Organic Chemistry In Biology And Drug Development
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Lecture – 48
Antimicrobial drugs

Welcome back to this course on Organic Chemistry in Biology and Drug Development. Last time, we have discussed, the chemistry of hypertension and what is the humoral cause for hypertension and mechanism for the production of angiotensin 2.

And then we have discussed, how the drugs like captopril, enalapril and other these type of drugs we were discovered based on the drug design principle; based on the mapping of the active site and ultimately we got to know that how this drug can be designed rationally. In fact, captopril is known as the first rationally designed drug.

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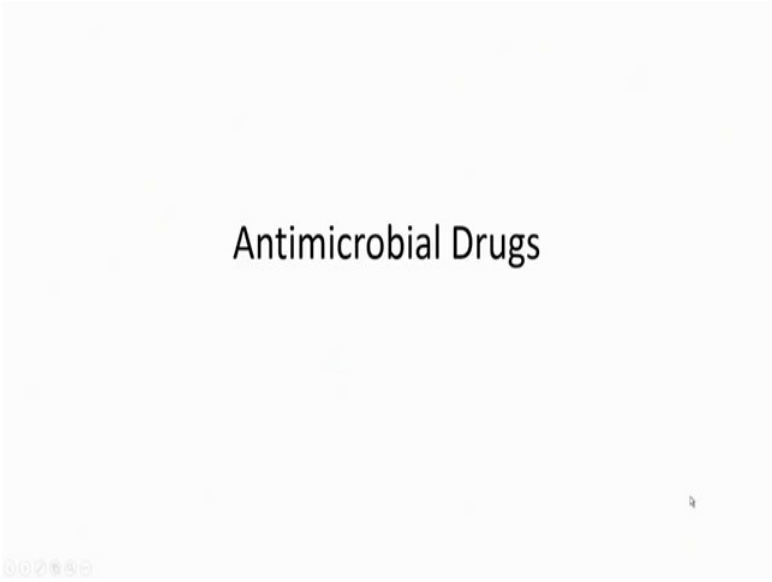

Today we will change the topic and go to a very important one which is affecting us all over the world, hypertension is also like that, but hypertension is usually affecting the more aged people than the younger ones. However, these antimicrobial drugs are very important, because throughout our life, at some point or other, starting from the childhood, we suffer from infections that are caused by small microorganisms, microorganisms are small, as the name suggests.

And these microorganisms include bacteria, virus, fungus all these. So, we will discuss the antimicrobial drugs and their chemistry and how actually they evolved to save the human lives.

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So, antimicrobial drugs are basically chemicals which work against infections caused by a microorganism. Now, every topic of research has a history behind it. So, drug discovery process especially the antimicrobial agents' related research has an illustrious history behind it. First it was Louis Pasteur, he believed in one idea that one microorganism survives against the attack of other microorganisms by producing some chemicals.

It is like the survival strategy of our country that we produce different weapons or procure different weapons and that is done to protect our own country against the invaders. Similarly, in the bacterial world, one bacteria wants to predominate on the other. So, the bacteria has adopted a mechanism by which they produce molecules surrounding them and these molecules basically can kill other microbes surrounding it, so that is the different survival mechanism of a microorganism like bacteria.

So that was Pasteur's theory. However, when Pasteur attempted this, after isolating some of these chemicals; he found that these are also toxics against humans. And if they are also toxic against humans that means, they are basically useless compounds. So, Pasteur's theory remained as a theory, it was never practically implemented.

Now, in the year about 1900, another a scientist whose name was Paul Ehrlich was a first one to believe that it is possible to develop chemotherapeutic agents in fact, the term chemotherapy was coined by Paul Ehrlich.

So, Paul Ehrlich thought that it is possible to develop chemicals which will if taken inside the body, then it will target the causative agent for the infection that means; if I am suffering from any bacterial infection, then Ehrlich believed that there is a possibility that you can design chemicals which if it is ingested, then that will not touch the host machinery, but it will go and attack the foreign organism and then it will kill that and he termed those chemicals as magic bullets.

