



University of Jordan  
Faculty of Medicine



Medical Committee  
The University of Jordan

Introduction to  
**Microbiology**

Title :

**Gram-Positive Cocci**  
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Professor:

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# : 13 .....

Slides

Handout

Sheet

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\*\*This lecture will discuss: Tetracycline, Chloramphenicol, Gram +ve cocci.

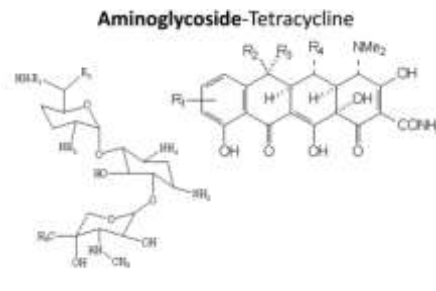
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\*Back to slide 16 / 17 :

## Tetracycline:

A complex structure which is different than the aminoglycoside structure (slide 16), tetracycline is composed of 4 rings associated with different hydroxyl, amino, methylene, methyl group, certain fluoro- or chloro- atoms. According to this combination we have different types of tetracycline; flour tetracycline, chlorotetracycline, oxatetracycline, deoxatetracycline.

In general; all tetracyclines have the same mechanism of action in relation to their antimicrobial effect on gram +ve or gram -ve bacteria.



Properties of tetracycline:

- -Wide spectrum antimicrobial drug; covers gram +ve and gram -ve.
- -Cover mainly facultative anaerobic bacteria not anaerobes.
- -Mechanism of action which is similar in all tetracyclines is: Inhibiting attachment of an amino acid to RNA on the 70s ribosomal subunit, which ends in inhibition of protein synthesis.
- -One of the most widely used types of anti microbial drug in the 1950s and 1960s and beginning of 1970s.
- -Used in huge amounts in animal husbandry; it is included in the food of animals to increase the weight of these animals; it does so by suppressing certain microorganisms in the intestines.

Unfortunately; tetracycline is still used in animals more than humans, but despite this fact there are new and developed types of tetracycline which are: Doxycycline and Minocycline.

Those two drugs are modified from the basic structure of tetracycline in order to reduce the amount of administration of the drug or to reduce the dose of the drug.

Ex: Instead of using 4g/day for treatment of gastrointestinal infection; by using doxycycline or minocycline we use 2g/day or even 1 g/day which may be enough to have the same effect as the basic structure of tetracycline. This means that the pharmacokinetics of those two drugs (doxycycline and minocycline) is more advanced, and they are smaller and more effective than the old drug.

Majority of infections related to treatment by tetracycline; e.g. cholera, certain upper respiratory tract infection caused by fastidious organism such as Mycoplasma and Chlamydia which can't be treated by other beta lactam drugs. And it still can be used in treatment of certain gastrointestinal infections caused by certain type of bacteria, especially salmonella, as well as urinary tract infections.

Ten years ago they have also modified minocyclin into a very effective drug with more specific treatment called *Tigecycline*, which (like other tetracyclines) has a wide spectrum, covers more gram +ve and gram -ve. But it might be associated with toxic effects; so it should not be used for treatment of a simple infection, usually used to treat *multidrug resistant organisms*; which can't be covered by other drug like aminoglycosides and fluoroquinolones.

It is very expensive drug with certain side effects. It can be used as a last resort for treating certain type of infections in relation to bones, meningitis, and other.

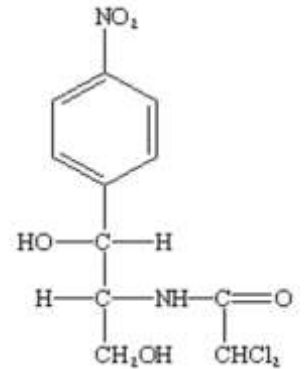
### Chloramphenicol:

Has a very small structure which allows it to penetrate well into the intracellular region of cells where some microbes cause localized intracellular infections; like *Salmonella typhi*; the causative agent of typhoid, and other organisms that cause meningitis, where this drug can easily penetrate the meninges in high concentrations and it covers a wide spectrum of bacteria gram +ve and gram -ve.

This means that Chloramphenicol is exactly like tetracycline: considered wide spectrum drug; covers large number of gram +ve and gram -ve, and as a result it may produce certain gastrointestinal side effects like diarrhea, and might produce mild side effects on blood stem cells in bone marrow and affect the formation and maturation of RBCs and produce aplastic anemia.

Considered toxic and should not be used widely for treatment of infections without a strong indication for using of this drug.

