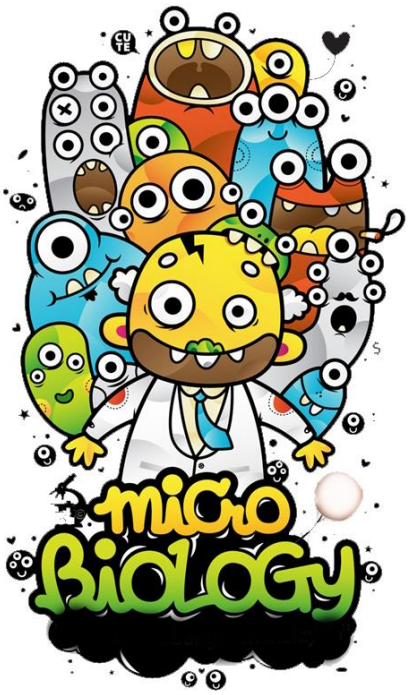

Subject: Gram +ve Cocci

Lecture No.: 18

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Last time, we talked about how we usually start identification of an unknown bacteria with gram staining, which gives is us something called dichotomous key, coming from the gram type and shape of the bacteria. This gives us the gram groups we talked about earlier.

We started talking about the first group which is the gram positive cocci, we have many types of these bacteria, but we have three medically important genres, which are:

1. Staphylococci
2. Streptococci
3. Enterococci

How can we differentiate them from each other?

First, we start by the catalase test:

- Catalase negative (does not produce catalase): streptococci or enterococci
- Catalase positive (produces catalase): staphylococci

If we get staphylococci, we're concerned with knowing whether it is *Staph Aureus* or not, so we do coagulase test:

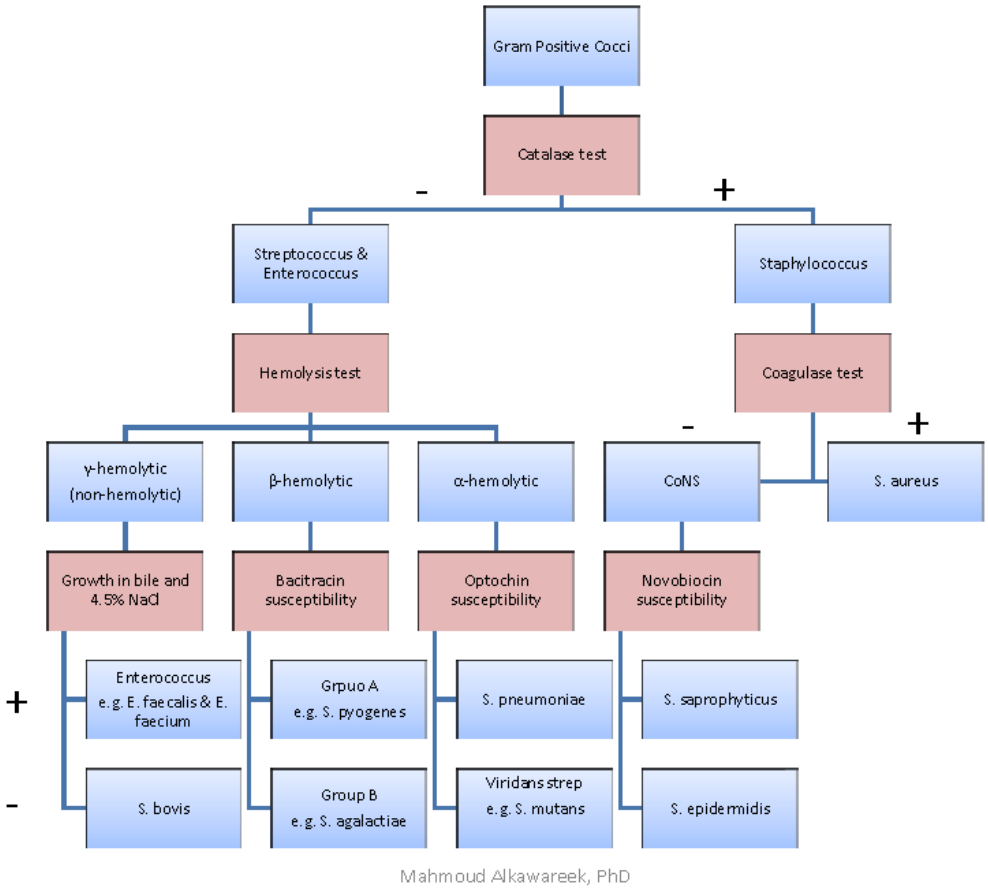
- Coagulase positive: *Staph Aureus*
- Coagulase negative: other staphylococci species which are collectively called "Coagulase negative staphylococci", we care only about two species of them and they can be differentiated from each other by an antibiotic susceptibility test. The antibiotic used is Novobiocin:
    - Susceptible: *Staphylococcus Saprophyticus*
    - Resistant: *Staphylococcus Epidermidis*

