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Introduction to microbiology and Bacteria

* Some pieces of advice for helping you in studying this course:

- Firstly, from the doctor's experience, attending classes is very important as this will help you through the semester especially in using terminologies, because microbiology is full of new scientific words. Moreover, keep in mind that this is an introductory course, we will not cover all aspects of each microorganism, only some details are required.

*What is the definition of microbiology (microorganism)?

- Microbiology: it includes all types of small living organisms which normally cannot be recognized easily by our eyes.
- Sometimes they require special types of cultured media. Stains are used for that, for example in **bacteria**, we use special **stains** to recognize the bacterial shape, to recognize what we call, the stain reaction.
- Sometimes we can observe the general growth of microorganisms on water surfaces, plants, food or on other material surfaces IF there is a large number of cells accumulating together on that surface.

Simple example:

- A type of Fungi called Penicillium can be detected on bread, fruit or cheese by the naked eye.
- But in the case of bacteria, it needs large numbers of cells (millions) in order to observe a change in the color of food or water (Turbidity) .

*But Viruses are different, how? They are:

- → Difficult to be seen and recognized in comparison with bacteria
- → NOT independent cells
- Tiruses can NOT be cultured or propagated (تتكاثر) in cultured media like in bacteria, they (viruses) need tissue culture or living cells



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** Electron microscope is used to observe viruses, on the other hand Light microscope can be used to see bacteria, Fungi and Parasites.

Microorganisms are classified into two major groups:

- 1- Eukaryotic cells
- 2- Prokaryotic cells
- All types of cells (prokaryotic and eukaryotic) are bounded by the <u>plasma</u> <u>membrane</u>, with a semifluid substance inside is called <u>cytosol</u>, in which subcellular components are suspended. All cells contain <u>chromosome(s)</u>. And all cells have <u>ribosomes</u> for protein synthesis.
- Prokaryotic cells contain circular double stranded DNA which is concentrated in a region that is *not membrane-enclosed*, called the <u>nucleoid</u>. They also have a special structure with appendages like flagella and Pili. On the other hand, **eukaryotic cells** have <u>true nucleus</u>, which is *bounded by a double membrane*, and have most of the DNA in it.
- Not all microorganisms are prokaryotes. For example Fungi are eukaryotes.
- Bacterial cells are prokaryotic cells.

	Eukaryotic cells	Prokaryotic cells
Presence of DNA & RNA	True	Double stranded DNA
Nucleus	True nucleus	Nucleus without membrane(nucleoid)
Chromosomes	Many	Only one chromosomes
Cell membrane	True	True
Special appendages	-	Flagella, fimbriae, different type of capsules
Examples	Animals, Humans	Bacteria



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Creepy fact: (read only)

"The number of bacteria in our tract is higher than the number of our living cells"

- Prokaryotic cells, like bacteria, generally have size ranges between (0.1-10)µm in length and (0.2)µm in width, and for that reason it is too hard to recognize it with the naked eye.

** What is the process that leads to the recognition of bacteria?

In order to see the bacteria, we are forced to culture it, which leads to the process of Growth (النمو), leading to the production of large number of cells forming the Colony (مستعمرات).

Colonies' morphologies give the opportunity to determine and see the different types of bacteria and also are useful in classification.

- * *Microbiology is divided into many branches according to its relation with human body and nature. And according to the function of the microorganisms in relation to the human body one of the branches is the science of medical microbiology which divides the bacteria in to two types; one type is the bacteria which reside in the human body and they are called Normal body flora or Commensal body flora.
- Common places to find such bacteria in human body: oral cavity, intestines, part of our genital tract, As well as skin.
- It resides in the human body without causing any type of illness or infection.
- These bacteria are so beneficial to the limit that human body would not survive without them, especially the bacterial cells which live in the intestine precisely in the large intestine.

* *Why are large intestines' bacteria essential and beneficial?