In the past it was widely used for treatment of typhoid fever and many infections, but now it has restricted uses, only for certain cases of meningitis or for topical treatment for conjunctivitis, or as skin ointment in association with bacitracin or in association with aminoglycoside. So it is not used widely in clinical practice anymore.



## ***Types of bacteria:***

According to gram reaction the bacteria is classified into gram +ve and gram –ve.

Gram +ve : cocci, bacilli, Gram –ve: cocci, bacilli

### ***\*\*Gram +ve cocci:***

This term means that once you prepare gram stain from culture of this bacteria like staph or strep or enterococcus, you'll recognize the presence of spherical cocci cells, and depending on the type of cocci they might be arranged in clusters (grape like structure); like in staph, or might be in form of chain of cocci; like in strep.

It is not typical to recognize this bacteria if you take a colony from a solid medium like the surface of blood agar, It is easier to demonstrate the presence of these clusters or chains (the morphological structure is more easily recognized) if you have a fluid culture medium like broth or by using clinical specimens. During replication this arrangement of cocci can demonstrate the type of the organism.

All types of facultative anaerobic cocci belong to group of Micrococcaceae. We have obligate anaerobic cocci belong to another family, so all cocci we will mention here belong to facultative anaerobic bacteria that belong to the family Micrococcaceae .

Within this family Micrococcaceae we have different genera, e.g. Staphylococcus genus, streptococci genus, enterococci genus. (We can also call them "groups")

Each genus is composed of many species, e.g. Staphylococcus is divided into two important species commonly associated with our commensal flora in relation to our nasal cavity and other. They are called: Staphylococcus aureus and staphylococcus epidermidis

(There are other species of less importance and found in fewer numbers.)

These two species are commonly found as part of our commensal flora and important causative agents of diseases, but there is also a difference between species, some species are considered more pathogenic than others.

**Staph. aureus:** term "staph" is related to the arrangement of cocci. Term "aureus" is related to the color of colonies on blood agar and nutrient agar; it is usually golden in color and aureus in Latin means golden.

### **Staph. epidermidis:**

- - whitish in color.
- -Term "epidermis" originated from our epidermis due to the fact that staph epidermidis is more common as type of our commensal flora than staph aureus. Sometimes, especially in hospitalized patients, in association with certain health professionals (physicians, nurses..etc) Staph epidermidis might form more colonies along with staph aureus in the nose, hands, and skin of patients due to close contact with patients who are often infected with these two types of organisms; particularly staph aureus.

All staphylococcus species whether staph aureus or epidermidis are *catalase positive*, which means they carry the catalase enzyme which converts hydrogen peroxide into oxygen and water, whereas all streptococci and enterococci are *catalase negative* which means they don't have catalase enzyme and cannot convert hydrogen peroxide into hydrogen and oxygen.

This can be easily done in the laboratory and is important as a test to distinguish between the staphylococcus, streptococcus and enterococcus as groups, and to classify easily the gram +ve cocci and give information to the physician about the causative agent.

Second important feature in relation to each type of these organisms whether staph or strep :

Staph aureus - compared with other staph species; staph epidermidis, citreus, albus etc. - , is considered more invasive and more pathogenic and more virulent. Invasive means it produces invasive infection like sepsis, wound infection, meningitis, localized abscesses, etc ... and means it easily can disseminate (spread) from the site of infection in the skin into lymph system then to blood stream.

If we compare the invasiveness of staph aureus and staph epidermidis; Staph aureus is more invasive by about a hundred times than staph epidermidis. So staph aureus can very easily penetrate from a simple wound infection to lymph system to blood stream and cause sepsis.

**\*\*Why is the staph aureus more invasive?**

Because of the secretion of certain virulent factors, which are responsible for virulence and invasiveness, and the feature for inflammation and other features which might be associated with the presence of toxins or specific enzymes.

Staph aureus contains in the cell wall a type of protein called *protein A*, which is responsible for production of specific antibodies and responsible for dissemination of infection. Second, it produces variety of enzymes formed of enterotoxins and exotoxins.

### **Keep In Mind:**

The term *exotoxin* means a type of enzyme which is excreted outside the bacterial cells and can be separated from bacteria cell. This exotoxin might be associated with variety of chemical reactions, e.g. Coagulase test which produces clumping of staphylococcus during the infection, and this can be demonstrated in vitro in the laboratory if you have a drop of serum and you mix a number of cells of staphylococcus with this serum. Prothrombin will be converted into fibrin and fibrinogen, and this causes coagulation of the staphylococcus into clusters in clumping colonies which can be easily detected in vitro.

