Microbiology

◉ Sheet ○ Slides

Number : 8

Done by : Abd alrahman I. alsliemieh

Corrected by: Abdullah Qaswal

Subject: Anti-Microbial Drugs

Doctor: Asem shehabi'

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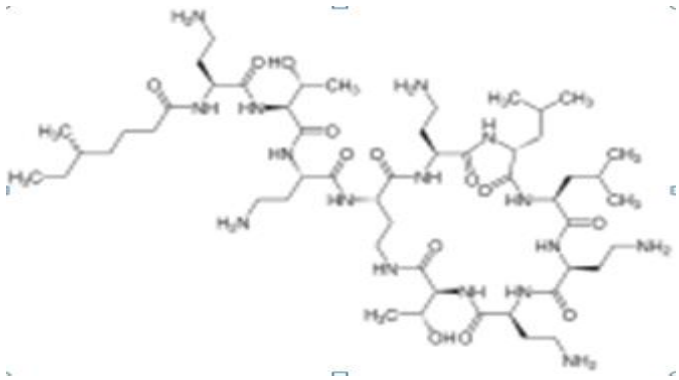
In this lecture we're going to continue the discussion of anti-microbial drugs. We said that there're many targets of the bacterial cell to be attacked by the drugs; the cell wall, cell membrane, protein synthesis and nucleic acids. We'll start talking about cell membrane since we've covered the cell wall in the last lecture.

Inhibition of the membrane integrity

There's a group of drugs known as polymixen that includes polymixen A, B, C, D and E (polymixen E also called colistin). as you see below, the structure of polyenes (the general name for this group of drugs in relation to its structure) is very complex consisting of many cyclic rings with amino, hydroxyl and carboxyl groups, therefore this group of drug is very dangerous; it's thought that it might cause cytotoxicity especially nephrotoxicity with patients who suffer from kidney failure. This drug is very usefull in treating any infection that caused be gram –ve bacteria that improved its effeciency in the resistence of penicillin group of drugs, but keep in mind that this drug must be the last bullet in treating the infection due to the fact that we mentioned before about the dangerous effects of polymixen.

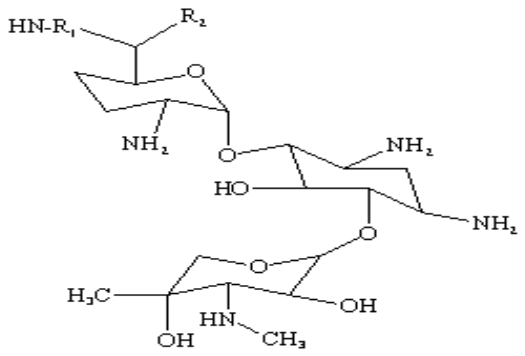


Polymixen E (colistin) is considered as a bactericidal.

Inhibition of protein synthesis:

In the cytoplasm of the bacterial cell we can find the ribosomes which are the mini-factories for protein synthesis. Ribosomes are composed of two subunits: the 50 ribosomal subunit known as 50S (larger) and the 30 ribosomal subunit 30S (smaller), together the two subunits form 70s ribosomes (the functional ribosome). We need the previous information due to the fact that some drugs attach the 50S inhibiting it and others attach to 30S. The attachment of the drugs to the ribosomal subunits interferes with the functions of tRNA and mRNA inhibiting the formation of a new polypeptide by the ribosome. Inhibiting the proteins synthesis in the bacterial cell can be done by several group of drugs including aminoglycosides, tetracycline, chloramphenicol and macrolides.

1- Aminoglycosides:



As you see in the figure, aminoglycosides composed of three amino cyclic rings associated with hydroxyl and carboxyl groups.

This group of drugs which was derived from soil bacteria is used mainly for the treatment of infection caused by gram –ve (facultative anaerobic bacteria but not obligate anaerobes) bacteria and few gram +ve bacteria by irreversibly binding to the 30S inhibiting it from protein synthesis. Examples of aminoglycosides are streptomycin, amikacin, gentamycin and tobramycin.

