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Microbiology

Doctor 2017 | Medicine | JU

◉ Sheet ○ Slides

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Mycobacterium

** Mycobacterium is a class of Actinobacteria , which are non-spore forming , non-motile and acid-fast bacilli (they remain stained with the **red** dye **carbofuchsin** and don't get decolorized when washed with alcohol). Also, they can't be stained by the gram stain since they lack cell wall.

**** There're 3 types of Mycobacterium :-**

1- **Mycobacterium Tuberculosis (MTB)** , which is the main cause of Tuberculosis .

2- **Mycobacterium Leprosy** , which causes Leprosy (Hansen's disease) .

$$ Don't worry , we'll discuss these 2 diseases later on in this sheet $$

3- **Non-Tuberculous Mycobacterium (NTM)** , previously known as environmental mycobacterium . This type of mycobacterium **doesn't** cause Tuberculosis , but they cause Chronic Pulmonary Infections .

** Tuberculosis in humans and any other living organism can be caused by any member of a **family** known as **MTB complex** ,which consists of 11 members , including Mycobacterium Tuberculosis and Mycobacterium Bovis (M.Bovis can cause TB in animals such as sheep and cattles , also we use it in the production of **BCG** vaccine (**Bacillus Calmette–Guérin**) – We take this vaccine 3-4 weeks after birth -) .

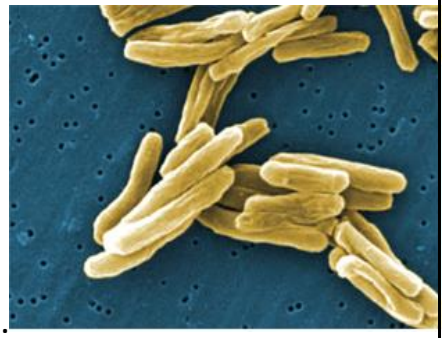
** Mycobacterium is slow growing bacteria (it needs 18-24 hours to divide) when comparing it with other types of bacteria, such as E.Coli (it needs 20 minutes to divide).

***-NOTE** :- Knowing the doubling time of bacteria is important in treatment and laboratory diagnosis.*

** Mycobacteria tend to be more resistant to chemical agents than other types because of their hydrophobic nature of cell surface and their clumped growth .

- NOTE :- Mycobacteria is an example of the obligate aerobes (they can derive energy from the oxidation of many simple carbon compounds).

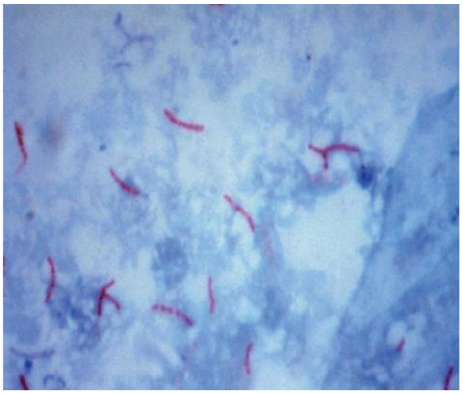
-As you can see in the picture , they are thin and straight rods.



- This is a high power micrograph showing the **acid fast bacilli** in the **sputum** (Coughed up material) of a patient **with TB** .

- **Sputum test** is usually done for **adults** since they're able to **produce sputum** .

- For **infants** and **children** , we use another techniques such as BronchAlveolar Lavage (**BAL**).



* To culture Mycobacterium , we can use Solid mediums (Semi-synthetic Agar Media and Inspissated Egg Media) **OR** Liquid mediums (Broth Media) .

-AGAR is a semisolid substance that contains agarose and agaropectin .

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* Let's talk briefly about the **mediums that are used to culture Mycobacterium** :-

((The doctor didn't talk about the components of each media so I think that we shouldn't memorize them)).

1- The semisynthetic agar media , it contains defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol . The commercially available SSAMs are 7H10 and 7H11.