This is an important thing during infection, because the presence of coagulase when there is an infection inside our tissues provides a protection to staph by forming a cover surrounding it and preventing access of antibiotic so it cannot reach the staph and kill it. In such a case we have to perform surgical drainage due to the presence of inflammatory cells or puss: you have to get rid of the puss in order to give treatment and cure the patient, otherwise the antibiotic will not give any result.

**\*\*you can refer to this link for more explanation of coagulase test:**

<http://www.microbelibrary.org/library/laboratory-test/3207-coagulase-test-for-staphylococcus-species>

Exotoxins can be manifested in the form of a clinical feature like food poisoning. About 30 % of staphylococcus aureus found in association with our body or in the body of animals might contaminate our food especially grounded meat or high protein food articles like white cheese, yoghurt and so on. And later produce a feature of food poisoning.

Food poisoning means you will suffer from a clinical feature of food poisoning which is normally recognized in the form of vomiting and diarrhea, but without the presence of fever because it is not an invasive organism (you are dealing with toxins and this toxin will be absorbed from stomach and will affect vomiting centers in the brain and later affect intestinal mucosa of small and large intestine and produce accumulation of fluid which results in diarrhea. Usually it is a mild form of diarrhea. )

Food poisoning from staph aureus is considered very common in Jordan and most developing countries due to the standard of hygiene, which is less than the developed countries. We wash our hands less, we use less disinfection methods, so a lot of food could be easily contaminated with staph and result in food poisoning.

According to our information from the ministry of health, 30% to 40 % of all types of food poisoning in Jordan are considered staphylococcus food poisoning cases.

### ***Definitions:***

**Exotoxin** : excretion of a specific toxin or enzyme outside bacteria cells.

**Enterotoxin**: a type of chemical substance which acts as a toxin but inside the intestine of the patient, so when we say staph produce exo and entero toxins; exotoxins are elaborated usually in food (formed outside our body) and will be responsible for food poisoning, without necessarily associating with staph in our intestine.

Whereas when staph reach the intestine, they will multiply and release enterotoxin, in such a case we say enterotoxin action.

**\*\*exotoxins and enterotoxins of staph are considered heat stable. (if you heat the food for a short period it won't be enough to get rid of them)**

**Endotoxins**: A feature of gram –ve bacteria in association with lipopolysaccharide, and this is manifested during infection with gram –ve bacteria associated with blood stream usually.

Again, staphylococcus aureus is considered more pathogenic more invasive than others due to presence of extracellular enzyme and toxin, from these we mentioned the toxin responsible for food poisoning and coagulase which is responsible for coagulase reaction or clumping factors, and have other enzyme of importance which contribute for dissemination of the organism like Hyaluronidase.

During infection in the tissue hyaluronidase is considered as spreading factor, which means it allows the staph to spread within subcutaneous tissue from one side to another side and produce more infection, and to reach the blood stream. And we have Leukocidin which affects cell membranes of white blood cells, destroying them.

All of these enzymes together are responsible for developing of inflammatory reaction in our tissues; which is manifested by presence and accumulation of WBCs and puss.

So in treatment of certain infections related to staph it is not enough to give only antibiotics you have to perform surgical drainage, which means if you have severe wound infection, and large amount of puss, you have to do cleaning; to open the wound to get rid of this accumulated puss and later to introduce directly the antibiotic in addition to giving oral or systemic drugs.

Staph aureus is always associated with the elaboration of the enzyme coagulase, so once we report in the lab that a sample is coagulase positive, you have to take in consideration that this might indicate that staph aureus is present. (It is not necessary to have a report from the lab saying that there is staph aureus; they might only report coagulase positive staph)

\*\*All other species of staph are considered to be coagulase negative.

*Staph coagulase positive* is very common cause of all type of infection in our body beginning from a simple abscess in any part of the body; hand, wound, it might easily spread from surface of our skin to deeper tissues to connective tissue to reach bones and cause osteomyelitis (inflammation of long bones) and this is very serious especially when there is a fracture.

- Or it might cause simple infection in the eye in form of conjunctivitis
- Might cause infection related to lung in form pneumonia usually following viral infection complication
- Might cause sepsis in blood, spread from blood to the meninges and cause meningitis especially in Immuno-compromised patients.

