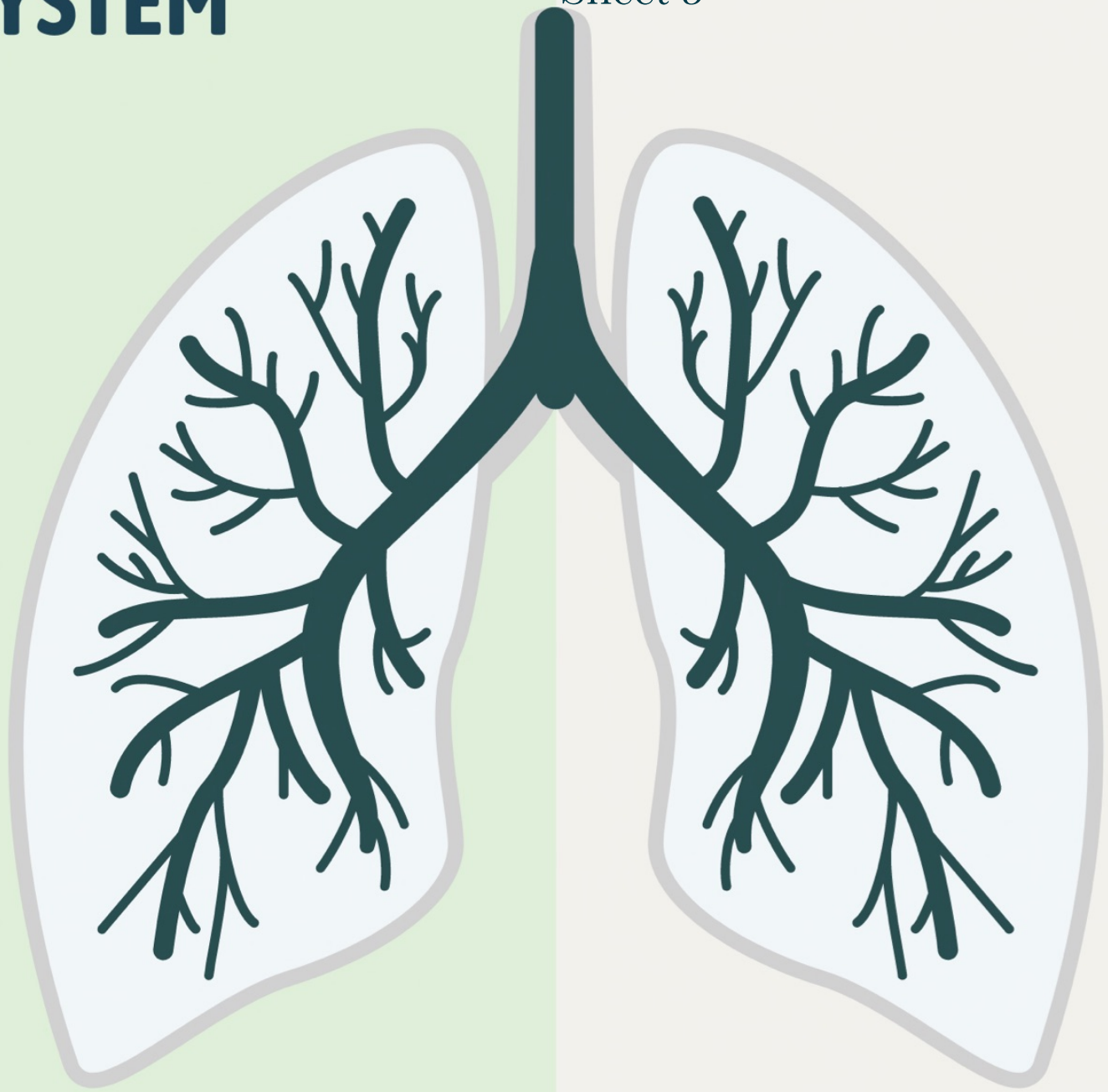
RESPIRATORY SYSTEM

MICROBIOLOGY

Sheet 5



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Today we are going to talk about Mycobacteria -The causative agent of tuberculosis.
~The underlined sentences were not mentioned by the doctor~

Enjoy <3

Acid fast bacilli 😂

Background

We don't use the gram description anymore (gram positive previously), we use acid fast stain instead.