So, magic bullets are nothing but chemicals, but it is why it is called magic? Because it it does not affect the host, only affects the microorganism which is the causative agents for the infection. So, these are called like bullets, like bullets you target a particular person and then you kill that person. Now, in the body, you are not seeing where these bacteria are, but it is possible to create magic bullets which will find the bacteria and kill it, so that was his theory and these magic bullets are nothing but the he called the chemotherapeutic agents.

So, he prepared different compounds, several compounds, 1000s of compounds were made on those days and he along the Japanese scientist S. Hata found that this compound number 606 was a very good antibacterial agent. Every compound had a number. And commercially it was named as Salvarsan.

Salvarsan is the first known antibacterial agent, it is an arsenic based compound. Ehrlich was awarded the Noble Prize in physiology and medicine because of his discovery of Salvarsan that was the compound number 606.

And then subsequently in our country, Sir U.N. Bramhachary in Calcutta, discovered a molecule which was called urea stibamine, which was an antimony (Sb) based. It was well known those days that arsenic, antimony and bismuth are having antimicrobial activity. So, people were starting to make these arsenic based compounds, antimony based compounds and bismuth based compounds. In fact, all these three are utilized to develop molecules which are drugs.

So, Salvarsan is an arsenic based compound and U.N. Bramhachary's compound was antimony based compound, this was an antibiotic not an antibacterial agent, because there is a difference in antibiotic and antibacterial; I will tell you later. It treated a deadly disease called syphilis, which was untreatable in those days.

The antimony based compound, this urea stibiamine, was utilized for treatment of black fever, which in Bengali it is called kala azar. So, these are the initial developments in the history of the antimicrobial drug discovery. Aspirin was discovered in the late 19th century, so this is early part of 19th century.

And however, since these are metal based compounds, these were later on found to be very toxic. So, apart from their beneficial action, they also have extreme toxicity. So, this compound containing arsenic, Salvarsan is now no longer used because of its toxicity. So, people started to look for different compounds. So, people although were killed of syphilis, but they might be also killed by the toxicity of the arsenic based compound, which is not desirable.

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In 1928 when Fleming discovered the penicillin and exactly what happened that Fleming had all these petri dishes, because antibacterial activities are determined by taking a covered plate like what is called a petri dish. And you grow bacteria inside the dish and you see the effect of different chemicals on the growth of the bacteria.

So, what Fleming observed? Fleming observed that one of his plates which was lying on his bench for quite some time, because Fleming went for a holiday and 2 weeks later he came back and without cleaning his petri dishes. So, he found one of his petri dish, he looked at it and he found there is a growth of a fungus and around the fungus there is no growth of any bacteria, this is a clear zone.

So, the bacteria were growing like this, because wherever there is a haziness that indicates that bacteria is growing, but around this fungal mold, no bacteria can grow. So, Fleming immediately realized, because he had a brilliant mind; he immediately realized the potential of this mold. And interestingly that Fleming was working on the first floor of the Queen's Mary hospital.

And in the ground floor there was another colleague of him who was working with different fungal strains and one of them was *penicillium notatum* that is the fungus, they flew from the lab at the ground floor, went out of the window and finally, came through the window of Flemings lab and it is just extreme sheer luck that the fungus went inside the petri dish.

Because when you put the bacteria you have to open the petri dish and then after spreading the bacteria, you close the lid; so that time fungus went inside the petri dish. So, there are many stories everything. This is a serendipitous observation that is true, but other people might have just washed this plate, but Fleming while checking all the plates, he found that the plate which he was going to wash clean an autoclave, but he found this miraculous observation and that gave the birth that is the birth of penicillin.

Then Fleming tried to grow this *penicillium notatum* fungus in flask, tried to isolate the compound that means, the compound which is secreted outside this fungus, he tried to isolate. He named the active compound without isolating it as penicillin, because it is being produced by penicillium fungus and, but he could not isolate penicillin.

But he found that the crude extract of the fermentation of this penicillium fungus, if it is grown and the filtrate is extracted, that filtrate had a lot of anti-bacterial activity; and but he could not isolate it, so it had along with that active compound, it had other compounds in it. He tried to work on mice that what is the toxicity level, you know all drugs need to be shown that they are not toxic.

