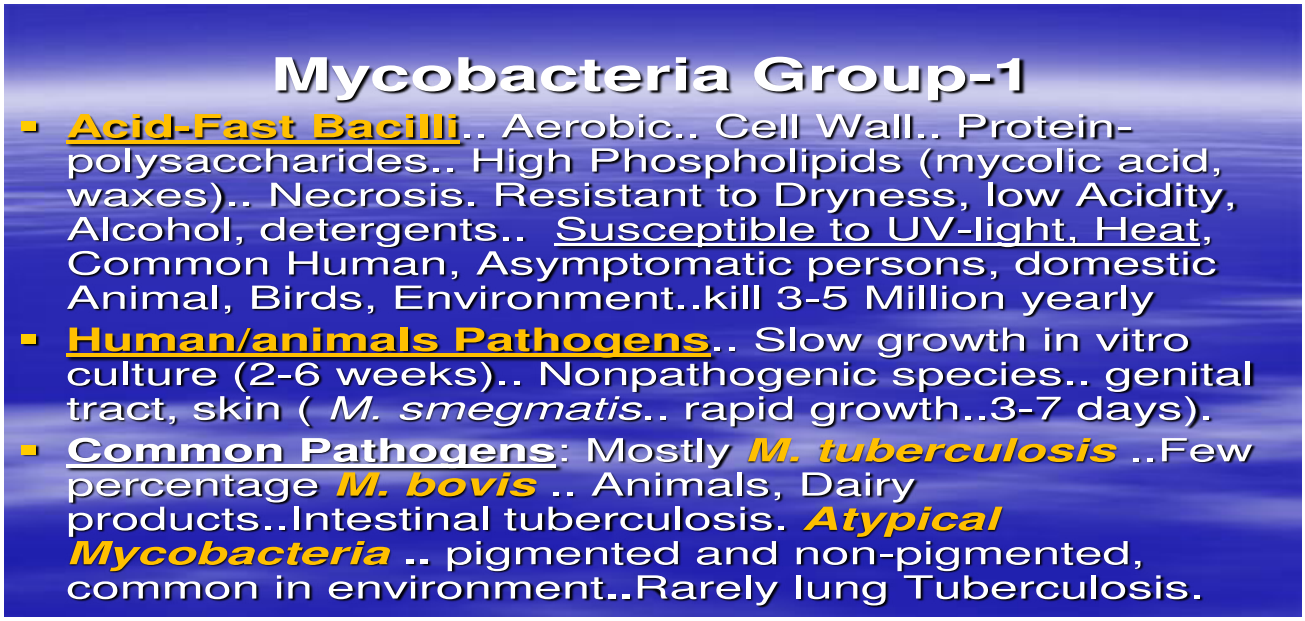
**Treatment.**

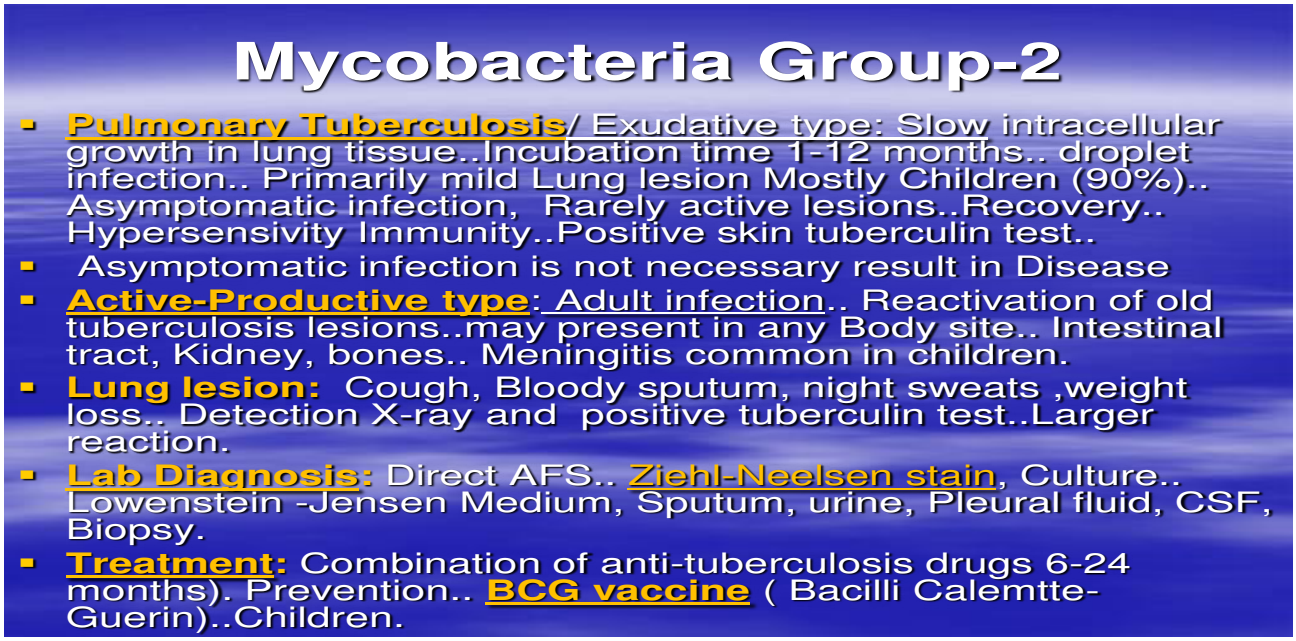
Treatment consists mostly of 2-3 types of drugs and continues for 3 (months, the word "months" was added by correction) - 1 year. Treatment depends on the severity of the case, site of infection, etc. Therefore, it is not easy to label any person as positive for TB, you must be 100% sure this person is infected. Otherwise, you will expose such a person to treatment for nothing. Treatment costs a lot of money and has lots of side effects. Many patients will not be happy taking these drugs because they will have GI problems and other side effects, etc. Therefore, we must be sure when we want to treat a case of TB. It must be true TB case, otherwise it would be a criminal act treating people without proof.

There is a vaccine available, and it is called the **BCG vaccine**. This vaccine is made of a subculture of mycobacterium TB cultured for about 50 times. This results in the autolysing of cells. What's taken is mainly the cell wall and the cytoplasmic membrane compounds to produce toxins.

These toxins may protect up to 50% in certain communities, it differs from

one country to another and it should be given better for children aged less than 10 years to be effective. Optimum age is 5-6 years. You will see many references saying this vaccine protects up to 70% and others 50%, it differs from country to country. Let us say for the sake of numbers 50% and not 100%. The vaccine does not provide complete protection like other types of vaccines. This vaccine was developed by 2 French scientists and is therefore called Bacilli Calmette-Guerin (BCG)




Good luck, and lots of thanks for Mohammad Abu Alia for correcting this.

Hasan Hammo

You are welcome bro – M. Abu Alia :P