

# Mycobacteriaceae

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9.04.2018

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# Classification

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Kingdom: Bacteria

Phylum: Actinobacteria

Order: Actinomycetales

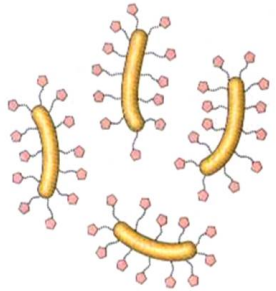
Family: Mycobacteriaceae

Genus: *Mycobacterium*

# The properties of Mycobacterium genus

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- Immobile, **non spore**, unencapsulated, strict aerobe, bacilli
- The cell wall contains mycolic acid (lipid)
- Resistant to acid and alkalines (in terms of vitality)
- Dividing time 15-20 hours, slow reproduction (more than 7 days)\*\*
- Hard stained



Mycobacteria

# Structure

- 0.2-0.6  $\mu\text{m}$  in width, 1-10  $\mu\text{m}$  in length
- Cytoplasm, plasma membrane and a cell wall rich in lipids

Lipids account for;

- 3 % of the cell wall in Gram negative bacteria
- 0.5 % of the cell wall in Gram positive bacteria
- In mycobacteria, **60 %** of the cell wall weight.

Peptidoglycan layer (N-glycolylmuramic acid instead of NAMA )

The layer adjacent to peptidoglycan is arabinogalactan;  
It's 35% of the cell wall.

Major polysaccharide.

Arabinogalactan is a branched polysaccharide composed of arabinose and galactose.  
The peptidoglycan is attached to the substrate via phosphodiester bonds.

Mycolic acids are attached to the side chains of arabinogalactans by covalent bonds.

The outermost layer is made up of peptidoglycolipids or phenolic glycolipids called mycosides.

Mycobacterium genus consist of nearly 70 species, some of which are saprophytic or compulsory parasites. Species, closely related to each other are named as "complex" in terms of bacteriological characteristics and DNA similarities.

***Mycobacterium tuberculosis***  
**complex**

Apart from this complex other bacilli are grouped as;

- Pseudotuberculosis bacilli
- Atypical mycobacteria
- Non-tuberculous mycobacteria (NTM)
- **Mycobacteria other than tubercule bacilli (MOTT)**

# *Mycobacterium tuberculosis*

**The main cause of tuberculosis in Turkey is *M. tuberculosis*.**

- Non-pigmented, non-motile, non-capsule, non spore bacilli, growth at 37°C, R colony morphology, main source is humans.
- They are not easily stained with bacteriological stains (cell wall showing hydrophobic character !!!)
- Stained with Ehrlich Ziehl Neelsen Method – acid-resistant bacteria
- They double their numbers in a mean of 18 hours. Since the growth is so slow, cultures prepared from clinical specimens must be kept for 6-8 weeks before giving a negative report.
- Their growth is well in glycerin (eugonic growth) and egg-based medium (such as Löwenstein-Jensen medium).
- Obligatoy aerobic. This explains why *M. tuberculosis* is located in highly oxygenated tissues such as the upper lung lobe and the kidney.



# How do you get TB?

- The bacteria spread through the air in the form of droplets, from one person to another. Bacteria get released into the air by some person who is already infected with them. When a person with TB of the lungs or throat coughs, sneezes, sings or talks, droplets containing the bacteria are released into the air. That is why people who think they may be infectious, may often hold something over their mouth when they are near to other people. People working in a health clinic may for this reason sometimes wear a mask.

- <http://www.tbfacts.org/tb/#sthash.nCnQqqfI.dpuf>

## PATHOGENESIS

Tuberculosis bacilli are phagocytosed by alveolar macrophages after reaching the lungs. At this stage, the tuberculosis bacilli is killed after phagocytosis if the immunity is strong.

If the immunity is weak, bacillus will multiply in macrophages.

After breakdown of infected macrophages, the released bacillus are phagocytized by new macrophages.

This continues with bacilli multiplying in macrophages, then macrophages breakdown, and then the released bacilli infect new macrophages.

**\*Primary tuberculosis:** At the onset of *Mycobacterium tuberculosis* infection, mostly the middle and lower parts of the lung are involved. Focus is often uniform.

Bacillus taken by respiratory ways escapes from host's immune system, inflamates lymph nodules and primary focus occurs.

✿ Cellular immunity develops in healthy people in other ways. Cellular immunity controls the disease and remains asymptomatic (Some bacilli remain dormant more than 20 years). The most important factors that effects *Mycobacterium tuberculosis* infection are; nutritional status and adequacy of the immune system.

✿ The period in which patients are most at risk for developing active tuberculosis is the year after the first encounter with the bacteria.

## **Active tuberculosis:**

Occurs as a result of progression of primary tuberculosis or reactivation of silent infection

**a) Progression of primer infection:** If *M. tuberculosis* is not kept under control in infection area, infection will progress. A lesion will occur in lung parenchyma.

