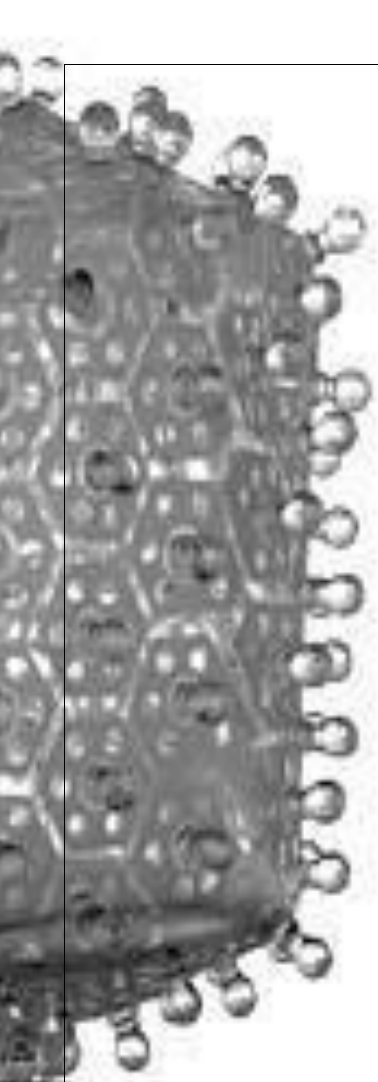


Microbiology

Lecture No: 16

Dr Name: Asem Shehabi

Done by: Yara Al-Kayed

Sheet ■ Slide □

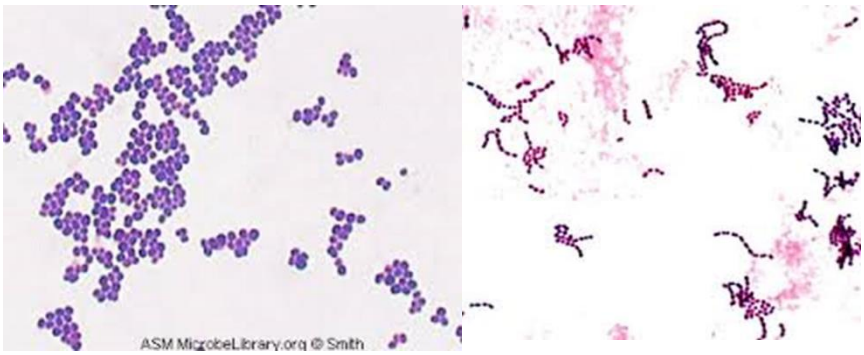
Gram-Positive Cocci

This is the **last lecture of the midterm material**

We had already talked about *gram+ve facultative anaerobic cocci*, and we had mentioned two types of microorganisms which are wildly associated with human infections;

1) staph aureus (staph coagulase positive)
2) staph coagulase negative, including the micrococcus which are rarely associated with infections in healthy people, but might under certain conditions cause infections in immunocompromised conditions or in association with certain severe underlying disease, which means that micrococcus are not a common causative agent of human infections.

Keep in mind the features in gram stain of streptococci. If you use gram stain on infected clinical material; respiratory tract, clinical site of infection, skin... You might not recognize long chains of cocci, you might recognize only few numbers of cocci (short chains), but if you use culture medium especially fluid culture medium then you can demonstrate the presence of streptococci. Therefore gram stain of specimens that are taken directly from infected tissue, contaminated swap or (for example from sinusitis in case of inflammation of sinuses) might not distinguish between staph and strep. So gram stain is mainly to demonstrate gram +ve cocci in general.



* Streptococci:

We have two important classifications which are used to classify the many types of species of streptococci:

1) Viridans streptococci group
2) Beta hemolytic streptococci group

* Viridans streptococci:

"Viridans": this group develops green pigmentation on blood agar. Once grown on blood agar; it'll produce an enzyme which converts the **red** color of hemoglobin -within RBCs- into **green**. This green pigmentation is known as Viridans and this group is known to produce viridans.

- Importance of viridans streptococci:

Viridans streptococci are widely present in our oral cavity and in the upper part of the respiratory tract especially in the tonsils. This group is important for protection of our respiratory tract mucosa against pathogens by producing certain end-products that are mainly types of acidity, like propionic acid and other acids which suppress the coat of pathogens to some extinct. Therefore this group is considered normally as part of commensal oral flora.

