Applied Environmental Microbiology
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Lecture – 42
Drinking Water Microbiology II

Dear students, in the previous lecture we talked about the basics of drinking water microbiology and then jumped into understanding the 4 classes of water associated microorganisms that cause diseases. We talked about the microbes that will infect us if we drink contaminated water of waterborne pathogens, we talked about microbes that will affect us and infect us.

If we cannot manage to maintain certain hygienic practices and then, we talked about microbes that actually require water to fulfill a particular part of their life cycle and they cause of diseases such as schistosomiasis and then, definitely the last of the 4 kinds of pathogens were the microbes that are transmitted to us by insect vector and that insect vector requires water for fulfilling a part of its life cycle. In today's lecture we are going to continue and go over the important pathogens that are, any of these 4 kinds of pathogens and then they infect us and are very relevant in Indian circumstances. So, let us proceed.

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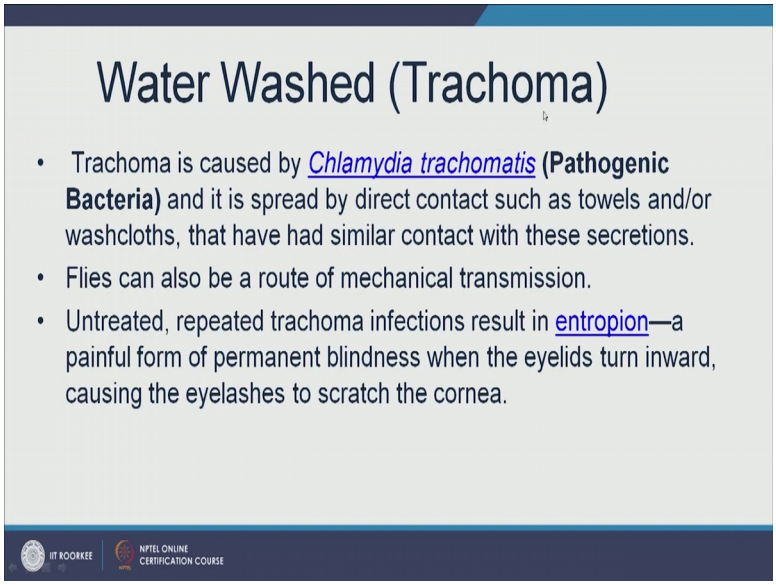

First, let us talk about a water washed disease caused by trachoma, if it is caused by Chlamydia trachoma, it is a bacteria and it is spread by direct contact, such as with contaminated towels, cloth or any other surface or for mites, these are called for mites, that have secretions in which this bacteria flies, can also be route of mechanical transmission, because what flies to is a contaminated surface and this microbes get stuck to it and then it transfers it to the other places.

Now, if we do not treat this trachoma and we get this disease repeatedly, it results in a very painful form of blindness, where the islets actually roll backwards and then rub off retina, it is very painful.

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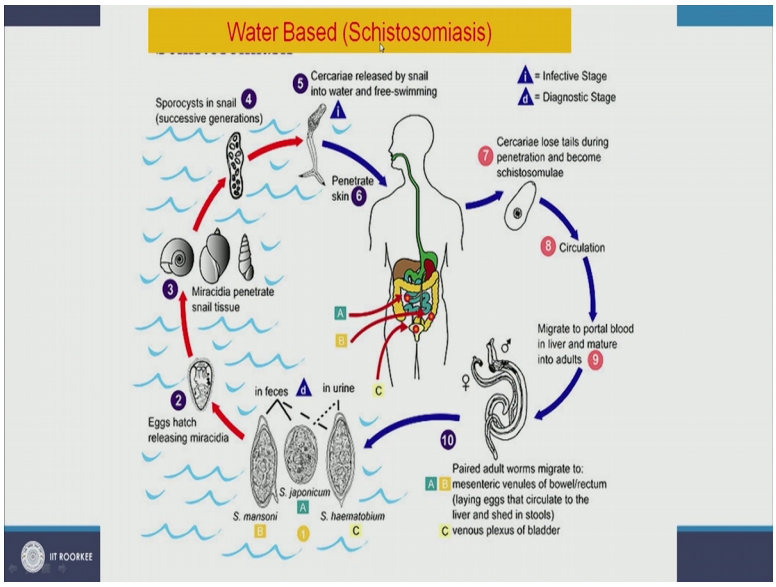

Now, we have water base diseases. So, both water based diseases are diseases, that we can catch by merely coming in contact with contaminated water and a typical example is schistosomiasis. So, in case of schistosomiasis, let us say a human being who has the disease, releases the microbes through their fecal matter and urine and these have eggs, and the eggs hatch, infectors snail fulfill a part of their life cycle and the snail releases it and then, they penetrate the human skin again or animal skin again infecting the human.

