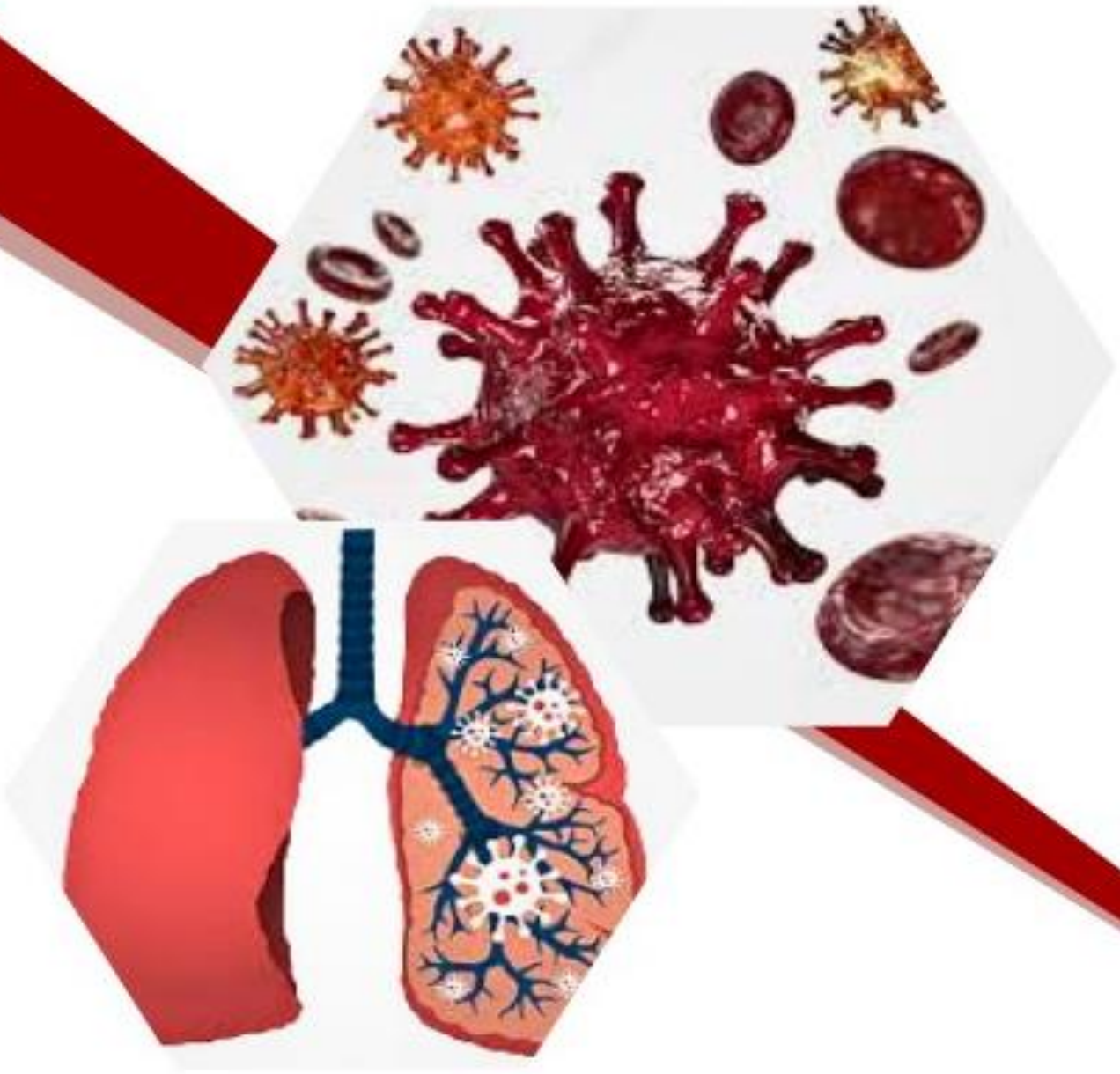
Doctor.021

no.

