University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Bacteria 2

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: 2

- ○ Slides
- ○ Handout
- ● Sheet

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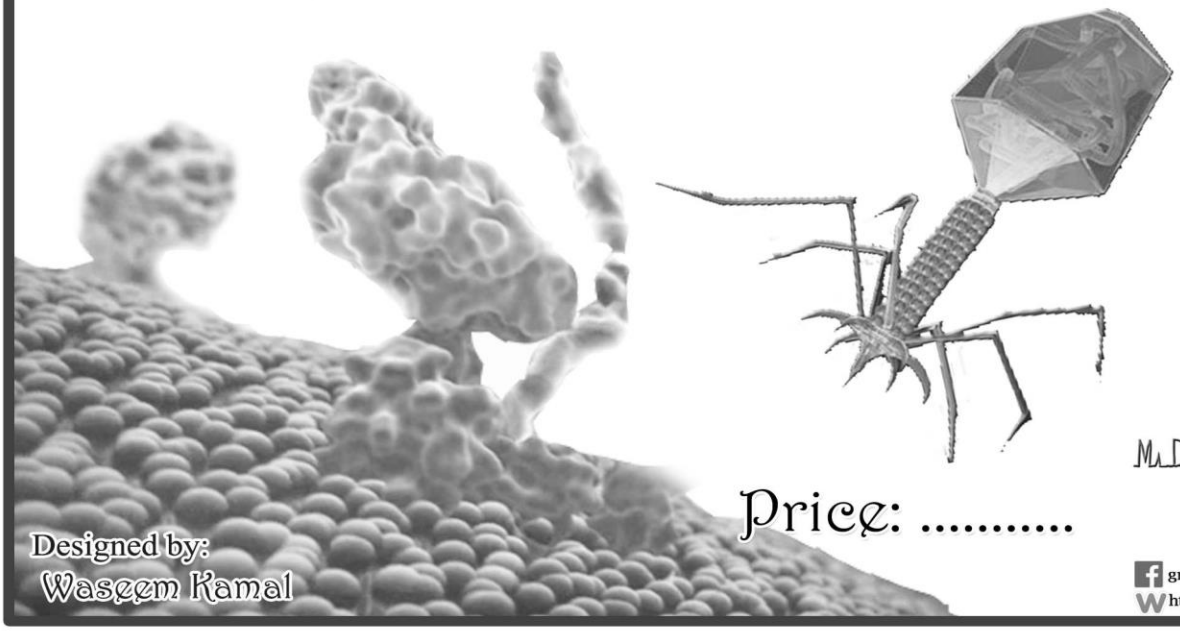

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Return back to our topic about the bacterial cells .

We have already mentioned the major structure of these bacterial cells " we'll concentrate on the major structures without going in many details".

As we said before, we classify bacteria to "Gram +ive & Gram –ive ", and this classification is mainly based on the bacterial cell wall.

In relation to this bacterial cells from outside to inside, we've already mentioned that the bacterial cells might be associated with a capsule " as slime layer ", surrounded the outer-most layer of the bacterial cells, which exposed to the environment.

We have capsulated and non-capsulated bacteria.

Capsule is composed of polysaccharide.

Capsule is important in pathogenicity, so during infection our body might have difficulty to digest the capsule.

#The second thing to be recognized is the appendages, which are:

1-large: it's the flagella, which composed of the protein flagellin.

Flagella are considered as antigen structure during contact with our body "H antigen, in return to the Latin word WHIP ", where in the case of the capsule it's called "K antigen, because the word capsule is written with K in Latin"

#Third type of structures that might surround "es p eci a lly the Gram – ive bacteria "is th e Fi m br i a e :

- Composed of protein specific protein "like the flagella "
- Contribute an antigenic structure, but of less developing antibodies
- Its function may be to attach to our mucosa in the respiratory tract, as well as in nature attached to the surface of materials to produce colonies and grow.
- It can associate in the outer cell body with a structure similar to fimbriae called "Pilli ". Which is in a few numbers "especially the Gram –ive bacteria " , and it's responsible for the future of conjugation between two cells , and this allow some of the genetic material to get transfer from one cell to another throw extra chromosomal DNA called " Plasmid ".

