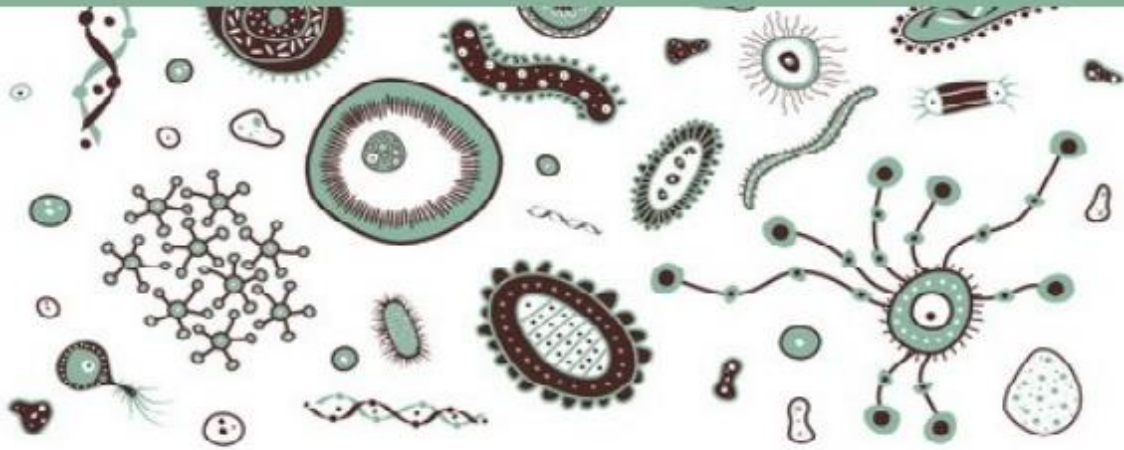




Microbiology



☒ Sheet

☐ Slides

Number : 9

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Subject: Introduction to Microbiology

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Gram positive cocci

The gram positive cocci is represented by gram positive cocci and gram positive bacilli.

Gram positive cocci belongs to a family called Micrococcaceae, in relation to spherical morphological structure of this type of bacteria, gram positive cocci mostly facultative anaerobes with certain obligate anaerobes, but the most common type causing infections to humans are the facultative anaerobic cocci, which grow in presence or absence of oxygen.

Accordingly we can classify the gram positive cocci into:

- Staphylococcus : (It's a genus belongs to the family cocacea)

Each genus is sub divided into species (types), and even these species will be divided into sub species according to certain physiological, chemical reactions, but at this stage of teaching we will concentrate on the genus and certain species only.

- **Genes are the ones responsible of certain characteristic found in this group of cocci.**

The Staphylococcus term is derived from the Greek language and means grape like fraction.

We can see them as cluster of cocci at least 5, 8, 20 clusters but not in chains, we can't distinguish one cocci from the other when gram stained and examined under the microscope, because they are closely arranged.

So the term of staphylococcus is in relation to its morphological structure as irregular arrangement of cocci.

- **One of the most important ways to distinguish between different cocci is using catalase in tests, catalase converts the hydrogen peroxide to water and oxygen.**

The presence of catalase in staph is important to distinguish this cocci from the cocci in the 2nd group, which is called streptococci.

- **Streptococcus:**

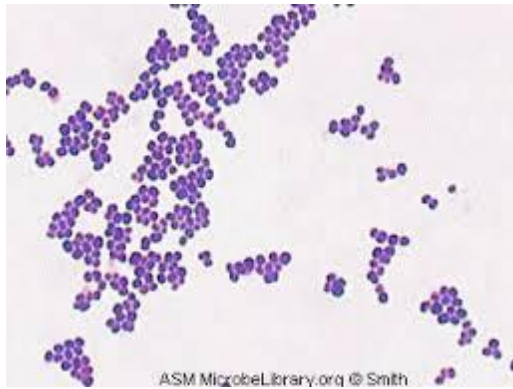
Morphologically are short or long chains, and are catalase negative, (there cell wall can't excrete catalase), catalase is used in the identification between staphylococci and streptococci, this is important for the treatment of infections and to understand the type of infection in relation to certain types of diseases.