RS MICROBIOLOGY



Writer: Doctor 018+020

Corrector: Rama Harb

Doctor: Nader Alaridah

Mycobacteria

Background

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

•The mycobacteria are rod-shaped,obligate aerobic, facultative intracellular bacteria that do not form spores, non-motile, non-encapsulated.

- 3 types of species that cause diseases in humans:

•.Mycobacterium tuberculosis complex (MTC) a genetically related group (they are 11 group) of Mycobacterium species that can cause tuberculosis in humans.

•Mycobacterium leprae causes leprosy.

•non-tubeculous mycobacterium(NTM) aka environmental mycobacterium: the most common type is Mycobacterium avium-intracellulare (M avium complex, or MAC) and other nontuberculous (NTM) mycobacteria frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.

Mycobacterium Tuberculosis (Mtb)

•It was not until the 19th century, when Robert Koch utilized s 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**.

•Tuberculosis ,aka consumption(because it consume patients ,by weight loss(used in diagnosis of the disease for cancer)), white plaque (extreme pallor, shortness of breath and cyanosis seen among patients).

•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.

Morphology

Again, non-motile, non-encapsulated, non-spore forming, obligate aerobic and facultative intracellular.

•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% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria.

•Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds.

•The **growth rate is much slower than that of most bacteria**. The doubling time of tubercle bacilli is about 18-24hours.

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, E.coli duplicate every 20 minutes.

•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.

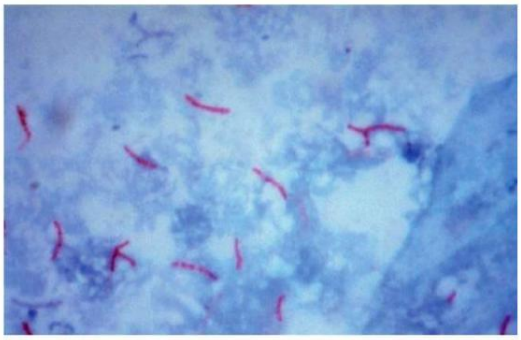
- They are resistant to disinfectants, so if the sputum that goes out of a TB patient lands in a cold-dark place, Mtb will keep viable in the sputum for up to 6 months and TB patients should be isolated - they become non-infectious after the 2-3 weeks after the beginning of the treatment.

- 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).

- We call the bacteria waxy because more than 60% of the bacteria's weight is lipids, virulence of the bacteria, low-multiplication rate, acid fastness: all of these features are related to high lipid contents of the bacterial cell wall.

- 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.



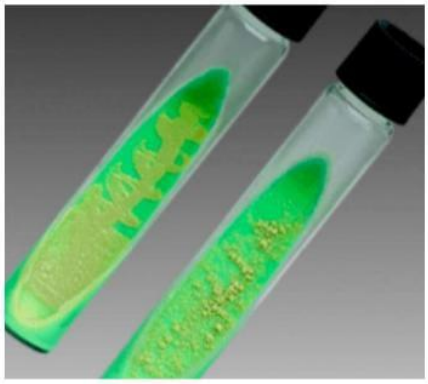
Mtb Culture

• The media for primary culture of mycobacteria should include a nonselective medium and a selective medium.
• Semisynthetic agar media, solid media— These media (eg, **Middlebrook 7H10 and 7H11**) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol .
• **Inspissated egg media,** semi-solid media— These media (eg, **Löwenstein- Jensen**) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations.
• **Broth media,** fluid media— (eg, Middlebrook **7H9** and **7H12**) support the proliferation of small inoculate.

- The growth of Mtb takes 4-6 weeks while 8 it takes 8 weeks to give negative culture

Löwenstein- Jensen media,Green stain called malachite green.

Inhibits most of normal flora contaminant, but NOT MTB



Dry rough colonies

Mtb Cell wall

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

•It consists of two layers:an inner layer and an outer layer that surrounds the plasma membrane.