#The fourth structure is cell wall.

Cell wall is divided according to the type of bacteria to

Gram +ive & Gram –ive.

We have a few numbers of bacteria which can't be classified as

NOTE:

In general, in relation to medical microbiology, around 99% of important signs of medical bacteria, which associated with infections or associated with our body as normal flora can be classified into "Gram +ive & Gram –ive " bacteria.

Gram +ive & Gram –ive bacteria, we classify them as “acid fast bacilli”.

We have another type of bacteria that can’t be demonstrated with Gram stain or light microscope,

Like “Chlamydia “.

This indicated that there’re some types can’t be stained with Gram stain.

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if we look for the composition of the cell wall in Gram +ive bacteria, like **Staphy** or **Strept** , we can recognize many layers of peptidoglycan layers.

#Peptidoglycan layers are composed of two chemical structures :

1- N-actylglucusamine bound to N-actylmuramic acid in alternating way, and these two compounds associated with cross bridges composed of protein “ up to 5 amino acids “, and these cross bridges are important to induce the rigid cell wall which can later protect cytoplasmic membrane, as well as the cytoplasm, and this prevention came from the fact that the osmotic pressure inside the bacterial cells is higher than the outside, and if there is no counter attack by the cell wall and the cytoplasmic membrane, this cell will not survive “ it will burse “ due larger osmotic pressure in the bacterial cell.

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Cytoplasm contains amino acids in the form of polypeptides, associated with RNA to produce ribosomes, in addition to double stranded DNA diffused from chromosomes, as well as minerals (anions , cations , phosphate ..etc) . And all of them due to biochemical interaction there will be high osmotic pressure which should be counter attacked by a rigid cell wall “ as mentioned before “ .

KEEP IN MIND:

The cytoplasm of the bacterial cells composed up to 90-95 % of water.

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#Gram positive bacteria:

- There are many layers of peptidoglycan layers, which fixed together with penta amino acid “ as mentioned before “ making cross linking , and it’s important in relation to antibiotics.
- We have two important substances, called “ teichoic acid & lipoteichoic acid “ .
  these two substances are important in Gram stain reaction …… HOW !!!!!!
  - if we have a growth of bacteria on culture media , we take one colony of this bacteria , we spread it over a special slide composed of glass “ we’ll see it in the lab :P "
  - the usual color of the slide is colorless or whitish color if we use a drop of saline.
  - when we use chemicals in Gram stain, we use two types of chemicals. one of them associated with blue color called “ Crystal violet “ , and the

in the culture media, we can recognize a variable colors, according to the type of bacteria

second one with red color called " Safranin ", and in between we usually use alcohol for discoloration.

Why do we use alcohol for discoloration ???

In order to see if the cell wall can fix the crystal violet or not.

So, in Gram positive bacteria crystal violet will interact with teichoic acid & lipoteichoic acid and form a complex that can NOT be washed out , and it'll be fixed in the outer cell wall, and because of that we can recognize the Gram positive bacteria in BLUE color .

But in Gram negative bacteria, crystal violet will be washed out,
because " as we will see later" that it's: a) lack teichoic acid & lipoteichoic acid.

b) it has less layers of peptidoglycan.

so it won't be colored with blue by the first dye, it will be colored with the second color " Safranin ",and appears red in color.

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#This simple procedure is highly important for:

1) Classification of bacteria to Gram positive OR Gram negative bacteria.

2) To recognize morphological structures if the bacteria "whether it's bacilli or cocci", and these morphological structures help use to classify the bacteria.

3) Help physicians to know the type of infection, whether it's Gram +ive OR Gram –ive, to choose the best antibiotic for this bacteria according to its type, because there is antibiotics are for Gram +ive bacteria, and others for Gram –ive bacteria, and there are antibiotics for both +ive & -ive ,but generally we recommend that our body should NOT exposed for wild spectrum of antimicrobial drugs, and might developed to what we call " Antimicrobial resistance "

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#Return back to Gram +ive bacteria , concerning the cytoplasmic membrane:

1) it's main structure is composed of two layers of phospholipids " phospholipid bilayer ".