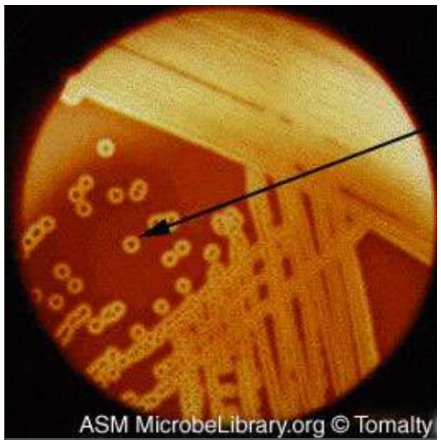
- Under certain conditions (especially some species) it might become pathogenic and might be associated with abscesses in the oral cavity especially following manipulation with the mucosa of the oral cavity especially during dental preparation.
- There are some few species -which are found also in the oral cavity that are classified as viridans streptococci- might also produce certain damage in teeth following the production of propionic acid or any other organic acid and produce **dental caries** تسوس الأسنان. Because it will affect the tooth slowly and produce a poring, this results in damaging the tooth, and will require removal of the tooth.
- Following any manipulation in the oral cavity by extraction of tooth for example or any dental process, might few of these viridans streptococci manage to reach the lymphatic system or the blood stream of our patient

and spread slowly to the heart valves, and there it will produce special extracellular product called dextran which is composed of polysaccharide and might produce endocarditis, especially if the risk genetically damage of the valves of the heart, which is called congenital heart disease. The streptococci might become active and produce such type of endocarditis or with age some damage can occur on the valves resulting in endocarditis, which is a serious disease that might be fatal if not treated with antimicrobial drugs and in some cases it might require valve replacement.

- Viridans streptococci are of less importance only in certain conditions.

* Beta-hemolytic streptococci group:

It's recognized in this picture, it produces complete destruction of the RBCs due to production of hemolysins (enzymes that affect the cell membrane of RBCs in blood culture) and produce severe damage in the RBCs and this can be demonstrated in vitro by the presence of beta hemolytic effect (complete destruction of the RBCs) and will have transparent colonies.



- Whereas viridans streptococci produce partial damage to the RBCs it converts the hemoglobin to a green substance (the dr. didn't say the name clearly but according to the book, it is methaemoglobin) and this can be manifested in the picture of green pigmentation as you'll see later.

This beta-hemolytic streptococci has unique importance in developing a variety of diseases, and it's considered so important and pathogenic to some extinct like staph aureus, due to production of variety of extracellular enzymes and toxins. It produces many clinical features which resulted in acute infection, sub-acute infection and post-streptococcal diseases or complications.

- Why are beta-hemolytic streptococci so important?

Beta-hemolytic streptococci is classified into A, B, C, D, E and F. According to their cell wall composition of polysaccharides, because there are special layers within the cell wall which are associated with each specific group of streptococci.

All types of beta-hemolytic streptococci produce complete destruction of RBCs, but group A is considered the most important, and secondly comes group B.

* Group A streptococci:

Group A is called streptococcus pyogenes.

"Pyogene" means: pus producing cocci, which means that they produce an inflammatory reaction especially in our mucosa, skin and tissues.

- It's considered invasive and pathogenic due to the production of various virulence factors. We'll only mention names not going into so many details. For example there're hemolysins that have 2-3 types, pyrogenic factors called erythrogenic toxins which are responsible for damaging skin surface (epidermis) producing superficial infection recognized in the form of scarlet fever الحمى القرمزية.

- For example, if there is an infection in the throat, there is presence of small rashes spread over the surface of the epidermis and this is associated with elevation of the body temperature and the name scarlet fever which is often recognized following tonsillitis in small children.

- In addition, group A streptococci is well known as one of the most causative agents of tonsillitis in children. For example, if we take the upper respiratory tract to look for the causative agent of tonsillitis (inflammation of the tonsils mucosa) from soft throat surface often we recognize group A streptococci associated with about 90% of all tonsillitis caused by bacteria, some other types of bacteria can cause tonsillitis especially in adults and less in children (staph aureus, haemophilus influenzae, Neisseria meningitides, corynebacterium

diphtheriae)→ they might produce tonsillitis while giving the impression that the causative agent is group A streptococci.

- We can differentiate clinically and by culture between these two types.

- Keep in mind that 90% of all types of bacterial tonsillitis are caused by group A streptococci. If we compare with viral infection, viral diseases are associated with about 90% of the total cases of sore throat or tonsillitis, with 10% only due to bacterial infections, and 90% of that 10% is caused by group A streptococci.

- In children, once there is tonsillitis the infection spreads easily to the ear and nose of the child, which means that it might develop otitis media (middle ear infection) due to the fact that children don't have enough barriers to prevent the dissemination of this group of organisms, it may cause sinusitis too. It might also reach the blood stream causing sepsis and meningitis (in very few percent).

- The real problem in group A streptococci is that it produces tonsillitis in children –very common-, but if this infection is repeated each few months → children will be exposed more and more to extra products of group A streptococci which will be accumulated in the body and induce the production of autoimmune response which might at the end produce post-streptococcal complications.