If we get streptococci or enterococci, we directly use the hemolysis test:

- Alpha hemolytic: we use the antibiotic Optochin as a susceptibility test to differentiate between two types of streptococci which are *S. Pneumonia* and *S. Mutans* (Viridans strep)
- Beta hemolytic: we use the antibiotic Bacitracinsusceptibility test:
    - Bacitracin positive(susceptible): *StreptoPyogenes* (group A)
    - Bacitracin negative (resistant): *Streptoagalactiae* (group B)

These bacteria are classified into many groups (group A, B, C, etc), this is called “The Lancefield Grouping System” which is used for grouping catalase negative bacteria (both streptococci and enterococci) based on the polysaccharide composition of the bacterial antigens on their capsules. This system is used along with the hemolysis test to identify what species of catalase negative bacteria we’re dealing with. However, it is mainly used to differentiate between different beta hemolytic bacteria. Most important groups are group A and group B.

- Gamma hemolytic:they are either enterococci (the medically important ones) or few numbers of streptococci such as S. Bovis. We differentiate between different species of gamma hemolytic bacteria by studying their growth in high concentrations of NaCl and bile salts, usually enterococci are highly resistant for bile salts unlike streptococci which are not. So, if there is growth, they are most likely enterococci, if there is no growth, they are most likely S. Bovis.



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Important notes:

- All enterococci are gamma hemolytic (no hemolytic activity), while streptococci could be alpha, beta or gamma hemolytic.

- **Bacteremia**: presence of bacteria in blood.
- **Septicemia**: presence and growth of bacteria in blood.
- **Sepsis**: presence and growth of bacteria with generalized inflammatory response, and has a high mortality rate.

Now we will start talking about the examples of the gram positive mentioned earlier.

First Genus: Staphylococcus

Staph Aureus

We use coagulase test to differentiate it from other bacteria, it is coagulase positive. Why do we care about it? Because it is the most virulent bacteria i.e. it is the most pathogenic bacteria to humans although it is present as normal flora in many individuals, especially on their skin, and sometimes in the upper respiratory tract and nasopharynx area (nose and mouth).

20-30% of humans' bacterial infections are mainly caused by S. Aureus, why? Because any compromise in the defense lines of the body (here we're talking about skin and immune system) such as skin abrasions or immune system suppression, or sometimes even when it reaches different places other than its place as normal flora, all these lead to infections, some of which are highly serious and life threatening. However, not all staph infections are serious; most of them are minor skin infections.

This particular bacteria has many virulence factors, we'll explain them in a moment, also, all strains of this bacteria produce polysaccharide capsule, which acts a protective method against the body's defense mechanisms especially phagocytosis, it is able to form biofilms (slime layer) which makes it much more resistant to antibiotics than planktonic bacteria.

