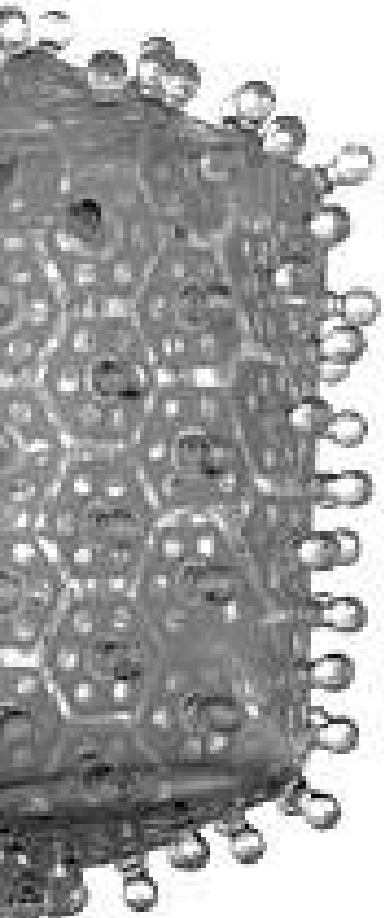


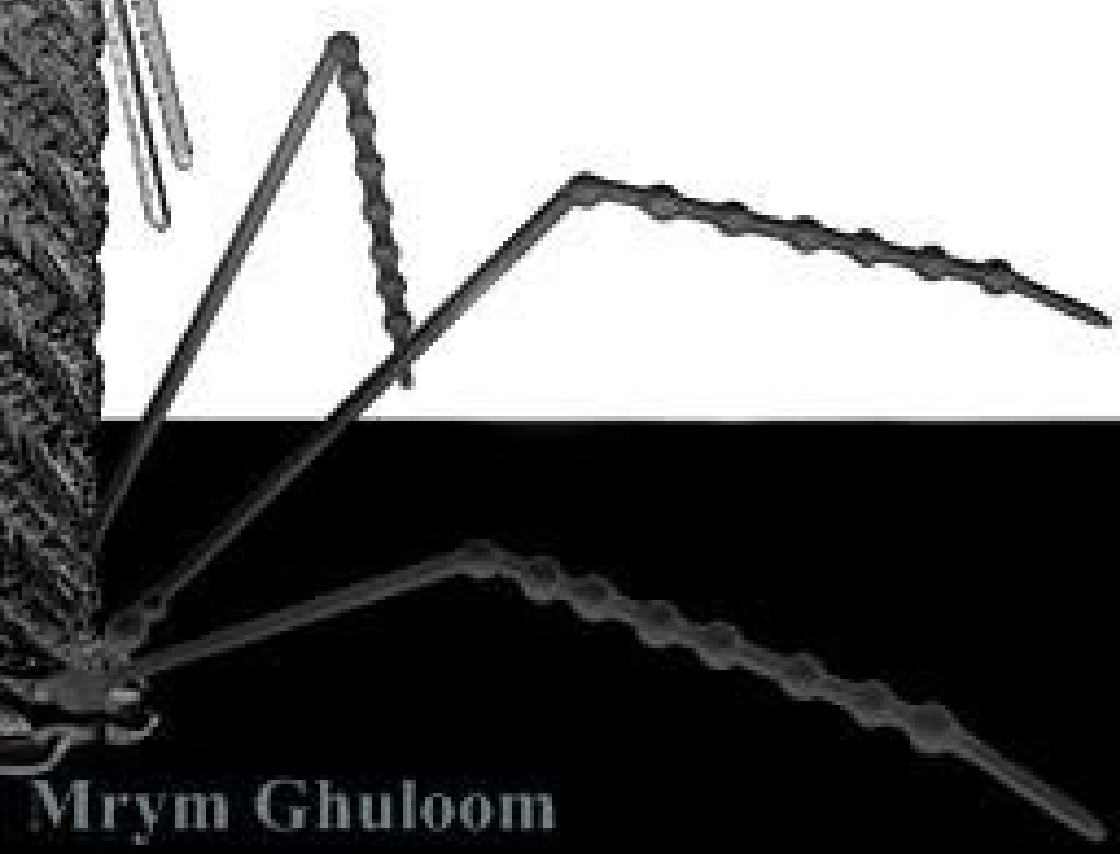
Microbiology

Lecture No:...14.....

Dr Name:Asem.Shehabi

Done by:..Aseel.Olaimat.

Sheet ■ Slide □



Mrym Ghuloom

Resistance of bacteria

In this lecture we will discuss 2 things :

part(1): Development of Antimicrobial resistance.

