Applied Environmental Microbiology
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Lecture – 50
Antimicrobial Resistance II

Dear students, welcome to the second lecture on Antimicrobial Resistance; in this lecture we will be focusing on three different things; first what is the extent of antimicrobial resistance in India is it a big problem for us is it really a problem. And the second question we will be talking about second focus would be on the how can we stop the spread of resistance and; obviously, in order to do that to understand that we need to focus on another aspect; the third aspect which is how does resistance work? What are the resistance mechanisms that a microbe uses to be resistance against particular pathogen sorry against a particular antibiotic or antimicrobial?

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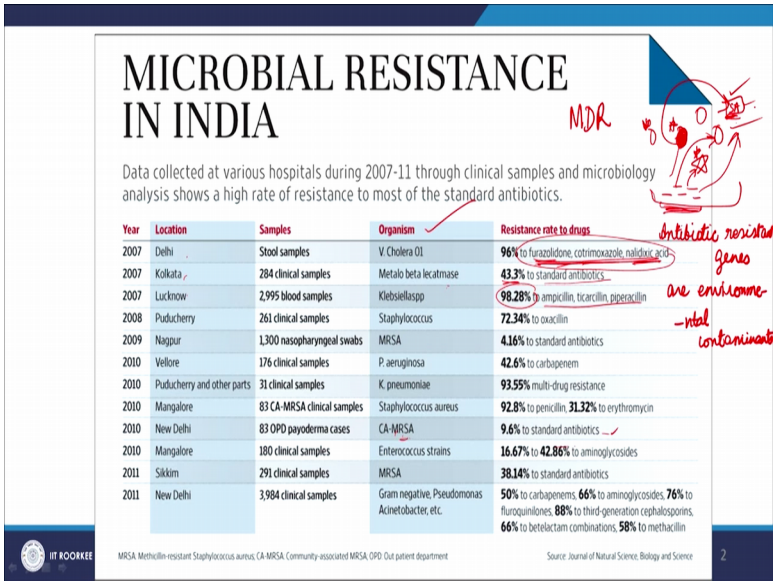

So, let us get started here this is the snapshot from a study it presented in journal of nature natural science few years ago and this is expressing the extent of antimicrobial resistance in India this information; these information were corrected from 2007 to 11 across various hospitals in India and let us look at the data. So, in 2007 in Delhi did stool samples and we were interested in cholera cases and we noticed that 96 percent of stools had pathogens resistance to nalidixic acid, cotrimoxazole and furazolidone. So, 96

percent of patients with cholera had red drug resistance and not just think resistance to a singular drugs, but multi drug resistance and this is a very good time to segue into very important terms one is simple drug resistance and then we have multiple drug resistance MDR.

So, multiple drug resistance is when a particular pathogen is resistant to many different drugs. So, hopefully if cholera is resistant to the first drug; we can use the second drug to treat it, but n if it is resistant to both and we can use a third drug, but what if it is resistant to all three? So, this is how we keep running out of our drugs to treat our diseases.

Then in 2007 Delhi, Kolkata, Lucknow were sampled and all of them had resistance and in Kolkata we notice that 43 percent had resistant to standard antibiotics and this is metallo beta lactamase. And I must have you bought metallo beta lactamase, this is a particular protein the enzyme that gives the ability to be resistant to most antibiotics. So, that is what they did not even care listing the antibiotics here because bed metallo beta lactamase means to almost all antibiotics. Then in Lucknow the blood samples showed resistance to ampicillin, ticarcillin and piperacillin 98.238 percent.

These are very high percentages of resistance and as we moved on the study included Puducherry, Nagapur, Vellore, Pudcherry and the surrounding areas Mangalore. Again New Delhi, Mangalore, Sikkim, in the New Delhi and every time we notice different kinds of pathogens that were resistant to different kinds of drugs.

Now dear students this is a very important thing one of the paradigm for public health is when we talk about antimicrobial resistance is give me a pathogen and give me a drug. Which means tell me the pathogen this is your pathogen and tell me the drug it is resistant to; what this paradigm this particular paradigm as presented here ignores is that if the resistance not see let us say 9.6 percent of cases MRSA cases method and metallicity resistant staphylococcus aureus revere resistant to standard antibiotics.

So, staph aureus 19.6 percent were resistant rest were not now, what this ignores is that let us say for rest of the population which is 90.4 percent of the population they had again a microbial community different shapes of microbes. Now if let us say this is staph aureus which causes infection this is not resistant, but other drugs can be.

So, the benign cells might be resistant to the drug. So, at the time of doing the study we did not notice MRSA staphylococcus aureus or methicillin resistant staphylococcus aureus to be resistant, but very soon it is very probable that these resistant other microbes in the body will give resistance to this pathogen and when that happens this will acquire resistance. So, instead of looking at which pathogen is resistant to which drug, we should look at which community is resistant to which drug because overall it is a community has the capacity to share genes as we talked about in last lecture through transformation, transduction or conjugation.

