Introduction to Complex Biological Systems
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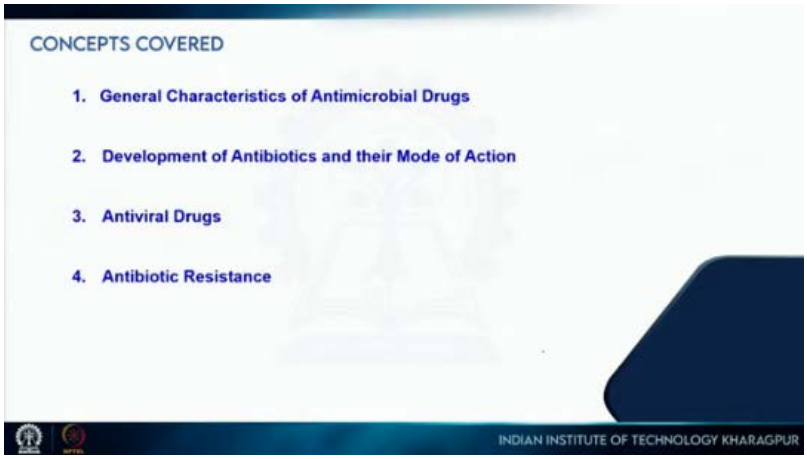
Lecture 45
Antibacterial and Antiviral Drugs

Hello everyone, this is the last lecture of Module 9, and in this lecture, I will be discussing antibacterial and antiviral drugs. So, particularly, I will focus on some major properties of antimicrobial drugs, followed by the development of antibiotics and their mode of action. I will also discuss antiviral drugs and, at the end, how bacteria develop resistance against different types of antibiotics. So, let us first discuss the general characteristics of antimicrobial drugs. So, there should be some important properties of an antimicrobial drug.



First of all, there should be selective toxicity because when we are trying to target microbes, for example, in this lecture, mostly we will focus on bacteria and viruses. So, we are targeting them when they are present inside our body, which means when we are getting infected. So we have to develop some kind of antimicrobial agent. So, an antimicrobial agent. So that they will not create any problem for the host cell.

So, this should be safe for the host cell, or I would say host physiology, but it should specifically target microbes. That should be a very important property of an antimicrobial drug. Only then can we utilize it. For example, when bacteria infect us, we can try to target some particular activity or metabolism inside bacteria. For example, we know that

ribosomes differ, eukaryotic ribosomes and prokaryotic ribosomes differ. So if we can develop some kind of antimicrobial agent that can specifically target the 70S ribosome. This is present in bacteria.

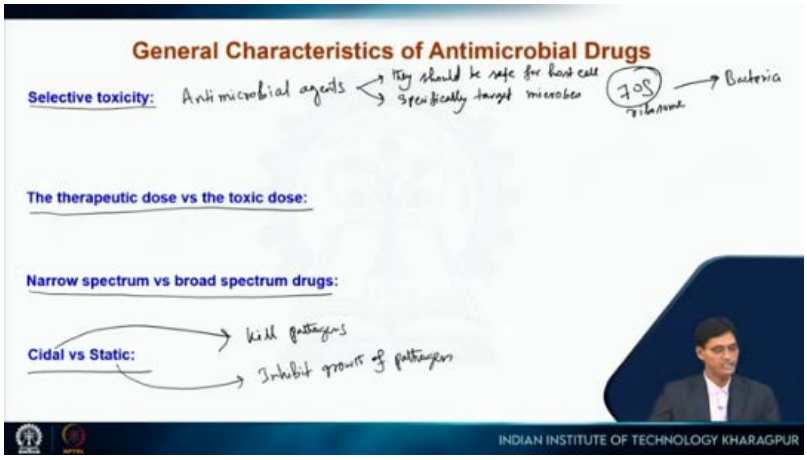
So, the 70S ribosome is made up of the 30S and the 50S subunit, small and large subunit, but in the case of eukaryotic ribosome, we have 80S ribosome in our cell, and it is a little bit different. So, as a result of that if we can target specifically this either 30S or 50S subunit that would be very beneficial and we can use that kind of agent for you know controlling bacteria. Then the next one is the therapeutic dose versus toxic dose, so we have to be very careful about the dose also because it should be functional; it should work at very low concentration because, as i just mentioned, there are some differences between eukaryotic and prokaryotic ribosomes, that means our ribosome and bacterial ribosome. But if you remember we discussed, the fundamental process of protein synthesis is the same. So there are a lot of similarities between our ribosome and the bacterial ribosome.