Part(2): Gram +ve classification.

First part :

As we've seen in relation to antimicrobial resistance, if we have a patient infected with any microorganism, we have to isolate the organism and identify it by using special culture medium and biochemical test and more advanced techniques in order to know if the isolated organism is the causative agent or not, so we collect specimen according to the site of infection:

-tonsillitis and pneumonia.. Specimen from upper respiratory tract. (Sputum)

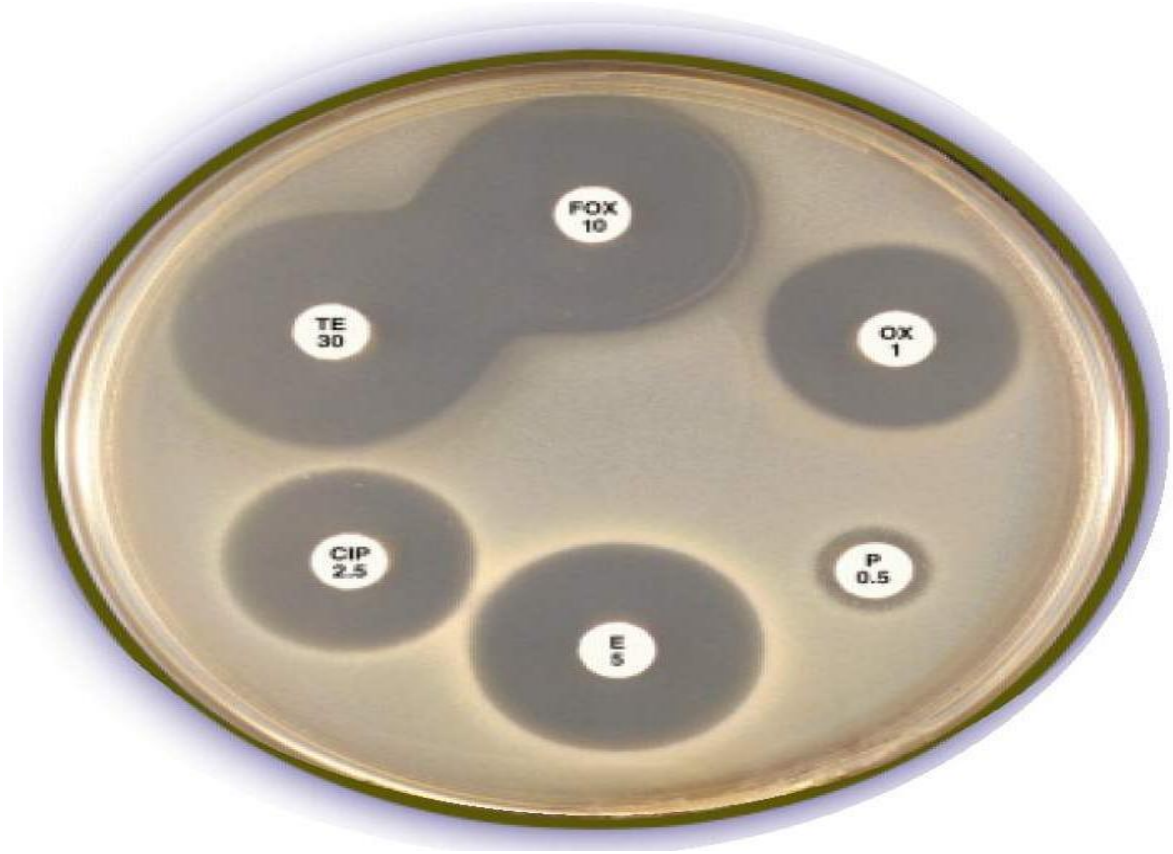
-sepsis ... Specimen from blood.

-urinary tract infection.. Urine from urine tract.

-wound infection ... skin... etc.

These clinical specimens must been sent to the labs without delay .. Within the lab ,they culture them to isolate the causative agent in order to know the type of the organism and antimicrobial susceptibility (if this org. Susceptible to this type of drug or not) In lab they use certain type of techniques and we have large no. of antimicrobial drugs , it's not necessary to test all types of drugs to check the resistance of the microorganism .. If the org. Isolated from urine, we use drug which used in treatment of urinary tract infection (anti-uropathogenic Drugs; ex: nalidixic acid , one or two types of cephalosporines, fluoroquinolones) in general in any antimicrobial susceptibility culture test we use around 8-10 types of antimicrobial drugs to check how much this org. is resistant. this slide illustrates an **antimicrobial disk diffusion test** (it's called like this cuz we have special disk filter paper which contain definite concentration of each type of antibiotic will be tested like : tetracycline(30ug) , erythromycin(5ug) ; these concentration have been calculated according to experiments in

Written by Aseel olaimat



animals and later in humans . for ex : If org. is susceptible it will produce large inhibition zone surrounding Erythromycin-test why do we do that ?! Cuz these concentrations which calculated in vitro (lab) will be translated to real concentration which doctors prescribe them to their patients in vivo (applied on human now) so patient respond and cure .