Streptomycin which was introduced in the 50s can be used with limitation in the treatment of tuberculosis caused by mycobacteria. Amikacin and gentamycin are used widely nowadays in clinical medicine more than tobramycin. But keep in mind that aminoglycoside drugs used in hospital only, that's because those drugs introduced only in the injectable form and the concentrations of them must be strictly maintained not to cause side effects.

Side effects

1- nephrotoxicity and ototoxicity

2-over doses of these drugs may associated with the damage of the 8th cranial nerve which causes hearing loss, this why we should control the concentration of these drugs strictly in hospitals.

Resistance

Despite the fact that beta-lactamase enzymes are not effective in the resistance of aminoglycosides, some bacterial species managed to develop a new collection of resistance enzyme including acetylate enzymes, phosphorylate enzymes and adenylate enzymes that attack the side groups in the drug structure. These enzymes can be chromosomal resistance or plasmid resistance*.

***plasmid resistance means that the genes encoding those enzymes are carried on the plasmid, so it can be inherited to the next generation of resistant bacteria.**

2-tetracyclines: **this drug has two mechanisms of action. Firstly, by accumulating in the cytoplasmic membrane and inhibiting the activity of metabolically active bacterial enzymes.**

Secondly, if this drug manage to reach the cytoplasm it acts in the same manner as aminoglycosides and attaches to the 30S of the bacterial ribosome and prevents the synthesis of proteins.

Tetracycline drug is one of the most widely used drug in the history of anti-microbial drugs. From the 1950s till now tetracyclines was used to treat a variety of intestinal and oral infections in animals and humans (wide spectrum drug). Also, it was used as a hormone to increase the size of the animal by mixing it with the food of the animal. But keep in mind when prescribing tetracycline that it must not used for a long time due to the fact that tetracycline kill some the body flora and enhancing the growth of the yeast candida which usually reside in the body in small amount, but when using tetracycline candida may over grow and produce some diseases. For example, over growth of candida in the oral cavity may produce filaments on the tooth of the children under 8 year-old and even adults when using tetracycline for a long time. also, candida can cause diarrhea and other intestinal diseases.

NOTE: tetracycline intake may be associated with preventing the production of pro-vitamins especially vitamin K. So it's recommended to describe vitamins with this drug to prevent complications.

Resistance :

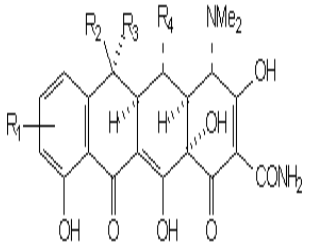
Although the bacteria slowly manage to develop resistance against tetracycline, it might be able to resist it by activating efflux pump system that expels the accumulated tetracycline out of the cytoplasmic membrane.

Classical tetracyclines (e.g. chlorotetracycline, florotetracycline or oxytetracycline) were given in large amounts (4-8 g) to treat the infections. But now, new tetracyclines have been introduced like doxcyclin and minocyclin which are given in small amounts (1-2g)

These modern tetracyclines are very beneficial in *reducing the side effects of the drug*. Another modified tetracycline had shown up in the recent years which is tigecycline. The benefit of this drug that it can be introduced to the patient intravenously to treat serious infections.

NOTES:

1.tetracyclines as the name implies have four fused rings:

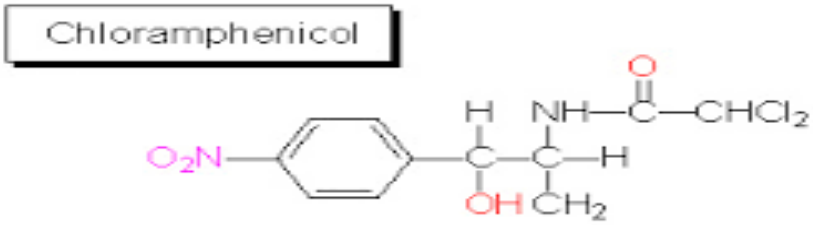


2.you never give tetracyclines to pregnant women or children under 8.