So, because it the potential of transferring the genes that are that give pathogens the resistance against an antimicrobial are present within the entire community regardless of whether pathogens has it or not; they should truly be alarmed and checking for the antimicrobial resistance in the community not just in pathogens. Now this brings me to another very important point is it the proteins that give antimicrobial resistant to a microbe at the pathogen or not that is our concern or is it the genes that it can transfer from one microbe to another.

And give a new microbe or a different pathogen or different microbe resistance is that a concern. Now look here let us say in this microbial community this over shaded red microbe has resistance to let us say most antibiotics that can be used for treating staphylococcus aureus; this cell will eventually die the person might continue being sick.

So, if the person is it this particular cell dies then its genetic material might still linger around. Let us say this cell dies without sharing somehow we could put an end to the horizontal gene transfer directly. Now this is its genetic material the lie down for some time in which because the gut flora tends to be very heavily populated, it is quite possible that at this time staphylococcus aureus might pick it up right other microbes might pick it up. So, basically even after the death of the cell the ability to share genes the ability to give gene then it resistance to other microbes has resisted.

This hints that it is not necessary it is not the antibiotic resistant antimicrobial resistant microbes which is the contaminant. In fact, it is your genes that are contaminant and Doctor Amy Prudent has done some great good work in and other scientists also in showing that antibiotic resistant they call them antibiotic resistance; I prefer using antimicrobial resistance because it is more broader term, but antibiotic resistant genes are

environmental contaminants. They do not even have to be present in the life cell even if they are just lying in the environment a life cycle can pick it up and get antimicrobial resistance.

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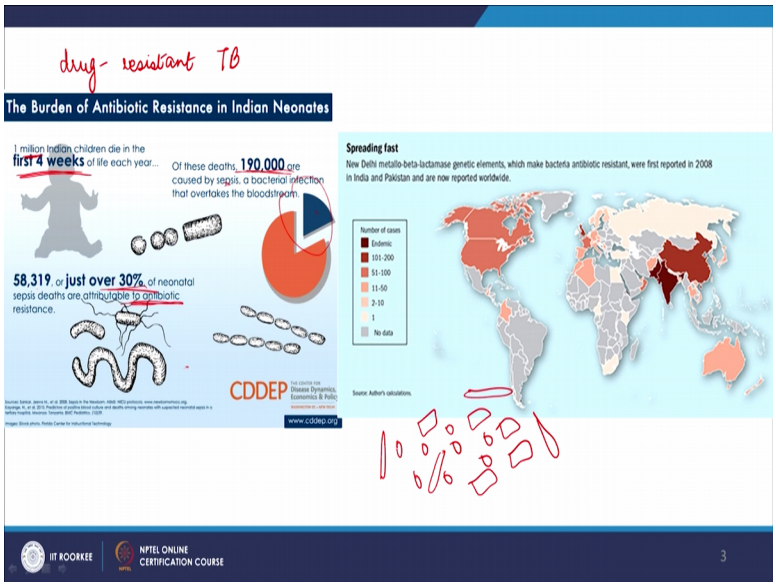

So, now let us look come back to India this is the burden of antibiotic resistance in Indian units. So, real infants 1 million Indian children die in first four weeks of life every year; now my dear students please do not take this as a mere statistics each child is; obviously, important this 1 million is a very large number most of us cannot even imagine how much how large it is. And this high rate of infant mortality within first four weeks the neonates is really alarming now out of these 1 million 190000 there is 19 percent are caused by sepsis deaths are caused by sepsis which was then an infection mostly in bacterial infection; it starts infecting blood cells.

So, it overtakes the bloodstream and then the person dies really fast now when. So, nearly 20 percent of infections happen because of sepsis now 58319 that is almost little more than 30 percent of neonatal sepsis death which is represented here by dark blue are attributable to antibiotic resistance. So, we can say 50 to 58319 children neonates every year just if we can control our antibiotic resistance.

Now this is only an example of sepsis in children; now if we look at other diseases for example, tuberculosis. Now in my generation many of us not many, but plenty of us had tuberculosis as kids and; I also had tuberculosis as a child. And then receiving the usual

treatment we got fine they were we were lucky enough to be in families that had access to good hospitals and good treatment and we recovered.

But over time the tuberculosis mycobacteria, which was susceptible to drugs started showing symptoms of resistance. So, what came up was drug resistant tuberculosis then the Indian government came up with the very nice strategy often doing a multiple drug therapy to take care of drug resistant tuberculosis cases. Now in this what we do is we cycle the different antibiotics over time so, that if myco recycling works this way.

Let us say we have a very diverse community of tuberculosis microbes and pathogens and their shape determines what antibiotic they are resistant to. So, we have big square looking we have this soft round looking and the let us make them really elongated. So, three different antibiotics; so, the three different kinds of mycobacteria to this is not how they look, but this is just for illustration purposes.

So, let us take an example here we have different kinds of mycobacterium tuberculosis in the body.

