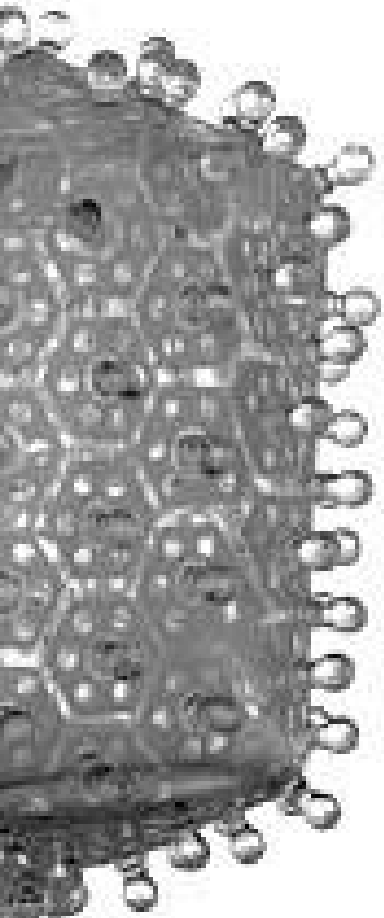

Microbiology

Lecture No: 2

Dr Name: Asem Shehabi

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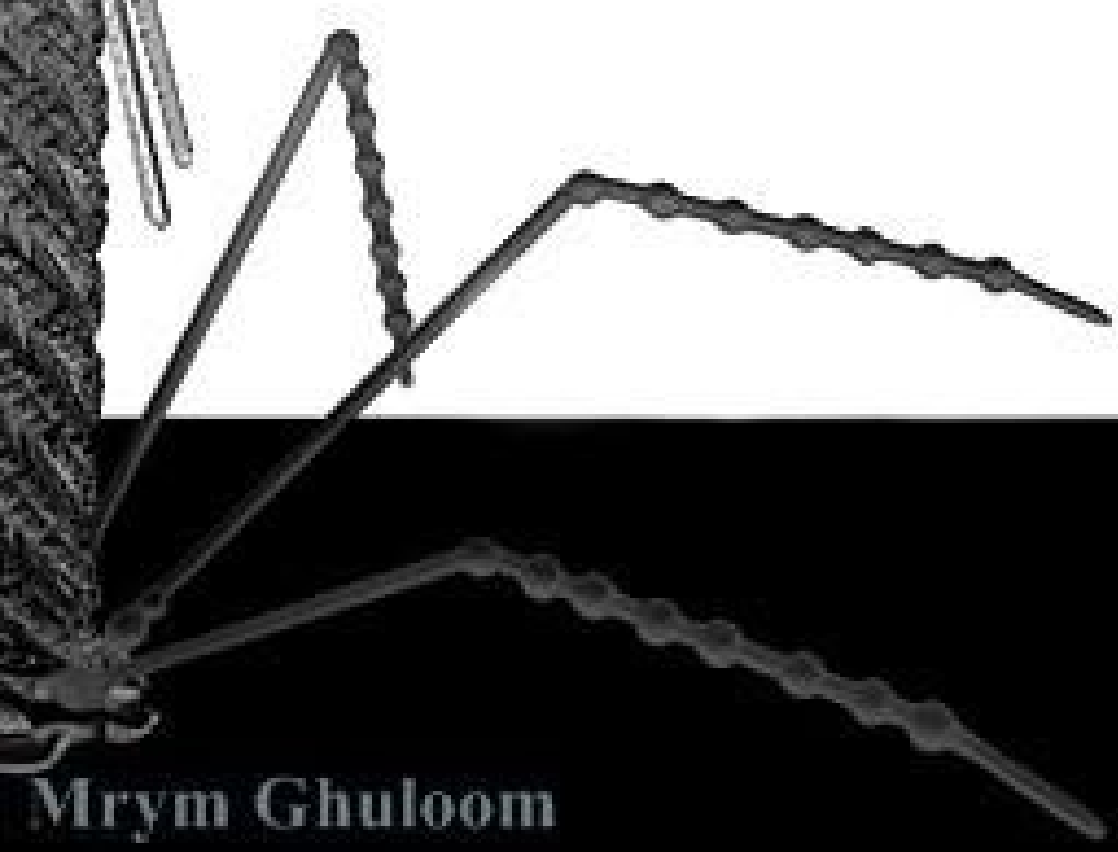
Sheet ■ Slide □



Mrym Ghuloom

بسم الله الرحمن الرحيم

Let's return to our topic (The bacterial cell structure). We have Gram +ve bacteria and Gram –ve bacteria.