- **Micrococcus:**

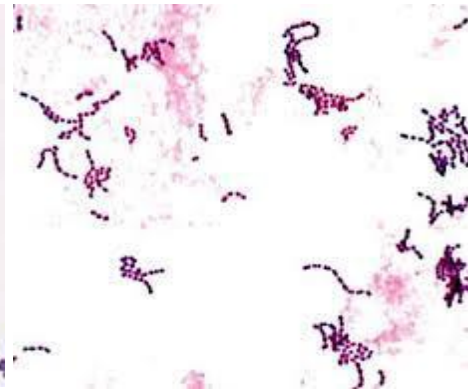
Which is a group between the staphylococcus and streptococcus.

Micrococcus is similar to staphylococcus, in the arrangement of its cocci in clusters and in being catalase positive but we can also see diplo cocci, tetra cocci and octane cocci.





Staphylococcus



Streptococcus

The major important human's pathogens which produce infections are classified into two groups:

- **The first group : (Staphylococcus group)**
- ***Staphylococcus* :**

We have 30 species of staphylococcus, the most common two species which mostly contribute to 95% of human infections are: staphylo-aureus and staphylo-epidermidis.

1. *Staphylo Aureus*:

It is related to the colour of colonies on the blood agar or nutrient agar.

The word aureus in Latin language means gold, and the colonies of *Staphylo aureus* appear in a golden yellowish to a slightly brown colour in cultures.

Its importance is not related to the colour of colonies, it is more related for the production of a variety of extra cellular enzymes and toxins.

Staphylo aureus is one type of the few microorganisms which produce more than 20 type of enzymes and toxins, each of these enzymes and toxin contribute directly and indirectly for the pathogenicity of the organism.

The second important feature of the *staphylo-aureus* is the presence of a slime layer known as capsule, this capsule might be thin or thick, and it depends on the type of strain.

The presence of the capsule, which is composed of polysaccharides and glucosamine(s) , helps in the adherence to surfaces of our skin, our mucosa and infected tissues.

Later *Staphylo aureus* will produce something like a net known as biofilm, which will cover the surface of a lot of medical devices such as a pacemaker ,cardiac valves etc.

This biofilm when it covers these devices, it allows the organism to persist and later on to produce infections.



In addition we have within the cell wall something called (protein A) and this protein is important for the resistance against the immune defence of our body, especially phagocytosis so killing the effect of macrophages and monocytes in our system and it may produce toxins.

There are toxins related to the intestines associated with food poisoning ,example: In our country approximately 40- 50% of all types of food poisoning are due to *Staphylo aureus* ,which means it is very common but why ? Simply because *Staph* is already present on our skin flora in our nose, oral cavity.

Approximately 30 to 50% of the population have *Staphylo aureus* or *epidermidis* especially in the nose, also in the oral cavity, intestines, and skin.

In the type of *Staphylo aureus* we have to expect 40 to 50 % of the *staphylococcus* strains carrying the gene responsible for production of food poisoning.

The enterotoxin which is known as (alpha toxin) may produce septic shock, there are other toxins related to blood septicaemia due to *staphylo-aureus* and the may produce a more dangerous toxic shock syndrome knows as toxic shock syndrome toxin number 1 number, 2 etc. which will result in the death of the infected person, due to kidney failure, disseminated intramuscular coagulation and so on.

In addition *Staphylo aureus* is the only species which produces the enzyme **coagulase** and the clumping factor.

Coagulase as an enzyme is either membrane bounded or extracellular, it contributes to the conversion of prothrombin which is a protein in our plasma into fibrin and fibrinogen.

This leads to the production of a net of polymers composed of protein, which will cover *Staphylo coccus aureus* during the infection of the tissue, in other words, *Staphylo aureus* gets protected by the fibrin net therefore preventing our body to resist the infection, because the phagocytosis system in our body cannot kill the *Staphylo aureus* when it is in association with the fibrin. It is a very important pathogenical factor in producing infection. Antibiotics will not reach it too, because it's protected by a this cover of fibrin. So coagulase positive is associated with mainly *Staphylo aureus* and rarely by another type of staph.

We have other important enzymes which are execrated during infection, depending on the type of infection if it's in the skin or blood or upper respiratory tract, especially joint infections which are very common.