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Because they contribute to get rid of certain complex organic compounds which are obtained from food. And also contribute to the detoxification of certain toxic chemical compounds, which are produced during fermentation and digestion in our intestinal tract. In addition to that, they play a role in insertion of certain components which are important to our health especially in the preparation of certain vitamins and essential elements to our bodies. (which our body cannot synthesize them).

- Conclusion-

No human or any living organism can survive without normal body flora or Commensal body flora.

*Caution Note:

In the normal conditions Commensal body flora reside in the human body without causing any illness or harm, but if these bacteria manage to reach internal part of the body due to different causes such as: perforation, damage in the intestine, injury or operation, etc.., after that the bacteria will reach the blood stream or any body fluid or tissue leading to **infection**, which means the presence of foreign bodies, usually living organisms, like viruses, bacteria or any other microbe causing disease and illness.

- **The science of medical microbiology is specialized in the study of:
- The positive agent of a disease, How the disease spread in the community, How we acquire infection (directly/indirectly), How to compete and kill these organisms during the infection.
- **And there is the **food microbiology** concerning about types of microorganisms which are found in food whether they were pathogenic or non-pathogenic, and the same goes to the agricultural microbiology and other branches of that science.



Sheet #1

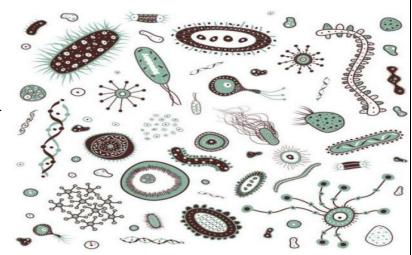
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In the upcoming lectures the doctors will cover only the important medical types of bacteria, fungi, parasites, and viruses.

- -- The picture on the right was taken from contaminated water.
- It represents large number of microorganisms with various shapes and structures, some of them are bacteria and some are parasites and the others are fungi.



- Bacteria as a word is fascinating, because it has large variety, bacteria can be observed as single cells, or large elongated cells, sometimes found as a cluster of cells, or they can be flagellated cells or non-flagellated...etc.
- **There are some types of bacteria can be found in the nature or in the soil and cannot survive in human, these bacteria usually utilize CO2 and Nitrogen from the atmosphere to produce necessary essential components for their growth.
- ** Generally, bacteria that resides in our body and pathogenic bacteria (which might cause disease) often require complex organic compounds > they need proteins and carbohydrates for example, they cannot utilize CO2 and Nitrogen from the atmosphere to produce the necessary components for growth.



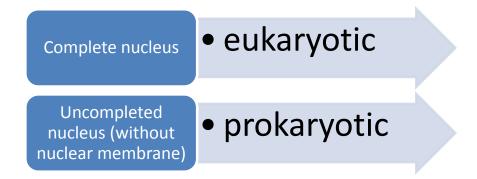
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Remember

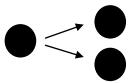
First thing to distinguish between eukaryotic and prokaryotic is to look at the nucleus.

If it was:



- <u>The genome</u> of a prokaryote is structurally different from a eukaryotic genome and in most cases has considerably less **DNA**. In the majority of prokaryotes, the genome consists of a circular chromosome.

So, Prokaryotes have a circular double stranded DNA in form of **chromosome**, therefore, the multiplication of the bacteria (as prokaryotic cells) is easier than that in eukaryotic cells. And one single cell can give rise to a large number of cells and unlimited growth by simple division, each single cell can divide into two cells with equal size.



- In the upcoming lecture we will take in more details
- In order to understand the different types of bacteria, they need to be cultured, and in order to recognize the shape and arrangement of the bacteria we need to use **Gram stain**.