So as a result of that, if you use a very high concentration of this drug, it might target our ribosome also. I'm just giving one example of taking the ribosome into consideration, but it is true for many other cases. So we should try some microbial agent that should work against microbes at very low concentration that is the therapeutic dose. The toxic dose that means it is very high concentration can create problems with the host as well. Then another very important point is narrow spectrum versus broad spectrum drugs because what happens for example if someone is suffering from some bacterial infection. All the time, it is not possible for us or for the medical person to directly recognize a specific bacterium. What is the specific bacteria which is causing the problem? From symptoms, you can guess, but without proper testing, it is not always possible to understand the exact genus or species of that particular bacteria. So as a result of that, there are different types of microbial agent, narrow spectrum and broad spectrum.

Broad spectrum will work against different types of bacteria, for example, gram-negative bacteria, gram-positive bacteria, I would say some antibiotics, if I say ampicillin, this is a type of penicillin. So ampicillin is a more broad spectrum compared to penicillin. Penicillin is the natural one first isolated and first discovered antibiotic but the ampicillin is a little bit derivative of that penicillin but it is a broad spectrum comparison to penicillin. The next

one is sidal and static that means this is sometimes bactericidal and bacteriostatic. So sidal means it will kill pathogens.

So, for example, killing bacteria and static means it will inhibit the growth of pathogens. Now the thing is sometimes I should mention that some of this cidal, antimicrobial drug if we use at very low concentration it can also act as static or bacteriostatic for example, against bacteria if we use. So, some of the antibiotics we use are bactericidal and few of them are also bacteriostatic. Now these are some general features about antimicrobial drugs.



Now, I will be discussing the development of antibiotics or first the discovery of antibiotics. So, here many of you know that Alexander Fleming in 1928 discovered penicillin.

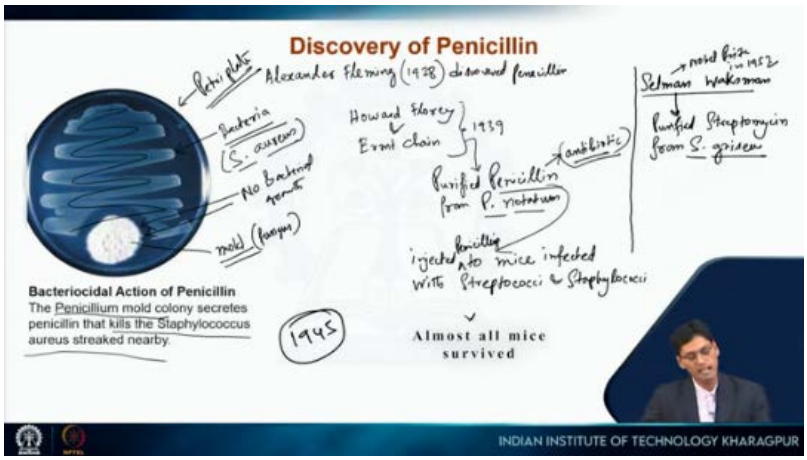
This is very important in terms of a revolution in the field of medicine, because this is a powerful drug like any antibiotic. But this is the first antibiotic discovered by Alexander Fleming. What he observed he was working with some, bacteria, and he found as you can see this is called a petri dish here if you streak some bacterial culture, that means you are putting some bacteria with some kind of sterile material and as you can see something like this way and then over time, this bacterium will grow and after a few hours, maybe after one day, you will be able to see some of those bacteria, as you can see this whitish substance, this is nothing but bacteria.

But what Alexander Fleming noticed was that in one of his plates, some mold or some kind of fungus actually grew inside the plate. And more interestingly, he noticed that if you see just surrounding the mold here in this position, no bacteria. No bacterial growth was observed beside this mold. So that some bactericidal agent or something is coming out from this mold, which is preventing the bacterial growth. This is kind of a great discovery.