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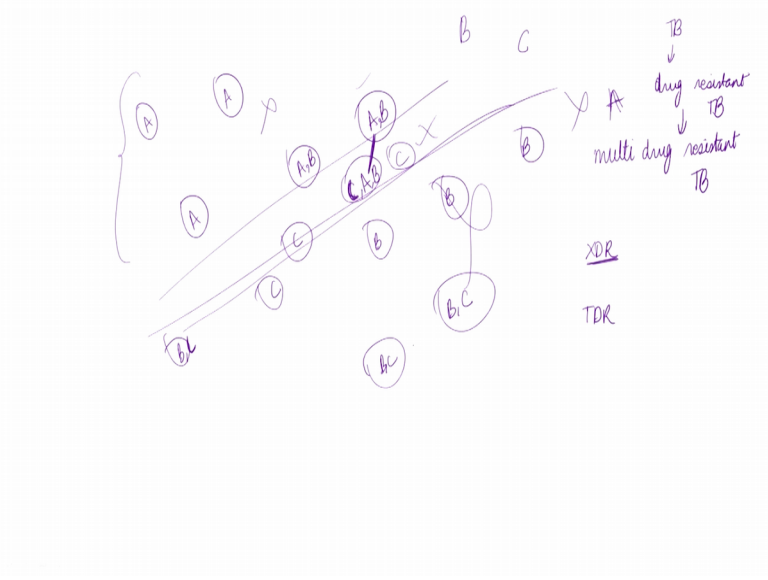

So, these are your tuberculosis pathogens in the body and let us say this is resistant to drug A this is resistant to drug A resistant to drug A resistant to drug A and B and this is resistant to B only these are resistant to B and C C only and to B and C.

So, let us say this is the case in the patients body where some to mycobacterium tuberculosis are resistant to only a some are resistant to A and B some are to C some are to B in C and some are only to B. Now this is a problem of multi drug resistance; if I give the patient drug A eventually these would die out the one that are not resistant to drug A, but that the ones that are resistant to drug A which have resistance to A and A and B will flourish and the person will die of tuberculosis. So, what the government of India came up with an approach was that we should recycle the drugs. So, for example, initially I give drug A and then ideally I get rid of all these microbes.

Let us say 1 microbe a resistance to C survives, but I kill all of them now in order to kill them. Now I will give them drug C now when because I have already given them drug A and I killed all these now I will them drug C and I will kill them too; now with drug C I have got rid of all these because they were not resistant to drugs. Now this one is left its resistant to drug C; so, now, I will give a third cycle of drug B and I will get rid of this pathogen also. So, as such I have treated the patient; so, this is my a dot program where we treat multi drug resistant tuberculosis.

Now many a time these drugs have other unpleasant or harmful effect on the human body. So, if when the patients they start feeling better they stop taking the medicine. And when they stop taking the medicine let us say in the first case when I took drug A and I got rid of all these pathogens; half of the pathogens were dead patient started feeling better, but patient also started feeling a pain in their liver and they decided when I am already feeling better I should you know stop taking the stop taking the medicine.

Now, when the stop taking doing the further course what we have is no overpopulation of other drug resistance. Now there are some survivors who when they got antibiotic A there are some survivors here who were first susceptible to A, but not to C like this one and now they will interact with this particular microbe and now they will B resistant to all A, B,C.

So, this break in the treatment is very dangerous because it promotes multi drug resistant tuberculosis. So, very initially tuberculosis was susceptible because of incomplete treatment and poor practices in our country; we had drug resistant tuberculosis. Then we started cycling the drugs to get rid of drug resistant TB and whenever there was a break in; the treatment we encouraged multi drug resistant tuberculosis.

Now, we also notice the cases of XDR which is extreme drug resistance and we suspect that we will soon reach total drug resistance also. So, extreme drug resistance is no matter how much I increase the dose; the tuberculosis bacteria is resistant to all microbes regardless of the all antibiotics or most the anti microbes regardless of the dose all righty.

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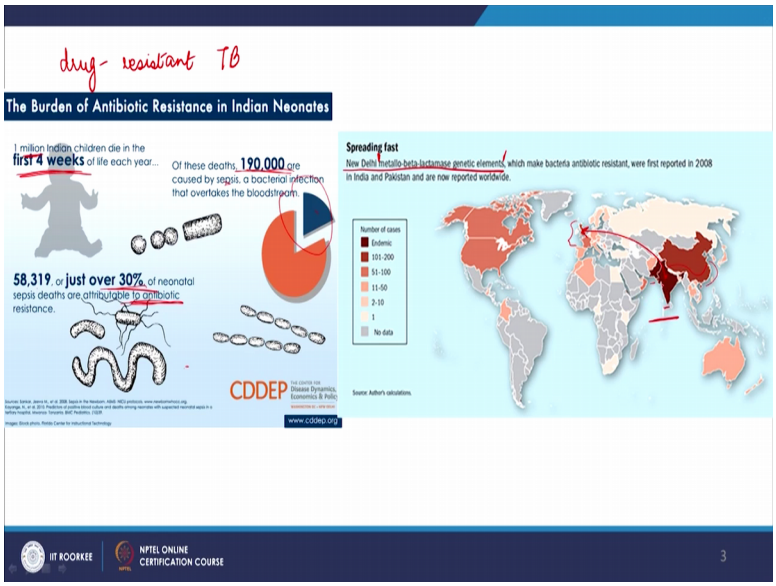

Now let us look at another case of a New Delhi beta lactamase gene. So, New Delhi beta lactamase obviously this illness is a genetic element and its horror is that it can be transferred from one microbe to another through plasmids. So, its cosmics are very easy to transfer horizontally. So, its names should suggest where it was first detected.

