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Part 1

Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

Done By

Laila Nazzal

Contributed in the Scientific Correction

Batool Bdour

Contributed in the Grammatical Correction

Laila Nazzal

Doctor

Nader Alaridah

This lecture is divided into 2 parts

Neglect the number of pages :P

This lecture is INTERESTING, its related to clinical situations, enjoy what you you're studying fellows!

Mycobacterium

Classification:

***Order: actinomycetales**

*** Family: *mycobacteriaceae***

Genus: *mycobacterium

There are three major species of this family:

A) Mycobacterium tuberculosis complex(MTC)→ That causes tuberculosis مرض السل

B) Mycobacterium leprae→ causes leprosy مرض الجذام

C) Mycobacterium avium intracellularie/ mycobacterium avium complex(MAI/ MAC).

NTM (non-tuberculos mycobacteria): also known as environmental mycobacteria. They are a group of mycobacteria that don't cause neither tuberculosis nor leprosy.

In other words, they are a nontuberculous, nongranulomatous category of mycobacteria that causes infections in different sites of the body (causing lung disease- other than tuberculosis, lymphadenitis, skin infections and others with **NO** "typical granuloma")

*(NTM) frequently infect patients with AIDS. Also, they are opportunistic pathogens in other immunocompromised individuals, and occasionally cause disease in patients with normal immune systems.

Principal pathogen that causes tuberculosis in human is ***Mycobacterium tuberculosis*** [Mtb]

BUT there are other species that can cause tuberculosis, collectively called ***"mycobacterium tuberculosis complex- MTC"***

This group (MTC) can cause **Tuberculosis** disease in humans and other livings. 11 members are in this group, they are for you to memorize or at least to be familiar with, try to make your own mnemonics for them☺ :

- M. tuberculosis (Mtb), Mycobacterium africanum, Mycobacterium bovis, Mycobacterium oryx

- Mycobacterium microti, Mycobacterium caprae, Mycobacterium canetti

- Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, and Mycobacterium pinnipedii.

Doctor Nader suggested the following question for those:

ALL OF THE FOLLOWING ARE MEMBERS OF MYCOBACTERIUM TUBERCULOSIS COMPLEX EXCEPT

*(choices will include one wrong answer, giving you for example one of **NTMs** that we mentioned before. The question may be introduced for you in the exam vice versa. i.e. all of the following are NTMs except one).

*Note: mycobacterium **bovis** was the major pathogen causing tuberculosis in the past, but when **pasteurization** method arised it has been abolished nearly completely yet it is still used in VACCINATION –**BCG vaccine**- to be discussed later in this lecture.*

MORPHOLOGY

Obligate intracellular, obligate aerobes, non-motile, non spore forming and acid fast bacilli.

An exception- motile mycobacterium: mycobacterium marinum was observed to be **motile inside macrophages. This bacteria causes 'fish aquarium granuloma disease'.

*In tissue, tubercle bacilli are thin,

straight rods measuring about

0.3 ~ 3 μ m.

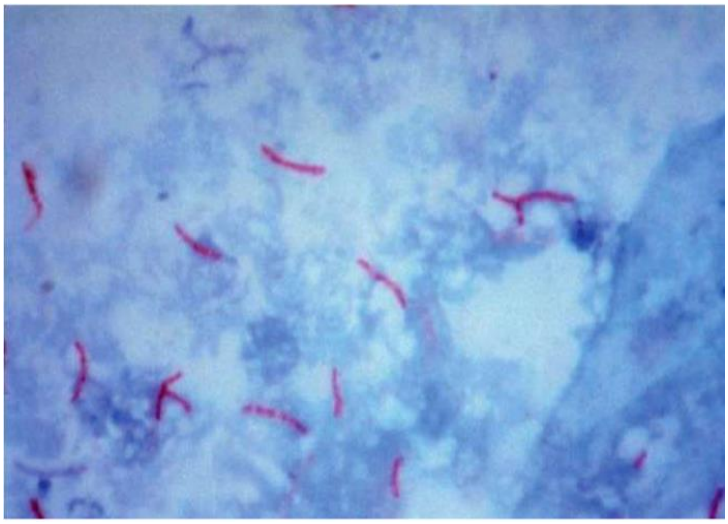
*Mycobacteria are ***obligate aerobes***

and derive energy from the oxidation of many simple carbon compounds.

Brainstorming/ controversial studies upon: active vs passive internalization of mycobacteria inside macrophages

Is it that mycobacteria favor macrophages and follow their steps to occupy them, or is it that alveolar macrophages were the 1st to pick it up?
who nagged the other frist?