*Gram stain is a method widely used in labs to help with the classification of bacteria that may be found for example in clinical specimens or in nature.

In order to understand why a certain type of bacteria is gram +ve while another is gram –ve, we should know the cell structure.

Knowing the cell composition of gram +ve and gram –ve bacterial cells helps us in understanding the process of infection, because the process of infection related to gram +ve bacteria differs than that related to gram –ve bacteria, due to the fact that each cell structure influences a specific response; once bacterial cells reach our body, they're lysed, damaged and broken down by phagocytosis and other factors to certain compounds, for example: Gram+ve bacteria will be broken down to a capsule, a cell wall, many parts of cytoplasmic membrane and the chromosome of the cell. Our immune system responds by producing specific antibodies (against the capsule, against parts of the cell wall and against the other components of the bacterial cell).

Some of these antibodies (which are composed of immunoglobulins) can be identified later by certain laboratory tests.

The most important components of bacterial cells (like capsules, the cell wall, the peptidoglycan layers, flagella and fimbriae) are considered foreign materials or antigens. These foreign materials will stimulate our immune response to produce specific antibodies against them. (So antibodies against capsules, antibodies against the somatic antigen) *The somatic antigen: is an antigen located in the cell wall of bacteria*

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Therefore, the structure of these bacterial cells should always be considered while studying, not only because it's important in determining the composition of gram +ve and gram –ve cells, but also to understand the process of infection in our bodies. For example, to know why a patient with gram –ve bacterial cell will suffer from what's called an endotoxic shock, it's because of the release of certain toxic compounds that are related to the cell wall.

Generally, bacterial cells consist of: (from outside to inside)
1- Flagella: (singular: flagellum), if one flagellum is found at one pole of the cell then the cell is called: monotrichous flagellated. If multiple flagella are present at one pole the cell is called Lophotrichous bacteria. If multiple flagella are scattered over the surface of the bacterium it's called peritrichious bacteria.

Usually, Flagella originate from the cell wall and they're localized there.

The number of flagella differs from one type of bacteria to another (ranges from a few 100's to a few 1000's).

Flagella are composed of subunits called flagellins (specific polymer proteins) (they differ from one type of bacteria to another but still serve the same physiological function).

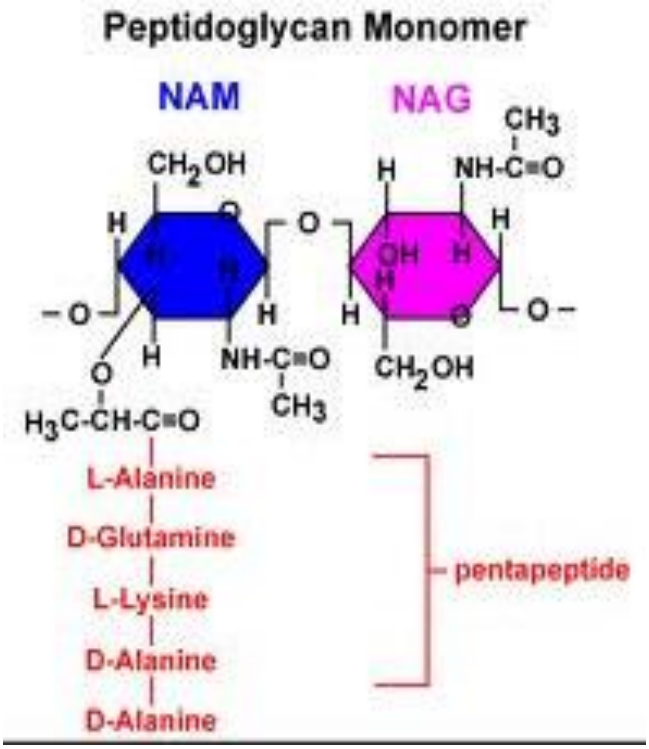
2- Fimbriae: (singular: Fimbria) they contribute to the adherence of bacteria to our mucosa.