So, a patient a person tourists from who visited New Delhi and when they went back to their country with infection and got treatment here they identified it as a pathogen that was resistant to all antibiotics that they could use. So, they could not treat the patient and when they typed it they found that there is a difference in this particular pathogen which is that it has this genetic element which is metallo beta lactamase genetic element. And because the patient had acquired the infection in New Delhi they called it New Delhi metallo beta lactamase genetic element.

And I want to repeat this; so, please listen carefully this NDMBL; NDML its often called New Delhi Metallo Beta Lactamase gene is gives a microbe resistance to almost all antibiotics. So, if person falls sick with the pathogen that has this gene or acquires this

gene, then there are chances of mortality are very very high. Now if you look at this map there are two countries here which have endemic cases of NDML gene.

So, for example, recently NDML gene was found even in drinking water even in bottled water was found in the drains in Agra, Delhi, Hyderabad. In fact, the two countries that have endemic levels of a New Delhi metallo beta lactamase gene in its environment and in drinking water and food everywhere are India and Pakistan. So, really we have the onus of treating antimicrobial resistance particularly when it involves severe cases such as NDML, we need to take initiatives we need to take leadership.

Now, look here initially the gene was found only in India and then we noticed high cases in Pakistan and then we started seeing them in wastewater treatment plants and in the sea and in the patients in China. And now we observe them at different concentrations across the world. So, there is a very strong sense of suspicion in scientific community that India and Pakistan perhaps the hot spots of antimicrobial resistance and because India is bigger in geography and in population; the fingers are often pointed that India.

And we really need to take leadership and we need to take responsibility for this. Now one of the unfortunate response of our government when this particular paper was published, where the NDML gene was identified and named was that you should not call it New Delhi because it brings poor name to our country. But well that is true and also there is no proof that it was in each region in India, but we do need to take responsibility; we do need to admit that we have a serious antimicrobial resistance problem.

In fact, every year thousands of people are dying because of multidrug resistant tuberculosis. Even when they are trying to complete their treatment at most sincerity also apart from tuberculosis staphylococcus aureus and other in pathogens have also acquired some very serious levels of multi drug resistance and extreme drug resistance.

Again what is so, common between India and Pakistan? Especially in India we have very high population density; we have very poor solid waste management and liquid waste management. And that makes conditions very amenable for antimicrobial resistance to grow and also we have a very poor control on the drugs available in the market freely.

So, the drugs that should be actually prescription only drugs are now available in medical stores for anybody to go and bid get them. The other thing is even after the doctors or the

medical community I did say we spread awareness among medical community and among the physicians and the pharmaceutical B pharma people the medical stores that; this is a major problem we should stop doing it.

Even if you can convince them the culture in our country is not amenable to completing the treatment exactly prescribed by the doctor. We either in between start modulating the treatment I am feeling better let us reduce the dose I am not feeling better so, let us continue dose for one month more. So, these kinds of tinkering that we do with our treatment makes us very very vulnerable to antibiotic resistance.

So, let us move ahead here.

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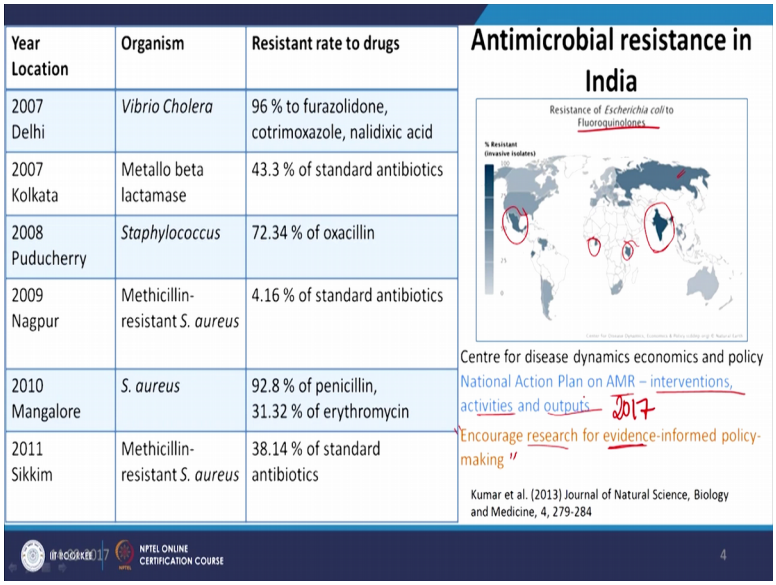

So, this is the summary of the same study that was presented in the first line. Now look here these are on the right panel we have E-coli and fluoroquinolones. Now fluoroquinolones are very interesting because they are very commonly used in our country and many a times they are the last resort antibiotics. And if you look at resistant or invasive isolates were very common very abundant in India some for good amount in Russia and in certain African countries also in Mexico, but definitely India is again a hot spot.

