Microbiology

◉ Sheet ○ Slides

Number : 16

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Subject: Gram negative bacteria

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Gram negative bacteria

We have already talked about campylobacter and campylobacter is associated with diarrheal diseases.

We have two important species related to campylobacter:

1. ***Campylobacter infantis***
2. ***Campylobacter jejuni***

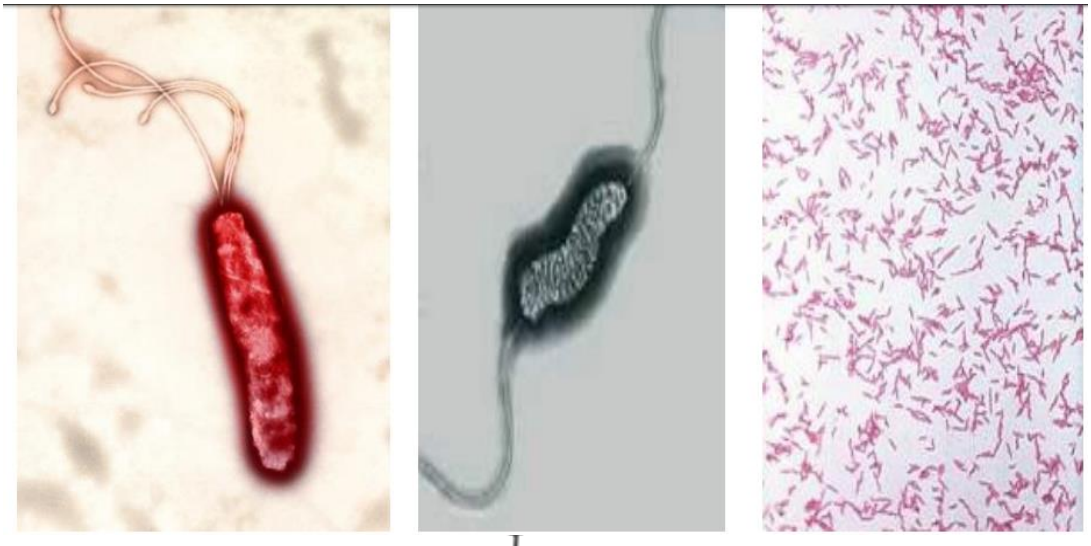
These are more related to <u>animals</u> than human beings, in animals they do produce abortions.

Also, by the presence of domestic animals ,especially cats and dogs ,the microorganism can contaminate people, other domestic animals like sheep's and goats the microbe might contaminate milk products by direct contact, it infects fresh prepared type of food.

Usually the contamination is self-limited which means it is rarely associated with complications, but it might in certain cases of patients because blood sepsis, so it might be invasive, especially in infants and immune compromised patients and rarely produce more severe infections.

Its incubation period differs from other microorganisms, we usually use **higher temperatures** and a **selective media** that contains 3 types of antibiotics , within 42 degrees under microaerophilic conditions in order to isolate the microorganism not like the *enterobacteria* under 37 degrees.

Figure(1): Flagella of Helicobacter pylori & Campylobacter jejuni , Modified G-stain



Campylobacter pylori (helicobacter pylori)

The second type which is related to campylobacter and previously called Campylo*bacter pylori* and now Helicobacter *pylori.*

It's found in the **intestinal tract** and its name is due to the fact that it's the only organism which can survive in the stomach, as we know the stomach is highly acidic its pH is between 1.2-2% , this organism can manage to live within the lining mucosa of the stomach and the duodenum, because it can produce high amount of ***the urease*** enzyme and this enzyme converts the amino group of the proteins in the mucosa into ammonia and CO_2 which result in neutralization of the HCL (hydrochloric acid) by the buffering system of the stomach and due to presence of ammonia and CO_2 we will have ammonium chloride plus bicarbonate as end products .

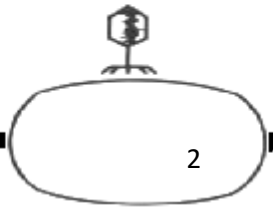
This reaction means neutralization of the medium surrounding the helicobacter which allows the helicobacter to reside in the folding of the mucosa of the stomach and duodenum in particular.

Generally according to various investigations young children become infected with helicobacter , 10% of them become colonized with it , and slowly the colonization increases with age until adulthood there it reaches up to 30-40% , in the age above 50 approximately 80% of the population become colonized with this type of organism.

Colonization is not necessarily associated with clinical diseases like gastritis or duodenal ulcer, between 1-3% of persons who are colonized might develop these clinical features due to other factors such as stress conditions or decrement in the acidity of the stomach and so on.

Conclusion : The colonization of helico backer is very high but the clinical disease is limited to few people.

The clinical disease of helicobacter can be treated with a combination of antibiotics and anti-acidic drugs, but the 100% eradication cannot be accomplished.