3-Pili: (singular: pilus) appendages similar to fimbriae but they are smaller, shorter and present in limited numbers. (*many bacterial cells can be classified to males or females). Pili may serve a sexual function in order to produce a "conjugation Pilus", which brings 2 types of gram –ve bacterial cells together and allows the transfer of certain genes from one cell to another.

4-Cell Wall:

- In gram +ve bacteria, it consists of many layers of peptidoglycans (thick cell wall and can reach up to 25 layers) the number of layers differs from one type of bacteria to another, it could be 8 in one type, 10 in another and can reach up to 25.

These peptidoglycan layers are composed of alternating subunits of (N-acetylglucosamine and N-acetylmuramic acid) and are usually bound to each other by a bridge of (4-5) amino acids.
This gives rigidity to the cell wall and helps in controlling what is inside the cell like the osmotic pressure within the cytoplasm of the bacterial cell (which is –the cytoplasm- composed of a huge amount of different small molecules and water, and where many biochemical reactions take place and affect the osmotic pressure).



Within these peptidoglycan layers we have at least 2 types of compounds called: Teichoic acid and Lipoteichoic acid, they are responsible for interacting with crystal violet dyes during the process of gram staining, the acids take up the dye and form complexes that give the gram +ve bacteria a blue color, and since complexes are formed, the bacterial smear can't be easily discolored by alcohols.

When preparing a smear we don't know if it has gram +ve bacteria or gram –ve bacteria, so we take into consideration that we may have +ve, -ve or both, and the detection of each type requires a specific method.

For Gram +ve processing, we fix the bacteria on the slide and apply a crystal violet solution, the bacterial peptidoglycan layers will from complexes with the dye and stain blue, and can't be discolored by alcohol.

Gram –ve bacteria has no Teichoic acid or Lipoteichoic acids, so it can't retain the crystal violet dye to form complexes and won't permanently stain blue (the color washes off upon adding alcohol), so it will be later counter stained by another type of dye called Safranin (it stains the peptidoglycan layers and the outer membrane and become fixed to the cell wall so the bacteria appear red).

*Going back to gram +ve calls, when we look at them, we recognize the **cytoplasmic membrane** which usually has two important layers of lipoproteins (phospholipids + certain proteins which normally contribute to the transfer of certain molecules from outside to inside. There are also hydrolases (enzymes) that control the flow of necessary molecules, nutrients and minerals by trans-oxidation or diffusion.

Actually there are many mechanisms that can be used by bacteria to allow the necessary molecules and nutrients to enter inside the cell and prevent the presence of excess materials there, the bacterial plasmic membrane and the periplasmic space control the intracellular environment.

The **periplasmic space** is a semi-fluid, gel-like structure that contains large amounts of enzymes and certain proteins that contribute to the transfer of necessary molecules and minerals through the permeable cytoplasmic membrane.

*Some gram +ve bacterial cells are covered with slime layers (composed mainly of polysaccharides).

There are two main types of slime layers; one type is solid (the capsules). *not all gram +ve cells are capsulated, the important type of cells: streptococcus pneumonia (this is capsulated) (the causative agent of pneumonia, the infection of the lungs الالتهاب الرئوي

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When capsulated bacteria reach our bodies, they release their capsules due to phagocytosis and lyses of the cells. The release of capsules (which are composed of polysaccharides) results in forming specific antibodies called Anti-Capsule Antibodies (AKA, Anti-K antibodies, "K" because "capsule" in Latin starts with a "K" not a "C"). Flagella influences the production of antibodies as well, they're called Anti-flagella Antibodies (AHA, Anti-H antibodies, "H" because "flagella" in Latin starts with an H).