In this way penicillin was discovered. Later on Alexander Fleming and other scientists established together that this is penicillin, secreted from Penicillium mold, this is Penicillium notatum and it prevents the growth or it kills the Staphylococcus aureus strictly nearby. So, this bacterium they are working with is S. aureus. So, this is some gram-positive bacteria, S. aureus. So, this is just the starting of penicillin discovery, but the thing is, if you see, I just mentioned that penicillin was secreted by Penicillium notatum, but Alexander Fleming did not purify this antibiotic; rather, it took a little bit more time, and later on, a few years later, Howard Florey and Ernst Chain in 1939 they purified penicillin from penicillium notatum, it is a fungus and penicillin is some microbial agent which is preventing the growth of bacteria here particularly staphylococcus aureus. So this is the antibiotic preventing bacterial growth and not only that what they have done is they injected this penicillin to mice infected with streptococci this is again another group of bacteria streptococci and staphylococci. So, they did it separately, but they injected this antibiotic penicillin to mice infected with staphylococcus as well as streptococcus bacteria.

What they found they found that So, this is very important so that means, purified antibiotic when it is injecting it is protecting host that means, in this case mouse from the bacterial infection. Later on human trials also happened and finally, I should mention here that in 1945 all of them, Alexander Fleming, Howard Florey and Ernst Chain, received Nobel Prize for their discovery of penicillin. As I already mentioned, it is a real milestone in terms of treatment, in terms of from a medical point of view and it inspired other scientists also to isolate different types of antimicrobial agents from microbes, particularly from fungus. Later on, what happened is another scientist, Selman Waksman, he also purified another important antibiotic called streptomycin from one fungus called Streptomyces griseus. So, this is a fungus. Selman Waksman also received the Nobel Prize because of this work in 1952.

So, as you can understand, this is a really important discovery for human society and streptomycin also acts against different groups of bacteria. Here is just the starting point and it continues and finally, there are many other antibiotics like chloramphenicol, neomycin, all are isolated from different types of microorganisms. Now I'll be focusing on how these antibiotics work, their mode of action.



So particularly cell wall synthesis inhibitors. So when I was talking about the specificity, as you know, in our body, in mammalian cells, in human cells, we don't have a cell wall. We have just plasma membrane, cell membrane. So as a result of that, if some drugs can specifically target the cell wall. So it is very good for us that we don't have a cell wall. So the first antibiotic discovered by Alexander Fleming is penicillin, particularly if you say penicillin G, this is the natural penicillin. So this antibiotic acts against peptidoglycan, so as you know by now that peptidoglycan is a major component of bacterial cell wall. Even in the plant cell wall, they don't have peptidoglycan.

So now, if we see that Penicillin G has high activity against most gram-positive bacteria as you know, in gram-positive bacteria we have a more peptidoglycan layer also, and it has little bit low activity against gram-negative bacteria. So, this is the penicillin structure as you can see, and this part is very important. This is the beta lactam ring. This is very important. I will discuss in more detail.

This is called the beta-lactam ring of penicillin. What happened? All are good. This is working against this peptidoglycan synthesis. But some bacteria have some machinery, some enzymes that can break this beta-lactam ring.

As a result, those bacteria are resistant against penicillin. This enzyme is called penicillinase. That means it breaks penicillin. That is why penicillin is or it is also known as beta-lactamase.