2) There're specific proteins called " carrier proteins ", they are responsible for transfer of:

a- electrons from outside to inside

b-nutritional molecules, which can be neutralized later in the cytoplasm to give the nessecery structure for the bacterial cells " proteins, sugar, … etc "

The cytoplasmic membrane has a semi permeability property help in controlling the fluid in the bacterial cells, and prevent the excess out of food from the inner site of the bacterial cell, so we have what we called " equilibrium "between outside the bacterial cells and inside it, otherwise the bacteria can't survive in solution or materials… etc.

The Gram positive bacteria are not so complicated as the negative one.

#There is some similarities that w e'l l m en ti o n :

1) As in Gram +ive bacteria, we might have capsules, and if it's there , it must be at the last outside of the bacterial surface, BUT if NOT , we have a layer called " lipopolysaccharide".

Lipopolysaccharide is composed of polysaccharide and a lipid called " lipid A ", and this lipid A is responsible for endotoxins " the toxicity of the cell wall of gram –ive bacteria ".

#Example:

Once any person infected with Gram –ive bacteria " like E.coli " in blood stream, the body will respond with the usual mechanisms, like phagosytosis, lysosymes, … etc , and this might resulted in lysing the cell wall, especially in ones where the polysaccharide is in the first. Release of polysaccharide which induce in developing of fever.

#Result:

Lipopolysaccharide is an important portion of Gram –ive bacteria, and responsible for endotoxicity of the bacterial cells, especially in association with the outer membrane.

The outer membrane -to some extent- is similar to the cytoplasmic membrane, and two layers of peptidoglycan "where in Gram +ive are more ", but instead you have the outer membrane layers.

Cytoplasmic membrane in the Gram –ive is quite more complex by having more carrier proteins, lipoproteins, nutrient binding proteins, etc…

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In relation to the **cell body of Gram –ive bacteria**, which means "t he out er membra ne assoc iat ed lipopolysaccharide and pe riplasmic spac e and pept idog ly c an laye r" , which can be easily expressed as "**O-antigen" which is called somatic antigen**

Now, let's connect everything together:

For a Gram +ive bacterial cell – as an example - , if available in human infection we can recognize the K-antigen, which is the capsule, but we don't easily recognize other antigens, but in Gram –ive we can recognize " if the capsule is absence " we'll recognize the somatic antigen (O-antigen) . And this is important in process of infection and in some diseases in diagnose of presence of infection or absence, because sometimes it's not easy to culture the organism because the patient had taken antibiotics and the organism will found died, and we can't contaminate the organism. In these cases we do a **"s er o l og i ca l tes t".**

#Serological test : to look for specific antibodies associated with presence of specific antigens.

So, in Gram –ive bacteria, we might look for somatic antigen " O-antigen" .

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#Gram stain is easily to be performed in fresh culture, but what is the fresh culture???

Fresh culture:

If you have a specimens " urine, blood, … etc " , and infected of type of bacteria, it takes 24-48 hours for the presence of growth, this growth can be identified now, by starting by a simple Gram stain to determine whether it's Gram +ive OR Gram –ive, and so according to the color and the morphological structures , but it's good to know that it's not easy to distinguish between Gram –ive species, were Gram +ive is more easy, because there're less differentiation.

Example :

We have a group of bacteria called "enteric bacteria ", which is in relation to our intestine, and we call it **"Fa cu l ta ti v e a n a ero b i c ba cter i a "** , which can grow under aerobic conditions and anaerobic conditions. and we have **"O b l i g a te a n a er o bi c b a cter i a ",** which can't live in the presence of atmospheric oxygen.

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In our intestines, anaerobic bacteria are about 95%, facultative anaerobic bacteria are 5%, and obligate aerobe are few, because in our intestinal tract, especially in the large intestine there is a lack of oxygen.

In Gram +ive bacteria, for organism which is widely associated in our body, and associated with infections, we have streptococci which can be found as long shaped.