<u>* Post-streptococcal complications mean the following:</u>

Children who develop repeated infections in their throat, especially if they're not treated properly with antimicrobial drugs like penicillin, they might after few years develop **Rheumatic heart Fever**, which means that it will affect their heart and produce complications and might produce killing effects.

Or it might also produce other inflammatory reactions in **joints**. Producing a form of rheumatism by affecting the connective tissue in many parts of our body.

In certain cases, it might produce **Glomerulonephritis**, which means that the extracellular product of group A streptococci will reach the kidneys and there it'll produce an autoimmune complex which produce damage in the glomeruli of the kidney.

- As a result, we have to prevent repeated streptococcal tonsillitis, by administration of antibiotics (especially penicillin; since group A streptococci is still highly susceptible for penicillin, it has not developed any resistance for penicillin).
- If the child is hyper sensitive for penicillin, then we have to use another type of drug, for example erythromycin "a type of macrolides" or cotrimoxazole.

** Prevention of group A streptococci is NOT possible. There is no vaccine available to protect against this type of infections.

The reason behind this is due to the fact that the cell wall of group A streptococci is composed of many antigenic structures (there are about 80 specific subtypes) related to specific proteins as M, T and R proteins, which we are not required to know about in this course. Therefore it's impossible to produce a vaccine to cover all these subtypes. So the only way to treat the disease is with antimicrobial drugs.

* Healthy carriers:

In winter months there is an increase in the incidence of tonsillitis due to the change in the climate (cold and moist) so bacterial and viral infections become more common than in summer months.

There is always in any community during the year, few percentage of the population that carries group A streptococci without the presence of any clinical symptoms. This percentage of people can be found in adults and children. These healthy carriers contribute for continuous infection in the community and continuous spread of the disease from one child to the other.

Generally, healthy carriers increase in winter months and decrease in summer months.

* Beta-hemolytic streptococci group B:

This group is related to the genital tract, where group A is related to the upper respiratory tract as we mentioned earlier. Group B streptococci is found in the vagina of certain percentage of the population between few percent up to 20%. It's part of the normal commensal flora of the genital tract in women.

Especially during pregnancy, group B streptococci might become important and might cause later a type of clinical infection, especially if there is rapture in the membrane that surrounds the fetus during delivery. This membrane rapture will cause the baby to swallow some of the amniotic fluids which is associated with few numbers of streptococci, which will cause the baby to develop a serious fatal disease called neonatal sepsis and neonatal meningitis due to group B streptococci.

If the newborn baby isn't treated without delay with appropriate antimicrobial drugs like ampicillin or amoxicillin, he might die within 24 hours.

At the same time, few numbers of these group B streptococci might (in the cases of injuries in the uterus during delivery) reach the blood stream of the mother and produce a type of disease known as Puerperal fever حمى النفاس. Which is a very serious disease especially for introduction of antimicrobial drugs due to the fact if it produces a severe inflammatory reaction inside the infected person and within a short period, the mother might die due to the infection.

So group B streptococci are important especially in pregnancy, and should be controlled before delivery by taking a vaginal swap to look for presence of group B streptococci.

- Group A is related to respiratory infections, while group B rarely causes respiratory infections or other kinds of infections, such as urinary tract infections.

* Detection of group A and B streptococci:

This procedure should be followed in the laboratory:

1) Populated swap; from the throat, tonsils, vaginal tract of the infected person
2) Culture on blood agar, we look for the beta hemolytic effect

3) See whether this group is susceptible to the antibiotic **Bacitracin**

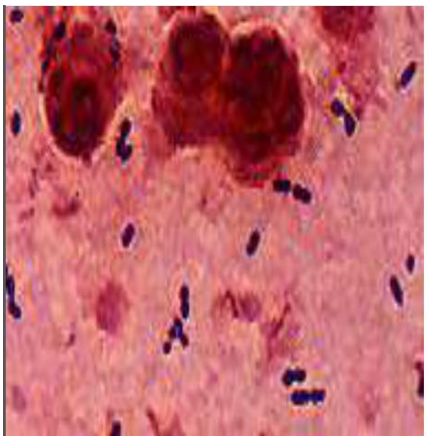
- If it's susceptible to Bacitracin → then it's group A streptococci
- If it's resistant to Bacitracin → it's group B streptococci or another type (any type other than A)

4) Later we have to do "agglutination" with specific sera against group A, B, C...

* Streptococcus pneumoniae:

"Pneumoniae" it's related to pneumonia → causes lung infection that can be so severe and associated with inflammation of the lung tissue, accumulation of fluid and death of the patient if not treated with antibiotics and other supportive therapy.