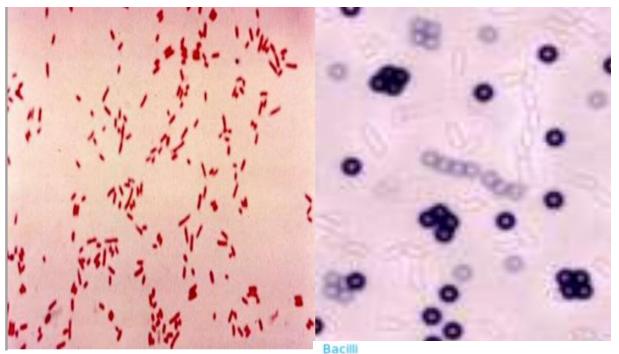
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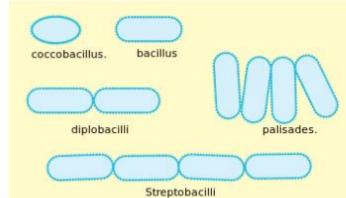
Glimpse on the Gram-stain

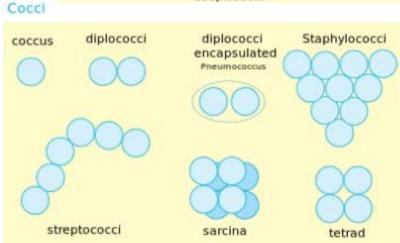
Gram - negative (Red)

Gram - positive (blue)



- *Gram negative (Red):- in the picture above, small cells known as rod cells or bacilli, they can be found as one, two or three cells living together.
- *Gram positive (blue):- in the picture above, small cells known as cocci, can live as single cocci (coccus), or double cocci (diplococcus), or in four cells known as tetrad cocci and sometimes seen as a cluster of cocci called Staph as an exampl, and finally it can be a Streptococci (Chain like).







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**The color of bacteria can be demonstrated in the laboratory by using Gram stain technique.

"In the lab we are going to see how to prepare gram-stain and how to recognize the shape of bacteria, and to distinguish gram positive bacteria and gram negative bacteria".

***In order to understand the Gram stain technique you need to keep in mind:

- 1- 99% of the bacteria (majority) can be classified into gram positive and gram negative.
- 2- Gram positive & gram negative \rightarrow shapes, colors and rate of reproduction and producing new individuals. For example, G+ve can or, (رباعیات) or be classified into diplococcic, streptococci, tetrads staphylococci.

NOTE: Bacilli can be found in both G-ve and G+ve stains.

- -In the gram negative (red) there is different type of bacilli, small bacilli, larger bacilli, and a type of bacilli but it's not clear in the record.
- -In the gram positive (blue) bacilli there are spore-forming bacilli, nonspore forming bacilli and normal bacilli.

**What is the importance of the gram stain? Why are we interested in knowing if it was gram positive or gram negative?

This is related to the antibiotic treatment, later on we will take it in specific.

* *There are some types of bacteria that cannot be observed by gram positive or negative, such as **Mycobacteria** that cause Tuberculosis السل and another type of bacteria called Mycoplasma pneumonia, we need a special stain to recognize them.



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*So Gram reaction is important as a first step in classification of bacteria.

* *There is different biochemical test other than gram stain, such as **Fermentation test**, this is used to recognize if the gram negative ferment sugar lactose for example or glucose ... etc.

These tests and techniques come together in order to classify the bacteria into similar or general groups and **genera (singular, genus)** groups. And in each genus there are **species.**

Example:

- Enteric bacteria or Enterobacteriaceae (scientific name) is a large family of Gram-negative bacteria that includes many genera: Klebsiella, Escherichia coli, Enterobacter...and so on
- Within each genus of these genera, there are species such as: kheqwdjhgseyusaknsia only **joking** the professor didn't give an example on species.
- → The information gathered from gram stain, biological reaction and biochemical reaction...etc, can lead to classification of bacteria into specific names, and these specific names are important to understand the type of infection in humans in order to use the right antibiotic.