- The inner compartment is 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, which add complexity to the cell wall.
- An outer layer: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.

Virulence factors:

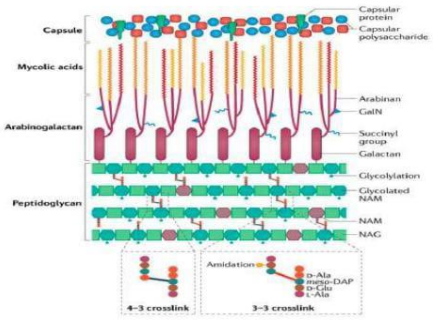
1. sulfatides are sulfur lipids present in the outer layer, they inhibit the phagosome-lysosome fusion and thus they evade the phagocytosis once they get inside the body, they will ingulfed by alveolar macrophages, inside these professional phagocytes they will evade the phagocytosis by blocking early autosomal autoantigen-1.
2. Lipoarabinomannan (LAM) and and lipomannan.
3. trehalose dimycolates, aka Cord factor, responsible for serpentine growth of bacteria.

Now, read what written in slides:

• Most of the arabinan is ligated with long-carbon-chain mycolic acids, which form the characteristic thick waxy lipid coat of mycobacteria and are major contributors to the impermeability of the cell wall and to virulence.

• 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.

• These mycolic acids are esterified to glycerol and trehalose where trehalose can contain one or two molecules of mycolic acids forming trehalose dimycolates (TDM) (Cord Factor) and trehalose monomycolates (TMM).



- Do not get confused, the capsule here not a true capsule, Mbt is non-capsulated.

Epidemiology

TWO TB-related conditions exist;

1.activeTB disease, If not treated properly, TB disease can be fatal.

2. latent TB infection (LTBI), People who have latent TB infection **do not feel sick, do not have any symptoms**, and,**cannot spread TB to others.**

- when they become immunocompromised, they will develop secondary reactive TB.

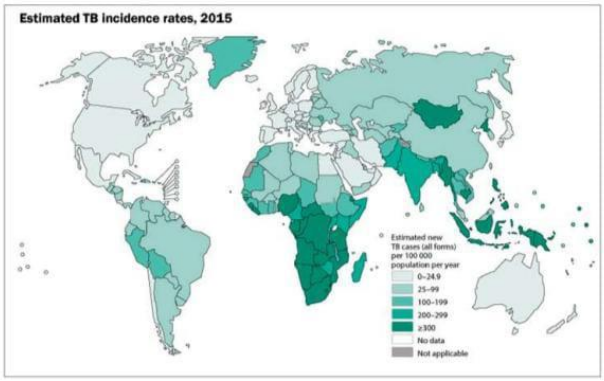
•WHO: About one third of the worlds population is infected with TB bacteria (TB latency).

• However, only small proportion of those infected will become sick with TB.

• TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen world wide.

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

- not everyone gets infected by Mtb will develop TB, that depends on the infectious dose, the main status of the patient and the environment
- 3 bacilli of Mtb are enough sometimes to establish the infection.
- immunocompromised patients (AIDS or immunosuppressive agents) are at higher risk to develop active TB and it is the leading cause of death in AIDS patients.



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

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) and it can develop to become Extra Pulmonary TB. The Extra Pulmonary can take place anywhere in the body.
- In pulmonary TB infiltration occurs all over both lungs: Miliary TB (millet seed-sized) and those lesions could erode to a blood vessel or lymphatics and access the systemic circulation.

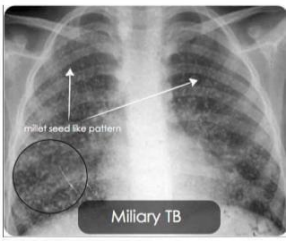
•The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.

• Spread – Lymphatic vs hematogenous (Miliary).