**b) Reactivation by exogen or endogenous infection:** occurs as a result of immunity break down against *M. tuberculosis* 20 years or more time after primary infection

c) Milier tuberculosis:

A necrotic tubercule lead to erosion on blood vessel, enabling bacterial spread by hematogenous way. As a result, infection occurs in all organ systems. In this type of tuberculosis the mortality rate is high.

## Clinical symptoms

Cough, hemoptysis (spitting blood), rising fever after lunch, night sweats (head and neck), fatigue, loss of appetite, weight loss

# Diagnosis-Treatment

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- Definite diagnosis = bacteriological diagnosis
- In the World Health Organization Tuberculosis Experts Committee report, «active tuberculosis (lung)» is defined as **a patient with bacilli in the sputum smear preparation.**
- Obtained by examination of sputum or pathological material in smear preparations and culture.

## Microscopic diagnosis

Sputum (crushes in two cover glasses and than stains) or pathologic material spread on cover glass without any processing and directly stain and examine. –

Direct preparation

Sputum or pathologic material homogenised by processing and bacillus is investigated by staining -the condensation method (important for the main culture)

**Ehrlich Ziehl Neelsen** is applied for both techniques. (Blue ground, red bacillus)

**Kinyaun method**

**Auramin-Rodamin method**



## Tuberculosis culture:

Tuberculosis bacilli grow very slowly. Different organic mediums are used for isolation of bacilli from sputum or pathologic material. (Synthetic semi-synthetic media for PPD)

**-Löwenstein Jensen medium**

**-Middlebrook medium**

# TREATMENT

Isoniazid  
Rifampicin  
Ethambutol  
Pyrazinamide  
Streptomycin

Para-aminosalicylic acid  
Etionamide  
Floroquinolones  
Cycloserin  
Kanamycin  
Rifabutin



**Primary infection**



**Secondary infection**

Treatment is divided into beginning and continuation period.

At the beginning, 4 antibiotics should be used as standard and at least two antibiotics should be used in continuation period.

### In Turkey

Rifampin+Isoniazid+Pyrazinamide\*+Ethambutol

### International Union Against Tuberculosis and Lung Disease (IUATLD)

Rifampicin+Isoniazid+Pyrazinamide\*

\*Pyrazinamide is administered during the first two months of treatment

**Beginning period:** Is the period when the fast-growing bacillus are cleaned. Four drugs are used in this period (5 drugs are used in recurrent and therapeutic treatments). Bactericidal activity and resistance-inhibiting activity in question. Usually 2 months in new cases, and 3 months in recurrent and relapse cases. If treatment is discontinued at this stage, treatment failure may occur and drug resistance is likely to develop.

**Continuation period:** Sterilization is performed in this period. That is, occasional activating, intermittent proliferating bacilli are cleared. It usually lasts 4 months in new cases. It lasts for 5 months in recurrent and relapsing cases. If treatment is discontinued at this time, recurrence can occur and bacilli are generally susceptible to drugs.

# BCG Vaccine

## Bacille Calmette-Guérin

In 1908 the vaccine was developed by two scientists, Calmette and Guérin, at the Pasteur Institute in France.

*Mycobacterium bovis* isolated from an infected cattle was cultured in a medium consisting of glycerin beef, bile fluid and paclitax. It was cultured in 3 week periods for 13 years, in a total of 231 passages to lose the ability to make the disease and to be used internally, that's how live-attenuated strain of tuberculosis vaccine (BCG) started.

This strain is still considered an international reference strain. Today, Glaxo, Japanese, Copenhagen and French Pasteur strains are used, which differ in growth techniques.

# Vaccine practice

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- Immediately after birth, a single dose is applied by intradermal (ID) route within one month at the latest.
- Tuberculin skin tests are performed in kids older than 2 months, and children with negative test results are vaccinated.
- Vaccine conservation for adults is low.
- There is no inconvenience in application in the same period with other vaccinations.

# Vaccine practice-2

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- Congenital immunodeficiency,
- Symptomatic HIV infection,
- Patients with leukemia - lymphoma,
- Pregnants,

**It is stated that BCG vaccine should not be used!!!**

# Ehrlich Ziehl Neelsen Staining Method

Preparation fixed

The carbol fuchsin is poured on the preparation and heated for 8-10 minutes without boiling.

The preparation is washed with water.

The preparation is decolorized with 3% acid-alcohol.

The preparation is washed with water.

The preparation is stained with methylene blue for 1 minute.

The preparation is washed with water, dried.

The preparation is examined with the immersion objective. The acid resistant bacteria are colored shiny dark red. The cells that make up the stamen and other bacteria are colored in blue.

# References

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- Murray Rosenthal Pfaller, Tıbbi Mikrobiyoloji. Yedinci baskı. Çeviri editörleri: Prof.Dr. Dürdal Us, Prof.Dr. Ahmet Başustaoglu, 2016.
- <http://www.who.int/immunization/policy/schedule.pdf>