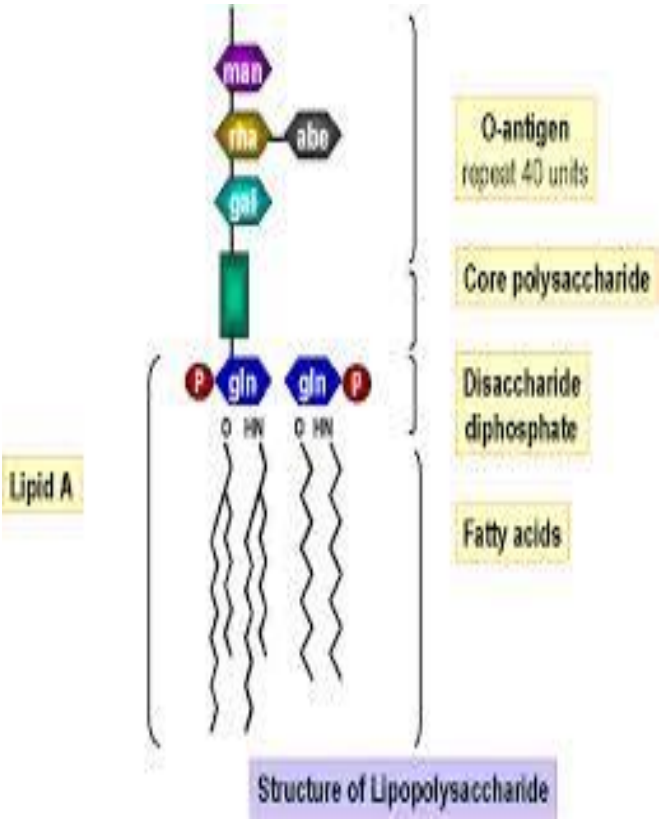
In Gram –ve bacteria, cell walls are more complex and the bacteria influence more severe infection signs (high fever and toxic effects) that can be easily recognized clinically.

- . Gram –ve bacteria consists of: (from outside to inside).

1- A **Lipopolysaccharide layer**, mainly composed of lipids, phospholipids and polysaccharides. These polysaccharides are composed of different sugars like (galactose, mannose, etc) and they differ from one type of bacteria to another. These lipopolysaccharides are also composed of 2 important compounds or parts; one of them is (Lipid A).

-LIPID A: phospholipid + certain disaccharide +long chain fatty acid. It's responsible for the development of fever during infection with gram –ve bacteria (endotoxic shock). This means that gram –ve bacteria is associated with more severe infection (high fever) than gram +ve bacteria.

The lipopolysaccharide layer is attached to a second layer that is not found in gram +ve bacteria, it's called:



Structure of Lipopolysaccharide

2- **Outer membrane layer**: it's composed of various proteins and lipoproteins, it contributes -like the periplasmic space- to the transfer of molecules from outside to inside the cell. It will also support the cytoplasmic membrane in controlling the flow of necessary nutrients and prevent the presence of excess materials or certain antibiotics inside the cell.

3- **The Periplasmic Space**, similar to that found in gram +ve bacteria.

*Note: Gram –ve bacteria can't be easily treated with antimicrobial drugs like gram +ve bacteria, due to the presence of the outer membrane and the periplasmic space.

In the periplasmic space there are many types of hydrolyzing enzymes that break/damage certain types of antibiotics during their access like: aminoglycoside drugs.

Often within the periplasmic space and cytoplasmic membrane, oxidation-reduction reactions take place, they are the major biochemical reactions that control the growth of the cell and prevent toxic oxygen from reaching the cell membrane. (Atmospheric oxygen is toxic to all types of bacteria. Aerobic bacteria which grow under aerobic conditions (with the presence of oxygen) have certain enzymes that detoxify the molecular oxygen and convert it to other harmless compounds).

*Related to gram –ve cells are the somatic antigens (somatic in Latin means the body of bacteria) but actually it's related to the LIPOPOLYSACCHARIDES that may be associated with certain amounts of periplasmic space. And can be used in detection of certain diseases due to the fact that the O antigen induces the production of anti O antibodies. Example of a disease: typhoid fever.