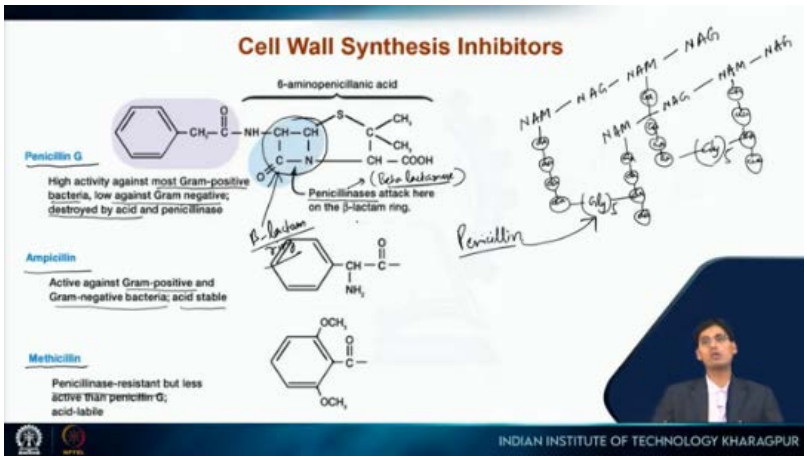
So this is some enzyme that can break this antibiotic. But now I should discuss a little bit more in detail. If you can remember a little bit about the peptidoglycan structure, we have this N-acetyl muramic acid and N-acetyl glucosamine. It is a repeating subunit of sugar and then another chain of the same repeating polymer, something like that. Now, if you see from the N-acetyl muramic acid, a short polypeptide chain is coming, although this amino acid is a little bit different from our natural amino acid; some of them are D-form, D-amino acid.

Similarly, from here also amino acids are attached with this N-acetyl muramic acid, and you can see some peptide linkages like cross-linking here. So, as you can see, I mentioned before that glycine 5 or some direct cross-linking is present in between this short polypeptide chain and that finally forms this mesh-like structure. So, similarly from here also amino acid is coming this way from this NAM and from here also coming like this, and again this kind of cross-linking is happening, but this penicillin prevents the synthesis of this step, it stops, so as a result of that, peptidoglycan cannot be synthesized, and that's why this is a very powerful drug.

Now, if I mention a little bit about different derivatives of penicillin, Penicillin G is the natural one. For example, ampicillin, ampicillin is active against both gram-positive and gram-negative bacteria and it is also acid stable. We have to be careful about this also because most of the antibiotics we take are oral. So, as a result, if antibiotics are acid stable, that is advantageous for us. In the stomach, we have very low pH and a very acidic condition. So, you can see that Penicillin G is destroyed by acid, so it is very difficult to take Penicillin G orally, but Ampicillin we can take orally and, on top of that, another modification happened in this penicillin group of antibiotics; this is called methicillin.

So, methicillin is very interestingly this is penicillin is resistant that means, the beta lactam is resistant, but less active than penicillin G and it is also acid labile that means, it is not resistant to acid, but one important feature here is it is beta-lactam is resistant as I

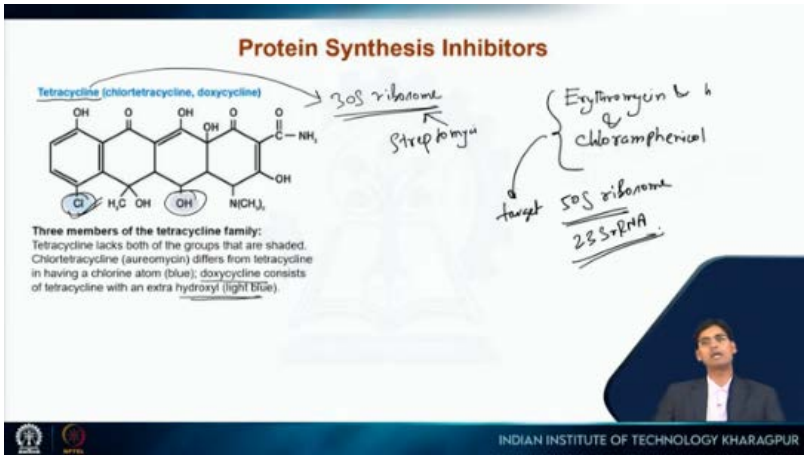
mentioned that this enzyme can break down beta-lactam ring. But unfortunately, when methicillin was used for a long time again, some bacteria developed resistance against methicillin also. So you can understand bacteria, they are also clever; they have many different types of strategies to combat antibiotic treatment. I will be discussing those at the end of this lecture. Now I should concentrate on protein synthesis inhibitors.



I just mentioned cell wall synthesis inhibitors, particularly peptidoglycan synthesis inhibitors, but if you see there are many antibiotics which target bacterial ribosomes and that is also very useful. So, as you can see Tetracycline, for example, is different from chlorotetracycline; doxycycline is, I would say, a derivative of tetracycline. so tetracycline works against bacterial ribosome, particularly, I would say, it works against 30S. ribosome. So during protein synthesis, as you know, the 30S and 50S bind together along with the mRNA, and therefore translation starts.