So, toxicological affect was fine, but because the active compound was present in tiny amount and he wanted to isolate it, then he found that it is very unstable; he could never isolate the penicillin, so he gave up the research. Just publishing two results; one in British general of pathology and another publication later on in 1932, the first one is 1929 and then the 32, so these two publications were forgotten by the scientific world, but later on it was two chemists in Oxford University, Howard Florey and Chain.

In 1939, they again went back and took the fungus from Fleming's laboratory, met Fleming, got the fungus and then grew it and then they isolated the penicillin. And tried to determine its structure; some proposal was there that this is the structure, but finally the structure was confirmed by Dorothy Hodgkins, the famous women crystallographer in oxford. So, she finally proved the structure of penicillin, so that is the story and that is the beginning of the antibiotic era.

Now, let me see what the difference between antibacterial agent and antibiotic is. Antibiotics are compounds which are produced by microorganisms for their own benefit and for their own survival to kill other surrounding organisms. So, antibiotics are produced from microorganisms, whereas antibacterial agents are a broad class of compounds. Any compound or any chemical which is having a power of destroying any microorganism that is called antimicrobial; if it is bacteria, it is called an antibacterial agent.

However, the general term should be antimicrobial agents, because antimicrobial will include everything antiviral, antibacterial, antifungal, ok. So, this is the difference, so basically antibiotic are isolated or they are produced by microorganisms. So, we do not have to do much, we just isolate and grow the microorganism and start isolating the active compound.

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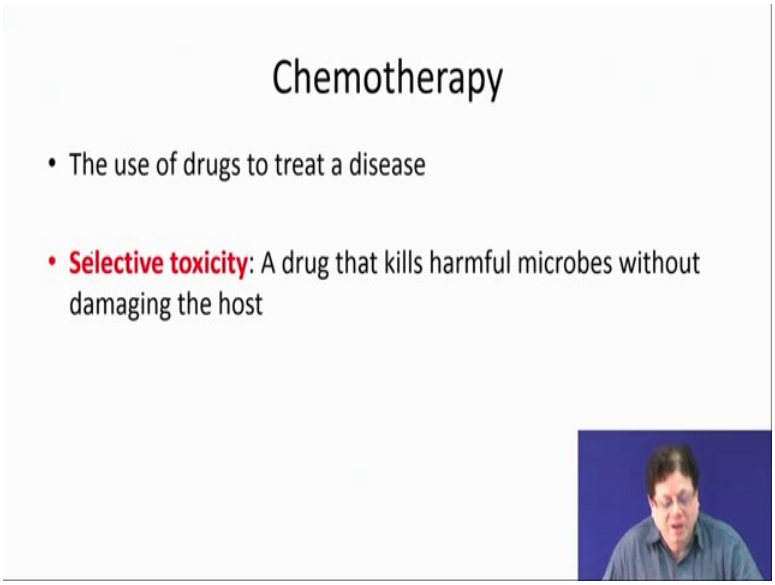

And there are many compounds which are in the market, they are all antibiotics. Now, let us again just quickly revisit chemotherapy; basically the use of drugs that treat a disease. And a drug that kills harmful microbes without damaging the host; see chemotherapy means that it has got a selective toxicity; all compounds are not branded as chemotherapeutic agents, if they have a high toxicity.

It's only that chemotherapeutic agents not only treat the disease, but at the same time, it should have less toxicity, less toxicity in the animal or human where it is being prescribed.