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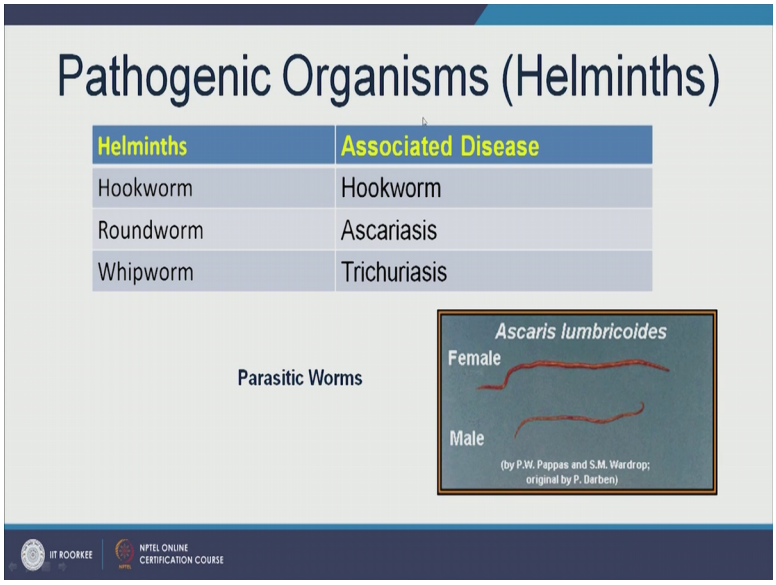

Then, in this human body what happens is that, they undergo another part of their life cycle undergo mating and then release eggs. Then, there are some pathogenic organisms which are not necessarily microbes, you can actually see them with the naked eye, they are multi cellular worms and I will briefly mentioned them. Some of them are hookworm, roundworm, whipworm and their diseases are hookworm, ascariasis, and trichuriasis. Ascariasis for example and all of them cause severe nutritional deficiency. Ascariasis, we call it as severe anemia. Many of these worms are hermaphrodites; some of them have male and female distinct from each other.

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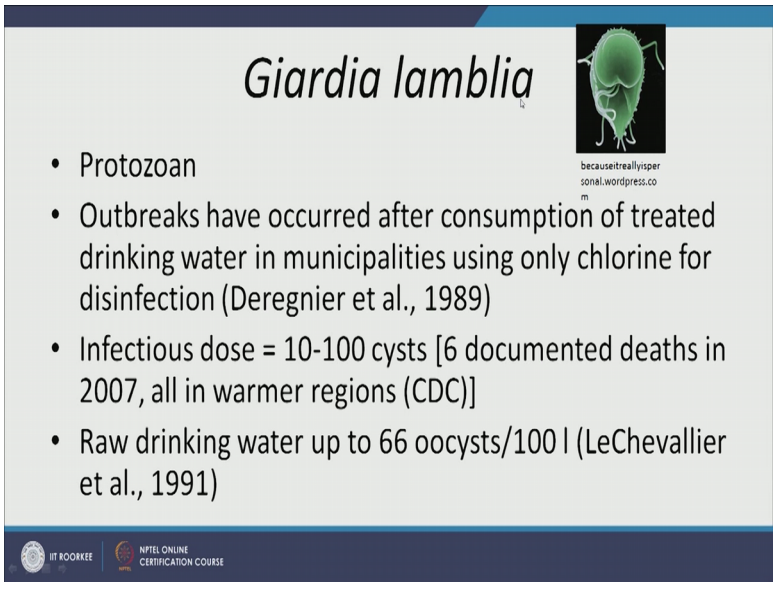

Then, we briefly talked about Cryptosporidium, another protozoa that is important is Guardia lamblia. Like cryptosporidium, it requires very low infectious dose to infect a human being and typically effects people living in warmer climates. That is why it is very - very important in India. Now, the thing with these diseases; Guardia lamblia or cryptosporidium is that, let us say I drink a glass of water and that entire glass of water might only have 10 of these protozoa and I will fall sick and these kind of protozoa are very - very difficult to find, if I put the entire glass of water in multiple slides and watch them with microscope, it will take a lot of time.