The treatment might relieve the patient for a short period of time (few weeks to few months), after that the disease will return back, this means the infections permits for a long time.

The presence of helicobacter pylori is not enough to indicate the presence of infection, we have to demonstrate the presence of the clinical infection in form of ulceration or duodenal ulcer which might later produce complications.
The growth pattern in the labs is like the campylobacters mentioned before, we use a selective media as well as 42° temperature to isolate the organism.

Generally helicobacter is not easily isolated due to two reasons :

1. **We must have a biopsy from the intestine**
2. **the organism cannot be excreted within the faeces or other type of specimens.**

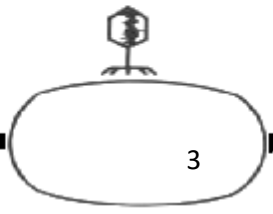
The organism rarely can reach the blood it (it's not invasive), so the only way to detect it is by the presence of urease and ammonia using a breath test which confirms the presence of infection. In addition to the presence of the clinical symptoms usually gastritis or ulceration in the stomach or duodenum and the treatment is not always successful.

Pseudomonas

Pseudomonas is another group considered to be related to enteric bacteria but it's not part of it, it is a separated group called pseudomonas or pseudomonadaceae and they are more related to nature , environment, water and vegetation etc.

There are about 20 species which might cause infections in humans one important specie called ***Pseudomonas Aeruginosa.***

●***Pseudomonas Aeruginosa*** **is the most common cause of nosocomial infections associated with hospitalized patients.**



This specie is more pathogenic (virulent) than other types of *Pseudomonas* because it resists the environmental factors which means it resists dryness , acidity and certain disinfectants, it might survive 70% of alcohol which means its not easily to be inhibited or killed by using disinfectant agents like other organisms.

In addition, they produce extracellular products which can be considered antibiotic-like substance known as biostiamin.

Biostamin is a colour dye which can inhibit other types of organisms like bacteriocins, which produce colistin from Escherichia coli .

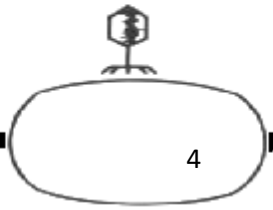
This pigmentation colours the infection of the tissue in burn cases ,so the physician recognises the presence of blue to green colour and the and fruity smell .

It is considered to be a more serious (dangerous) gram negative bacteria, because it developed resistance to antimicrobials very rapidly. Often the isolates in hospitalized patients will be multi drug resistant ,making it hard to eradicate the infection.

It is found in the environment of the hospital, so it it's the first type of microorganism which produces nosocomial infection (hospital acquired infections), wound infection dissemination, meningitis and sepsis.

During spring it can cause external otitis media which means inflammation of the external part of the ear. So it its highly dangerous especially to pulmonary infections during the usage of inspiration equipment which might be contaminated with the organism and later on producing dangerous or fatal pulmonary pneumonia.

To sum up : this microorganism is more dangerous than the others. In addition it cannot be easily eradicated from hospital floors, rooms and surgical rooms and they will colonize the patient and cause infections.



Acinetobacter

The last type is Acinetobacter and it is not part of the enteric bacteria, rather than that it is a special group related to some extent to our skin flora.

It can be found as part of our skin flora without any significance, but it might under certain conditions especially in immune-comprised conditions with malignancy and immunodeficiency's cause severe infections.

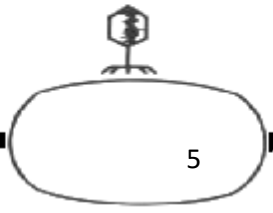
The infections are exactly like *Pseudomonas Aeruginosa,* they may cause pulmonary infections while using the respiratory equipments or by using catheters.

The organism might be found on the external genitalia and might be introduced during the usage of Foley catheter (a thin, sterile tube inserted into the bladder to drain urine) or urinary catheters in the urinary bladder and cause localized infection.

Generally the Acinetobacter is less virulent than *Pseudomonas Aeruginosa,* it rarely produces extracellular enzymes, but it is more resistant to the environmental factors exactly like *Pseudomonas Aeruginosa* it can survive in the hospital and the hospitalized patients especially the ones who undergo surgeries might get infected, the infections is established in form of pulmonary infection or blood sepsis and it will be very very difficult to be eradicated by antimicrobial drugs ,the organism often develops multi drug resistance.

As an example ,in the JU hospital at least 20 % of the patients died following the infecting with Acinetobacter also in the Hussien medical center and other private hospitals.

Due to the fact that all available drugs cannot kill the microorganism ,the only drug available is called colistin, and colistin is a very toxic drug, if it's given more than two weeks it will lead to kidney failure and this will complicate the treatment and there is no vaccine available against it .