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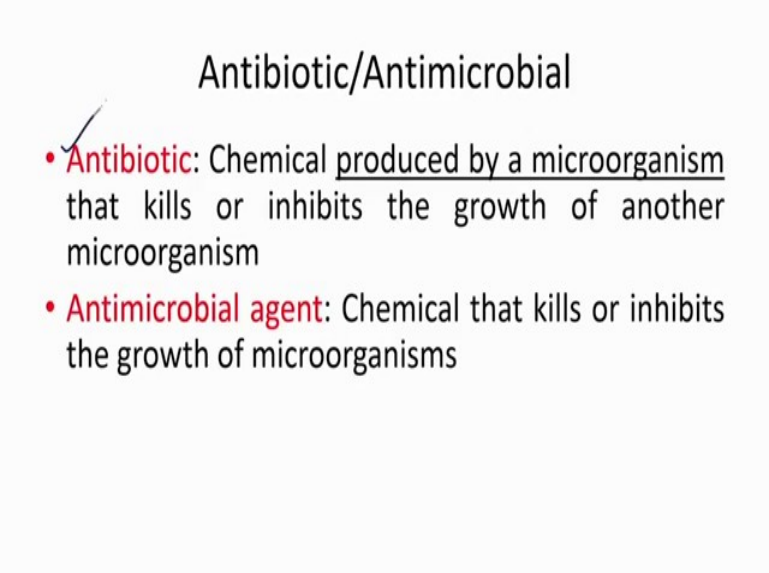

It is again the same thing, I just already told what is an antibiotic; chemicals produced by a microorganisms that kills or inhibits the growth of another microorganism. And antimicrobial agents is a basically a chemical that kills or inhibits the growth of microorganisms. So, I can say that all antibiotics belong to the class of antimicrobial agent, but all antimicrobial agents are not antibiotics, because some could be synthetic compounds.

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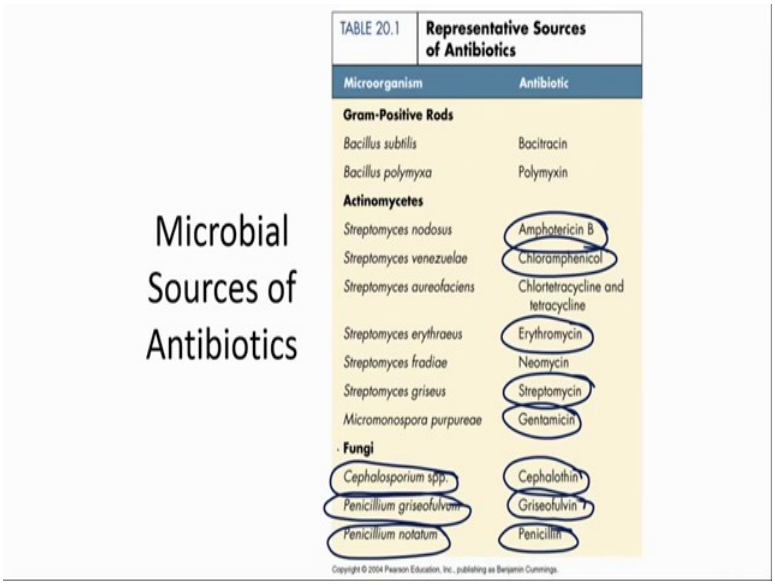

Now, these are some of the different antibiotics that have been isolated from different microorganism sources. I will say this penicillin, I have already told you that it has been isolated from *penicillium notatum*. Griseofulvin is an antifungal compound, which is isolated from another penicillium sources. Cephalosporin is a compound which is isolated from cephalosporium species; gentamicin these are very well known streptomycin, erythromycin, and chloramphenicol means Chloromycetin, and then amphotericin B. And interestingly many of these are associated with Noble Prize winning work. So, these are whole lot of compounds that have been isolated from microorganisms and there is no end to it, because if you today want to enter into this field antibiotic, what you have to do? You have to see a new microorganism, you have to isolate and then see what type of chemicals it is generating.

However, it is hard to beat the multinational pharmaceutical companies, because they are having a collection a huge library of microorganisms and then try to see what type of compounds they produce. So, usually this is in the domain of pharmaceutical industry.