*don't forget that the cell wall of gram-ve bacteria has only one or two layers of peptidoglycans. (While in gram +ve the layers reache up to 25 layers).

*Concerning the capsules in gram –ve bacterial cells, they're rarely recognized but few types of bacteria may have slime layers (semi-fluid, not solid as in gram +ve), and this enhances the pathogenicity of the bacteria. So there are no true solid capsules as in gram +ve cells.

The Gram stain method was developed by the scientist Gram to help with the classification of bacteria into gram +ve and gram –ve (it involves using 2 different dyes and alcohol).

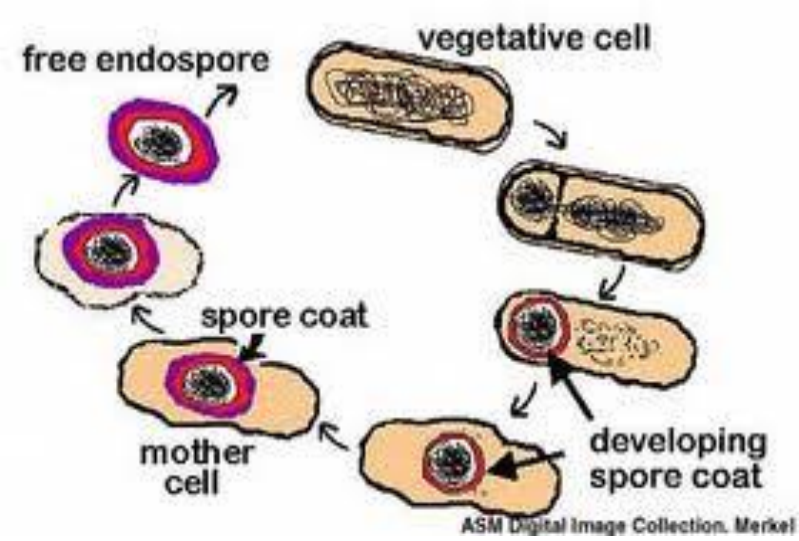
*the blue and red colors aren't always so accurate, it's important to use fresh culture (24-48 hours old) in order to determine the true type of the bacteria, because in old culture; gram +ve may become gram –ve and vice versa. Cytoplasmic membranes in both gram +ve and gram –ve cells have oxidizing and lysing enzymes that are activated after a certain period of time and begin to damage the cell wall, because bacterial growth has limitations and bacteria can only survive for a short period of time (time varies according to differences in bacterial growth curves).

-Examples of gram +ve bacteria: Staphyloccocus, Streptocooci, Bacillus.
-Examples of gram –ve bacteria: Enteric bacteria group E.coli, Klebsiella, Salmmonella Pseudomonas.

** During the growth of gram +ve bacteria, there is a process that is called: **endospore formation**:

In general gram+ve and gram –ve bacteria can't survive for a long time, and must always produce new generations/cells. But there are some types of bacteria found in nature and might also be found in our intestines called: spore forming bacteria (usually they're gram +ve bacilli). They manage to survive under harsh conditions (lack of nutrients, water, etc) *-water is very important for the growth of any type of bacteria-.
For example some types of bacteria can't survive outside our bodies for more than 30 minutes like Haemophilus or Neisseria, other types can survive outside the body for 24-48 hours if cultured on dry surfaces like Escherichia Coli (E.coli).

But spore forming bacteria can survive for hundreds of years without water or nutrients and this is so dangerous, because for example these spore forming bacteria may reach a damaged tissue due to an injury, and with anaerobic conditions (may be aerobic but anaerobic is more dangerous) they become vegetative and start dividing and increase in number.



*In dry conditions, spore forming bacteria lose the majority of water from its cytoplasm and produce a double cell membrane and concentrate the genetic material within the cell, AND THIS WHY THEY CAN SURVIVE FOR A LONG TIME UNDER HARSH CONDITINS.