This group has a special characteristic; demonstrated in this picture:




- Observe the green pigmentation; streptococcus pneumoniae produce green pigmentation in blood agar.
  - Keep in mind that the green pigment is associated with both viridans streptococci and streptococcus pneumoniae.
- We can differentiate between streptococcus pneumoniae and viridans streptococci by using a test called **Optochin test**.
- Optochin is an antibiotic-like substance, where optochin can inhibit streptococcus pneumoniae and cannot inhibit streptococcus viridans nor group A streptococci (doesn't produce an inhibition zone).

- If we prepare gram stain from an infected material (sputter, for example) we can demonstrate presence of diplococci surrounded by capsules, these capsules can be demonstrated by the presence of empty spaces

surrounding the diplococcic→ indication of the presence of polysaccharides.

- Streptococcus pneumoniae are known to be associated with large capsules, which are important in invasiveness and pathogenicity of the organism, because these capsulated diplococci resist the phagocytic mechanism in our immuno response

- These anti-phagocytic activity are associated with other virulence factors which contribute for the pathogenicity and invasiveness of this organism.

- Streptococcus pneumoniae (like group A streptococcus) can be part of our respiratory tract normal flora; within the same range (5-20%).

- Under normal conditions, streptococcus pneumoniae cannot cause infections; it only initiates infections if there is damage in the mucosa of the respiratory tract, especially following the development of viral infections.

- Viral infections especially influenza cause a decrease in the resistance of the respiratory tract normal flora. Where there will be a sort of damage to the mucosa and this allows the pathogenic streptococcus pneumoniae (if present in the respiratory tract) to produce an inflammatory reaction and entail of disease, but the inflammation is not related to tonsils or pharynx, it might spread from the pharynx or tonsils producing more severe infections in the lung tissue and produce a feature called pneumonia.

- Pneumonia is a very severe disease, and within 1-2 days if the patient has not received any supportive therapy by respiration, he might develop severe lung infection and death.

- In children (especially less than 5 years old) streptococcus pneumoniae is highly invasive, once it's activated in the respiratory tract; it might reach the blood stream producing sepsis and meningitis. So it produces fatal outcomes in children under 5 years of age, unless they receive proper and rapid antimicrobial drugs.

- In elderly, due to the suppressed immunity (lack of specific immunity), streptococcus pneumoniae might also cause death.

- So streptococcus pneumoniae is a major causative agent of pneumonia and death in children and elderly.

- Exactly like group A streptococcus, streptococcus pneumoniae might spread in children from the local inflammatory reaction in the respiratory tract (especially in the pharynx, were it isn't easily detected unlike group A streptococcus) and reach the middle ear causing otitis media, or the nose causing sinusitis, it might also produce conjunctivitis, or an infection in any part of the body.

- Therefore, 10 years ago they have developed vaccines for streptococcus pneumoniae, which weren't easily prepared. Those vaccines have types specifically for adults and other types specifically for children, due to the fact that within streptococcus pneumoniae we have about **85 capsular types**. So they collected the most common capsular types that are prevalent in the western world and made a vaccine that is composed of antigens for 23 capsular types for adults and children, and this vaccine is protective for 1-2 years.

- The vaccine should be given especially for children who are suffering from an underlying disease (like malignancy, lymphoma, leukemia) to lower the incidence of developing pneumonia, since they're highly susceptible for developing pneumonia following viral infections.

* Differentiation between **viridans streptococci** and **streptococcus pneumoniae**:

1) Using Optochin test

2) Gram stain

3) Anti-serological test

These procedures can be easily done in any laboratory, but they're essential since each type has its own treatment methods.

* Enterococcus:

They were classified as group D streptococci previously, but now they're separated from the streptococci and have their own genus (group) called enterococcus.

They're present in the intestines of animals more than in humans, but due to the connection between animals and humans, enterococcus can be found in the intestines of 20% of the population (starting from young infants, children…)

Enterococci under normal conditions are of low pathogenicity, they rarely spread from the intestines to the internal organs, or they might produce skin infection.

Enterococcus following the use of cephalosporin especially the second and third generation, as we mentioned before enterococcus are naturally resistant for cephalosporin, there is no specific penicillin binding protein found in the membrane, therefore they will be increased and accumulated after using cephalosporin and might if they manage to reach the urinary tract for example, produce urinary tract infections.

Recently studies showed that 1- 5% of urinary tract infections are caused by enterococcus. While 20 years ago, enterococcus urinary tract infections were very rare. And this indicates that the use of antimicrobial drugs changes our intestinal flora and affects the biological equivalence in our intestinal tract.

There are two species of enterococcus;

1) Enterococcus faecium:
2) Enterococcus faecalis:
   - It's more than enterococcus faecium.
   - Mostly found in the animals intestines, but can be found as well in the intestines of humans.
   - It can be associated with certain cases of wound infection but more with urinary tract infections.
   - It's hardly treated compared with others types of streptococci.

Best of Luck!