Staphylo aureus will produce Hyaluronidase which is a spreading factor during tissue infection causing damage in the connective tissue allowing the *Staphylo aureus* to disseminate and produce more severe infections, reaching the blood stream later.



Another important enzyme called leukocidin which destroys the white blood cells.

We have many extracellular enzymes and toxins related to the *Staphylo aureus* which is the most pathogenic one in its family, these enzymes and toxins contribute to its pathogenicity, this is why the *Staphylo aureus* is mainly the cause of skin abscess and wound abscess, if other types of *Staphylo coccus* are the cause they can be known later but they are less pathogenic.

The reason we can acquire infections is because our skin flora, nose etc can easily be spread and because the infections, the spreading occurs by contact (hands, clothing).

Staphylo aureus can survive in any excretion of our body associated with white blood cells or tissue etc up to a few months in good conditions, and it will not get killed, which means it can spread on the floor of the hospital on the clothing and so on.

It is easy to acquire infections in the hospital and it can cause very serious infections beginning with skin abscess, wound sepsis and bacteraemia according to the number of bacteria we are exposed to.

Bacteraemia as an expression is used in relation to gram positive more than gram negative, in gram negative we prefer to use Septicaemia, but in our country they don't distinguish between these two expressions.

In literature Bacteraemia means less number of organisms in the blood stream or wound and is often used in relation to gram positive rather than the expression Septicaemia.

Staphylo aureus is the common causative agent of types of infections approximately from up to 20% of them such as sinusitis, osteomyelitis, pneumonia and infection of the bone, in outpatient clinic and inpatient clinics, so it is very common, it depends on which age, gender etc.

2. *Staphylo epidermidis*:

Epidermidis refers to the epidermis, in culturing its colonies are white in colour, they called it in the past staphylo Alba and in Latin Alba means white.

It is also widely distributed in our body, nose and skin, it is to some extent similar to *Staphylo aureus*.

In certain persons *staphylo epidermidis* over numbers the *staphylo aureus*, but hospitalized patients mostly are associated with *staphylo aureus* more than outpatients infected with *staphylo epidermidis*, because *staphylo aureus* in hospital is more selected to resistance and associated with infection during surgical procedure And treatments with intra venous fluid ,anti-microbial drugs etc .

Staphylo epidermidis is coagulase negative they can't produce toxins they can produce only a few numbers of extracellular enzymes which might contribute to infection, especially with patients who suffer from immune deficiency or immunosuppression



(compromised), patients with leukaemia or malignancy and treated with toxic drugs, that's why they are susceptible to any type of microorganism including *Staphylo epidermidis*.

On the other hand, in a healthy person with intact immunity the *Staphylo epidermidis* rarely can produce infections, it might produce but only if there is damage in the skin, *Staphylo aureus* is more pathogenic.

Biofilms (adherence) can be associated with a certain number of them, but not all of them, capsulated ones are more infections than the non-capsulated, especially when we use implant devices.

All type of staphylo coccus can be very easily cultured in simple media, blood agar is enough, after 24 hours you can discover the organism by colour later on using catalase and coagulase tests.

To sum up coagulase negative indicates for *Staphylo epidermidis* or other non staphylo coccus because we have more than one type of them, and coagulase positive for *Staphylo aureus*.

Less number of *Staphylo epidermidis* are resistant to B-lactams and more and more numbers of *Staphylo aureus* developed resistance to it due the use of penicillin drug and later on the resistance to Methicillin/Oxacillin-resistance, *Staphylo aureus* (MRSA), but they are all still susceptible to Vancomycin.

In the hospital if we want to measure the level of hygiene, by knowing the percentage of *Staphylo aureus* or *Staphylo epidermidis*, if the hospital has high incidents of MRSA it means lower standards of hygiene, and a lot of infections and misuse of antibiotic. MRSA are used in international guide lines, for example the percentage of MRSA might reach 70% in Jordan university hospital but in Al-Basheer 100%.

Increased rate of isolation of MRSA is a purpose to the usage of more toxic/expensive drugs, such as Vancomycin, it is very effective, but we rarely do have staphylo aureus resistant to vancomycin, recorded numbers 2 or 3 cases called Vida staphylo coccus, Vida means medium resistant not complete.