Now we need to understand this that this particular study that was presented by center for disease dynamics this study call talked about only isolates which we could culture in the

lab. Many environmental microbes cannot be cultured in the lab many pathogens also struggled to culture them in a lab. So, this is a very small snapshot of what actually might be the gravity of the problem is not captured perfectly in the snapshot, but it is still a very helpful information.

So, as a result finally, in 2017 earlier this year we are when I am recording the lecture Indian government launched national action plan on AMR; antimicrobial resistance to come up with interventions, activities and outputs for controlling antimicrobial resistance in the country. And one of the very important parts of this national action plan was to encourage research for evidence informed policymaking. So, if you are the young researcher; there is a lot of scope in developing in doing more research to develop evidence that will inform the policy to contain AMR ok.

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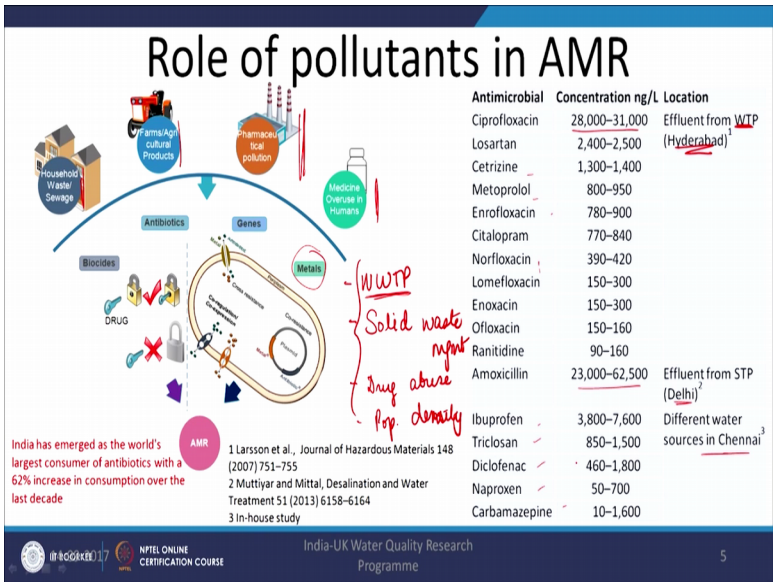

Now, let us look at how does AMR spread and white is such a big problem in India? So, here we want to talk about role of pollutants in antimicrobial resistance. Now we know antimicrobial resistance if the antibiotics present they will eliminate the susceptible microbes and then the ones that are resistant will go and multiply and they will share those resistance with pathogens and with other microbes that were first susceptible.

And when microbes had when in the microbial community some microbes are susceptible to different kinds of antibiotics, they share and after a while we can how we

can see that the entire microbial community may have become multi drug resistant. So, we know these antibiotics will promote sub therapeutic levels of antibiotics.

So, antibiotics that allow certain members to survive will result in antibiotic resistance being increased over time, but there are other things too we talked about good core resistance and cross resistance in the previous lecture. So, please go and look through that my last slide of previous lecture again it is not just antibiotics their presence that promotes antibiotic resistance, but there are many pollutants many xenobiotics that are released every day in our household waste and sewage, in our farms like pesticides, insecticides and then; obviously, the pharmaceuticals and abuse that we do by humans like you know medicinal overuse abuse and misuse. And we also have other xenobiotics and in fact, the list is very long.

In fact, even temperature shock might induce antimicrobial resistance. So, all of these are very likely to promote increase in antimicrobial resistance. So, we get our biocide antibiotics and antibiotic resistance genes and heavy metals are a big cool selection of factor. And finally, what we have is antimicrobial resistance and look here India has emerged as the world's largest consumer of antibiotics with 62 percent increase in consumption over the last decade 62 percent increase.

So, we are increasing our consumption at a very very fast rate and we are also increasing our problem of antimicrobial resistance at a very very fast rate. Now let us summarize what I have already said India is particularly susceptible to AMR resistance because our basement wastewater treatment plants are either do not collect all the waste water from the entire city entire town.

So, it is not it does not have a very good coverage and even when it has a coverage this many of them are operated and maintained poorly and not up to the standard. As a result lot of fecal matter lot of antibiotics; lot of other materials are exposed to the environment exposed to the other microbes and to pathogens and then we have a serious antimicrobial resistant problem.

The other part where WWTP is very important is that our WWTPs are not designed to remove these triggers of antimicrobial resistance, they are not designed to remove heavy metals, not designed to remove antibiotics and others xenobiotics. And it requires very low concentration of these compounds to trigger antimicrobial resistance. So, we need to

think about one statement here. So, India one of the problem is wastewater treatment plant is not are not up mark our solid waste management is really poor.

So, nowadays there is a big push by the government to clean the cities, but the question remains you keep the cities aesthetically and visually clean what happens to all the waste you have collected? You dump it in a landfill and now that landfill becomes a hot spot for anti microbial resistance. We need to have a very nice way of segregating our waste properly composting when it we can compose recycling reducing when we can and then only what cannot be to use recycle.