• The mycobacteria are rod-shaped, obligate aerobe, facultative intracellular bacteria that do not form spores. *Non motile , not capsulated*

- 3 types of species that cause diseases in humans:

1. Mycobacterium tuberculosis complex (MTC): a genetically related group (they are 11 members) of Mycobacterium species that can cause tuberculosis in humans.
2. Mycobacterium leprae: a causative agent of leprosy (This is outside the scope of the lecture)
3. Non-Tuberculous (NTM) Mycobacteria: The most known type is Mycobacterium avium-intracellulare (M. avium complex, or MAC) They are opportunistic infections and mostly infect AIDs patients. (We will talk about this briefly, at the end of this lecture)

Leprosy has two major types, tuberculoid and lepromatous
these types are different by the type of defense (innate or adaptive) cell mediated immunity

Today, the disease is rare. It's also treatable

Also called environmental mycobacterium

Main rout of transmission: Ingestion
Other routs : inhalation
specially in immnocomprimised people

Mycobacterium Tuberculosis (Mtb)

- It was not until the 19th century, when Robert Koch utilized new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus
- This is a very old disease which humans were never able to eradicate. Now, incidence rate in western countries is very low but it is still present in developing countries, so they call it "Disease of the Poor".
- It is also called: called consumption- it causes weight loss (used in diagnosis of the disease for cancer) and White plaque – patients become pale. But now, it's mostly called Tuberculous Bacillus, TB.

patients become pale due to hypoxia

• The family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis(TB) in humans and other livings.

• It includes: M. tuberculosis (Mtb) (the principle pathogen), Mycobacterium africanum, Mycobacterium bovis (previously more important, comes from cows before milk pasteurisation begins), Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium canetti. (others differ depending on geography) - 11 in total.

Any Mycobacterium of these can cause TB in human

Morphology

• In tissue, tubercle bacilli are thin, straight rods measuring about 0.3 ~ 3 µ m.

• True tubercle bacilli are characterized by "acid fastness"—that is 95% ethyl alcohol containing 3% hydrochloricacid (acid-alcohol) quickly which decolorizes all bacteria except the mycobacteria's primary staining- Carbon fuschin- a red dye.

Acid fast bacilli ?

- *related to cell wall complexity (more than 50% of the bacteria's weight is lipids{ mycolic acid - long chain fatty acid }*
- *if we put the red stain the stain resist*

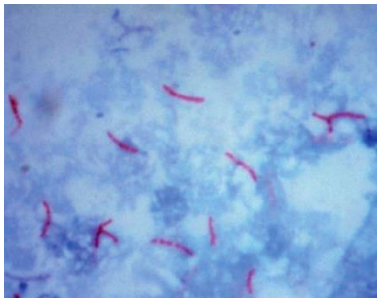
~Hence, mycobacteria are acid fast bacilli~

This technique is called The Ziehl-Neelsen technique

• Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Its main living micro-environment are macrophages, but sometimes it is extracellular- hence it's a facultative intracellular.

• The growth rate is much slower than that of most bacteria. The Doubling time of tubercle bacilli is about 18 hours. (We wait up to 8 weeks in labs, when diagnosing, to find colonies, and takes 6 to 12 months for treatment). This has to do with the complexity of the cell wall.

- We call the bacteria waxy because more than 60% of the bacteria's weight is lipids.



باختصار سريع للي بصير هون :
بنجيب الsputum
بنحط عليه الصبغة الحمراء
بعدين بنجيب الكحول و بنحطه
الكحول رح يشيل الصبغة عن كل الاشياء باستثناء الmtb
بعدين بنرجع نصبغها مرة تانية و بنحطها تحت الميكروسكوب و بنشوفها بالشكل اللي زي الصورة

As see in the diagram, Acid fast staining- carbon fuschin stain still retains its red colour even after decolourisers. So, it appears this way. This is called smear positive- sometimes found- found in the sputum. Smear negatives are less infectious, but the patients might still have tb.

The Gold standard diagnosis is culture. But if there is a strong suspicion that the mycobacterium is Tb, immediately start the treatment and isolate the patient because he is infectious (The multiplicity of infection here is less than 10 mycobacteria).