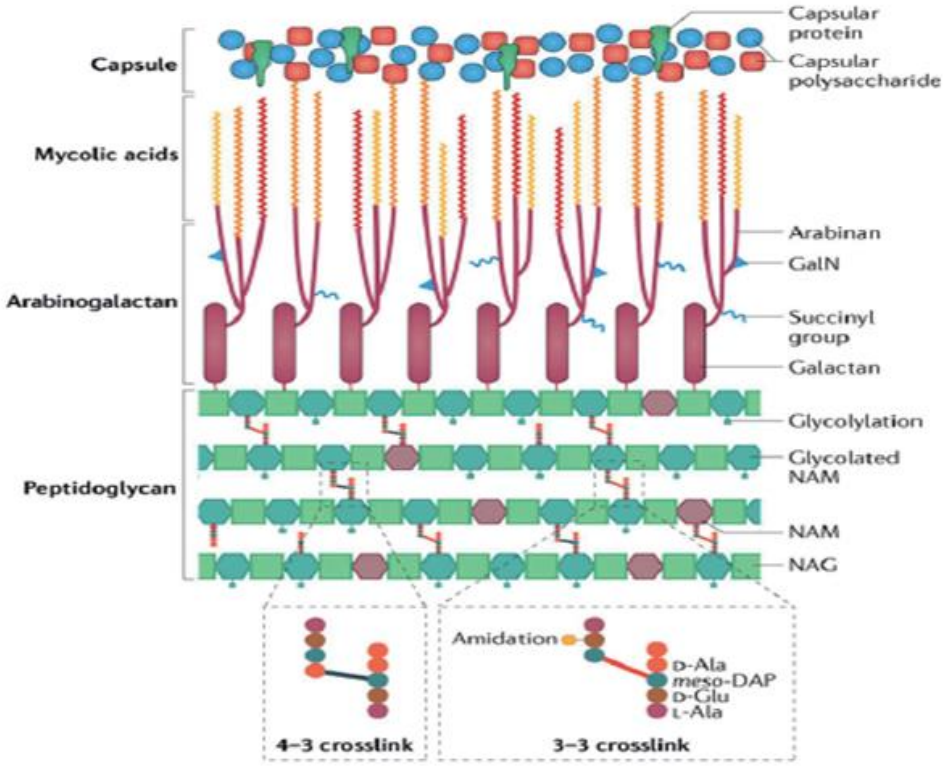
2- The inspissated egg media (Also called Lowenstein-Jensen) , it contains defined salts, glycerol, and complex organic substances (eg:- fresh eggs or egg yolks, potato flour, and other ingredients in various combinations) .**IMPORTANT :-** When we use this media we add Malachite Green to inhibit the growth of other types of bacteria.

3- Broth media. The commercially available BMs are 7H9 and 7H12.

- Here, you can see the **colonies** of **MTB** on **IEM** , notice that the background is green and the colonies are not pigmented , puff and irregular .

Mycobacterium Cell Wall

- Consists of two layers :- inner layer and outer layer.
- The **inner layer** is composed of **three** distinct **macromolecules** covalently linked to each other (Peptidoglycans , Arabinogalactans and Mycolic Acids). They form a complex known as **MA-AG-PG complex** .
- **PG** is composed of repeated units of the disaccharides **N-Acetylglucoseamine** and **N-Acetylmuramic acid** which are linked to each other by peptide bridges .
- MAs are very long chain fatty acids (C78 – C90).
- Most of the **AGs** are ligated to **MAs** forming a thick **waxy** lipid coat which contributes to the **impermeability** and the **virulence** of the Mycobacterium.
- Sometimes , the MAs get esterified to trehalose forming Trehalose Dimycolates (TDM ,Trehalose with 2 MAs) and Trehalose Monomycolate (TMM , Trehalose with one MA).
- **TDM** is an important virulence factor , it allows MTB to **form** cord like structure , thus called the **Cord Factor**.
- The **outer layer** which represents the **capsule** of the MTB is composed of capsular proteins , lipids and polysaccharides , and is **non-covalently** linked to the inner layer.