But you not be composted only that is put in the landfill; this case we will reduce the biological activities in the landfill and we will be able to contain AMR better. The other problem we have is our drug overuse abuse and misuse; so, combined and then; obviously, if you have high population density. So, everything is compounded by our high population density and as a result India is the hot spot of antimicrobial resistance.

Now these are the amount of antibiotics which were found in the right panel that were found in different environmental samples. So, here on first we have effluent from a water treatment plant in Hyderabad.

So, this is what is being supplied to people to drink in Hyderabad; a lot of ciprofloxacin, losartan, cetrizine all these antibiotics and other drugs and there some of them are not antibiotics, but still they are pharmaceuticals that can trigger AMR.

And in fact, this is interesting in Hyderabad some water bodies the amount of antibiotics and xenobiotics found in some of their lakes surface water was more than therapeutic dose. I hope you understand the gravity of the situation, there is lot of pharmaceutical waste in certain parts of our country. And this is really bad for environment really bad for public health for many reasons anyway and then this is efferent from STP in Delhi lot of amoxici amoxicillin and in different water sources in Chennai ibuprofen and triclosan diciofenac, naproxen and carbamazepine.

So, our water sources are already contaminated with antibiotics and pharmaceuticals and hopefully we will find a way to remove them from our water and contain AMR. Now, look at let us look at the resistance mechanism; so, this is the second focus of today's lecture some microbes they develop an ability to reduce the permeability of the cellular

membrane. And thus they are able to resist antibiotics such as penicillin and usually this ability to reduce permeability is present in their chromosome.

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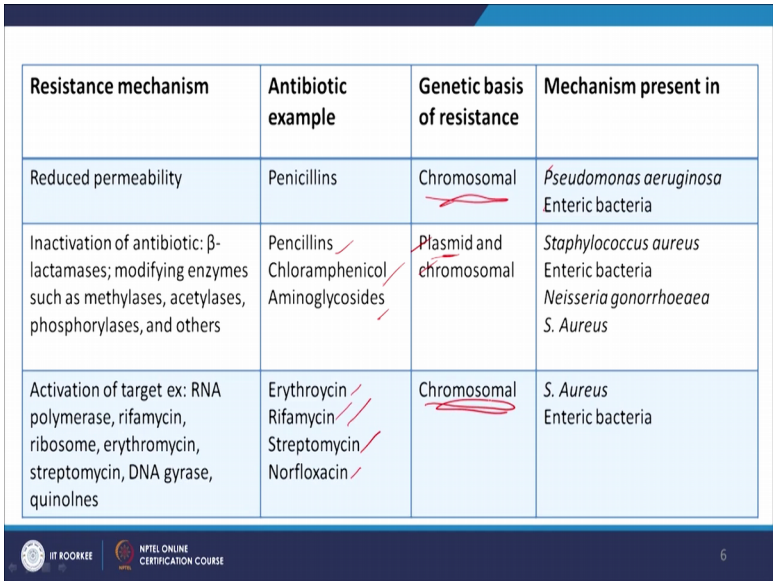

So, this is again genetic, but this is because it is in chromosome it is not extra chromosomal genetic element. So, it is slightly harder to share it through horizontal gene transfer is found in enteric bacteria and pseudomonas hydrogenism.

The next is that there are there is a mechanism through which my groups can inactivate antibiotics which as beta lactamase; they can modify enzymes such as methylation we will talk about this. And in this way they are able to resist pencillins, chloramphenicol, aminoglycosides or antibiotics and this ability is present in both plasmid and chromosome. So, plasmid one would be very easy to transfer chromosome would be little hard to transfer through horizontal gene selection.

Then we have the other resistance mechanism where we can actually modify the target. So, we modify the antibiotic and it no longer harms us; so, in this particular case inactivation of target the antibiotic it targets a particular part of your cell it might target RNA polymerase deactivated and then the cell dies. So, what it does is it modifies the interaction between the target and the drug and make sure that antibiotic cannot kill. This kind of resistance is found again streptomycin rifamycin, erythromycin norfloxacin and this is often chromosomal.

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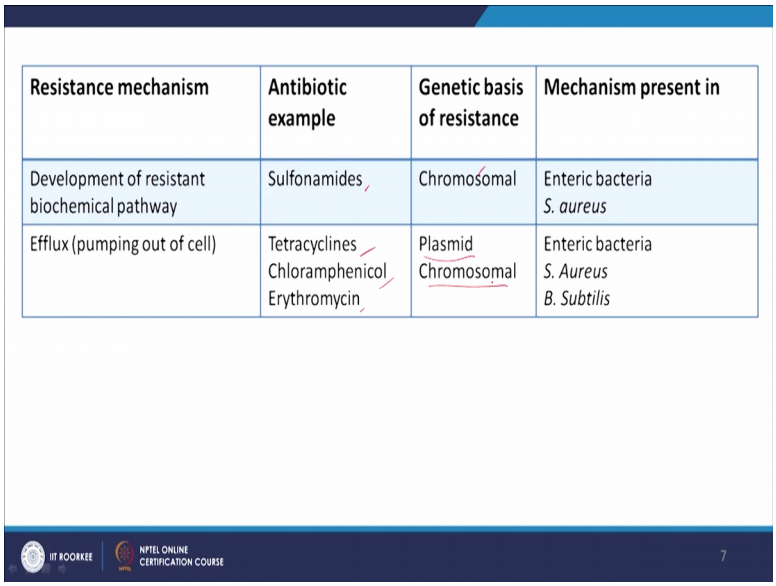