By the time, I am probably going to be very thirsty and in my frustration I will drink the water instead and thus, what I am trying to highlight is that Guardia and cryptosporidium are really hard to detect using conventional microbiological techniques, and here is an important thing. Outbreaks have occurred after consumption of treated drinking water, when they only use chlorine for disinfection and Indian chlorine is the major form of disinfection.

So, chlorine is not sufficient to kill Guardia and cryptosporidium and thus, these diseases are major concern in India and then, this is just showing you Guardia life cycle, how it undergoes changes within host and without host, and now, we are going to talk about aeromonas species.

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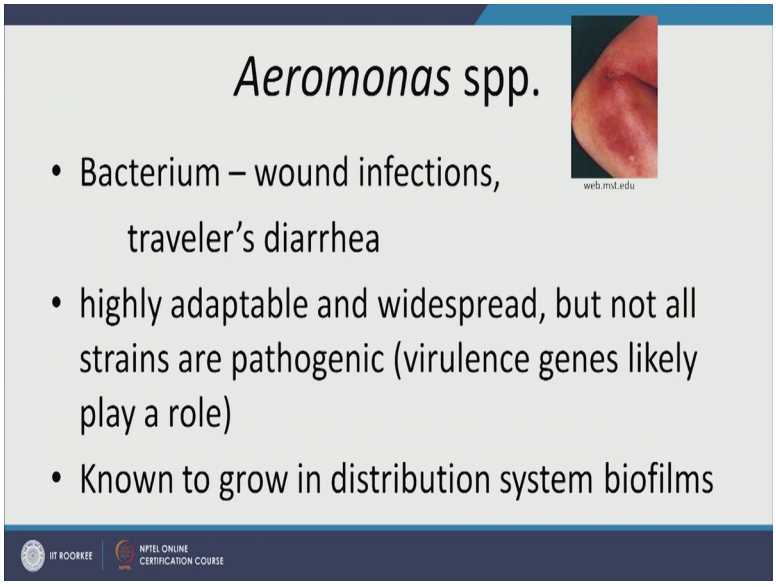

So, here is an elbow that is infected by aeromonas, this is a bacterium that causes wound infection. So, if you have a small wound, aeromonas will go inside and infect. It also causes travelers' diarrhea. So, people believe that Delhi - belly is often caused by this. It is highly adaptable widespread. So, it affect all the cells in our skin, it can also affect our intestine, causing travelers' diarrhea, but not all strains in aeromonas species; SPP dot by the way, is a shorthand for species are virulent and this is the saddest thing.

They are they are known to grow in bio films. So, if I have drinking water system which allows nutrients and microbes to leak in from somewhere and the water flow is intermittent. So, it creates ideal conditions for bio film to grow and the aeromonas species can come and hurt us; can grow there in the bio film and then we can get infected by it.

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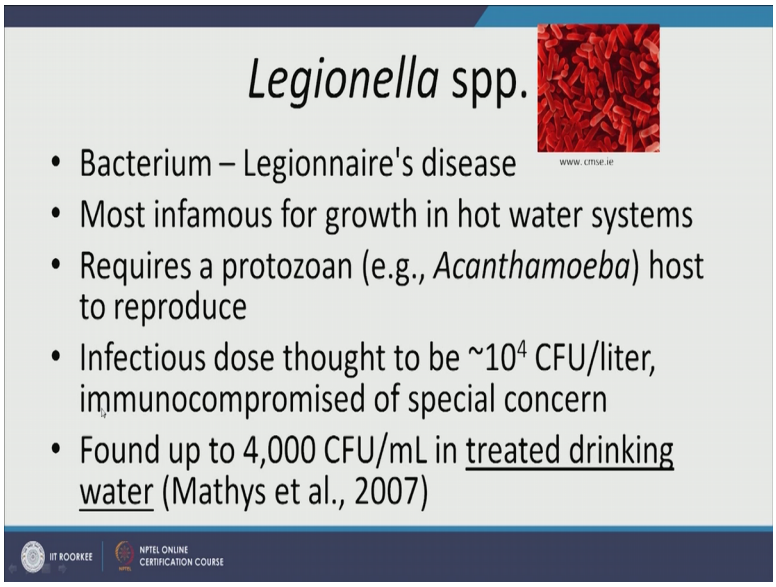