And the most important group is the bacilli, which is divided into aerobe and anaerobe, were the anaerobe bacilli called **"Cl o s tr i d i u m "** , were the aerobic one called **"A er o b i c Ba ci l l us ".**

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Generally, under normal conditions all types of bacteria, during their growth, replicate in process called **" simple division "** OR **" binary division "** , which means that each cell can produce elongation and separation to produce two cells, each one of them have a copy of the original chromosomes.

Chromosomes must be found in double stranded DNA, were single stranded DNA can't survive inside the cytoplasm, due to the fact that inside the cytoplasm of the bacteria we have an enzyme called **"en d o nu cl ea s es "**, which usually cut the **single, non circular** stranded DNA inside the cytoplasm, were the double stranded and the circular DNA is protected from the restriction of endonucleases.

So, during the process of grow we may observe a type of bacteria which can easily replicate, but if there is unfavorable condition, there are certain type of bacteria produce something called **"endo spores**".

Which means that in order to supply the uterines, if there is no enough water, we recognize a concentration in the core of the cells, and this process is quite slow, but it's important in survival of certain important types of bacteria (especially anaerobic baccilus)

And the spore forming bacteria highly involved in causing diseases in humans, because during injuries or accidents, any contamination of our body with the soil might be associated with the spore forming bacteria, and this type of bacteria once it reaches subcutaneous tissue, converted from spore forming to vegetative forming " which needs living cells ".

In order to understand more details about growth of bacteria, we've to consider the bacteria as exactly as humans and animals, so as humans. we can't survive under very cold temperature, and the same for the bacteria, were – for example- the requirement of growth is related to the presence of oxygen (for aerobic), as well as the water and the pH, etc … and all these need to be controlled

KEEP IN MIND:

Pathogens which cause disease requires special culture media

Pathogens manage to survive in our bodies by the presence available nutrients.

In relation to clinical microbiology or medical types of bacteria, we divide

it generally to aerobic, like mycobactiruim tuberculosis, which is the positive agent to cause the

disease tuberculosis which found in lungs.

This type of bacteria proliferate if they exposed to the oxygen in our lung tissue, so they will not survive in our intestine, where there's a lack of oxygen.

Aerobic bacteria mean that the end electron acceptor should be the oxygen, during the process of oxidation reduction reaction.

Why this bacteria can survive in presence of oxygen, even that the oxygen is a toxic substance, and the oxidation reaction can kill the bacteria, especially if increases the amount of oxygen !!!

The answer is that the aerobic bacteria have specific enzymes which control the concentration of oxygen and to control the neutralization of oxygen in biochemical reactions, they have an enzyme within the cytoplasmic membrane like **"S u p er o xi da s e dismutase",** which usually reduce the active form of oxygen "radical form ", which is very dangerous for the tissues into hydrogen peroxide and oxygen.

And there is another enzyme called **"Ca ta l a s e ",** which convert hydrogen peroxide into water and oxygen

And these two enzymes are NOT found in the anaerobic bacteria, so once they exposed to the oxygen, they can't survive.

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Within the aerobic bacteria there is an important group called **"Neis s er i a " .**

Neisseria cause a sexually transmitted disease known as **"Go n o rr h ea " .**

Neisseria despite its aerobic, but in culture media can NOT survive under the atmospheric oxygen, because it requires a cretain amount of carbon dioxide, so it's considered as **"Mi cr o a er o ph i l i c Ba cter i a ".** which means that it requires certain less amount of oxygen.

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Facultative anaerobe:

It's a very important group cause :

1) It can be found in our intestine.

2) Important as positive micro agent in many diseases, which accumulate about 70-80% of all bacterial infection in our body.

They found mainly in our intestine associated with obligate anaerobe.

Why do we call it facultative anaerobe ??

Because these bacteria can be switch from aerobic condition to anaerobic condition, according to the environmental condition, and they can manage to survive in any place in our body.

How do anaerobic bacteria manage to survive without oxygen ?

In order to produce energy, they must have a system depends on a process of glucose fermentation.

which allow producing energy