3.tetracyclines are considered as a bacteriostatic.

3-chloramphenicol:

This drug which was introduced in the 1950s has a small chemical structure with nitrogen atoms and carboxyl groups attached to its carbon atoms.



Chloramphenicol is considered as a bacteriostatic and acts by binding to the 50S ribosomal subunit and blocking the formation of the peptide bond.

It can be produced by bacteria or produced synthetically. Also, it has a wide spectrum in treating almost all infections caused by gram +ve and –ve bacteria especially meningitis (due to its small structure, chloramphenicol manage to reach the menings and treat the infection there unlike other drugs with complex structure.)

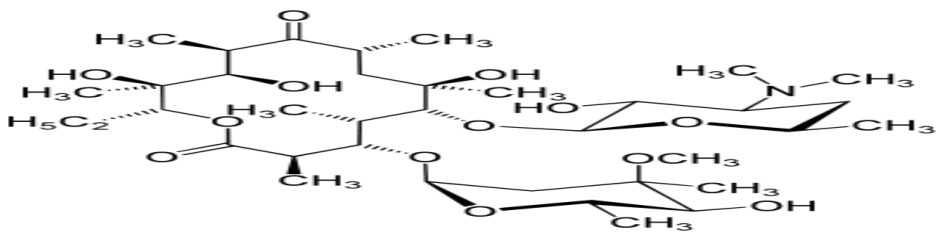
<u>Side effects:</u>

1- **It can reach the bone marrow and affect the stem cells there and produce anemia.**
2- **It can affect the intestinal flora and enhance the development of infection there.**
3- **Highly toxic.**

NOTE: because this drug is cheap, it isn't used usually in medicine. And it's used mainly in animal feeding to enhance its growth.

Don't forget that we're still studying the anti-microbial drugs that inhibit the protein synthesis.

<u>4-macrolides:</u>



This group of drugs **which can be obtained from the bacteria cell then it can be modified, inhibit the protein synthesis in the bacterial cell by attaching to 50S ribosomal subunit. It's characterized by having huge ring called *lactone ring*. It acts mainly on gram +ve bacteria especially that cause respiratory infections. Also, to some extent it affects the gram variable* bacteria and the bacteria without the cell wall like Chlamydia and mycoplasma.**

The most widely used macrolides:

Erythromycin which is the firstly-introduced macrolide. But erythromycin was the modified to produce clarithromycin and azithromycin, these two drugs can be taken one or two times daily instead of having erythromycin 4 times daily (the aim of developing calrithromycin and azithromycin is to be long-acting drugs-12 hours).

NOTES : -macrolides can be taken orally or intravenously.

-macrolides are similar to penicillin regarding to safety, pharmacokinetics and having wide spectrum of effect. But they're different in the mechanism of action (penicillin acts on the cell wall but macrolides on protein synthesis).

- relatively non-toxic drugs.

There are another groups of drugs act as macrolides:

Lincosamides **Include clindamycin and lincomycin.**

It's considered as a part of macrolides because it has a similar chemical structure and they act on 50S like erythromycin "similarities".

The differences between erythromycin and lincosamides is in the targeted bacteria. Erythromycin acts on aerobic and facultative

anaerobic gram +ve bacteria. On the other hand, lincosamides affect facultative anaerobic and obligate anaerobic gram +ve bacteria.

<u>Side effect:</u> pseudomembranous colitis:

Due to excessive usage of clindamycin or lincomycin to treat different infections which the patients in hospital suffer from, this results in reducing the number of anaerobic bacteria(normal intestinal flora) that give the chance to a spore-forming bacteria called *clostridium difficile* to grow to form about 70-80% of the intestinal bacteria. Clostridium difficile start to produce exotoxins that enhances large intestinal necrosis results in damage of the mucosa and eventually bloody-diarrhea. This how pseudomembranous colitis develops.

vancomycin

It's a large glycopeptides drug that used mainly to treat infections caused by gram +ve bacteria that is resistant to beta-lactamase drugs and macrolides especially clostridium difficile. As we mentioned before clostridium difficile can't be affected or killed by lincosamides, so using vancomycin to treat bloody diarrhea caused by this bacteria proved to be effective.