*Mycobacteria are **ACID-FAST bacilli** i.e. the stain which we use to identify mycobacterium is acid-fast stain (or zeihl-neelsen stain). Robert Koch utilized (ZN stain) and discovered the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus.



Good to know: we refer acid-fast bacilli to two genera; MYCOBACTERIA and NOCARDIA.*

→Some **details** regarding **acid-fast staining** method (just understand the general idea because the doctor explained it in the lecture, Not in the slides):

a red stain called "carbol fuchsin", is used to stain the sample which is usually taken from the **sputum** of the patient.

-heat off the sample – to facilitate penetration of the stain

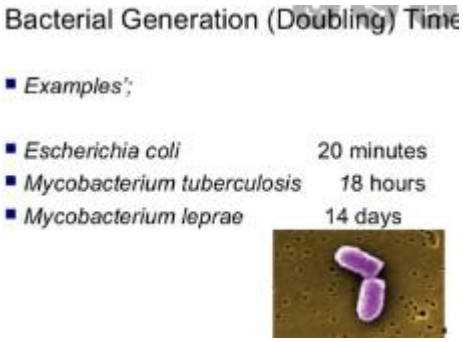
-add hydrochloride acid – to wash off the stain, "decolorization" in other words.

-counterstain the sample with methylene blue

Now, acid fast bacilli will RETAIN the 1st dye(carbol fuchsin) and resist the acid treatment (they fast from the acid), so they appear red under the microscope and don't counterstain with the blue stain→ True tubercle bacilli are characterized by "acid fastness" i.e. resistant to decolorization by **acids**—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) which quickly decolorizes all bacteria *except the mycobacteria.*

Just keep in mind, MYCOBACTERIA are ACID-FAST BACILLIIIIII

MYCOBACTERIA INSIDE HUMAN BODY ARE OBLIGATE INTRACELLULAR



Its mentioned in the slides that it is facultative, only to illustrate the idea that it is **culturable** and to explain other situations regarding mycobacteria that you are not required to know now

(as in, if it's not facultative OUTSIDE the human body, it won't be cultured)

Inside human body? Mycobacteria are OBLIGATE Intracellular.

Keep in mind that they prefer the niche of alveolar **MACROPHAGES**.

The growth rate of mycobacteria is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18-24 hours, comparing it to E.coli for example which divides nearly every 20 min clarifies the long doubling time.

This slow doubling time has many consequences on many areas. In diagnosis for instance: **culture results** are lately revealed, preventing fast diagnosis

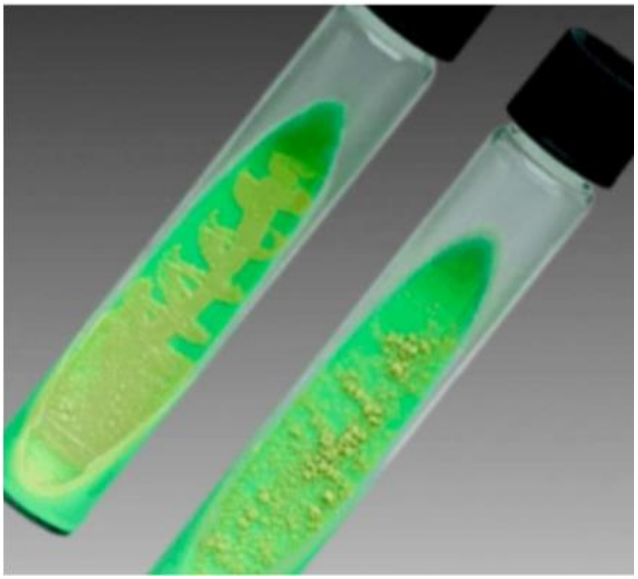
Treatment: **prolonged** period of medication administration (up to 2 years)

Mtb CULTURE

The media for primary culture of mycobacteria should include a nonselective medium and a selective medium, it's of 3 types:

-**Semisynthetic agar media**— eg, Middlebrook 7H10 and 7H11. These media contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol.

-**Inspissated egg media**— eg, Löwenstein- Jensen. these contain defined salts, glycerol, and complex organic substances (e.g. fresh eggs or egg yolks, potato flour, and other ingredients in various combinations



*We add Malachite green along with it, which will inhibit the growth of bacteria other than mycobacteria, notice the green color in the figure aside→

-**Broth media**— (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inoculates – unfortunately it has low specificity and sensitivity.

تأمل الشكل المرافق ثم صِف الشكل النموذجي لمستعمرات البكتيريا المسببة لمرض السل وغيره من الأمراض:

This is a typical mycobacterium colony, its unique in a way.It's described as **raised, rough and CLUMPED.**

A problem we face in culturing, is that it doesn't always give us positive even though the bacteria is there!