Tuberculosis (TB)

- TB is an infectious disease shaped by an interaction between the pathogen and the host.
- The pathophysiology of TB is in the formula of 10-3-1 , which means that in every 10 infected people , 3 of them develop latent TB and 1 develops active TB , while the rest can clear the infection by their immune system.
- 90% of the infections result in Pulmonary TB (Since MTB targets the lungs mainly) and the other 10 % result in extrapulmonary TB where it can attack pleura , pericardium and other organs.
- The spread of TB occurs through the lymphatics and once inhaling the bacteria , it'll induce the infiltration of PMNs and then Macrophages (If the spread occur hematogenously - through the blood –, it can affect any organ and cause Miliary TB).

- **NOW we'll talk about the types of TB :-**

- TB may be one of two :-

1- **Primary Infection** , which is the **active** one and usually occurs at the **base** of the lungs , and it includes inflammation and infiltration of immune cells.

2- **Reactivation Type** , which is the **latent** type of TB and usually occurs at the **apex** of the lungs. In this case , the patient may have the bacteria but it's not active and it gets activated after a certain time usually in patients with compromised immune system.

- **Miliary TB** is an example of **Primary infection** and is characterized by the wide spread in human body (as a result of the hematogenous spread) in addition to the tiny spots that look like millet seed.

- About the **transmission** , TB is considered as an **airborne infectious** disease (so patients with TB must be kept isolated) also it can be spread through unpasteurized milk.

- **PATHOGENESIS OF TB :-**

- The virulence factors of MTB allow it to multiply and survive when phagocytosed by PMNs and Macrophages , and that is the definition of **intracellular infection (IC Infection).**

- The hallmark of TB infection is the formation of Granuloma *(By definition , Granuloma represents a protective mechanism , in which the tissue gets remodeled by the host , due the host's inability to clear IC infections , and it looks like a necrotic center*

surrounded by a zone of immune cells) , then the blood supply becomes less (because of the presence of this zone of immune cells) leading to caseous necrosis (Cheesy like appearance) which may then rapture causing hematogenous spread of TB , ending with Miliary TB.

- **Inflammation of TB** could be **Exudative** (Resulting from **accumulation of PMNs** , and their killing by MTB , which give rise to caseous necrosis and edema) **OR** **Productive** (Characterized by the formation of **granuloma**).

REMEMBER that granuloma can be healed through the resolution and absorption of the exudate by lymphatics. HOWEVER, if not healed , it may lead to massive necrosis of the tissue and may end up with the reactivation type of TB.

** The pulmonary signs of TB are coughing , weight loss , anorexia (loss of appetite) , fever , night sweats , haemoptysis (coughing blood), dyspnea (Chest pain) and general fatigue , while the extrapulmonary signs depend on the affected organ.

➡ **DIAGNOSIS OF TB :-**

- In the diagnosis , you need a smear microscopy to detect Acid Fast Bacilli (AFB) (Remember that even if the results were negative , the patient could still have TB , because if you want to culture MTB , you'll need a long time since its doubling time is 18-24 hours !!)
- From each patient with suspected TB , three specimens should be examined microscopically for AFB (or Mycobacterium) , and both liquid and solid cultures should be performed for every specimen. Culture of AFB is the most specific test for TB identification and determination of the susceptibility of causative organism.

-There're three fast tests that are commonly used in the diagnosis of TB :-

1-Nucliec Acid Amplification Test (NAAT).

2-Tuberculin Skin Tests (TSTs) :- TST is a method used for determining whether the patient is infected with MTB or not. TST is done by injecting 0.1 ml of tuberculin purified protein derivative (TPPD) in the forearm. The injection is intradermal and should produce a circle of paleness and redness of skin around the injection site accompanied by induration (We measure the diameter of the induration , not the redness). Further, TST should be read between 48 and 72 hours after injection.