So, again hard to transfer through horizontal gene transfer; next is development of resistant biochemical pathway. This is often found in sulfonamides and chromosomal the fifth one here is efflux where they actually have efflux pump and a pump it out of cell; this is very different scary because if not in sometimes they have efflux pumps to get rid of heavy metals, but they also can use it to get rid of antibiotics and this is in general more generic it can pump out more other different kinds of compounds. So, tetracycline, chloramphenicol, erythromycin this is both abilities in plasmid in chromosome it is easy to transfer alrighty.

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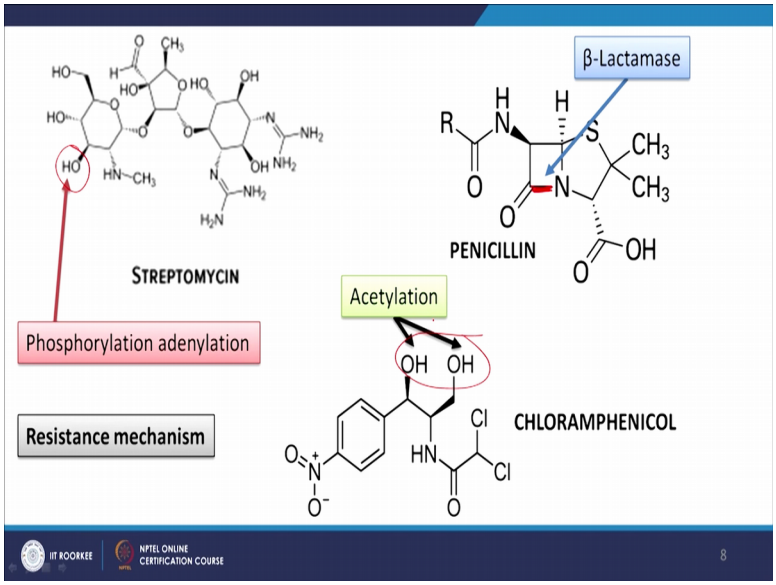

So, we talked about modifying the target now here we have streptomycin; now what the cells can do is they can they can do phosphorylation adenylation of this particular OH and then render streptomycin useless.

So, you keep giving streptomycin to the body to the cell and the body has the itself has bacteria has the ability to phosphorylate adenylate this particular OH and streptomycin is benign. In pencilin what we can do is we can modify this particular bond and then the problem does not exist and this is done using beta lactamase enzymes. Now in chloramphenicol we can do acetylation these two OH and render the chloramphenicol benign throw pathogens.

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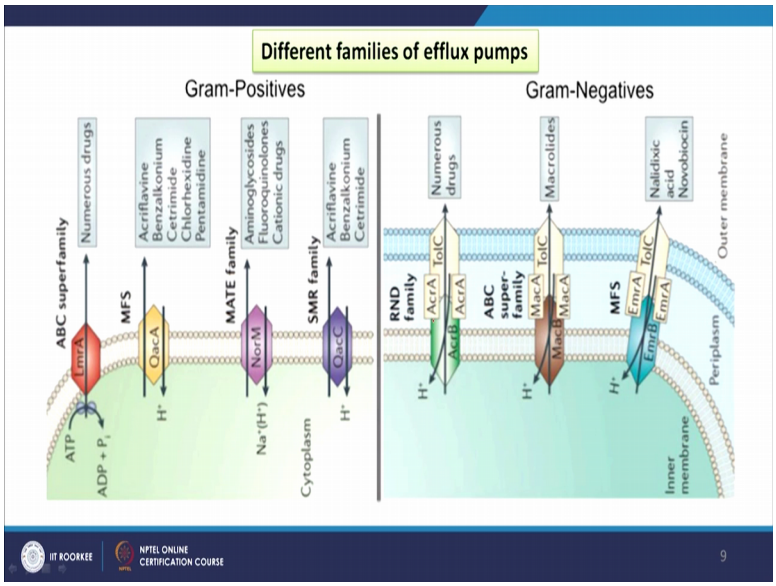

And then we look at efflux from there at least five different families of efflux pump we have the ABC families the ATP based transporter families we have MFA family, the mate family, the SMR family and RND family. And we notice that they are different from gram positive and gram negative and some of them can evict multiple drugs; usually all of them do for multiple drugs some of them can do for many different kinds of drugs alright.