So staph coagulase positive is a very common cause of infections, therefore it must be considered serious and should be treated with a specific type of antibiotic, and this requires collecting clinical specimens from site of infection of wound or blood or any part of the body and send them to the lab and culture them and add gram stain and test them for catalase and coagulase.

In our country as in most other developing countries, all isolated staph aureus are resistant to penicillin drug and have a wide spectrum of beta like lactamases, ampicillin, and amoxicillin, 1st generation of cephalosporine and maybe 2<sup>nd</sup> and 3<sup>rd</sup> generation.

Therefore you have always to test for Methicillin and Oxacillin susceptibility. If Staph is resistant then it is considered as MRSA (Methicillin/oxacillin resistant staph aureus), and the only thing to be done is to use more toxic drug and more expensive like vancomycin.

In our country usually all staph coagulase positive are considered susceptible for vancomycin and fucidic acid and these drugs are used in treatment of the infection.

### **Staph epidermidis:**

S. Epidermidis is less pathogenic, often not associated to severe infection or to food poisoning because it doesn't excrete exotoxins and fewer enzymes are responsible for invasiveness like glycosidase and hyaluronidase.

It might be associated with skin infection, might produce bacteremia (the presence of bacteria in the blood) especially in immune-compromised patients or very young patients like newly born babies.

It is considered as an opportunistic pathogen not an obligate one like S.aureus.

The same methods (biochemical tests) are used in order to know if it is S.aureus or S.epidermidis.

\*\*In relation to staph we have a type of organism known in the past called

### ***Micrococcus:***

-It has many species.

-recently included within coagulase positive staph.

-less associated with clinical infections, wound infection, sepsis...etc

- Might be associated with immune-compromised condition,

Until now there is no vaccine available to protect our human body against infection with staph

### **Second group: streptococci:**

Classification :

We classify it according to hemolytic activity on blood agar, which means we have to recognize if the isolated organism is hemolytic or non-hemolytic.

In addition all the streptococcal species are considered catalase -ve in contrast to staph.

- Picture in slide 7:

Streptococcus produce damage to RBCs in blood agar; it produces an enzyme which causes damage to cell membrane of RBCs and this is considered as hemolytic reaction.

It is of two types:

**1-Beta-hemolytic streptococci:** produces complete destruction of RBCs, which means if you look at the culture of strep it is transparent (no RBCs), the hemoglobin has been broken down and there is no red color.

**2- Viridans Streptococci Group (also known as Alpha-hemolytic or non-hemolytic) :** produce partial destruction of RBCs. Red hemoglobin will be converted to verdohemoglobin (green) and this can be used in classification and to give the term "viridans streptococci" . Depends on conditions (ex: presence of CO<sub>2</sub> during incubation in the lab).



What is the importance of differentiation between alpha and beta hemolytic strep?\*

Reasons: alpha hemolytic strep is part of our respiratory flora; very common in **all** human beings, there is no human being that has no viridians strep in his oral flora; whether in the oral cavity or in association with the tonsils and throat.

And rarely these viridians strep are associated with infection due to lack of virulence factor or elaboration of toxins which elaborate outside the bacteria cell (No coagulase, hyaluronidase...etc).

Therefore, mainly, viridians strep might produce infection in association with other microorganisms, like causing dental decay when associated with various types of microorganisms.

It might also under certain conditions reach our blood stream, and if there is any defect in the heart valves due to a congenital defect or due to a heart attack, it might reside on the injured heart valve and produce endocarditis, which is a severe infection and results in the death of the infected person if not treated with antimicrobial drug or even certain heart surgery.

**Beta-hemolytic strep:** this group causes a complete lysis of RBCs which is easily detected in the laboratory. There is an enzyme elaborated from these bacteria called streptolysin enzyme, which is responsible of destruction of cell membranes of RBCs.

Second; Cell wall of these strep is complex, composed of a variety of carbohydrate groups and according to the organization of this carbohydrates we have different antigenic or serotypes: A, B, C, D, etc...

The important groups for us are A and B groups; because they are responsible for very serious infections in human beings, especially group A in children which cause tonsillitis (sore throat).

E.g. To understand the importance of beta-hemolytic strep in clinical practice: if we considered 100 cases of sore throat (inflammation in tonsils and pharynx), from these 100 cases: 90% are caused by viruses and 10% by bacteria.

From these 10% caused by bacteria: 90% are caused by group A beta-hemolytic strep.

So majority of sore throats in children particularly up to age 13 are caused by group A beta-hemolytic streptococci.

"Education is not the learning of the facts, but the training of the mind to think" . Albert Einstein

Study well and keep smiling!