Fusidic acid

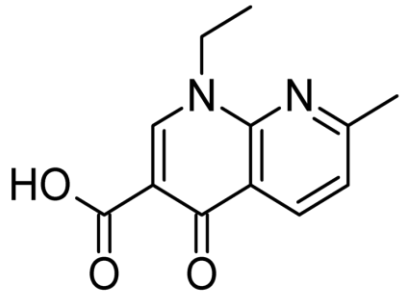
It's known as fucidine which is used to treat injuries.

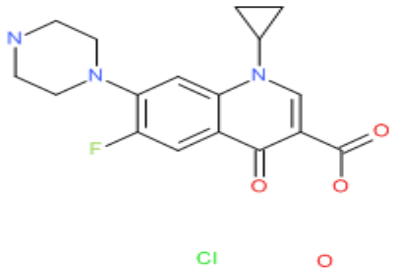
This drug inhibits protein synthesis in gram +ve bacteria – especially staph- that is responsible for producing 70-80% of wound infection and inflammation in skin and mucosa. Unfortunately, this bacteria managed to develop against fusidic

acid, so fusidic acid must be used with strict indications from the physician.

Inhibition of nucleic acids synthesis:

1- Nalidixic acid and floroquinolone





On the left: the structure of nalidixic acid

On the right: the structure of floroquinolone

Nalidixic acid, as you see above, contains two cycles with two nitrogen atoms one for each cycle. It may be attached to hydroxyl or carboxyl groups. But the problem with nalidixic acid that it's much secreted by the kidney (up to 95-96% of the drug might be secreted). So it's used mainly to treat urinary tract infections UTIs, mainly gram –ve bacterial infections.

From that point, the scientist was trying to develop another drug used to treat several infections till the 1980s, they managed to add another cycle to the compound to form the basic quinolone (it's named like that due to the presence of hydroxyl group in its structure).

Floroquinolone: it's a modified nalidixic acid in the 1980s with increased potential energy in treating infections. It has a wide spectrum in treating gram –ve bacteria like E-coli and gram +ve bacteria like staph and strept. There're about 10 floroquinolones

that you can distinguish them from the name; norfloxacin and ciprofloxacin.

Both nalidixic acid and floroquinolone affect the enzyme DNA gyrase that plays a vital role in bacterial cell replication through completing the DNA structure.

➔So this enzyme will inhibited➔ transcription of the DNA and replication of the bacterial cell will stop➔ the bacterial cell stop growing➔*bactericidal effect.*

NOTE: nalidixic acid and floroquinolone are characterized by their natural sources, both of them can be extracted from plant or specific bacteria.

Nitrofurantoin: this drug was introduced in the early 1950s as an anti urinary tract infection "anti-UTI".

It produces its bacteriostatic effect by damaging the bacterial DNA in a specific mechanism that we're not supposed to know it exactly. As we mentioned, it's a bacteriostatic drug that doesn't contribute 100% in the treatment (recall that bacteriostatic means to inhibit the bacteria), so the human body have to contribute in the treatment of the infection by its immune system.

NOTE: nalidixic acid and nitrofurantoin both used as anti-UTI, whereas floroquinolone used in the treatment of variety of infections.

Rifamycin: this group contains many drugs but the most clinically used is *rifampin*. This drug affects the RNA polymerase preventing the transcription of DNA into RNA and eventually the production of important bacterial proteins, so it's considered as

bactericidal. It has a wide spectrum of activity against gram +ve and gram –ve bacteria.

This drug is recommended in the treatment of tuberculosis caused by mycobacterium tuberculosis and other similar bacteria species. So it's considered as anti-tuberculosis drug.