So, if it binds to the 30S ribosome it prevents protein synthesis. So, as a result, this antibiotic specifically will inhibit bacterial growth because they cannot synthesize their own proteins. So, this is tetracycline, and here, as you can see, there is a little bit of modification, for example, this OH group and this chlorine group, which are not present in tetracycline, tetracycline lack both of this group. But in doxycycline this is you know, now we are also using this antibiotic, doxycycline, which consists of tetracycline with an extra hydroxyl group here. On the other hand, chlorotetracycline has an extra chlorine group here, but their mode of action is the same. So, in this group, another antibiotic I can name is streptomycin.

This is another antibiotic that acts against the 30S ribosome. Their target is 30S ribosome again and now not only 30S ribosome there are other antibiotics for example I would say erythromycin and tetracycline already I mentioned that is not the right one. So, erythromycin and chloramphenicol. So both of these antibiotics target 50S ribosomes of bacteria. So, specifically they create problems with 23S rRNA present in 50S ribosomes. So, that is why they are powerful antibiotics, and there are many antibiotics available in medicine shops; they are actually targeting bacterial ribosomes. Now those two are, I would say, the major class of antibiotics. One of those groups inhibits peptidoglycan synthesis, and the other group inhibits protein synthesis in bacteria. Some other antibiotics are also available, for example, inhibitors of nucleic acid synthesis, as you can see that norfloxacin and ciprofloxacin. So here, this is the norfloxacin; they inhibit some enzymes, particularly topoisomerase enzyme, and topoisomerase is important for bacterial replication. So, as a result, if they inhibit topoisomerase, then again bacteria cannot replicate themselves. So, this is one way this antibiotic works. Some other groups of antibiotics are called antimetabolites. for example, trimethoprim, dapzone and isoniazid.



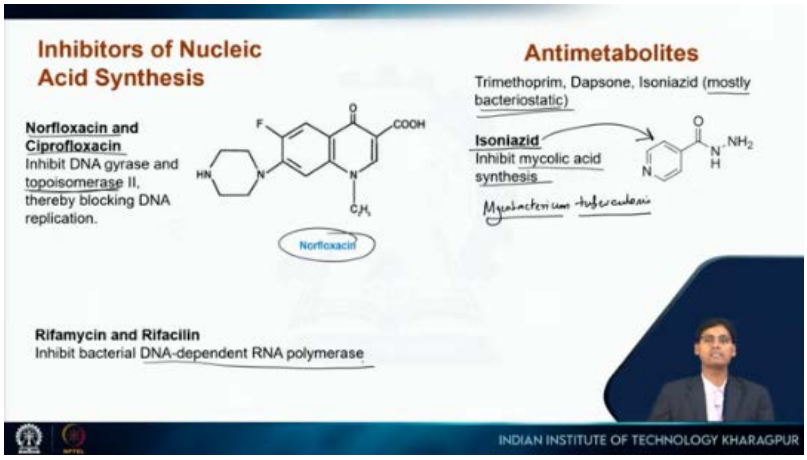
They are common examples of antimetabolites. and I should also mention that most of them are bacteriostatic. They just prevent the growth of bacteria. And particularly I would focus on isoniazid. So, this is an isoniazid structure.

It inhibits mycolic acid synthesis. So, what is mycolic acid? So, as you know, Mycobacterium tuberculosis. It causes TB or tuberculosis disease in humans. But this

bacterium is a little bit different from classical Gram-positive bacteria as well as from classical Gram-negative bacteria.

So, they have some special features. One of the special features is that they have mycolic acid on their surface. But this antimetabolite agent actually inhibits mycolic acid synthesis and that's why this drug, isoniazid, is used against tuberculosis. This is a very important drug against tuberculosis.

Now, rifampicin and rifampin also inhibit, as you can see, DNA-dependent RNA polymerase. So, this is bacterial DNA-dependent RNA polymerase because we also have human RNA polymerase, but this drug should not impair the function of human RNA polymerase. This is bacterial DNA-dependent RNA polymerase. Now, before discussing these antiviral drugs, I should mention that, as you can see, antibiotics target different steps of bacterial growth, for example, protein synthesis or cell wall synthesis.