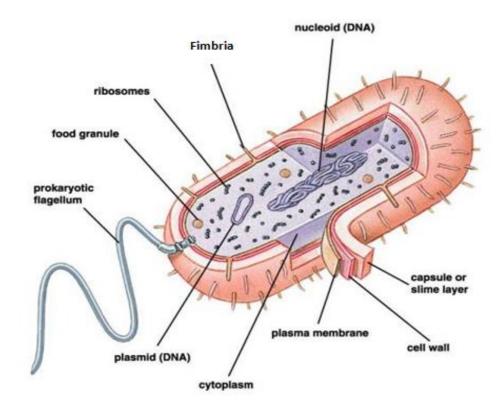
((because the most important aspect to know, after isolating an organism (ex:bacteria) from blood, from urine or any part of the body, is the type of that bacteria. And without knowing that we cannot start using any antimicrobial drug))

*In community physician, they give general antibiotic to cover many types of organisms, and this can be accepted in some cases, but in general it's a wrong way of treating the patient, because it's not enough, especially in serious cases, for example a patient that suffers from blood Sepsis, he can't be treated by giving him any available drug like Ampicillin, Amoxicillin. Physicians need to find out exactly what are the different organisms responsible for the disease to be treat patients properly.

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Prototype of Gram negative cells:



- * Gram positive cell may differ in some certain characteristics
- -Starting the structure from outside to inside-
- → Flagella (singular, flagellum): organs of motility, elongated appendages (10-20 µm), can be absent or present, they are composed of the protein flagellin, and important in relation to bacteria environment and to the human body.
- *Why are Flagella important or efficient to the bacteria in their environment (water)?
 - 1- Flagella help bacteria to attach and adhere on the surface of materials.
 - 2- Flagella direct the bacteria towards nutrition, and they are responsible for motility (الحركة) of the bacteria in fluids.



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*How Flagella are important or efficient to the bacteria in relation to the human body (respiratory tract/intestine)?

Flagella allow the bacteria to attach to the surface of our mucosa especially in the respiratory tract or intestine, and that's very important to the maintenance and adherence of bacteria in our body, without Flagella the bacteria will be washed out.

→ Fimbriae (singular, fimbria): appendages smaller in size compared to the flagella (less than 10 µm)

*Why are Fimbria important or efficient to bacteria?

- 1- They play a major role in adherence of bacteria, (but flagella for motility and adherence).
- 2- Some specific type of Fimbria are called **Pili(singular, pilus)**, which are appendages that pull two cells together prior to DNA transfer (certain genes) from one cell to the other.
- * *The transference of the genetic material from one cell to another through Pili, remarks and its part of the evolution and mutation of the bacteria because it is changing the characteristics of the bacterial cell itself, and that may lead to develop an antimicrobial drug resistance (مقاومة المضاضات الحيوية). As well as, transference of toxic genes could occur, that would result in producing pathogenic bacteria (which have toxic genes associated with more pathogenic strain). To sum up, Pili contribute to the variation of characteristics of bacteria, and may **convert** non-pathogenic (non-toxigenic) type of bacteria into pathogenic (toxigenic).
- → Cell walls: the cell wall is the outer layer that surrounds bacterial cells, and it is <u>rigid</u>, composed of several layers.
- The cell wall is important because it protects the bacterial cell, maintains its shape and size, prevents it from explosion (or bursting) in a hypotonic environment, so it keeps the inner contents inside the cell, and it protects the cytoplasm and the plasma membrane of cells.





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*Why is it important for bacteria to protect their cytoplasm?