Now, let us talk about legionella. This is a very interesting microorganism, even in developed countries, where they have a really good water treatment system often suffer from legionella outbreaks and the reason for that is, that legionella grows in a distribution water system, very happily inside an amoeba and no matter how beautifully we clean and how much amount of residual disinfectant is present in the water, as long as the bio film can protect legionella from that disinfectant, it will be fine.

Again it is a bacterial disease and it is very infamous for growing in hot water system. It lasts temperatures that are near human body temperature, such as 37 degree Celsius. It

requires a host, a protozoan host and usually in bio films it is acanthamoeba and infectious doors is 10 to the power C 4CU per liter. CFU here is colony forming unit. So, if I take this water and I plate it in and (Refer Time: 07:12) plate it, then I should find up to 10 to the power 4 colonies. If that is the concentration, then people who have already compromised immune systems - such as children, elderly or people who are under autoimmune drugs for autoimmunity and other children, people who already sick are more likely to get infected by legionella.

So, for healthy people may be legionella is not a problem. We can get continually exposed to it because remember, it grows on bio films within acanthamoeba and other protozoan host. So, if I turn on my tap water and I drink it, I might be drinking a lot of legionella, but it will not hurt me, or if I am taking a shower, then the water vapor might be the legionella bio error source, but it would not hurt me because, let us that say I am really healthy, but if I am in compromise, it will drape my immune system. Now, not here we need up to 10 to power 4 few per liter, this is not a lot. Yeah! So, this is like 10 CFU per ml, but people have seen up to 4,000 CFU per ml in treated drinking water, in developed countries. Cannot even imagine for situation we have in countries like India.

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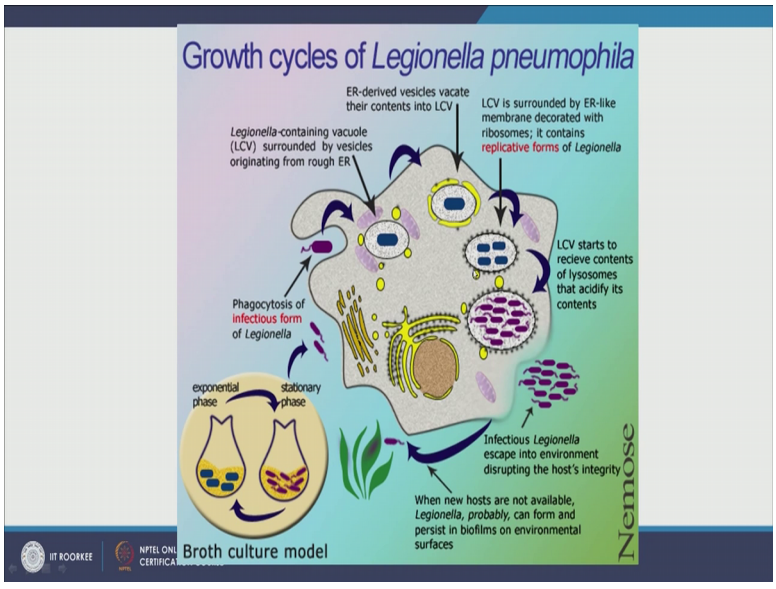

This is the growth cycle of legionella pneumophila. It is a very interesting case, where it is like a predatory microbe for acanthamoeba. What it does is, it enters our beautiful legionella and amoeba eats it, thinks that it is undergoing fasciitis is, but there is

legionella, it protects itself inside and it is in the vacuole right now and then, it grows in the vacuole and then, when it has grown enough, it acidifies itself, causes lyses of this boundary and then leaves the amoeba.