In fact not easily always the result which we have calculated it in vitro (lab) correlated with the result in vivo ; more explaining : if we have test certain organism to drug (X) and show large inhibition zone .. We prescribe this drug to our patient cuz the organism is susceptible and as a result the patient cure. But this not always happen.. In other words, the org. Might be sensitive to the antibiotic in vitro but when patient take it.. Doesn't respond ! This may happen due to many things for ex:

*infection is localized in certain areas of the body due to presence of abscesses which prevent antimicrobial drug to reach it .. So we have to remove the damage tissue then we give the antibiotic

*Or maybe the Drug can't reach the cerebrospinal fluid in meningitis cases . So there are many factors which control and limit action of antimicrobial drugs.

But generally correlation between the result of vitro and vivo exceed 95% (majority of organisms which usually proved to be susceptible in vitro should respond to treatment) now in order to get accurate result of this test ,the org. must be isolated in pure culture , we can't test mix culture(staph and E-coli as an example) .. So we take few colonies similar in morphological and biochemical test.. Etc. And later we prepare solution called inoculation solution and it's spread over the surface of the culture medium in confluent growth (cover the surface of the plate not in small places) we wait few hours and we place number of discs (6 or 8 .. Up to 10 , usually between 8-10 discs for 8-10 antimicrobial drugs which will be tested) . Inhibition zone for each drug should be measured by a ruler and there is a guide line prepared from special community by WHO microbiologic society which indicate:

* if inhibition zone >= 16 .. Org considered susceptible

* if inhibition zone < 16 .. Org considered resistant

-sometimes we might have intermediate sensitive (between resistant and sensitive) , in this case we report to physician .. So he has to increase the amount of antimicrobial drug to ensure treatment. But this shouldn't doing for all cases of intermediate organisms , just for certain types of antibiotics cuz some drugs we can't increase their doses due to their side effect and toxicity in relation to gastrointestinal and kidney.

Look again to the pic. : Drug P (is a specific drug not often used, called cefepime, 3rd acco. To dr and 4th generation acco. to ecnyclopidia of cephalosporine) it's conc. Is 0.5 ug .. No inhibition zone around it so we report the organism is resistant to it.

* Laboratory report should write the following :

1-name of the organism

2-a genus or species; genus for ex: if we write only staphylococcus species (SP) and streptococcus species, genus and species* for ex: if we write staphylococcus aurous or staphylococcus epidermidis .

*sometimes not necessary to do full test to identify the species ,cuz it's difficult and take time , we only test the genus, and according to the result, physician can responded and give the antibiotic .

Remember : the result doesn't necessary to be always associated with the cure of the patient or as we said results of vitro might not collateral with vivo results due to many factors as we mentioned earlier.

Now ; in relation to blood sepsis (presence of an organism in the blood), we will use drugs like : bulkomycin (against G+ve) or gentamycin or amikacin (aminoglycosides drugs) , these drugs are toxic , so the physician want to know exactly the minimal inhibitory concentration MIC (least amount of antibiotic which inhibit the organism) and it was done in past by using dilution tube and it was taking 22 tubes order to see exactly the MIC, recently we use E-test : strip of filter paper carrying different concentrations of antibiotics, these concentration begin with minimum 10ug,12ug ... to maximum 32ug.

Here : we have growth of bacteria , lower one is ampicillin strip, no inhibition zone whether at low concentration or high concentration .. So in short the organism is resistant to ampicillin, so we report it as resistant.

Above one: other drug (fluoroquinolone) .. there is inhibition zone and it's 1ug/ml of MIC ; and this number is the concentration of drug which later will be the actual dose which given in prescription .

This method (MIC) help us to determine exactly the amount of drug and prevent the development of toxic effect and we do it in specific cases in relation to septicemia and

meningitis in order to avoid toxic effect especially to patients who have problem in kidney and liver , so we dont treat them with over doses of antibiotic drugs.