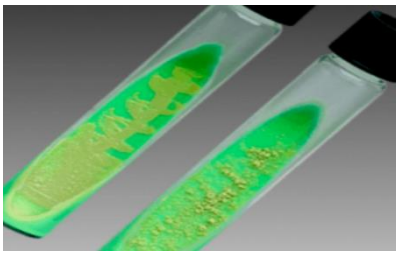
• Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth,

Mtb Culture *Need 2 to 3 months to be seen*

There are 3 options for culturing in labs:

1) Löwenstein- Jensen (oldest, most known): contain defined salts, glycerol, and complex organic substances (e.g., fresh eggs or egg yolks, potato, flour, and other ingredients in various combinations.

   Inspissated egg media and malachite green dye is added- which inhibits the growth of most contaminants but permit only Mtb.



less sensitive but faster then Semisynthetic agar media

2) Semisynthetic agar media— These media (e.g., Middlebrook 7H10 and 7H11): contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol.
   Selective medium- colonies that are white, creamy, fuzzy.

Used to grow mtb in semisolid media

3) Broth media— (e.g., Middlebrook 7H9 and 7H12): less sensitive and specific than agar culture, but faster.

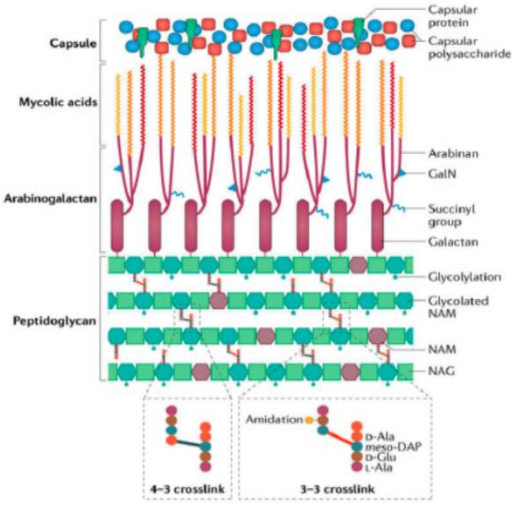
Mtb Cell wall *It has :*
1.plasma membrane
2. Cell wall composed of 2 layers :
** inner & * outer layer*

• The mycobacterial cell wall is a complex structure that is required for cell growth, resistance to antibiotics and virulence.

• It consists of 2 layers:

- An inner layer: composed of three distinct macromolecules— peptidoglycans (PG), arabinogalactans (AG) and mycolic acids (MA)

covalently linked together to form a complex known as the MA-AG-PG complex. These add the complexity of the cell wall. The peptidoglycan layer surrounds the plasma membrane and comprises long polymers of the repeating disaccharide N-acetyl glucosamine–N-acetyl muramic acid (NAG–NAM) that are linked via peptide bridges.



Long chain fatty acids

- An outer layer that surrounds the plasma membrane: other lipids and other polysaccharides, not required for exam purposes. (we took in 2nd year)

Virulence factors:

2.Secretion system
3.sulfatides
4.[phosphatidylinositol myelocytes]
مش متأكدة منها ٪١٠٠ :)

1. Lipoarabinomannan (LAM)

5. -trehalose dimycolates- Mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides, can be found in Mtb cell wall and make up 50% of the dry weight of the mycobacterial Cell envelope, also called Cord Factor. It causes clumping of the bacteria, which deprives the bacteria from nutrients and allows it to enter the dormancy stage inside the human body.

In vitro : causes clumping of the bacteria
In vivo : causes granules fomrmation

Epidemiology:

• Two TB-related conditions exist:

-latent TB infection (LTBI): The MTB is present in the body but it's not infectious and shows no signs or symptoms, occurs in 2/3 of the world's population. *Living dormantly*

-Active TB disease. If not treated properly, TB disease can be fatal.

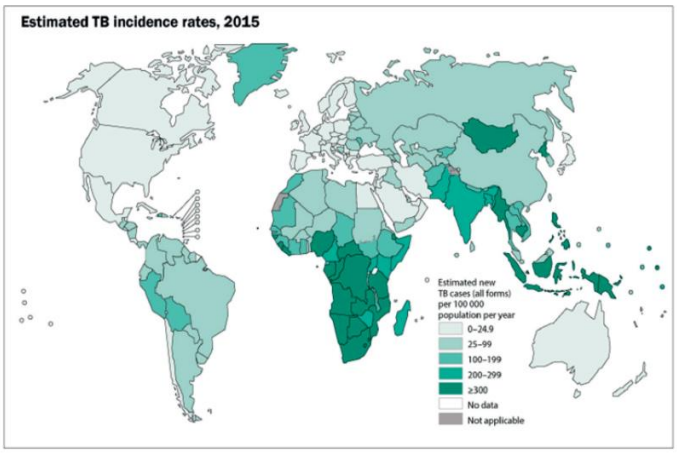
Only small proportion of those infected will become sick with TB.