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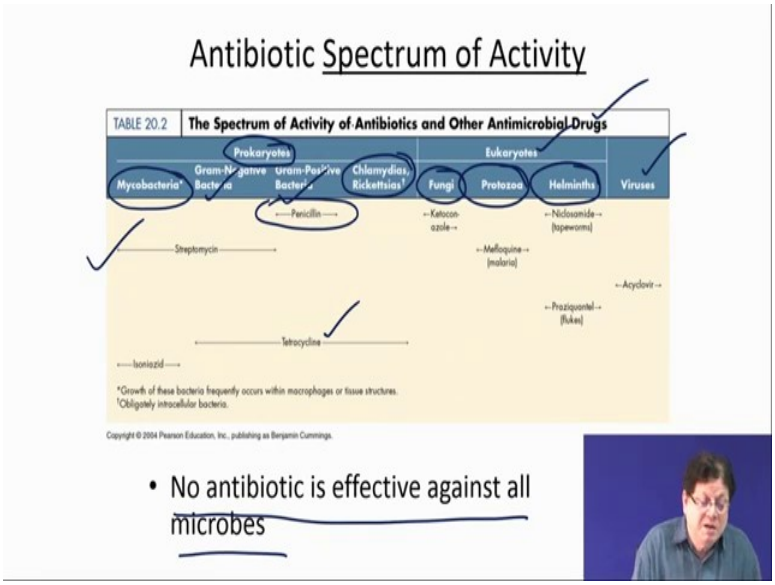

And not academic, academically it is very difficult, because you can only touch a few of the microorganism, but this is not the way, this is the same thing like combinatorial library of different compounds gives you a possibility of identifying the hit quickly. Similarly, a common library of different strains has a better chance of getting a new

antibiotic compound. These are some of the spectrum of activity of the antibiotics and other antimicrobial drugs.

There are different agents that cause diseases, like mycobacteria which is the causative agent of tuberculosis and leprosy. Then you have prokaryotic bacteria; beating the prokaryotes you have gram negative and gram positive that is the gram sensitivity to gram reaction. Then you have there are other types of chlamydia, that could be the fungi means fungal infections, which are actually eukaryotes that is the higher organisms; protozoa in protozoa is the malaria one.

Then helminth, helminths mean actually related to people suffering from this worm (tape worm) infection. So, these are little bit longer in one direction and then you have viruses. So, this is the spectrum of different micro different organisms that we suffer from. No antibiotic is effective against all the microbes. It is not that you have one antibiotic that is going to kill all these, from fungi to virus to mycobacteria, which is not possible.

Mycobacteria that is tuberculosis drug, one of the earliest drug in streptomycin. For gram positive infection, penicillin was the drug of choice, then you have mefloquine that is for malaria. You have a tetracycline which has got a broad spectrum. You know, narrow spectrum antibiotic, broad spectrum antibiotic. Broad spectrum means which actually covers many of these microbes, like tetracycline is a broad spectrum antibiotic, and the penicillin.

Some of the penicillin are quite narrow spectrum, they just kill gram positive bacteria, but we will cover that; there are now different types of penicillins which can also kill gram negative bacteria, but gram positives are the real targets of penicillins. Then isoniazid is a synthetic compound that works against tuberculosis, so that is the spectrum. So, when we talk about antimicrobial chemistry, you have to touch these prokaryotes infections and the eukaryotes infection and the viruses.

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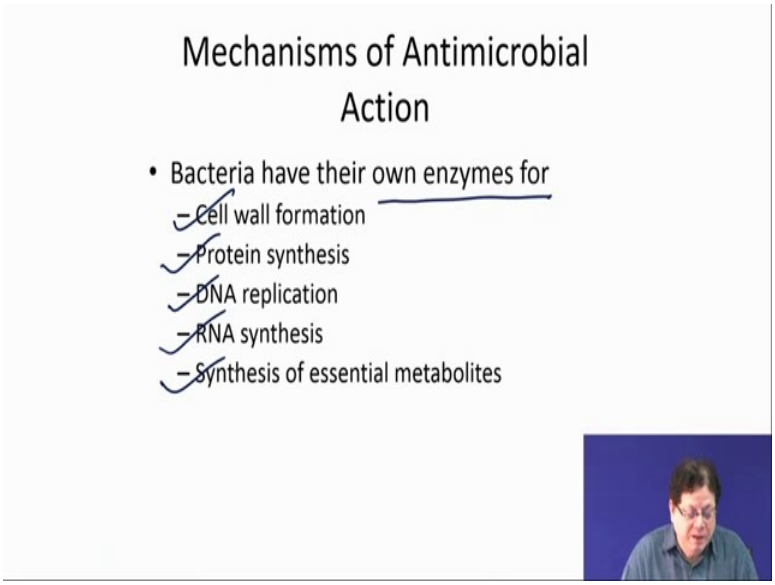