Note : under normal condition without using any type of antibiotic , normal person might under certain condition developing resistant -not due to using antimicrobial drugs- , there is a lot of chemicals available in our humans which may contribute under certain condition in developing resistant in intestinal flora..etc.

So developing of Resistant is not due to using antimicrobial drugs alone, but antimicrobial drugs will increase the resistant too, where it started slowly.

Ex: in our intestines we have billion of cells from different type of bacteria, if we have a patient infected with E-coli in his intestine and we take a specimen of stools which have E-coli bacteria.. and test 10 antibiotic drugs, we will find it is susceptible to all of the drugs like ampicillin and the patient cure, if the patient reinfected by E-coli again after a month and we do the same test , we will find few cells became resistant and this resistant increase slowly and accumulate especially if E-coli has a transferable plasmid which contribute in dissemination of resistant to other cells.

So it's not necessary to recognize the **developing of resistant** cuz it's slowly progress due to accumulation of more and more resistant for one or other type of antibiotic , but **at the end** we recognize large amount of resistant .

Once a physician asked our doctor: 'you have reported to us this organism is resistant to antibiotic(X) and we gave this antibiotic to the patient and he responded!!'

Dr. Answred him : 'it's correct , the patient might respond, but he responded one time not in the 2nd time or 3rd or 4th times if he reinfected with the same org. again .

So again , keep in mind : it's not necessary always recognize the presence of resistance .. It might be recognized later .

let us take an urinary tract infection as an example : numerous cases especially ladies who came with E-coli which were at the 1st susceptible level to majority of anti-uropathogens and they cure , after few months they might acquire repeated infection , in 2nd time of infection we recognized the E-coli is resistant to 2 antibiotics , after 1 year ; repeat of infection associated with resistant of E-coli to many drugs and so on .. Until the infection be a chronic case (very difficult to treat orally so we use IV type of antibiotic - in hospital - which is more expensive and toxic).

Summry of all wt doctor want you to know : Developing of antimicrobial Resistant is a slow process requires weeks, months and years to be developed and recognized.

Last slide illustrate that the antibiotic drugs - which used in treatment of patients – are the only drug which considered non-individual Drugs

What do we mean by 'non individual Drugs' ?

Means we've to treat the patient and at the same time treat the community where the patient is living , why? cuz development of resistant - in any person whether inside or outside hospitals - will be exacerbated to community soon or later by defecation and contact ... etc.

So resistant will not be eliminated from one single person. it will include community , so overuse or misuse of drugs will increase the resistant and spread through community ,so it's not relate only to one person cuz developing of resistant in intestines tract of any patient means this antimicrobial resistant might disseminate by defecation and direct contact with contaminated food or water .

So It's complex process involves animals and humans , by the way 70-80% of all types of antibiotic which produced are produced for animals more than for humans, cuz they use these antibiotics to feed chicken and animals to increase their weight not related to medicine (they don't use antibiotics to protect animals and treat their diseases) so presence of animals are a source for increasing the resistant , and this must be monitored and controlled by ministry of agriculture and Health.

- Development of resistant in any type of organism considered stable (not easily we can return resistant org. to susceptible one), we rely only in mutation ➔microorganism cure their self by release antimicrobial resistant gene (this long lasting process takes 100s of years like nuclear elements in the lumen ,not easily can be eliminated)

second part :

We will start talking about G+ve cocci and will concentrate only in most important features like morphological structures in relation to epidemiology and organisms which cause diseases and some hints how we can isolate the diseases and a little bit about pathogenicity but not in details and the doctor will not ask us about the specific drugs which used for every type of bacteria.

In G+ve bacteria we have a family called MICROCOCCACEAE family (because the organism is cocci in morphological structure which demonstrated by gram stain) within this family we have Different subgroups or different genera, in each genus there are group of organisms who have similar biological characteristics to some extent especially in terms of genotypes

Note: ALL Gram Positive bacteria belong to the family Micrococcaceae

Genus:

1-staphylococcus

2- streptococci

3-enterococcus

*there is specific region in bacterial chromosome called **16S RNA** (considered as a marker for each group of bacteria) which can be detected in lab and it's essential to classify bacteria according to 16S RNA

<1> **staphylococcus**

Has certain important characteristics and there are about 15 types (species) but just 2 types who have medical importance especially: staphylococcus aurous and staphylococcus epidermidis (coagulase negative staphylococcus).

Characterization of streptococcus and staph is only done by Gram stain according to their morphological structure in G-stain

Morphological structure : means if they are cluster or strips and this require certain experience cuz sometimes you might not recognize cluster of cocci as grape-like structure or as a chain of cocci , you may recognize single cocci which not easily demonstrated e.g. in wound or blood infection, but it easily can be demonstrated if you have growth in culture medium ; so culturing then using G-stain is the only way of demonstration.