In 2015, an estimated 10.4 million new, active TB cases were seen worldwide, of which 1.5 million died. It is the single most killer infecting agent in humans and the number one killer in AIDS patients (because it's an intracellular bacterium, so its main defence is the cell mediated immunity which is low in HIV patients) This is a deadly combination.

Worldwide: every 100,000 person there are hundreds cases yearly

In Jordan, in every 100,000 person there are 25-35 cases yearly, which increased later with the presence of more refugees, which is a good incidence rate in a developing country.

• TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurised milk, and direct inoculation.



- As we can see in the picture , the countries with a dark color have TB incidence rates that reach hundreds.

Imp Examples on countries with high rates : South Africa, Switezerland and the Soviet Union countries.

People who came from the Soviet Union countries were at the top in regarding to TB diagnosis. These people suffered from an issue called MDR (multi drug resistance TB) and also suffered from extensively drug resistance TB which will be talked about later on.

- INFO : No antibiotic works on extensively drug resistance

Tuberculosis TB

As a disease entity we have 2 kinds of TB : Active TB and Latent TB

But depending on sites we have Pulmonary TB and Extra Pulmonary TB , Pulmonary TB is the most common form(more than 90% of the cases) *80-90%* and it can develop to become Extra Pulmonary TB. The Extra Pulmonary can take place anywhere in the body.

- The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.

• Spread – Lymphatic vs hematogenous (Miliary).

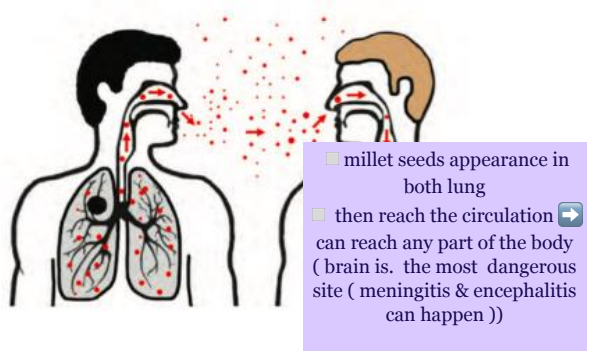
• TB bacteria can attack any part of the body such as the pleura ,L.N.(when TB happens here we call it Scrofula) ,pericardium, kidney, spine (we call it Pott's disease) , brain (TB here can happen in meninges , it happens in children and is

considered the most serious one) and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.

• Primary Infection(Active) and Reactivation Types (Secondary) of Tuberculosis.

- If someone is infected with the mycobacterium of TB and containment of it happens , it doesn't develop into an active disease and if the immune status got compromised for some reason after a year or 10 years , Reactivation happens to the dormant bacilli.
- There are clues to know which type of Tb it is , for example : Primary(active) happens in the middle and lower lobes while Reactivation happens in the apex of the lobe.
- Mycobacterium TB dissemination can be direct for example in abdominal TB : the TB in lungs can be disseminated through the diaphragm and reach the abdomen.

Transmission



• TB is considered an airborne infectious disease(means even when the droplets evaporate the bacteria is still alive which makes it dangerous), heating and ultra violet light are used to disinfect the mycobacterium although M. tuberculosis complex organisms can be spread through unpasteurised milk, direct inoculation and other means.

• The underlying pathophysiology of TB is the "10/3/1 formula : every 10 people exposed to mycobacterium TB , 3 of them will develop latent TB and 1 will develop Active TB which means that 6 people got rid from the mycobacterium through innate immune system or adaptive or both.