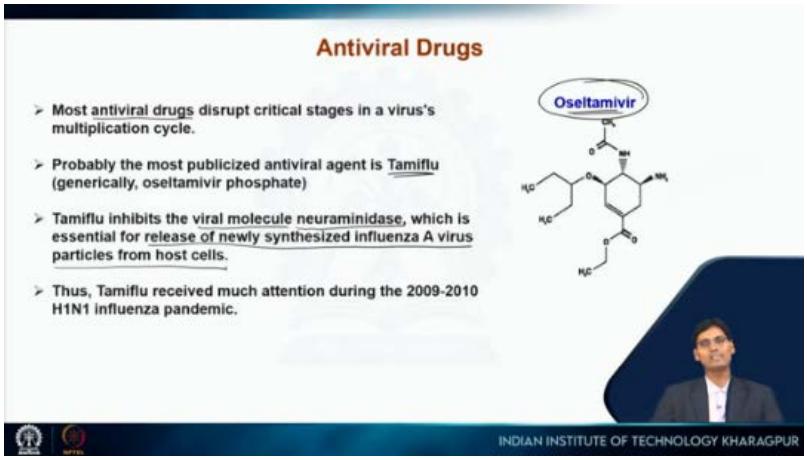
But I mentioned in some other lectures that we have a huge amount of beneficial bacteria in our body. In our body, a huge amount of bacteria is present, which actually help us through different methods, so unnecessarily we shouldn't take antibiotics unless it is absolutely required. If a doctor prescribes antibiotics, then only we should take them because these antibiotics act against peptidoglycan synthesis or against protein synthesis. They are also killing those good bacteria; those beneficial bacteria present in our body. So that is one problem also. So that's why we have to take antibiotics very judiciously, not randomly sometimes without any reason; we shouldn't take antibiotics.

It will be more problematic than a better result and now, antiviral drugs are a little different from antibiotics because bacteria have their own machinery, their own system, and we are targeting them, for example, peptidoglycan or their ribosome. But as you know, viruses have very little; they have a little bit of their own genome, either DNA or RNA, and some specific proteins present on the surface. But mostly, they are utilizing the host machinery and that is the problem in targeting viruses compared to bacteria.

So this is kind of a little bit challenging for us to target a virus and to combat the virus. So let's see how we can do that. So most antiviral drugs disrupt critical stages in a virus's multiplication cycle, and probably the most publicized antiviral agent is Tamiflu; this is the chemical component, the main thing present in Tamiflu. So, now what does Tamiflu do? So, this is antiviral; many of us know it.

The Tamiflu inhibits the viral molecule called neuraminidase, which is essential for the release of newly synthesized influenza virus particles from the host cell. I discussed during the virus lecture that viruses hijack our system, enter inside our host cell, make their own parts, and finally generate virus particles. They then leave the host cell and infect nearby or surrounding cells. But nueraminidase they are preventing the release. See, release of newly synthesized influenza virus particle from the host cell. So if you stop the release of this virus, newly generated virus from the host cell cannot infect the surrounding cells.

So that way we can also prevent viral growth and that is why Tamiflu received much attention during the 2009-10. During that time, we had the H1N1 influenza pandemic. So that is why this is a very famous Tamiflu. There are many other antiviral agents also available in the market now.



But now I will mostly focus on the mode of action of different anti HIV agents. There is a lot of progress happening with HIV disease and how to prevent that. Now there are many good remedies available against human immunodeficiency virus. So as you can see, this is the HIV virus. So now there are many important steps we have to take.

I am just taking HIV as an example, but this is true for many other viruses as well. But we also have to develop virus-specific drugs. So now, the infection begins with HIV fusion. As a result of that, we can use some fusion inhibitors so that the virus will not fuse with the host cell itself. So that is one important drug that can work against HIV.