Anti-tuberculosis drugs:

The cell wall of mycobacterium tuberculosis is special. unlike gram +ve and –ve bacteria whose cell walls composed of peptidoglycan, the cell wall of mycobacterium tuberculosis is composed of mycolic acid (long chain of fatty acid) and phospholipids which give the cell wall waxy appearance.

So such cell wall not easily penetrated and needs special drugs in combination to be penetrated especially that this cell wall doesn't contain pores.

Isoniazid (INH), Ethambutol, Cycloserine, Streptomycin and rifampin are examples of anti tuberculosis drugs that, as we mentioned, must be given in combination of two or three drugs to produce synergistic or additive effect. Also, if one of these drugs was given alone, it's easy for the bacterial cell to develop resistant against it.

NOTE: these drugs can be taken for a long time (3-12 months) unlike other antibiotics which can't be taken for more than two weeks, that's because the body can tolerate this drug and its effects on the intestinal and skin flora are normal.

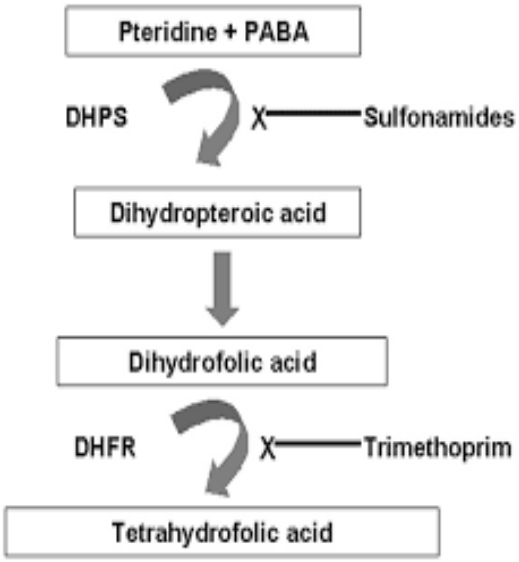
Metronidazol known in the markets as flagyl.

It's the only available drug against obligate anaerobes as well as its anti-protozoa effect. Protozoa like ascharis caused gastrointestinal tract infections. This drug is not efficient (not used at all) in the treatment of infections caused by aerobes due to the fact that it's easily oxidized by the aerobes' enzymes. It affect the DNA (cause DNA damage).

Inhibition of the synthesis of essential metabolites:

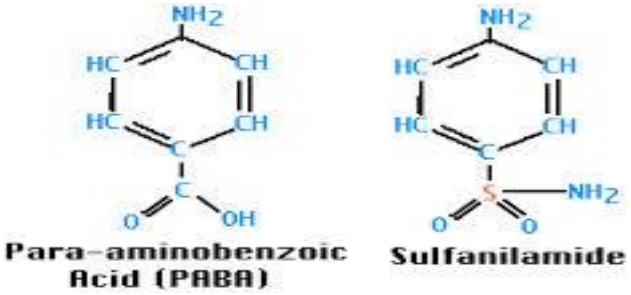
as we know the human body can't synthesize all necessary vitamins but with the enhancement of the normal intestinal flora we'll be able to synthesize some pro-vitamins like pro-vitamin K, so as a result our food must contain all type of vitamins.

Whereas the bacteria can't utilize preformed vitamins, instead it synthesizes its own vitamins using what it's called essential metabolites. For example, folic acid which is essential for the enhancement of protein, nucleic acid and vitamins B synthesis can't be produced by human body, so our food must contain this material. Bacteria on the other hand, can utilize specific essential metabolites to produce folic acid.



The production of folic acid in the bacterial cell follow this metabolic route. It depends on the presence of essential metabolite called para-aminobenzoic acid PABA. So if the culture media in which the bacteria is growing contains PABA, it will use it to produce folic acid "tetrahydrofolic acid".

The scientists develop a material that mimics PABA and competes with it for the active site of the enzyme responsible for the first step, this material is known as sulfa drugs "sulfonamide".



As you see in the figure above, sulfonamide has a very similar structure like that of PABA. So depending on the concentration these two materials compete with each other for the active site of the enzyme. So it has a bacteriostatic effect.