- Another problem with the spore forming bacteria is for example when we want to sterilize surgical instruments. Generally, the majority of bacteria can be killed within few minutes at temperature between 60-100 degrees, while this spore forming bacteria may need more than 2 hours to be killed (at 100 degrees); this means that it's resistant to heat due to the presence of the double cell membrane and the spore that contains the nucleic acid.

The spores in the spore forming bacteria (that contain the chromosome or the circular double stranded DNA) could be concentrated in one part of the bacilli -central- or terminal and this helps in determining the bacterial type.

* NOW we'll talk about the cytoplasm; the cytoplasm of the majority of bacterial cells is composed mainly of water (80%) + Ribosomes.
The Ribosomal structure in prokaryotic cells is refered to as (70S), it's composed of 2 subunits (a 30S subunit and a 50S subunit) even though they add to 80, the ribosome is still considered 70S due to the configuration of proteins.
(The letter "s" means Svedberg (the Swedish scientist that developed the method to differentiate between prokaryotic and eukaryotic ribosomal structures, and a unit to measure the rate of sedimentation) (*sedimentation describes how quickly particles settle down when a force, such as centrifuge or gravity acts on them.)

*Eukaryotic cells (fungi, parasites), have 80s Ribosomes.
*Prokaryotic cells are mainly (bacteria and some types of algae).
*Ribosomes are important in the process of protein synthesis.

Within the cytoplasm we have circular double stranded DNA represented by one single chromosome that may be small or large and carries (1000-4000) genes. (Each gene has a specific function).
Keep in mind that this chromosome is found concentrated not necessarily in the center but may be elongated over the surface of cytoplasm.
Recent discoveries state that not all bacterial DNA is circular, some is linear (the majority is circular though).

Without the presence of DNA and ribosomes, bacterial cells can't produce any structures or materials needed for their growth and functionality.

So the process of replication and synthesis of proteins depends on the genes found on the chromosome and on the ribosomes. There are necessary enzymes for this process like RNA polymerases for the production of polypeptides and nucleases for cutting off the proteins.

*Certain types of bacteria can carry storage compounds like phosphate (in environmental bacteria) or proteins or glycogen (in some Saprophytic bacteria)
In human bacteria, the storage material is mainly in the form of polypeptides (may be associated with phosphate or other materials) but generally it's rare to recognize the storage materials in pathogenic bacteria. Storage compounds are easier found in Saprophytic bacteria found in nature.

Growth of bacteria

It doesn't mean the increase in size or function, but it is the increasing of number of the cells. For example, instead of having 1 cell, you can have 1billion cells in order to recognize the growth of bacteria, and this can happen in less than 18 hours if we offer the necessary conditions (water, growth conditions, and oxygen). So if bacteria is cultured in favorable conditions it will undergo rapid continuous division, for example it's been calculated that 1 E.coli cell if cultured in favorable conditions might produce (within 24 hours only) 1 ton of bacterial cells.
But luckily continuous culture is very difficult to control and is not easy to occur in nature or within our bodies because there are limitations whether

due to nutrients or water or the role of the immune system which all contribute to the control of bacterial growth, however in industry continuous culture can occur.

*Bacterial Growth Requirements

Any type of pathogenic or nonpathogenic bacteria requires certain organic or inorganic compounds for example.

Oxygen, if there is no oxygen available; there must be inorganic compounds or minerals to produce the energy needed for the oxidation-reduction rxns during the bacterial growth.

But generally, bacterial growth (whether commensal or pathogenic) requires Carbon sources and Nitrogen sources and often both of these come from sugars –polysaccharides- and proteins.

*for eg) Meat is essential for the growth of most pathogenic bacteria.

*In addition to these organic materials, there are vitamins and inorganic minerals that might be needed for the growth of some types of bacteria (eg: Na, Mg, K, S, P).

*regarding the infection of blood, most pathogenic bacteria find all necessary nutrients because the blood is rich with water, minerals and nutrients.

Bacteria produce energy through 3 different processes, depending on their need of oxygen.

-obligate aerobic bacteria

-obligate anaerobic bacteria

-Facultative anaerobic bacteria

And we will talk about them in the next lecture ☺

بالتّوفيق ☺

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