The next step is, once inside the host cell, HIV encodes, and its reverse transcriptase forces the host to make DNA from the viral RNA. As you know, HIV has RNA as its genetic material, and the virus itself encodes the reverse transcriptase enzyme. So as a result of that, first it will make RNA into DNA by reverse transcriptase. So this step is called reverse transcription.

Now there has been a lot of progress with RT inhibitors. That means the reverse transcriptase inhibitors that block this step. So this is a very useful drug. One of the most important antiviral, anti-HIV agents is azidothymidine or AZT. So as you can see, it is very similar. This is, I would say, a nucleoside analog. As you can see, some sugar is there and the thymine group is also there.

The only thing that is very carefully noticed here is the 3' prime here; it should be OH in order to extend the chain during the polymerization reaction; instead of that, it has an AZT

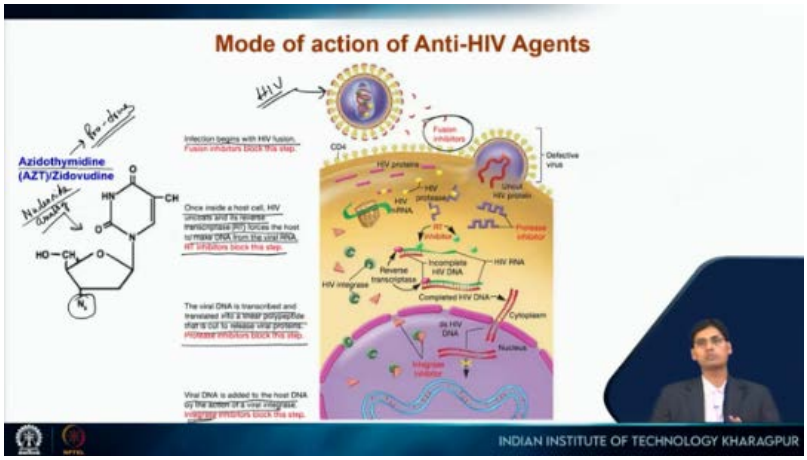
group here instead of OH. Therefore, it will stop the synthesis of the chain. So there is some similarity when you are learning about the dideoxy chain termination discovered by Sanger for DNA sequencing. But here, because of the agile group, it will not be extended by enzyme polymerase.

Another important thing I should mention is pro-drug means prodrug, this is a nucleoside analog. But for polymerization or for DNA, RNA, the monomeric unit is the nucleotide. But the nucleoside analog we are using here is pro-drug because the permeability is better. So that this drug can go inside the cell in a much efficient way.

So pro-drug, permeability is better. But after entering inside the cell, the phosphate group will be added. Then it will be behaving like nucleotides and therefore it will work against these viruses. But now the question is: if you carefully listen, the question will come to your mind that whether it will impair the polymerization by our human DNA polymerase because then that will inhibit the synthesis of human DNA also. I would say yes; theoretically, it is possible. But, the thing is human DNA polymerase is more powerful. It has proofreading activity whenever it will check that some wrong nucleotides are there.

So, it can remove it and properly adjust accordingly, but compared to that, I would say reverse transcriptase is not that powerful compared to human DNA polymerase to take care of this thing. So as a result, it will stop the function of reverse transcriptase specifically and then some protein inhibitors can also be used because the viral DNA is transcribed and translated into linear polypeptide, which is then cut to release different viral proteins so that virus particles can be assembled. But now if we can use some protease inhibitor specifically, so that this viral protein will not be processed, and it can also be used as a drug. All those things are actually being used nowadays.

The last one here is the viral DNA, particularly for HIV, is added to the host DNA by some enzyme called integrase. So, if we can develop an integrase inhibitor, it can also be powerful against HIV. So, these are the different approaches to target human immunodeficiency virus. This is the last slide here. I would like to discuss antibiotic resistance, which is a real problem now.



So, as you can see, this is bacteria. Now, if you see, we are targeting some critical states inside bacteria. For example, if you are targeting a critical enzyme, this is the critical enzyme here, as you can see in bacteria for example, topoisomerase, as I mentioned now, when you are applying an antibiotic, it goes inside the bacteria, binds to the critical enzyme, and inhibits the function of the critical enzyme; therefore, the bacteria cannot survive or grow. This is the mode of action, but bacteria are also very clever. What can they do? Some resistant bacteria can increase the expression or production of this target molecule.