Unfortunately, the bacteria managed to over come the effect of sulfonamides. So we need to use another drug called "cotrimoxazole=sulfamethoxazole+trimethoprim "which affects the second stage of the production of folic acid. So here is another example of how we use more than one drug in the treatment of a specific infection "synergetic or additive". These two drugs(sulfamethoxazole + trimethoprim) used together as a cotrimoxazole to treat gastrointestinal infections, urinary tract infections and respiratory tract infections.

NOTE: sulfamethoxazole is a sulfonamide antibiotic.

But again the bacteria was able to develop resistance against these two drugs.

Antibiotics susceptibility test :

The clinical medicine used to be practiced based on the physicians' observations and experiences. For example, penicillin was used to treat sore throat and so on.

but with the development of the resistant in bacteria, the need for being specific in prescribing a drug showed up and the antibiotics susceptibility test started to be used.

For example, if a patient suffers from UTI, we take a urine sample to recognize the causative agent, we grow this sample in a culture media then we isolate this agents in a pure culture media to be studied. The next step is to start testing several drugs and observing whether it's effective or not against this agent. The efficiency of a specific drug is judged by two factors:

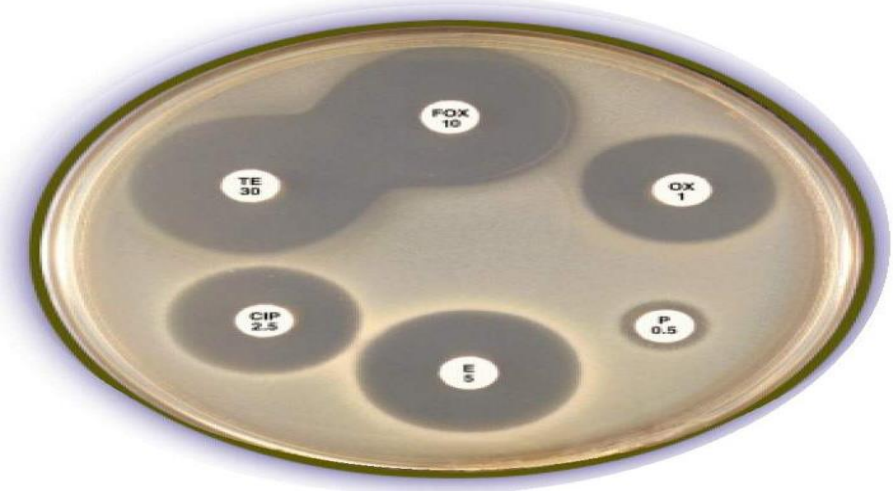
1- **The susceptibility of the causative agent of the disease for the used drug.**
2- **The ability of the used drug to reach the site of infection.**

For example, doing the antibiotics susceptibility test for nalidixic acid in the treatment of blood infection has no meanings due to the fact that nalidixic acid is highly excreted by the kidneys, so it can't reach the blood which is the site of infection.

By looking at the figure in slide #25 that represent antibiotic disc, this disc contains only one type of bacteria with many type of drugs applied to it with certain concentrations and certain inhibiting zone for each type of antibiotics* (the number under the name of the antibiotics used represents the concentration).

***the inhibiting zone -which appears like a circle surrounding the area where the drug was administered in the disc- is an**

indicator for the susceptibility or resistance of the bacteria toward that drug. Each drug has specific parameters, that means that that for X drug ,let's say, the inhibiting zone must be wider than 60 mm to say that this bacteria is susceptible for X drug. But for Y drug the inhibiting zone must be wider than 10 mm and so on.
But the question now... how to determine this specific concentration to be used? This done by another test called minimal inhibitory concentration MIC.



<u>Minimal inhibitory concentration</u>: it's the minimal concentration of the drug to produce the desired effect by inhibiting the bacteria responsible for the infection without side effect. The aim of prescribing a drug with the minimal effect not to induce undesired side effects. The importance of such test arises in some conditions where the over-dose may be dangerous. (recall what we've learnt about narrow therapeutic drugs in pharmacology).
For example, some patient categories like neonatal suffering from blood sepsis or meningitis, the over dose of the drug may be fatal for them.
Generally speaking, the mechanism to determine MIC includes in vitro experiments and in vivo experiments.