But we cannot actually cover all these in this course; we will just take some examples which became the land mark in discovery of antibiotics. Now, mechanisms of antimicrobial action; remember microbes have a life, so they have a life cycle and they also utilize some of the processes that are also in-built and also going on in our body. However, there is a difference, there are some differences between the enzyme systems in the microbes *vis-a-vis* the humans.

Like bacteria have their own enzymes, which is not present in the host; host means I mean the animal, the human, etcetera. Like cell wall formation, we will talk about what is this cell wall; that means in bacterial cell there is a wall surrounding the cytosol that means, whatever the components inside that is protected by the wall. Then protein synthesis, we also have protein synthesis, we have already read this replication, transcription and translation.

However, their enzymes might be slightly different from the enzymes that we have, and if that be the case then you can exploit that difference. Remember when we talked about the folic acid chemistry, we encountered an enzyme which is called dihydrofolate reductase, this is present in bacteria, this is also present in human, but the dihydrofolate reductase has different from in a microorganism versus the human. So, you can selectively target the DHFR of bacteria.

DNA replication, see these are all processes which are common to the host; RNA synthesis, transcription and the DNA replication, this is translation and synthesis of essential metabolites. However, these can be the targets for devising an antimicrobial agent. And the reason is that I said that although some of the enzyme systems are common or processes are common, but there is a difference in the enzyme structure, primary structure that means the amino acid and finally, the tertiary structure is also different. So, you can exploit that difference

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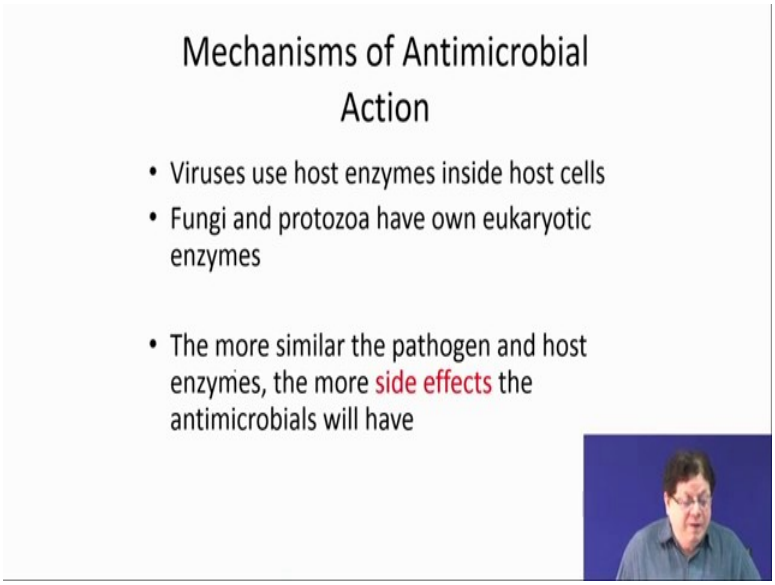

I think we will talk about these that how you can develop antiviral compounds, antifungal and but this is very important. Whatever may be the target, the more similar the pathogen, you are going to have more side effects and if the similarity is less, then you will have less side effects and if there is no similarity, then you can expect that there is very few side effects. Pathogen means the microorganism which is causing the infection to the host body. In fact, penicillin is one of them that it has got extremely less side effect, because if targets an enzyme which is not present in the human system, it is not required in the human system.