It escapes into the environment and this pure ameba collapses and then, when the hosts are not present, then the legionella surviving bio films, it is pretty happy. So, the moment protozoan host comes, it will stand near, “hey! come and eat me” and then once, amoeba has taken it in through phagocytosis, it will survive in a vacuole, grow and then acidify and kill ameba and get out.

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This is amoeba pulling the legionella and it is a really neat picture.

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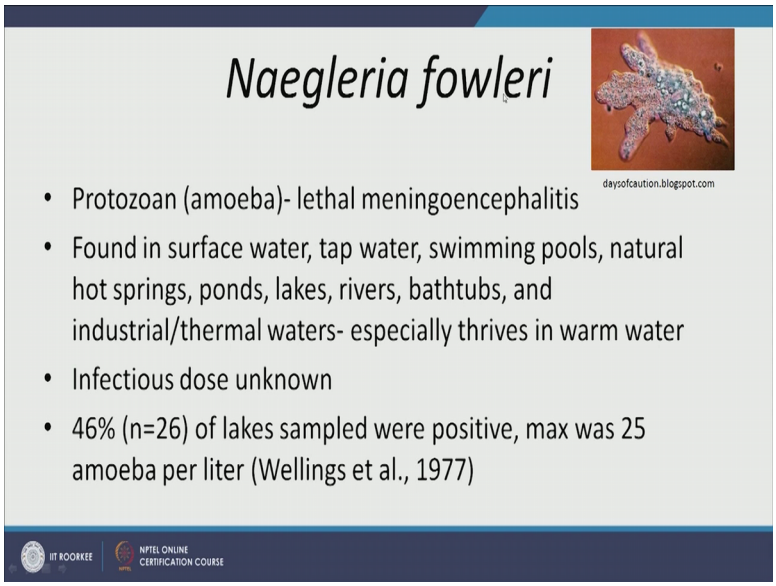

Alright! In next protozoa in India – Pakistan, is a big issue, it is naegleria flowery. Some years ago in Pakistan, there was this gentleman, who got infected by naegleria flowery and many of you might be aware of this practice; that many people do in our country and other countries it is called neti pot technique for cleaning the nasal cavities.

So, you put warm water from 1 and with some salts in it and allow it to come out from another nostril. Now, the most cases that we know of naegleria flowery in Pakistan and some in India are of people who practice this technique. It was found that, because naegleria flowery loves warm water, it was actually growing in the neti pot that had warm water in it for substantial amount of time.

So, please if you practice neti pot technique, please make sure your pot is clean and your water is changed regularly, every time you use it and the reason for that is naegleria flowery is also called as brain eating amoeba. It is a serious disease. Now, what it does is, if it enters your body, it will go to your brain and start consuming your brain cells and you would not even know, until it has done enough damage, that now the body is showing symptoms.

So, it causes lethal meningeal encephalitis and then of course, the ménages of the membrane gets swollen. It is found inside (Refer Time:10:58) the way also there is a warm lake, warm water in hot heat in hot water tanks, it is found in subsurface water, tap water swimming pools, natural hot springs, borne, lake, river, bathtubs, industrial, you

just name it. Wherever the water is warm and it is stationary naegleria foolery can grow in it, specially thrives in warm water. Infectious doses are unknown. In fact, it is not very common disease.

So, I do not want to scare you, but people have got sick in our subcontinent and we do not know the infectious dose because it is not very common, so it is not very easy to study and it is very hard to detect. Usually, people get symptoms, only after the damage is too much. Long time ago, in 1977, 46 percent of lake that were sampled was positive. So, do not swim in, like that is a very high number. Now, this is brain tissue and you can see this Naegleria flowery growing inside the brain and eating it, it causes extensive destruction of brain cells.