- An induration of **5 mm** is considered **positive** in **HIV** patients and patients who had recent contact with TB patient.
- An induration of **10 mm** (or little more) is considered **positive** in **laboratory personnel.**
- An induration of more than **15 mm** is considered positive in **any person**.

**In the US and Europe , TSTs and NAATs are abandoned. Instead, they use :-
3-Interferon Gamma Release Assay (IGRA) :- Here we measure the release of IFN-ɣ from monocytes.
-The IGRA and TS tests are screening tests which means that they tell you whether the patient is exposed to TB or not , without giving information about the state (Active or Latent).

-Now , we'll talk about the **TREATMENT** of TB :-

- One of the major problems when we come to the treatment of TB is the Multiple Drug Resistance (MDR , that is , the resistance for at least 2 types of the drugs used in the primary therapy of TB , also called First Line Drugs) and the Extensive Drug Resistance (ExDR , the resistance for all of the First Line Drugs and at least one of the Second Line Drugs) of the MTB.

- For patients with active TB , they should be given an intensive phase of 4 drugs (Mentioned below) for 2 months , followed by 4-6 months of a continuation phase of 2 out of the 4 drugs *(The Dr. didn't mention what are the two drugs)* so the whole duration of treatment is 6-8 months.

-The four drugs are :-
1-Isoniazid (Pronounced as IZO – NAZIDE , Abbreviated as INH since the true name is IsoNicotinyl Hydrazide).
2-Rifampin (RIF). **3- Pyrazinamide** (PZA).
4- Either **Ehambutol** (EMB) **or** **Streptomycin** (SM).

★ For patients with latent TB , they should be given INH and Rifapentine for at least 9 months.

Non-Tuberculous Mycobacterium (NTM)

- Also called **Environmental Mycobacterium** , this bacteria do NOT cause tuberculosis. Instead , they causes Chronic Pulmonary Infections (CPIs) . These infections mimic Pneumonia.

- They target people with immunocompromised (eg ; HIV or Diabetic) patients rather than immunocompetent people.

- NTM can be classified as Rapid growers (Grow in < 7 days) and Slow growers .

** Mycobacterium Avium-Intracellulare Complex (MAC or MAI) , this complex causes (CPIs) in immunodeficient patients (eg; patients with AIDS).

Other types of NTM include :-

1-M.Kansasi → Causes Pulmonary Infections that mimic the classical TB.

2-M.Scrofulaceum → The most common cause of lymphadenitis (Enlargement of the lymph nodes).

3-M.Marinum →This type of Mycobacterium affects patients who work with fish in marines.

4-M.Ulcerans → Causes ulcers in the skin and the subcutaneous tissue.

5-M.Fortutium

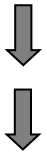
6-M.Chelonae-Abscessus →It's able to form abscesses in the skin.

Mycobacterium Leprae

- An example of an Acid Fast Bacilli (Rods).
- This bacteria is the main cause of Leprosy (Hansen's Disease) (The severity of the disease depends on the host's immune response).
- One of the major characteristics of M.Leprae that it's not cultivable in vitro , it can be only grown in vivo.

- **There are three types of M.Leprae :-**

1-Lepromatous Leprosy (LL) , which is the severest form.
2-Borderline Lepromatous (BL).
3-Tuberculoid Leprosy (TL) , which is the least severe form.



LEPROSY

-An infectious disease caused by Mycobacterium Leprae.

-The clinical manifestations of the disease include pale patches of the skin that are insensitive to temperature and pain along with problems in nerves , nose , pharynx and many other organs.

- The **diagnosis** of the disease → A **biopsy** of skin or nasal mucosa is smeared on a slide and then stained by Ziehl-Neelsen stain *(The biopsy will give the typical -granulomatous- histological appearance).*

- **Treatment :-** → Sulfones such as dapsone are the First Line Drugs used in the treatment of tuberculoid and lepromatous leprosy.

→ Rifampicin or Clofazimine can also be used.

☺ Good Luck ☺