So, if they can increase the amount of target molecule, particularly here, the critical enzyme, a lot. Then, how much antibiotic will we be giving? That particular dose, a very high dose, can be a problem for the host cell also. So, this is one strategy that bacteria use, and they become antibiotic-resistant. There are many other methods by which they can develop antibiotic resistance.

They can alter the critical enzyme itself. For example, because of some point mutation, now your antibiotic is not acting against the critical enzyme, your target. So, for example, mutation in the particular gene that produces target protein or enzyme and then a protein that degrades the drug; this is also very important. Some bacteria are resistant because they have some proteins which degrade antibiotics; I already mentioned beta-lactamase, so beta-lactamase breaks. Beta-lactam ring is present in the penicillin group of antibiotics, so those bacteria are now resistant against penicillin. Now I would particularly mention some strategies; not just one thing that you have to use. Now humans are also dealing with this problem in a different way, for example.

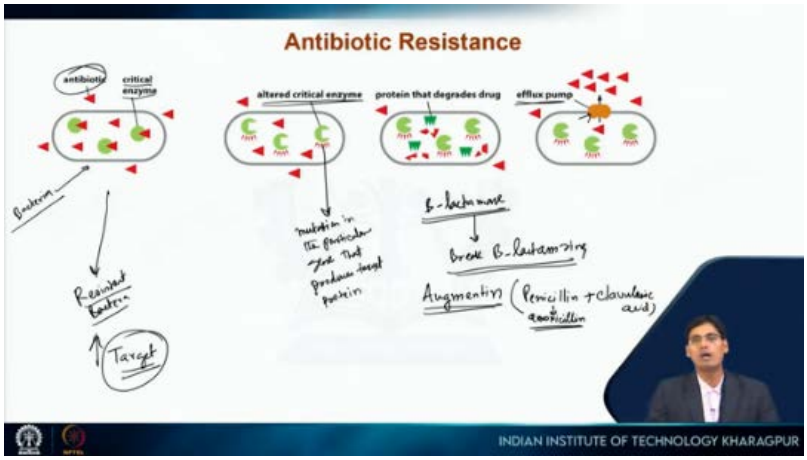
Now one antibiotic, you might know this name, Augmentin. Generally, if someone is suffering a lot, then Augmentin can be used. Doctor might prescribe this Augmentin. So Augmentin is just a mixture of penicillin plus clavulanic acid. So, this is particularly amoxicillin.

So, amoxicillin is also a derivative of penicillin. So, amoxicillin and clavulanic acid. So, what does it do? The amoxicillin or penicillin prevents cell wall synthesis, but the problem is some enzyme beta lactamase is present in some resistant bacteria, that is why they are destroying this penicillin or amoxicillin whatever you say. But the thing is, this clavulanic acid, which is present in addition to amoxicillin in this drug augmentin, will destroy the beta-lactamase.

It will break down beta-lactamase. So as a result of that, now that particular bacteria is not resistant to the antibiotic itself. So I hope that you understand that in augmentin, we have both the penicillin as well as the beta-lactamase inhibitor and that is why this is a very powerful antibiotic. Another strategy very often used by bacteria is that we are trying to treat by antibiotic reagent, antibiotic drug. So it should go inside the bacteria, and then they should stop the critical step inside the bacteria. Now bacteria will try to survive. So what will they do? So this is an efflux pump.

As you can see, this is an efflux pump So they will increase the amount of efflux pump on the surface of these cells in the bacteria itself. So therefore, we are trying to give more and more antibiotics, and it is going inside the bacteria, and the bacteria is removing those antibiotics from inside to outside. Therefore, this is another very important strategy of bacteria so that's why antibiotic resistance is a big problem.

So properly, antibiotics should be used because misuse of antibiotics can lead to more antibiotic resistance. In the last lecture, I mentioned that bacteria can transfer their genetic material in a horizontal gene transfer manner. So, as a result of that, some antibiotic resistance genes can be moved from one bacterium to other bacteria, and that is increasing bacterial resistance. So this is all about antibiotics and antivirals, and the resistance against antibiotics.



That's all. Thank you very much.