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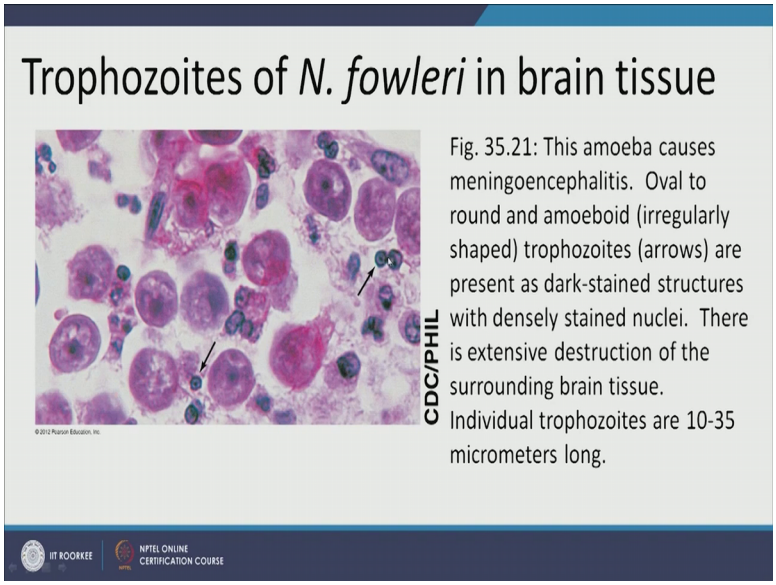

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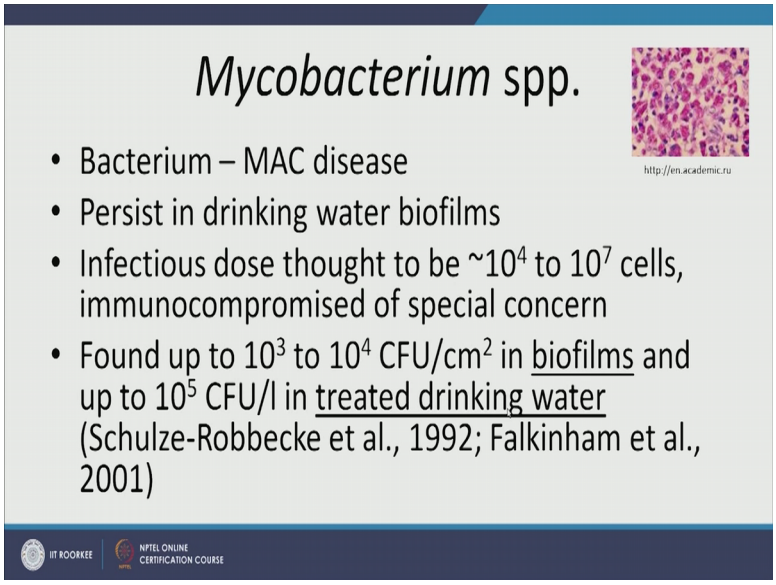

Next, we have mycobacterium species and tuberculosis mycobacterium is also a member of these species, but we are talking about the mycobacterium that will grow in our bio films. So, these can survive in drinking water bio film. They do not cause a very severe sickness, but they affect especially immune compromised people. The infectious dose is comparable to that of legionella pneumophila and they are usually found in bio films, up to 10 to the power 3 to 10 to the power 4 CFU per centimeter square. That is a very high concentration and up to 10 to the power 5 CFU per liter in treated drinking water.

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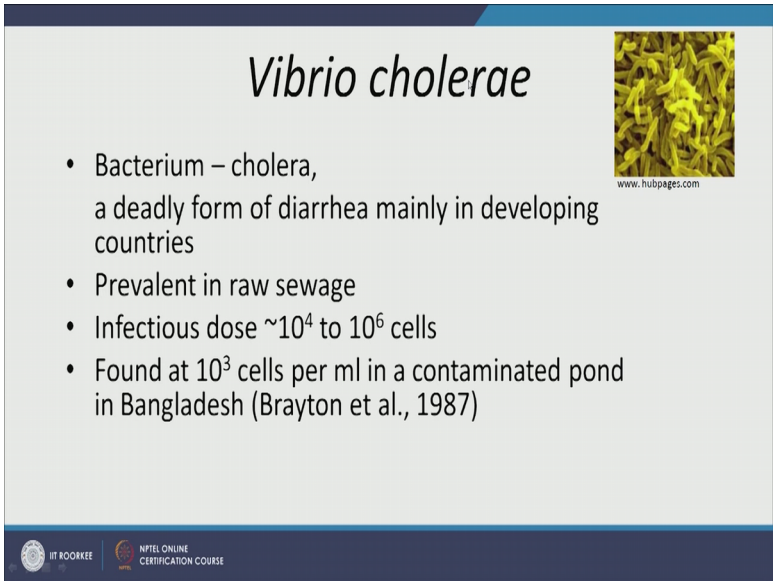

The next disease you want to look at is vibrio cholera. It is caused by bacteria and caused a serious cholera, which is a deadly form of diarrhea and in this slide I have written

mainly in developing countries, but I want to note here that, cholera is spread through fecal oral transmission which is, fecal oral is a fancy way of saying, if the fecal matter somehow through some for mite, through environmental contact or contaminated food and water finds its way to enter the mouth of the healthy person, it will infect.