Although it is a normal flora it is also considered as an opportunistic organism and pathogenic bacteria that causes a wide range of infections. Virtually, it can infect almost any organ system, and causes a wide range of **skin infections** such as boils, carbuncles, and abscesses. It can also cause **respiratory infections**, such as hospital acquired pneumonia. Also, it could cause **blood infections (bacteremia)** followed by septicemia and septic shock which has a very high mortality rate, it can **infect bones**, cause**urinary tract infections**, some **hospital acquired infections, endocarditis,** and many other infections (remember that it causes quarter of human infections).

In term of antibiotics resistance, S. Aureus is one of the most common to form resistance. Penicillin G was discovered at the same time as S. Aureus, originally, all of its strains were susceptible to penicillin G, but within a couple of years (before penicillin G was even commercially available) new strains became resistant for penicillins by producing B-lactamases. So scientists came up with a special group of penicillins called the B-lacatamase resistant penicillins e.g. methicillin and oxacillin. Within few years, other strains became resistant to these antibiotics, not all strains of staph aureus, some of them only.

Many strains became already resistant to normal penicillins (natural and broad spectrum penicillins such as amoxicillin and ampicillin), but most strains are still susceptible to the methicillin group (B-lacatamase resistant penicillins).

Methicillin susceptibility test is not done in hospitals, because methicillin is not used anymore. Usually, oxacillin orcloxacillin are used instead, if it is resistant to any of these two, we consider it resistant to the whole group, in this case, we call the S. Aureus "Methicillin Resistant S. Aureus i.e. MRSA".MRSA strains are still relatively low in number, but unfortunately it is increasing.

Most important concern of doctors when a patient turns up having S. Aureus related infection is find out whether is it methicillin resistant or not, because MRSA treatment is totally different than normal S. Aureus treatment. **Vancomycin** is the drug of choice in treating MRSA.

Very rare cases (around 13 published reports only) show that some strains of MRSA are becoming more resistant to vancomycin (VRSA), the drug of choice is **linezolid**. Streptogramins and tigecyclines could also be used.

VISA (vancomycin intermediate resistant staph aureus) could be treated with vancomycin but at very high concentrations, remember that vancomycin toxicity is high and it is dose dependent, it causes ototoxicity and nephrotoxicity, in this case, we also change to linezolid.

Now, why are staph aureus capable of causing many infections? Because of its many virulence factors, which are the "weapons" used by the bacteria to increase their pathogenicity, they are mainly enzymes and exotoxins. Not all S. Aureus strains produce them all, but collectively, all strains together produce

these factors, in other words, some strains produce half of them others all of them, and some of them are common in all strains.

Note: there are many other **virulence factors**, but those are the most common.

a. Coagulase**:** common in all strains of S. Aureus. Why is it a virulence factor? It causes clot formation, now because bacteria have a very low number when it infects a body part; it uses the clot as a protective mechanism.
   Clot → no blood flow → no leukocytes → no immune response

b. Enterotoxins:mainly in the intestines and shows food poisoning symptoms. If the bacteria were present in food and produced high concentrations of these toxins, ingestion of this food will result in foodpoisoning.We have many bacteria that cause food poisoning, one of them is staph aureus. Enterotoxins cause diarrhea, vomiting and usually no fever.

c. Hemolysins: cause hemolysis i.e. rapture of RBCs. S. Aureus is hemolytic, but remember that we don't use hemolysis test to identify it.

d. Leukocidin: damage (lysis) of leukocytes, it can also damage RBCs. Phagocytes for example are killed after phagocytosis of the bacteria, this could also lead to bacteremia and septicemia, which means that they can survive even within blood that has high immune components especially WBCs.

e. Hyaluronidase: degrades hyaluronic acid, which is a very important component of connective tissues.

f. Epidermolytic toxins: exfoliation of the skin.

   As we said, there are many other virulence factors, and these are the most important.