3. Micrococcus:

Is one of our skin flora found in the intestines , as a single bacteria they rarely induce skin infections or bacteraemia , but in combination with *Staphylo epidermidis* or *aureus* they may cause infections , we are lucky that it is easily discovered in the lab due to the high susceptibility to beta lactam.



- **Second group (They are more difficult in classification)**

1. ***Streptococci***:

They can be recognised in gram stains by presence of long or short chains, and using catalase test (they are catalase –ve), facultative anaerobes organism and uses dismutase oxidase convert hydrogen peroxide into water and O_2 (as antioxidant mechanism, notice they don't have catalase).

They are classified according to their haemolytic activity on blood agar in vitro, we can use human blood or sheep blood, rabbit etc.

Accordingly haemolytic enzymes might produce complete destruction to the RBC's, which produce beta homiletic reactions produce damage in haemoglobin and release and inactivate it so RBC's become colourless.

If the reaction converts hemoglobins to **viridoglobin** or cause partial destruction to green (viridian) pigmentation occurs to the RBC's in the culture, which means it is alpha homiletic streptococci. By this mean we can distinguish between the types of streptococci that causes infection like group A and the groups which don't cause infections is NON homiletic.

- a) **Viridans Streptococci Group (Alpha haemolytic cocci) :**

They are found in the normal flora, this group is very useful for the oral cavity due to its protection by producing organic compounds (as end products) thus preventing the attachment of pathogen to our gum and mucosa. It is considered harmless, only if there are injuries in the oral cavity and entered the blood stream (might cause bacteraemia) then if it reached the heart tissue and valves it causes endocarditis disease, which means it will produce damage in the cardiac valves and death.

So it is more common in the oral cavity it might be found in few numbers in the intestine, and rarely induces infections only under certain conditions such as surgical manipulation in the oral cavity.

In dentistry they produce dental caries (تسوس) in teeth, If the plaque on the teeth is not well cleaned might later produce worms and inter-carries (inside the teeth) and rarely bacteraemia in other parts of the body.

- b) **Beta haemolytic Streptococci Group :**

We have many of them not only one, they completely kill the RBC's and homiletic reaction with the blood agar, they can be classified according to the composition of the



polysaccharides in the cell wall (Cell wall specific carbohydrates) (serogroups) into A B C D E F.

These groups is important in research to understand at least 2 or 3 types of infections caused by beta haemolytic Streptococci.

Group A is important due to cell wall antigenic structure (carbohydrates) and proteins, we have 85 sub-groups (serogroups) why? Because these subgroups are related to epidemiology of diseases and there immunology (resistance).

Group A is the most common cause of sore throat (inflammation in the throat and tonsillitis), and the most invasive, up to 90% of respiratory infections are caused by viruses and only 10% are caused by bacteria, 90% of the 10% are caused by group A particularly in children aged from 2 to 13.

In adulthood people rarely develop tonsillitis due to group A, because it is related to antigenic structures and our immunity.

We are exposed only to specific numbers of the 80 serogroups, in other countries they are exposed to different serotypes, it is difficult to be immune against all of them, and we only develop specific antibodies 1, 3 and 9 to specific serotypes which we are immune against, thus preventing pre occurrence of tonsillitis in our country. If we travel and we got infected we will develop immunity against the different serogroups, these groups can be easily cultured and recognized and test for their presence.

Group A which is found in a healthy carrier person is from 2 to 30% according to the season, so in summer we have a few number of people carrying group A streptococci, whereas, between summer and winter for example the percentage of carriers increase due to the cold temperature and humidity, it might reach 50% in December, we carry this organism and adults spread them to children.

Children are more susceptible to group A *Strepto cocci* and get infected quickly due to lack of specific anti-antibodies.

Streptococci group A is also called strepto pyogenes, pyogen means puss producing infections, it is similar to *Staphylo aureus*, and it produces 20 types of toxins and extracellular enzymes.

Most important is the presence of protein M in the cell wall which contributes to its pathogenicity, protein type R and L are also present but M is the most important one, it produces haemolysis.

They tried to invent vaccines against group A but they didn't succeed. So we must protect people who have acquired repeated infections but the vaccines didn't succeed to protect them.

***Staphylo aureus* and group A they have no vaccines.**