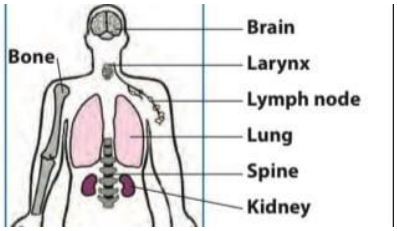
• TB bacteria can attack any part of the body such as the pleura ,L.N .(when TB happens here we call it Scrofula) ,pericardium, kidney, spine (we call it Pott's disease), brain (TB here can happen in meninges, it hapens in children and is considered the most serious one)and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.

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

- 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.



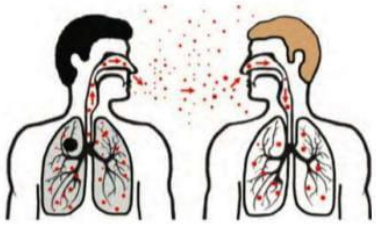
- When MTB enters they will ingulfed by alveolar macrophages.
  They inhibit the phagosome-lysosome which result in acute exudative lesion in MTB site, called ghon focus, if ghon focus reaches drainif lymph node that will result in ghon complex.



- Ghon focus/complex may resolve by absorption of exudative lesion then fibrosis and calcification occur so active TB not occur **or** it may disseminate then active TB occur.
- Not every extra-pulmonary is accompanied with a pulmonary lesion or inflammation.

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.



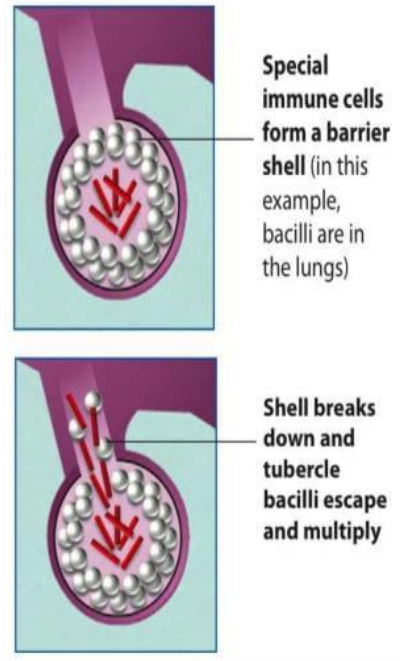
• The underlying pathophysiology of TB is the "10/3/1 formula. That's mean: every 10 people exposed to mycobacterium TB , 3 of them will develop latent TB and 1 will develop Active TB which means that 6 people got rid from the mycobacterium through innate immune system or adaptive or both, the risk of Reactivation of LTBI is high in the first 2 years (in 50% of cases).

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

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

- 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.

  In the lecture there is a slide titled with **PATHOLOGY,** I did not find it in ppt. or in 020 sheet. anyways, I added what the doctor said about it below:

  Pathology
  There are two types:
  1.exudative type: consist of inflammatory rxn with **edema**; mainly polymorphonuclear cells, the first lesion happens due to presence of MTB in alveolar macrophages.
  2.productive type: active or latent TB will develop into chronic productive type(granuloma), involves 3 layers:
  1st layer→containing tubercle bacilli and giant cells.
  2nd layer→containing monocytes and macrophages.
  3rd layer→containing T cells that differentiate to T-helper cells.

Primary Infection and Reactivation Types of Tuberculosis

• 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

the progress is very slow, and symptoms takes months to develop.

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

• Tuberculosis is usually a chronic disease; it presents **slowly with weight loss**(significant weight loss)**, 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.

Laboratory diagnostic methods

ϖSmear microscopy

• Three specimens from each patient with suspected TB should be examined microscopically for **Acid Fast Bacilli** AFB (classically **Ziehl-Neelsen**) or mycobacteria can be demonstrated by yellow fluorescence after staining with auramin.