Now, we have staphylococci bacteria that are coagulase negative, which are all the staphylococci bacteria that are not S. Aureus. We care about two of them:

1. **Staphylococcus Epidermidis**
   Present as normal flora on skin, it is also an opportunistic bacteria, and under certain circumstances it can cause certain infections especially in immune compromised individuals. This particular bacterium causes what we call **medical device associated infections,** we are not talking about medical equipment used in hospitals, instead, we are talking about implantable devices such as prosthetic heart valves, hip replacement surgery, artificial pacemakers, etc.

   This bacterium is very common in causing biofilms on surfaces, especially these medical devices. Immune componentsare continuously attacking, which means in deed having an inflammatory response on the surface of these devices, which also declares the failure of the device, so it must be replaced, this is not easy because it requires having the patient another surgery . Many bacteria can do this, mainly S. Epidermidis.

2. **Staphylococcus Saprophyticus**
   It almost exclusively causes one type of infection: urinary tract infections (UTIs), especially the community acquired not the hospital acquired.

Second Genus: Streptococci

Most species of streptococci are present as normal flora, mainly in the nasopharynx area (mouth and nose) and upper respiratory tract. Still, they have the ability to cause wide range of infections some of which are serious and life threatening. Also, in theory they can be present on skin as normal flora but this is much less common. And collectively, (same as S. Aureus) they can produce a wide range of virulence factors.

Classification of streptococci into different groups is based into two major aspects:

- **Patterns of hemolysis**: alpha, beta, or gamma.
- **Lancefield grouping system**: we could apply it to all streptococci, but it is mainly used with beta hemolytic bacteria.

1. Alpha Hemolytic Streptococci

a. Streptococcus pneumonia (known as pneumococcus)
It is present as normal flora in the upper respiratory tract in many humans. One of the most common causes of **otitis media**, **sinusitis** (both are upper respiratory tract infections), and **pneumonia** (lower respiratory tract infection).

It has a vaccine, mainly against its ability to cause **meningitis** (very common for this bacterium to cause it), which is a serious emergency and has a high mortality rate.It can also cause bacteremia, septicemia, septic shock, and **endocarditis.**

It is less resistant to antibiotics than S. Aureus; most of its strains are still susceptible to penicillin G, V, and broad spectrum penicillins such as amoxicillin and ampicillin. However, numbers of resistant strains are increasing (this is a general trend in all bacterial species nowadays).

It has more **than 80 serotypes**(serotypes: same species of bacteria with different antigens on the surface), it can form capsules with different polysaccharide composition, and accordingly, they are divided in these serotypes. Not all of them cause the same risk, which means that they differ from each other in term of virulence and pathogenicity.

The serotypes that have risk on children are different from those who have risk on adults. Brevnar vaccine is used for strains that have risk on children, whilePneumovax protects against 23 serotypes that have risk on adults.

Note: Brevnar is not a compulsory vaccination in Jordan; it is only given for those at risk, for example: people who have favism. Adults who have taken it during childhood and need further protection, need to take Pneumovax. Both of them are not cheap.

b. Viridan Streptococci (Known as viridians)
Streptococcus mutans is one of the most common normal flora in the oral cavity and upper respiratory tract. It is one of the most common colonizers of teeth (dental plaque: 100-200 types of bacteria have been identified, one of them is viridians streptococcus).

In term of susceptibility it is still susceptible to natural penicillins and broad spectrum penicillins. It causes **dental carries** and **periodontal (gums) diseases**. Although these conditions cause irritating pain, it is not as serious as you think. This bacterium is able to cause **endocarditis**, especially for those who have heart valve problems, so any dental procedure requires an antibiotic prophylaxis e.g. amoxicillin, it is a must. If endocarditis happens, amoxicillin is not used any more, instead, penicillin G is used (still one of the most potent gram positive antibiotics), in combination with gentamicin (an aminoglycoside).