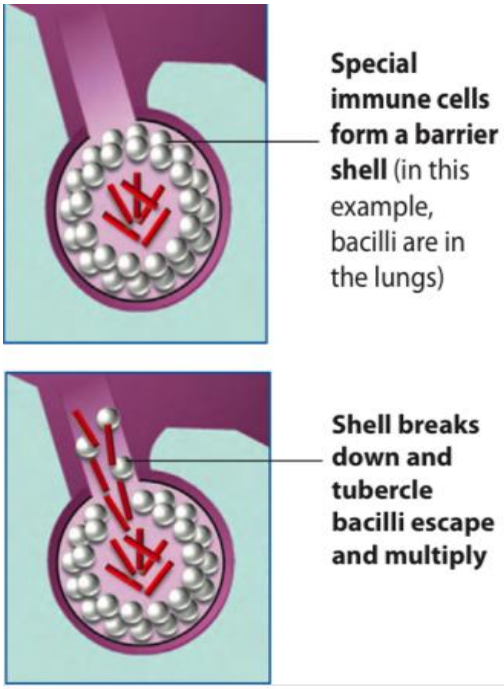
Pathogenesis

• Mycobacteria are in droplets when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli then they get internalized into macrophages

• Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages.

Mycobacteria they get inside endosome then phagosome ,it inhibits the fusion of phagosome and lysosome and inhibits the acidification of phagolysosome.

• Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli.



The thing that is considered a defense mechanism done by our bodies and the hallmark of pathogenesis of mycobacterium is granuloma formation which happens when an infected macrophages and another recruited macrophages are differentiated into epithelioid cells and then the recruitment of lymphocytes fibroblasts.

• The cells form a barrier shell, called a granuloma, that keeps the bacilli contained and under control (LTBI).

We have ghon focus and ghon complex
• granuloma formation occurs in the node
•If calcification happen then we called it ghon focus •
•if ghon focus affect drainge lymphnode we called it Ghon complex
• Ghon complex = ghon focus + adjacent lymph node

• If the immune system cannot keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (TB disease).

Primary Infection and Reactivation Types of Tuberculosis

Sometimes , the first infection is latent , some time the first infection is active and then transform into latent stage

• An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly.

• In primary infections, the involvement may be in any part of the lung but is most often at the base. • The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion

• The reactivation type almost always begins at the apex of the lung, where the oxygen tension (PO2) is highest.

Clinical manifestation

You should be a good doctor 😄
So sometimes the pateints will come with lower back pain or bloody urine [extrapulmonary tb]

• Classic clinical features associated with active pulmonary TB are coughing, weight loss/anorexia, fever, night sweats, haemoptysis (coughing blood), dyspnea (chest pain) and malaise/fatigue.

Productive cough with sputum , shortness of breath

Remember the difference between haemoptysis and hematemesis
hematemesis :Vomiting blood

• Tuberculosis is usually a chronic disease; it presents slowly with weight loss(significant weight loss is seen), low-grade fever, and symptoms related to the organ system infected. Because of its slow course, it may be confused with cancer. Whenever you have an infection of any organ system, tuberculosis will be somewhere on your differential diagnosis list.

• It is one of the great imitators

To diagnose Mtb there 2 not definitive and 1 definitive **diagnostic test** :

-**Both not definitive**
1) complete Blood count ➔ Rise on WBCs
2) X-ray ➔ You can see the Ghori complex Hilar lymph node are commonly involved and Miliary TB if present of course you will see it

Positive TB depending on the diameter of the induration:

If induration size > 15 mm(normal healthy individual)
-Induration size > 10 mm (intermediate risk group like health care providers)
-Induration size > 5 mm (HIV patient) [which makes sense as we don't expect patient with HIV to have large induration due to compromised immunity].

-**And Definitive ➔** through culture

We can also use 2 tests (screening tests, not diagnostic): Tuberculin skin test (TSTs) and Interferon-gamma release assays (IGRAs).

Type 4 hypersensitivity reaction
used in America because the vaccine isn't given there

TSTs test => purified protein derivative that is taken from Mtb and given to patients intradermally. The patient is asked to come back after 48 hours , if he was sensitized to the mycobacterial antigen, he will develop erythema and raised skin. *We measure the induration not the erythema*

But we have to know that this test has a lot of false positive results, especially in the vaccinated people and the people who were already infected with one of the environmental mycobacteria.

- IGRA test => we take a blood sample from the patient, we mix this blood with mycobacterial antigen, which we already have in the lab, and we measure the Interferon-gamma release and based on certain cut of values, we can determine if this patient has been expose to mycobacterium before or not.

No false positive results in vacccinated people so it more commonly used in countries that give the vaccine

Positive IGRA test : >25

Note: these 2 tests don't tell us if the infection is active or not. They just tell us if this patient has a history with this disease by recognizing the mycobacterium antigen.

Treatment

The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.