- Highly specific but with low sensitivity, if no acid fast bacilli were found in the septum, this doesn't mean that there is no infection.
- The smear correlate with disease severity and infectiousness.
- The worse the disease the more chance to get positive Smear.
- The more positivity of the smear the more contagious the patient

ϖCulture→the most specific diagnostic test

• Both **liquid and solid** mycobacterial cultures should be performed for every specimen, and recovered isolates should be according to standard criteria (**Lowenstein-Jensen** or Middlebrook **7H10**), Radiometric **broth culture** (**BACTEC** radiometric system) and mycobacterial growth indicator tube (**MGIT**).

• Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism

ϖA nucleic acid amplification test (NAAT), Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well.

We can also use 2 tests (screening tests, not diagnostic):

- Tuberculin skin test (TSTs) and Interferon-gamma release assays (IGRAs). - TSTs test => purified protein derivative that is taken from Mtb and given to patientsintradermally. 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. 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)

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 usually involves a drug cocktail, or a mixture of multiple drugs, with an intensive initial 2-month phase followed by a slower 4- to 6-month **continuation phase.**

the main anti-tuberculosis drugs used in the chemotherapy of TB are(for intensive phase): **isoniazid(INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or** streptomycin (SM).

The continuation phase-4 months continue with two drugs: **isoniazid (INH), rifampin (RIF).**

- If the patient has a resistance for isoniazid and rifampin, we call this case a multi-drug resistance(high mortality). 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 and fluoroquinolones , this is called **extensive drug resistance**, which is most likely **not treatable**.

- 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.

• Isoniazid preventive therapy IPT is the recommended treatment for LTBI but the regimen's main drawback is the duration of therapy.

Prevention

The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (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), a live **attenuated vaccine derived from M. bovis**, is the only licensed vaccine against tuberculosis (TB).

- 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(0%).

OTHER MYCOBACTERIA

- environmental mycobacteria
- They cause mild disease in normal individuals, but severe ones in the immunocompromised patients, mainly HIV.
- Contagious, person to person.

•The nontuberculous mycobacteria (NTM) is a diverse group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens.

•The NTM can be further classified into the **rapid growers (grow in <7 days)** and slow growers**(grow in >7days).** Each group can be subdivided on the basis of pigment production. photochromogens (produce pigment in light) ,

scotochromogens (produce pigment in darkness) and nonchromogens (don't produce pigment).

•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)

1-Mycobacterium kansasii ➔ Cause Pulmonary disease, TB like

2-Mycobacterium marinum ➔ Cause Aquatic Granuloma

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), most common cause of scrofula in children.

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

6-Mycobacterium fortuitum Complex ➔ Causes Pulmonary infection

7- Mycobacterium chelonae-abscessus ➔ Causes skin infection (6 and 7 are fast growing).

V2

Note that:

Mycobacterium is a partially acid fast bacteria that causes pulmonary infection.

There is another type of bacteria that has these features; which is **nocardia.**

How to differentiate between them?

Nocardia is **a filamentous branching bacteria.**

المعلومة هاي ذُكِرت عالهامش بالمحاضرة، بس على ذمة أحد الرواة إنه الدكتور جاب عليها سؤال بالسنوات السابقة، دورت عليه وحضيفلكم ياه🫶

A homeless, malnourished chronic alcoholic presents with severe headache and dyspnea. Physical examination reveals a disheveled man with poor hygiene. His temperature is 41.0 C (105.8 F), blood pressure is 110/78 mm Hg, and pulse is 96/minute and regular. Auscultation of the chest reveals absence of breath sounds over the left middle lung fields. A chest x- ray confirms left lobar pneumonia. Sputum stain reveals partially acid-fast bacilli with **branching rods**. Which of the following agents is the most likely cause?

A. Mycobacterium avium-intracellulare
B. Mycobacterium kansasii
C. Mycobacterium leprae
D.Mycobacterium tuberculosis
E. Nocardia asteroides

Answer: E

Clear now?

Good luck🫶🏽