2. Beta Hemolytic Streptococci

After doing the hemolysis test, we are concerned with the knowing the Lancefield classification. Most important groups are group A and group B.

a. S. Pyogenes (group A)

Present as normal flora in the nasopharynx in many individuals, it is one of the most common causes of **pharyngitis(sore throat or strep throat)** in adolescents and children (pharyngitis has other causes in adults). Although strep throat is a minor issue, its treatment is a must and it should start immediately, why? Because it is very common for this bacterium to cause post infection complications, for example, if pharyngitis is not taken seriously, a patient could develop an autoimmune disease for the rest of his life.

One autoimmune disease is **rheumatic fever**(which is much more complex than rheumatoid arthritis); it includes the destruction of joints as well as the heart tissues. So, the immune system is attacking the body, not the bacteria. Why does this happen? Because S. Pyogenes contains a protein called M protein, the immune response is to form antibodies against different antigens, including the M protein. The antibodies formed for the M protein react with self-antigens; especially those present in joints and heart tissue: inflammation in joints (arthritis), endocarditis (heart valves) and myocarditis (cardiac muscles). So if you treat the bacteria with an antibiotic, it is useless, because the body is producing antibodies against itself, the patient has to live rest of his life on immunosuppressants. So always treat pharyngitis immediately (empirical treatment).

Other post infection complication is **glomerulonephritis** (nephrons inflammation). Certain strains have super antigens; these antigens are not cleared easily by the reticuloendothelial system (mainly spleen and liver), because when they react with their specific antibodies they remain circulating in blood for a long time in the form of immune complex i.e. antigen-antibody complex.These complexes precipitate in the kidney tissues, and their accumulation will trigger a strong inflammatory response which destroys the kidney resulting in kidney failure. This phenomenon is called immune complex hypersensitivity.

Other strains of S. Pyogenes secrete a toxin called erythrogenic toxin, which causes yet another complication called **the scarlet fever** i.e. bright red rash all over the body.

Virulence factors of S. Pyogenes:

- Hemolysins: remember it is B-hemolytic.
- Leukocidins
- Hyaluronidase
- Erythrogenic toxin: causes erythematous rash in scarlet fever.
- Streptokinase: it dissolves clots, because the bacteria need to spread to the body through blood (it's a spreading factor).

Note: streptokinase is used medically as a drug. It is extracted from S. Pyogenes in very low amounts (that's why it's very expensive), and for long years it was the only drug of choice to treat MI and strokes, because in these conditions the patient has 1-2 hours before he dies or have permanent disability. Streptokinase is on WHO list of essential medications that must be present in any country in the world; even the poorest health centers must have it. It costs around 600 JOD/syringe. Alteplase (also an enzyme) is also used, and costs 1000 JOD/syringe.

b. <u>S. agalactiae (group B streptococci)</u>

It is present in less than 20% of humans as normal flora in female's genital tract without causing any harmful effects. It can cause serious infections in newborns during delivery, including **neonatal meningitis;** in fact, it is the most common cause of neonatal meningitis which has a high mortality rate.

It does not cause serious infections in adults, it only does when the individual is severely immunocompromised. In theory, it can cause infection in the female during delivery causing sepsis, which is deadly.

Note: any types of bacteria could cause serious infections in immunocompromised patients.

Screening is done for this bacterium, if it is present, we give an antibiotic during delivery (IV penicillin G) and sometimes administration starts approximately 4 hours before delivery.

***The doctor did not explain the following, you must study them.*

3. Gamma Hemolytic Streptococci

S. bovis

- Group D streptococci
- Mainly present in animals GI (i.e. cattle and sheep)

- Causes some human infections such as **endocarditis** and **UTI**
- It has also been associated **with colorectal cancer**

Third Genus: Enterococci

- γ-hemolytic bacteria that can grow in presence of high concentrations of NaCl or bile salts.
- Most important species are E. faecalis & E. faecium
- Normal flora in GIT but can cause some serious infections such as **endocarditis**, **UTI** and **abdominal wound infections** (many of which are hospital acquired)
- Known for its high resistance to several antibiotics including vancomycin (i.e. VRE)

Good Luck