Mycobacteria

Mycobacetria is a large group composed of bacteria that has a special type of cell wall in comparison to all gram positive and negative bacteria.

Mycobacteria cannot be identified using gram stain as the positive or negative bacteria ,due to the fact that its cell wall is special ,it's **not** composed of lipopolysaccharieds or many layers of peptidoglycans like gram positive.

Its cell wall is composed of a **mixture of protein polysaccharides**, fatty acids and a high amount of long chain fatty acids and this is known as **my colic acid.**

Mycolic acid gives rigidity to the cell wall ,so it cannot be easily stained with methylene blue or other stains. It has to be stained by a special stain called **acid-fast stain**, that has a special procedure.

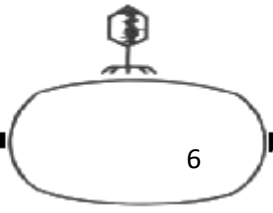
Mycobacteria, in comparison with all others, is very highly resistant to environmental factors so it can survive for a long period of time in the environment like the spore forming bacteria, due to mycolic acid which is like wax it resists the dryness and to some extent the acidity and alkaline conditions and other factors.

This means it's a very important epidemiological factor to understand why acid fast bacilli *Tuberculosi*s is dangerous.

The infection might resist for a long period in the community because of the presence of the acid fast bacilli which has the ability to reside in the sputum of the patient for a few years, and contribute to infections by dust particles which reside in the respiratory tract and later might developed pulmonary tuberculosis .

The most important species associated with pulmonary tuberculosis:
We have Three groups of mycobacteria;

1. ***Mycobacteria Tuberculosis****:* which is highly pathogenic to humans producing infections related to the respiratory tract or any body part .



2. ***Mycobacteria Bovis*** : related to animals which produces infections similar to tuberculosis in animals.

> ***Mycobacterium Tuberculosis*** **cannot produce the same clinical features in animals .**
> ***Mycobacteria Bovis*** **can infect humans but** ***MycobacteriumTuberculosis*** **cannot infect animals.**

3. ***Atypical mycobacteria***: they are widely distributed in the environment associated with wild animals birds etc. And some of them might be also associated with our skin without significance; it's found as our normal flora and rarely associated with infections.

   The difference between Obligate pathogenic *Mycobacteria Tuberculosis* and *Atypical Mycobacetria* in relation to the incubation period is that *Mycobacteria Tuberculosis* generation time for a single cell to become two needs at least few hours and sometimes up to 24 hours, whereas for all *Enteric bacteria* is between 20-22 minute, which means increasing the number of the culture of the *Mycobacteria* is slow.

   This means once we have to culture the clinical specimen from the respiratory tract sputum or any body part we have to incubate at least 4-6 weeks to recognize the presence of the *Mycobacterium* and identify it .

   The *Atypical mycobacteria* incubation period is normally within 3 days others within 1 week and others rarely grow after 2 or 4 weeks.

   The growth (incubation period is important to identify between pathogenic and non pathogenic in other words among *typical* and *atypical mycobacteria*).

   Like *Mycobacteria smegmatis* which might be found in our skin especially in the genital area ,it has no significance and if it is identified in the urine it has no importance, despite the fact that its acid=fast bacilli.

If the incubation period for *Mycobacteria smegmatis* is only 2 days ,it's enough to say its ***Atypical*** ad not important as a causative agent for a disease because it rarely induces it.

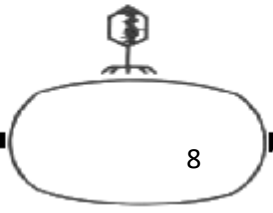
The important one is *Mycobacteria Tuberculosis* associated mainly with pulmonary tuberculosis.

In general in our country and most developing countries, infection with tuberculosis occurs during childhood ,we got infected with a few numbers of *Mycobacteria Tuberculosis* especially through the respiratory tract, the few numbers they reside in the tissue of the lung they cause small minor lesions in the form of small granulomatous lesions and the patient might not develop any clinical features as child.

Up to 95% of the children they got infected without any symptomatic signs . The other 5% might develop clinical symptoms in relation to pulmonary tract in form of fever or production of purulent sputum which means sputum containing blood and pus cells etc.

Following the asymptomatic infection of tuberculosis, the infected persons develops something called cell mediated immunity or general immunity not humoral but cell mediated immunity, and this to some extent protects the patient, it's not an absolute protection but its only partial the patient might later develop pulmonary infection or disseminated infection with *mycobacteria Tuberculosis* during his life time especially as a young adult or elderly person, because there is weakness in the general health condition of the infected person, this might now develop the reactivation of the old lesion which will result in active *Tuberculosis* often starting in the lungs and disseminate to any part of the body it might reach the kidneys, gastrointestinal tract , central nervous system and cause meningitis and so on .