Mtb Cell wall

Most of the properties of this sophisticated bacteria are referred to the COMPLEXITY OF ITS CELL WALL, mainly the lipid component in it.

The mycobacterial cell wall is a complex structure that is essential for cell growth[1], resistance to antibiotics[2] and virulence[3].

It consists of an ***inner layer*** and an ***outer layer*** that surround the plasma membrane.

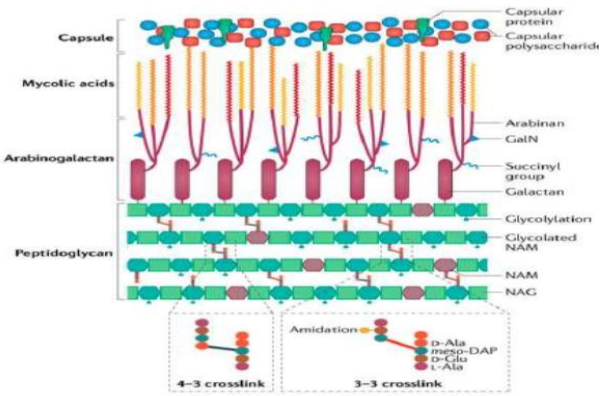
The ***inner*** compartment is composed of three distinct macromolecules —

1)peptidoglycans (PG)

2) Arabinogalactans (AG)

3) Mycolic acids (MA)

— covalently linked together to form a complex known as the MA-AG-PG complex.



*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.

*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.

Outer layer: is a capsule- like that contains polysaccharides, lipids and proteins. It contains a lot of bacterial virulence factors such as **LAM- lipoarabinomannan and LM- lipomannan.**

ركز معي شوي

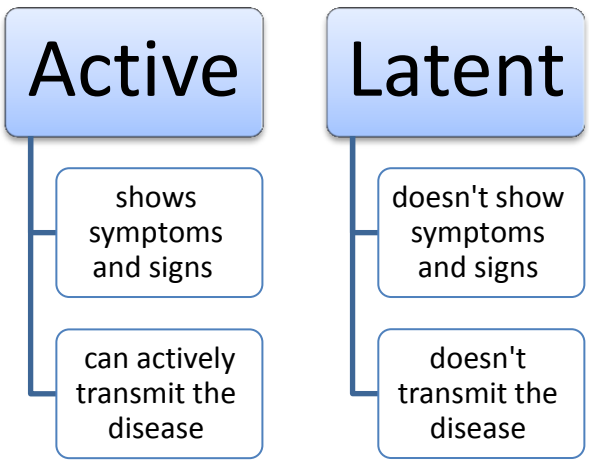
Usually these mycolic acids are esterified to glycerol and trehalose. Trehalose (a disaccharide) can bind one or two molecules of mycolic acid forming trehalosedimycolates ***(TDM/ Cord Factor)*** *and trehalose monomycolates(TMM).*

* Trehalose dimycolates (TDM) (Cord Factor)= are important virulence factors. They are also responsible for the CLUMPING morphology of mycobacterial colonies.

Have a break, have a KitKat 

Epidemiology

Before we proceed here you need to differentiate between 2 clinical entities of TB (tuberculosis):



*Latent TB could **reactivate** and cause the disease, mainly in the first 2 years of latency.

*Keep in mind: IMMUNOCOMPROMISED PATIENTS such as AIDS patients are at high risk of REACTIVATION…WHY?

Remember that TB is an **intracellular** pathogen, so the MAIN mechanism of elimination by immune system is **through CELL-MEDIATED IMMUNITY** (CD4+ cells), so in the case of AIDS patients, cell mediated immunity is not there to get rid of it.

Combination of AIDS + TB = fatal situation!

So, ACTIVE TB can arise in two types,

1)*primary* active disease **2)*secondary***-from reactivation of latent TB

In primary infections, the involvement may be in any part of the lung but is most often at the base, **well oxygenated areas= where the oxygen tension (PO_2) is highest**. i.e. **mainly the lower part of the upper lobe and the upper part of the lower lobe of the lung which makes sense because they are obligate aerobes**. (Remember, they're obligate aerobes)

*risk factors of Mtb:

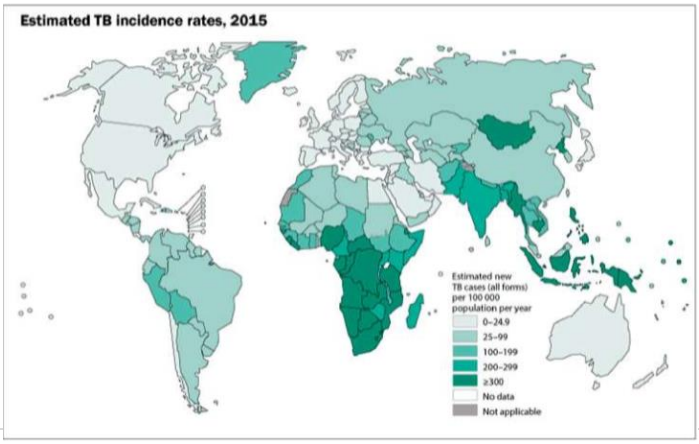
poverty, malnutrition, overcrowdedness (we observe many cases of TB in **jails**), patients who utilize immunosuppressant drugs(are at high risk of REACTIVATION of latent TB) as in the case of **rheumatoid arthritis patients.**

Now lets go over some statistics:

*Incidence rate of TB in Jordan is 25-50 cases per 100,000

*WHO, 2015: about 11 million new cases of active TB are recorded, 1.5 millions of them died. Actually this is not a good indicator as we are in 2019 and yet an INFECTIOUS agent is still causing these huge numbers.

-About one third of the world's population is infected with TB bacteria (latent TB). However, only small proportion of those infected will become sick with active TB.



Have a look on the map, lighter the color -> less incidence rate.

*South Africa and Swaziland are major countries having high incidence rate for TB, in Swaziland for example it reaches 10,000 per 100,000!!!

*One of the reasons for such high number is due to high HIV rates there.

Transmission

TB is considered an **airborne** infectious disease. Although, *M. tuberculosis* complex organisms can be spread through unpasteurized milk, direct inoculation, cough, sneezing and other means.

Patients with TB should be isolated for 2-4 weeks after we start the treatment.

The underlying pathophysiology of TB is the "10/3/1 formula", which states the following:

= if ***10 people*** are exposed to *mycobacterium* TB, **three** of them will develop LATENT TB, and **one** will develop ACTIVE TB

=notice that 6 of them cleared the bacteria somehow by their immune system and didn't develop anything.

Note: less than 10 bacterial particles are enough to establish the disease (virulent)

Mtb can withstand dryness and weak DISINFECTANTS that droplets out of patient may face!!

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Pulmonary vs extrapulomonary tuberculosis

The primary site of TB is usually the lung "***pulmonary***", from which it can get disseminated into other parts of the body.

The other routes of spread can be **1)contiguous involvement,** in other organs by adjacent tuberculous lymphadenopathy or **2) primary involvement** of extrapulmonary organ. * 90% of infections by TB are **pulmonary** TB.

Extrapulmonary TB are infections that affect other organs than lungs, such as: the pleura, pericardium, kidney.

some of which are given special names, examples:

-TB can affect lymph nodes(**cervical tuberculous lymphadenitis** is called **scrofula**), *other NTM can cause scrofula (called *Mycobacterium scrofulaceum*, to be mentioned at the end of the lecture).

-TB can affect the bones = **Pott disease**, in which TB infects the vertebrae of the vertebral column

-It can affect the abdomen = **abdominal TB**

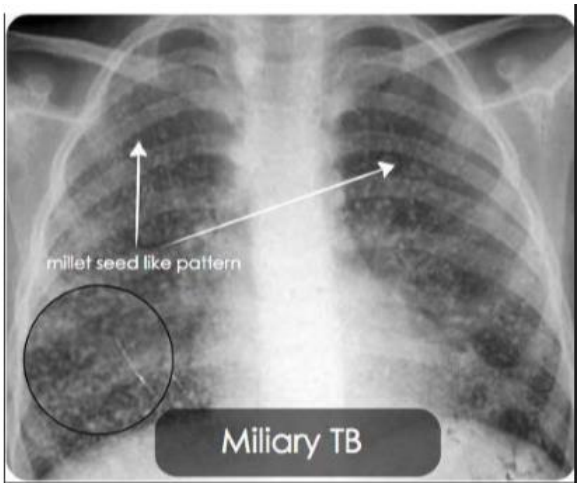
-It can affect the brain, causing **tuberculous meningitis**

-It can spread through the blood circulation, causing **miliary TB**

MILIARY TB AND TUBERCULOUS MENINGITIS ARE VERY SERIOUS CONDITIONS!

**Pulmonary and extrapulmonary can happen together or each on its own.*

Meliary TB



It is a condition where TB enters the blood circulation (i.e. **hematogenous spread**).

→One suggested mechanism by which this occurs is that TB *erodes* from its GRANULOMA to adjacent blood vessels.

Diagnostic feature of meliary TB: MILLETs- which are seed like patterns in the lungs, each of which is *a mycobacterium TB surrounded by a granuloma*.

يلاع الثانية :P