- 1- Mainly the cytoplasm is composed of water (80%), and there are certain organelles and essential components that are crucial for growth, like ribosomes (for protein synthesis), Mesosomes and storage materials,.. etc.
- Mesosomes (small segmented membrane) they are infoldings in the plasma membrane, these are rich in enzymes that help to perform functions like cellular respiration, DNA and cell division (most important function).
- 2- The cytoplasm is the place where most biochemical reactions develop and occur in order to produce the necessary components that are needed for cell growth, survival and replication. ((Just like us; we cannot survive without nutrients, and also bacteria cannot survive without the needed biochemical reactions, or some kinds of bacteria need the absorption of water and minerals, others need certain compounds; proteins, carbohydrates,..etc))
- These contents in the cytoplasm of bacterial cells should be kept in a saclike structure, and that what rigid walls do actually, they play a major role in maintaining the osmotic pressure, without rigid cell wall it will be very high, so that bacteria would not be able to live in water or any physiological fluid or even our bodies.

The cell walls of Gram positive bacteria differ in structure from those of Gram negative bacteria.

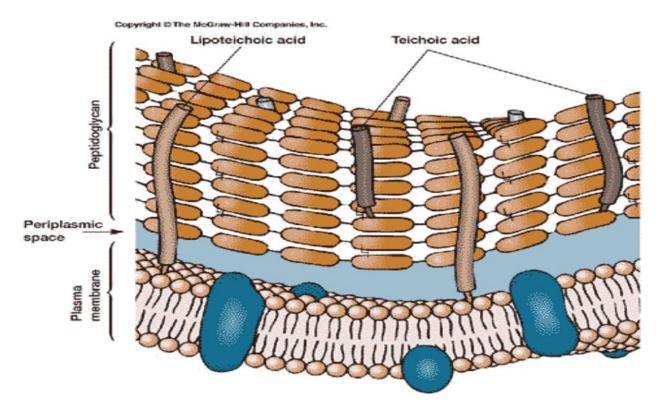
- *It's essential as a medical student to know this Point for many reasons:
- In order to know how the cell wall can be damaged in relation to the infection so we can determine which type of drug can be used.
- Processes of the infection in relation to the cell wall differ from Gram positive to Gram negative bacteria.





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Cell wall Gram-positive bacteria-3



- **The cell wall of G+ve bacteria is made up of many **Peptidoglycan layers**, composed of two important components: N-acetylglucosamine associated with N-acetylmuramic acid.
- * * N-acetylglucosamine and N-acetylmuramic acidare **cross-linked** by oligoprotiens (not more than 20 amino acids, usually tetra- or pentapeptides) and that will lead to a fixed rigid cell wall capable of supporting the cell and protecting it from explosion.
- -These layers are associated with other certain chemical compounds like: *Teichoic acid and Lipoteichoic acids, and these are responsible for the staining color of the gram positive bacteria, when we use the crystal violet color for staining, it will react with these two compound and will be fixed in the cell wall, after that when we use alcohol for discoloration it would not change color, because the stains attached to the Teichoic acid and Lipoteichoic acids.



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- **Teichoic acid-Lipoteichoic acids: Both are found **only** in Gram-positive bacteria.
- *To sum up, Gram-positive bacteria have a thick cell wall made of many peptidoglycan layers (could reach 80 layers), which trap the crystal violet. The alcohol rinse does not remove the crystal violet, which masks the red safranin dye.
- → Periplasmic space: it is a semi fluid space (it is not empty), has some specific proteins dissolved in the fluid, to allow the attachment of the Peptidoglycan layers to the plasma membrane.
- *Plasma membrane in gram positive and gram negative are nearly the same.
- → Plasma membrane: composed of phospholipid bilayer, associated with specific proteins as well steroid similar to the cholesterol in plasma membrane of human cell.

Clinical note:

- -During **infection** when gram positive bacteria reach human blood or other fluids (causing develop of <u>Fever</u>), **lysosomes** (found in oral cavity or blood .. etc) will start the process of <u>Phagocytosis</u>, leading to the breakdown of cell wall components into small parts.
- -But fever in gram positive bacteria is less severe to human body in comparison with fever caused by gram negative bacteria, why?

Because it is related to the difference between the composition of the cell walls.

-Pressure makes Diamonds- ed3ole @



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