Firstly, we need to calculate the MIC in vitro then we apply the result to the body of the patients (in vivo) in respect to the variables like weight, gender and age.

The traditional way to calculate MIC was by applying the antibiotic in a gradually-increasing concentrations on the culture that contains the desired bacteria(we may need about 10 test tube in each one there's a culture for the bacteria then we add the drug with a specific concentration on the first tube, next we increase the concentration and apply it on the next tube and so on) until we reach the concentration of the drug that induces inhibition in the growth the bacteria in the test tube as low as 5 bacterial particle in 1ml. So it's a time-consuming method need to be improved, this why we now use what we call E-test.

<u>The mechanism of E-test:</u>

We need a pure culture with a single bacteria species, its age must not be more than 24 hours. Then we apply what it's called antibiotic strip that contain the antibiotic in an increasing concentrations.(this strip is like a stick that as we go forward to the tip of it, the concentration of the antibiotic loaded on it increases). Then we wait for 24 hours to have the result, by looking at the graph in slide 26 you can notice when or at which concentration on the scale the bacterial colonies start to disappear (simply this concentration is MIC).

The anti-microbial resistance:

In the clinical years we'll learn how to deal with this problem, and what antibiotics to use in each case, for now we'll learn the basic about this subject.

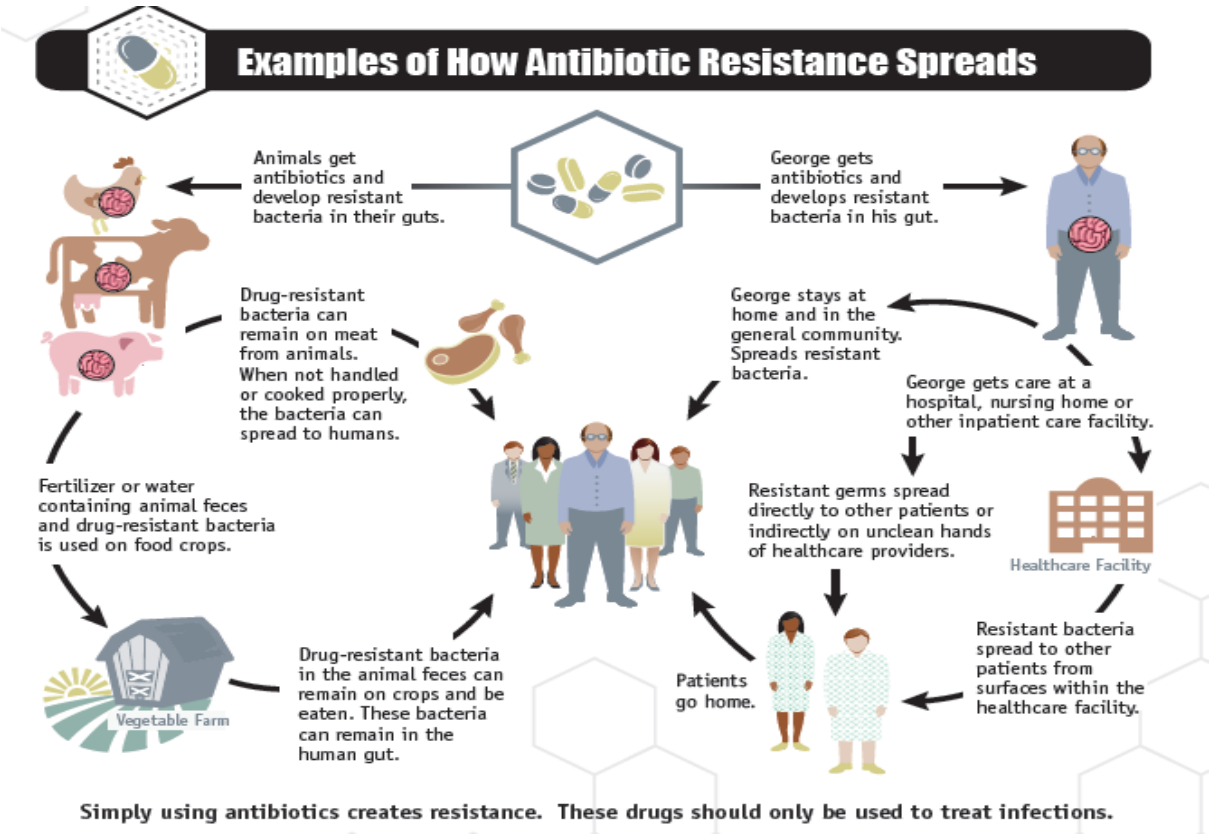
First, you must keep in mind that if a bacteria develop resistant against one type of drug, this process *isn't reversible*. Developing resistance introduced what it's called superbugs or multi-drug resistant bacteria MDR that kill thousands of patient around the world. For example acinetbacter is a 100% resistant bacteria. The treatment of such superbugs is a high cost thing that need to put the patient in the hospital and treat him/her with more than one drug for a long period.