<2> **streptococcus** : it's known to be in short chain or long chain or small cocci .

<3> **Enterococcus** : it's name is related to intestine in human and animal

It's not arrange in a strip , it's arrange in diplococcic .. 2 cocci, 4 cocci , and it's difficult to distinguish between streptococcus and enterococcus .

We've very simple test for cocci to know if it is strep or staph or enterococci and its called *catalase test* (recognize if hydrogen peroxide in bacteria produce O_2 and H_2O) ➔ staph (catalase +ve),strep and enterococcus (catalase-ve) ; so G-stain and catalase test help us in classification .

This link illustrates the catalase test http://www.microbelibrary.org/library/laboratory-test/3207-coagulase-test-for-staphylococcus-species

Now, we will concentrate on the 1st group again (staphylococcus): 2 species of staph. Form 20-30% of all types of infection of human body (common):

1-staphylococcus aurous : aurous (gold colour of staph which result in the culture media.)

2-staphylococcus epidermidis (albus): white in color, related to skin

Both or one of them can be found in most of us ,in nose and skin (mainly), folding of skin, lower part of urethra and may be found in oral cavity too.

Under normal condition they are a part of normal body flora and not associated with infection , but if there is any damage in dermis, epidermis of skin ; few no. Of them might reach the subcutaneous tissue and produce inflammatory RXN and infection.

- **staphylococcus aureus** inflammation is more common and intense due to :

✓ it is highly pathogenic

✓ it releases a large no. Of extracellular product and enzymes (like hyaluronidase and DNAase and haemolysins) and toxins whereas staph. Epidermis doesn't do that.

✓ it has a capsule in its cell wall and protein A which make it more invasive .

- most important enzyme produced by staph.auerus is *coagulase enzyme with clumping factor* which responsible for the major pathogenic potential of this organism. It attaches to damaged tissue and produces large molecule which associated with inflammation reaction which attract WBCs and other cells. And then, by converting

prothrombin to fibrinogen and then fibrin, it produces a nest which surrounds staph and prevent the antibody from reaching the staph in the infected tissue .

In such a case ,physician should do a surgical deprivation or elimination of a wound infection for example to release the presence of these accumulation of damage cells in order to let the antibiotics to reach the target of infected tissue and produce its action.

- **staphylococcus epidermis** : widely distributed in our body ,easily isolated in lab but it is less pathogenic , which means not involved in developing of the infection especially in invasive* infection .

What do we mean by *not involved in the invasive infections* ? any infection in our skin or mucosa or oral cavity will be localized (rarely disseminate by lymphatic channels and produce sepsis) whereas staph.auerus often associated with developing of sepsis and severe type of infections due to it's toxins and enzymes .

Note : auerus is common and associated with serious and invasion infection , due to that, physician use antibiotics to treat it , and this org. is developing it's resistant, therefore 70-80% of all aurous isolated from clinical specimens considered as B-lacatam resistant ,which means we have to use penicillinase resistant Drug like oxacilline or methicillin (but usually we use oxacillin cuz methacillin is not stable) and might even the penicillinase resistant drug are not enough , because it will still resistant . So we should give another **toxic** drug called *vancomycin* (given only by physician in hospitals and is a LAST RESORT) and the patient will cure. Therefore in lab it's very important to know if staph.auerus is methicilin resistant or not (resistant to B-lactamase resistant drugs or not) if it resistant ➔ we use vancomycin (we can use another drug with vancomycin, but vanocomycin is the most common one).

In relation to staph genera we have 4th specie called *micrococcus* which is not highly pathogenic (associated mainly with skin) rarely associated with infection , may produce infection in certain type of patient which called Immunocompromised patient (who suffering from leukemia and lymphoma and low immune response) , so micrococcus can't produce infection in healthy persons.

- Note: Differences in microorganism don't depend only on pathogenic potential of the organism or releasing of enzymes and toxins ; it depends also on the immune status of the patient :

Low immunity➔ more organisms produce infection

Recently physicians don't use micrococcus term .. they say staphylococcus coagulase –ve (more simple to them to understand it's pathologic potential) so staphylococcus epidermis and micrococcus are similar (both are coagulase –ve)

So micrococcus (in past) or staphylococcus coagulase –ve(recently) ➔ less pathogenic, doesn't require treatment in Healthy patients , but required for immune compromised patient .