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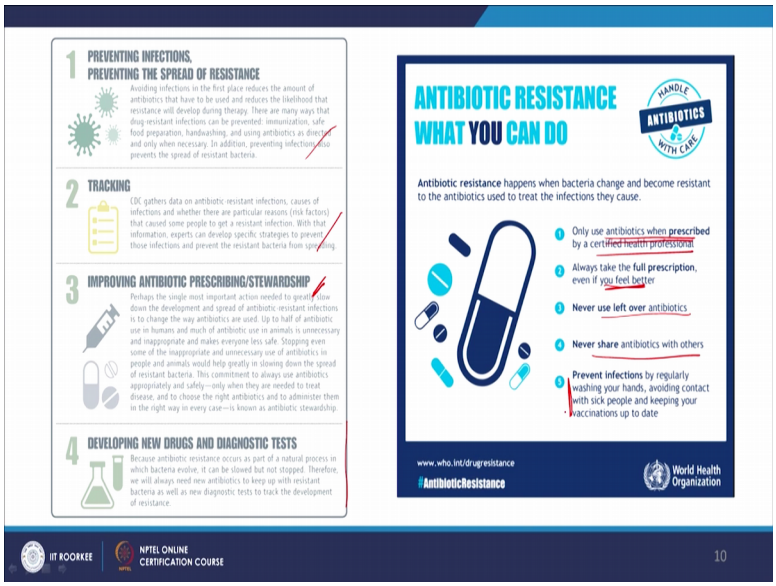

Now, how do we prevent this problem? First we can prevent infraction. So, that is vaccination for example, being more careful practicing good housekeeping techniques better hygiene cracking the infection. So, every time there is an infection we test for resistance and then we track it how much time it takes to cure where did the patient go how did the patient interact with.

Next is this is very important we need to improve antibiotic prescription and stewardship. So, our pharmaceutical people and our medical store people are informed and they are very strict about when they hand over antibiotics and when they do not hand over antibiotic same with the doctors. For example, there is absolutely no need to prescribe antibiotics for a flue viral flue, but still they are.

So, these doctors they need to be made aware and the next is we need to push for developing new drugs and doing new faster diagnostic tests. So, as the patient comes with an infection we can find out right away what kind of infection it is it a stranger or not what drug should I give. So, instead of increasing the drug resistance by giving the wrong drug I will give the right drug and get rid of the infraction.

So, and for a human being like for a general public what we can do is we can use antibiotics only when prescribed by certified health professional; even if we start feeling better do not stop your treatment, never use leftover antibiotics. So, if you had extremity biotics and doctor said stop and you start feeling better; stop, do not eat them again.

Once you have stopped there done never share antibiotics with others and prevent infection by practicing very good hygiene alright.

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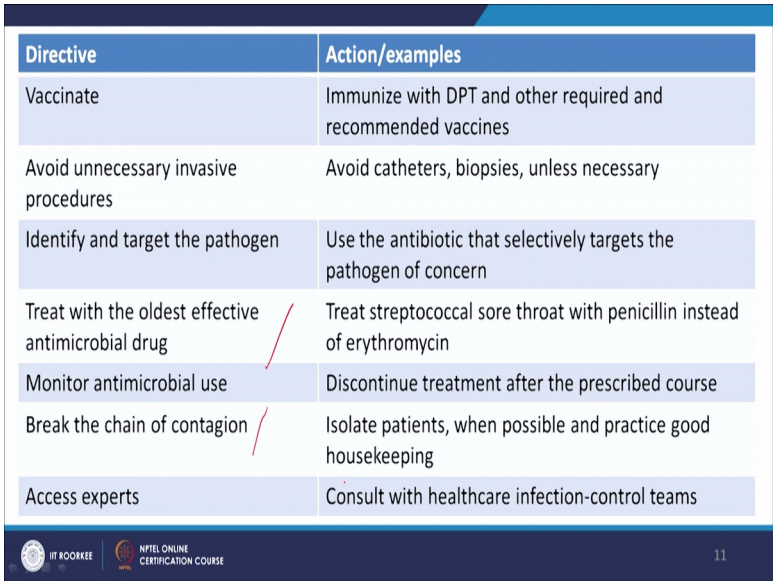

So, these are so, this is summary of some actions we all can take we can vaccinate; we can avoid unnecessary invasive process processes. So, when catheters are biopsies can be avoided avoid them because in invading our body; we are making it more susceptible to catch the infection especially with catheters what we the bio (Refer Time: 30:56) can develop on catheters and people can get really bad infection.

The next thing is we should be able to identify and target the pathogen right away. So, we use an antibiotic that only is particular for that pathogen and does not kill good bacteria. And also we want to start treating with the oldest effective antimicrobial drug for example, for streptococcal sore throat we start with penicilin instead of erythromycin even though penicillin has side effects because we would rather first develop resistance to penicillin than to erythromycin.

Next we need to monitor an antimicrobial use very well and once the prescription ends and we stopped using them. And we break the chain of contagion this is very important. So, here it is isolate the patient we contain the infection; we practice good housekeeping, but it is also important it beeps prevent, we contain the spread of antibiotic resistance genes, the anti microbials through our environment. And we talked about experts and we

have a good solid team of researchers that can stop spread of antimicrobial resistance to environment. So, dear students this is all for today.

Thank you very much.