So we have two forms asymptomatic form and the symptomatic form the symptomatic form is generally due to reactivation not due to new infection.



In our country approximately 70% acquire the infection without development of clinical features. But why do we have 70% in comparison to countries like Sweden and Germany where the children rarely got infected with tuberculosis?

The reason is that we have certain numbers of infected persons in the respiratory tract usually and these persons normally contribute for the circulation of the disease in the community, by spitting there sputum in the streets or floors.

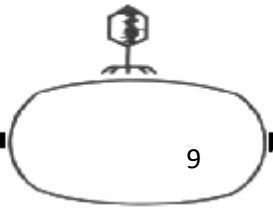
Mycobacteria survive normally and this surviving means we might later find them in dust particles and these particles will be inhaled producing infection in children and later reactivation in young adults or elderly persons leading to the case of pulmonary diseases (pulmonary tuberculosis) which might get sever leading to death if no treatment is acquired.

The treatment of pulmonary tuberculosis is managed from the ministry of health, its free in order to reduce the incidence if it in the community.

The second important feature in relation to immunity ,partial immunity is acquired by direct contact with the organism ,but to increase the immunity against it there is a vaccine BCG vaccine (Bacilli Calmette-Guerin) it gives to some extent protection but it doesn't exceed 70% .

In our country we used this vaccine until the 90s and then we stopped but now we started using it again in order to have certain protections, because we have cases that came from the emigrants.

Lab diagnosis: *Tuberculosis* from sputum or other specimen requires a lot of experience .first we must have a direct stain (acid fast bacilli) its scientific name is (zhiel-nelson stain) the name of two German scientist who developed it.
If the test is positive we have to report it to the physician in order to start the treatment, and we must use a special type of medium called (Lowenstein-Jensen medium) which contains antimicrobials and a special type of dye which inhibits the presence of contamination in



the sputum and can be used after incubation of 6 weeks or report if its present or not.

Chlamydia

A special group which cannot be demonstrated with gram stain despite the fact it's classified as gram negative bacilli.

In fact, the amount of lipopolysaccharides in the outer membrane within the cell wall of *Chlamydia* is few and not enough to help in the staining procedure, so it cannot be stained with gram stains.

We have to culture the *Chlamydia* not on an artificial medium like nutrient or blood agar we have to culture it exactly like viruses on tissue cultures, there is a special type called **McCoy cells**, Its identified by **the presence of inclusion bodies within the culture** so it doesn't identify the presence of the organism itself rather than that it identifies the effect of it inside the tissue culture.

Why do we see the effect of the organism?
Because it has a special replication cycle it's not like other types of gram negative or positive which replicate by simple division of a cell into two, a special cycle of growth including inclusion bodies, produce inclusion in the infected tissue or culture where there is replication.

There are two types of new forming cells:

1. Infection bodies responsible for increment and later for transition from one cell to another.
2. Inclusion bodies within the cell which require certain incubation period to divide to 4 parts or 8 in the infected tissue

The inclusion bodies with the infection bodies are considered as two replication forms of *Chlamydia*.

We have species which are related to the respiratory tract:

●***1) Mycobacteria pneumonia*** associated with a type of disease called *typical pneumonia*.

Classical (typical) and A*typical pneumonia*, classical *pneumonia* which is caused **by streptococcus pneumonia or klebsiella pneumonia** are associated with severe inflammatory reactions and with purulent sputum, but the atypical *pneumonia* is associated with minor inflammation and dry cough which might not be serious, but it can be serious in certain persons when it is a longer type of coughing, mild fever but its less dangerous.

- ***2)Mycobacteria trachomatis***: this species is associated with the cornea causing the disease trachoma which results in blindness. It's mainly in the genital tract and it produces non-specific urethritis.

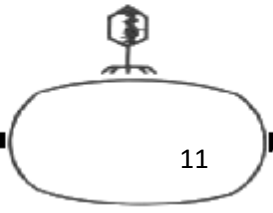
We have specific and non specific urethritis:

1.The specific one is associated with sever inflammation in the urethra which can be recognised in the form of discharge of fluid like by *Neiserria gonorrhoea*.

2.The non-specific is associated with irritation of the urethral mucosa, burning sensation without discharge of fluid.

- ***3)Chlamydia trachomatis*** :might cause damage to the genital tract of women , affect the fetus and might contaminate the newborn babies. Resulting in conjunctivitis (inflammation of the conjunctiva of the eye), which if not treated will result in damage of the cornea and blindness.

-There are at least 1 million new born babies in the world infected with *Chlamydia trachomatis* who developed blindness following the infection.



-The importance to diagnose Chlamydia is for the new born babies to be free of this organism. Also *Chlamydia* might be associated with a form of *pneumonia* if it reached the lungs like *Chlamydia pneumonia*.

The End