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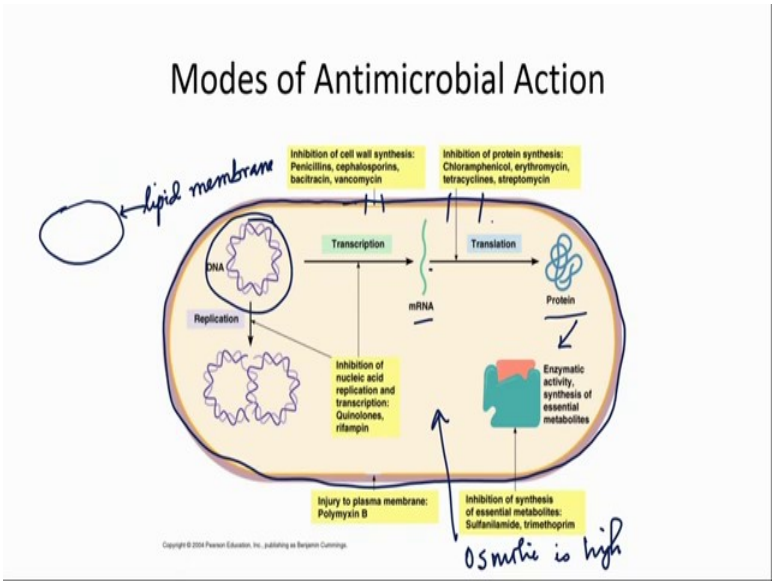

Now, this is a schematic diagram, I told you what are the targets earlier, but this diagram makes it little bit easier to understand that; this is the bacterial cell. And in the bacterial cell there is this DNA and you have this transcription process mRNA, mRNA goes to protein. The protein makes different small molecules, which are essential for the survival. Now, where you can target, which can be the target for your drug discovery process which are antimicrobial agents.

So, one is cell wall, because the cell wall is not present in the human cell. The human cell is protected by what is called a membrane, a lipid membrane inhuman cell. In the bacterial cell, apart from the lipid membrane, outside there is coat which is a very rigid type of system and that coat is extremely important, because inside the bacterial cell, the osmotic pressure is very high.

If the osmotic pressure is very high so that means, they are trying to go out. And so outside osmotic pressure is low that means, when I put the cell in a biological medium, outside the osmotic pressure is low, inside it is high. So, the water from outside wants to enter into the cell. If water goes here, then is has to swell, because it has to accommodate the water and there is no space; already it has got a lot of cytosolic material inside which creates this high pressure. So, as water goes inside, it swells and then it bursts.

But the question is how water will go inside, so you create cavities that means, you make molecules which will not allow the bacteria to form this cell wall. So, one good target is

cell wall and this cell wall humans does not have. So, cell wall synthesizing enzymes can be very good targets for antibacterial agents. Then you have inhibition of protein synthesis, so there are molecules like chloramphenicol, erythromycin, tetracycline, streptomycin, they stop the translation process.

This cell wall is inhibited by penicillin, cephalosporins, bacitracin and vancomycin, very important life-saving antibacterial agents. Then you can stop the bacterial transcription process using the quinolones. Like you must have heard of ciprofloxacin, norfloxacin or rifampin; that is again a tuberculosis drug, they stop the transcription process.

So, you can stop the production of mRNA by these quinolones, you can stop the translation process by chloramphenicol, erythromycin etcetera. You can stop these enzyme activities and hence stop making of important small molecules, like your folic acid; remember sulfonamide is a PABA analogue.

And then trimethoprim is dihydrofolate reductase inhibitory in bacteria, so all these are the targets. Bacterial cell wall, translation machinery, transcription and then you can also target functional aspect where the protein which synthesizes different small molecules, is inhibited. Dihydrofolate reductase was one example.