How antibiotics resistance happens Antibiotics resistance develops slowly, it needs a long time to spread within the body.

The procedure:

1- **When we use a specific antibiotic to treat an infection, this antibiotic kills many bacteria including pathogenic bacteria *and some of the normal body flora*. But the used drug isn't effective against resistant bacteria which reside the body in a small amount.**
2- **Then the drug-resistant bacteria allowed to grow and increase in number. Also, this drug-resistant bacteria transfer the genes responsible for drug resistance to another bacteria that was not able to resist the bacteria. So at the end the number of drug-resistant bacteria increases.**

NOTE: read the last slide that talks about how antibiotics-resistant and infections spread in the community.



Test yourself!!

1.all of the following about chloramphenicol are true except:

a.broad spectrum with small structure.

b.acts by binding to the 50S ribosomal subunit.

c. necessarily, it kills gram + ve and – ve bacteria for treatment.

d.highly toxic.

e. none of the above.

answer: c (it does not kill bacteria because it acts as a bacteriostatic not bactericidal).

2. the common characteristic between macrolide and chloramphenicol :

a. both of them have the same number of rings.

b.both of them are highly toxic.

c. both of them bind 50S ribosomal subunit.

d. more than one of the above.

Answer: c

3.all of the following drugs affect cell wall or cell membrane synthesis except:

a.penicillin

b.cephalpsporin

c.polymixen E

d.tetracycline

e. more than one of the above

answer: d (tetracycline inhibits protein synthesis through two mechanisms one of them is binding to 30s ribosomal unit).

4.about nalidixic acid and floroquinolone, what is the wrong:

a. both of them inhibit DNA gyrase.

b. floroquinolone has wider spectrum than nalidixic.

c.nalidixic acid has 2 rings while floroquinolone has 3 rings.

d.both of them have bactericidal effect.

e. none of the above.

Answer: e

5.the main difference between nalidixic acid and nitrofurantoin is:

a. nalidixic acid inhibits nucleic acid synthesis while nitrofurantoin inhibits protein synthesis.

b.nalidixic is anti-UTI while nitrofurantoin is anti- RTI

c.nalidixic acid less dependent on immune system than nitrofurantoin in terms of treatment.

d. nalidixic acid has bacteriostatic effect while nitrofurantoin has bactericidal effect.

e. c +d

answer: c

6. about sulfonamide and cotrimoxazole, what is the wrong:

a. sulfonamide + PABA = competitive inhibition of the enzyme of the first step of folic acid route in bacteria.

b. sulfamethoxazole + trimethoprim = synergetic or additive effect= cotrimoxazole.

c. sulfonamide has a bacteriostatic effect.

d. cotrimoxazole has a broad spectrum.

e. none of the above.

Answer: e

(The End)