The standard regimen treatment of TB is 6 months and usually involves a drug cocktail, or a mixture of multiple drugs. We start with 4 drugs during a 2-month intensive phase, followed by a slower 4- to 6-month continuation phase. The main anti-tuberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM)

Rifampin side effect :changes the color of body fluids such as urine into [orange or red]

Side effects of these drugs : Hepatotoxicity , nephrotoxicity , ototoxicity

These drugs have a lots of side effect and a very low compliance. The patient starts to feel good during the first 3 to 4 week, then the bacteria will develop a selective resistance. That is why we give the patient multiple drugs.

Some countries treat it in 9 months and others in 12, and some countries use a type of treatment called DOT treatment (directly observed treatment), which makes the patient the medication in front of a medical person to that he takes it.

If the patient has a resistance for isoniazid and rifampin, we call this case a multi-drug resistance. In this case, we use the second line of treatment: fluoroquinolones and injectable anti-tuberculosis drugs.

If the bacteria develops resistance for isoniazid and rifampin and one of the injectable drugs, this is called extensive drug resistance, which is most likely not treatable .

Isoniazid preventive therapy(IPT) is the recommended treatment for latent TB but the regimen's main drawback is the duration of therapy (but if you have a suspicion of tb, initiate the treatment immediately and isolate him).

9 months treatment

Prevention

As we know, prevention is better than cure and the best way to prevent TB is to diagnose and isolate infectious cases rapidly, and to administer appropriate

treatment until patients are rendered non-infectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.

Additional strategies include BCG vaccination and treatment of persons with LTBI who are at high risk of developing active disease.

Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine, derived from M. bovis, is the only licensed vaccine against tuberculosis (TB), but this vaccine can be effective from 0% to 70%. Scientists don't now why this happen exactly but we still take this vaccine because its effective to protect from the most serious one,TB meningitis, which occurs in young children. The low efficiency vaccine occurs in the most common form, pulmonary TB, on adults.

The doctor said the efficacy of the vaccine is 0-80%

OTHER MYCOBACTERIA

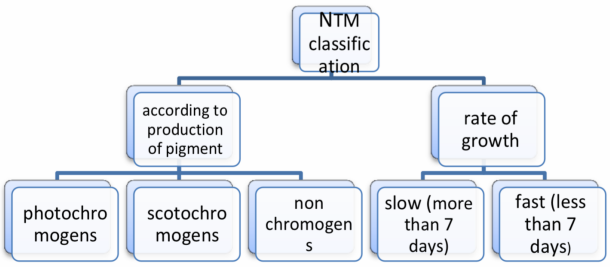
They cause mild disease in normal individuals, but severe ones in the immunocompromised patients, mainly HIV.

The nontuberculous mycobacteria (NTM) is a diverse group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens *In water and soil mainly*

The NTM can be further classified into 2 groups: the rapid growers (grow in < 7 days) and slow growers (grow in > 7 days). Each group can be subdivided on the basis of pigment production to (photochromogens (produce pigment in light) , scotochromogens (produce pigment in darkness) and nonchromogens (don't produce pigment)))

Note :The most common type of **nonchromogens** is mycobacterium tuberculosis

Now , we will talk briefly about Mycobacterium avium Complex (MAC or MAI) :



- MAC organisms infrequently cause disease in immunocompetent humans. MAC infection is one of the MOST COMMON opportunistic infections of bacterial origin in patients with AIDS.

The nontuberculous mycobacteria (NTM) more than 100 type but the most important ones are :

TB like illness

1-Mycobacterium kansasii ➔ Cause Pulmonary disease *Has highest isolation rate in America*

2-Mycobacterium marinum ➔ Cause Aquatic Granuloma *In pateints who work with fish*

skin and soft tissue infection

3-Mycobacterium ulcerans ➔ Cause skin and soft tissue infection

(1 and 2 and 3 are slow growing and Photochromogen)

4-Mycobacterium scrofulaceum ➔ cause lymph node inflammation (slow growing and Scotochromogen) *Without lung infection*

5-Mycobacterium avium complex, or (MAI). ➔ Most common in AIDS Most one isolated

- *nonchromogens*
- *Slow growing*
- *multi drug resistance*

6-Mycobacterium fortuitum Complex ➔ Causes Pulmonary infection

7- Mycobacterium chelonae-abscessus ➔ Causes skin infection

(6 and 7 are fast growing).