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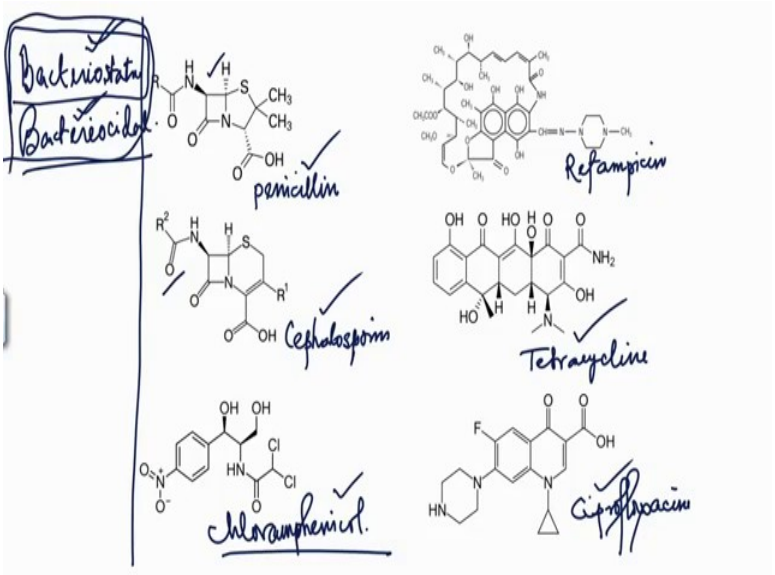

These are some of the structures which are isolated from the microorganisms. This is the structure general structure of penicillin, see these structures; only similarity is between

the cephalosporins and the penicillin, but other structures are entirely different, this is called rifampicin. And this is what is called tetracycline, you do not have to remember the structures; my only intension is to show the variety of structures that these microorganisms make including stereochemistry, everything is very stereospecific. This is your ciprofloxacin and this is the chloramphenicol.

Apart from that, there are other molecules which are having different type of structures. We are going to cover penicillin, we are going to cover cephalosporin, they come together and we will also discuss some of the mechanisms of possibly chloramphenicol if time permits.

Another term which has to be made clear is bacteriostatic and bactericidal. What is bacteriostatic? Bacteriostatic is basically that if somebody suffering from some bacterial infection and you add some chemicals some drugs, which stops the further growth of the bacteria.

Like if a bacteria is already there, say 100 bacterial cells are inside my body, now what they want to do? They want to divide; 100 will be into 200, then 200 will be 400, but for the division to occur you have to utilize the machinery of replication, transcription, and translation. Now, there are molecules like chloramphenicol which actually stops the protein synthesis by inhibiting the translation machinery.

So, what will happen, the existing cells will remain, because they are already matured, but when they try to divide that is not possible, that means, I will have this 100 bacterial cells, I will not kill them, but I will not allow them to grow; so that is what is called bacteriostatic that means, the bacterial population remains the same.

But then the question is how do I cure myself? Because if the bacteria does not grow, that gives enough time for the body to make antibodies and then antibodies ultimately take care of this whatever microorganisms are there; so that is bacteriostatic. On the other hand, compounds which are called bactericidal in nature; bactericidal means they go and kill the existing bacteria that means, the 100 bacterias will go to almost 0; so you can see the difference.

So you are killing the bacteria with bactericidal agent; and penicillin is one example which kills the bacteria and bacteriostatic is which does not kill the bacteria, but stops

the growth; both are antibacterial agents. But definitely if somebody has very weak immunity, like somebody may be suffering from HIV infection, HIV is a viral infection which ultimately attacks our immune cells and breaks down the immunity.

So, if anyone is suffering from HIV, bacteriostatic will never work, because he does not have the immunity; so how can he take care of the remaining population of bacteria. Even in case of transplantation, say kidney transplantation, your external organ, that foreign kidney is given from the donor to the acceptor.

However, since it is not the part of the body system from the very beginning, so the body thinks that it is a foreign agent and whenever body thinks something is foreign it generates antibody. And then that means, there is a immune response and that rejects the kidney; so there are many cases where the kidney transplantation is not successful, because there the body does not accept the foreign kidney as its own.

So, what it usually practiced that at that point of kidney donation, you lower the immunity of the body, practically to a very low level. And then you do the transplantation. So, at that point, you slowly increase the immunity, see immunity was very low slowly you increase the immunity and ultimately say after 2 to after 3 months, 4 months the body thinks that it is my own material. So, at that time if there is an infection, you cannot treat that person with bacteriostatic drug, you have to give bactericidal ok, so that is an important difference I told you.

I think in the next session, we are going to start with the chemistry of penicillin. So, it is a very background introductory remark of antimicrobial compounds and then we will slowly go to the actual drugs, their mechanisms, their discovery and all these things.

Thank you.