The thing about cholera is that, it lasts really long. It can survive long in the environment. So, most diseases like let us say HIV, they have very short, half life in environment. They come out and they wither away in short time, but cholera survives for long time. The other thing is, its infectious dose is very little, up to 10 to power 4, which is very small. It is secreted in large quantities in fecal matter and the diarrhea that causes cholera is very severe form of explosive diarrhea and without proper treatment, people can die very fast.

Now, as I brought to your attention here, that people have said, that this deadly form of diarrhea is mainly in developing countries. The reason is, because the countries those are developing or underdeveloped, they do not have sufficient and good coverage of wastewater treatment plants. They do not manage their sea well enough and then the human beings in the community are more likely to get in contact with the untreated sewage. Now, this is an important part. Not only is cholera is a developing country disease, but now we are also finding out that cholera is a refugee disease too, because in our world right now, one of the major human problems we are facing is the refugee crisis.

Large number of people, who are being displaced from their original homes, their original cities, towns and villages across all continents are suffering this problem and what happens when these people displace population, they live in makeshift towns, makeshift tents, these places usually do not have good wastewater treatment systems.

So, we have millions of people who are displaced in a place, let us say and these people, their poop cannot be treated and as such, they are very likely to get exposed to fecal matter and if there is one person who is carrying cholera bacteria, more people will get exposed to it, more cholera will release into the environment and sooner than later we see a cholera epidemic and if you Google it, you will see that, how in many middle eastern countries and many other refugee camps across the world. In the recent few years including Haiti for example. So, the refugee camps are not always because of

anthropogenic reasons, but mostly right now, in many refugee camps across the world cholera is a big - big serious issue.

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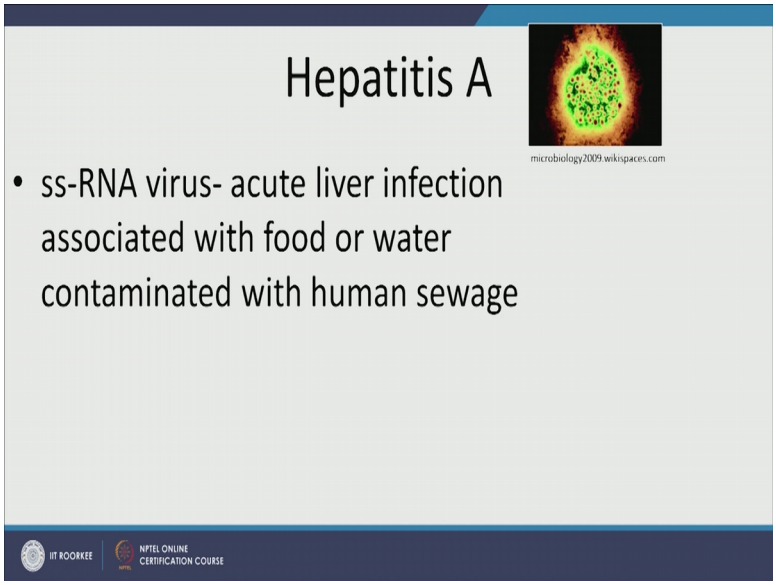

Now here, I am talking about only hepatitis A, but hepatitis B, belongs to a different family of the same virus are both waterborne and are an issue in any country. This is a single stranded RNA virus it causes acute liver infection. Sometimes, we call it as jaundice. So remember, jaundice is a symptom, jaundice is not a disease, but liver infection is an infection and it is again fecal - oral.

So, if there is fecal matter that carries hepatitis A, it comes into contact with the human being, the person might get hepatitis A or E. Now here is the thing it requires very little amount of viral particles for hepatitis A to happen. For hepatitis A disease to blow up same is the case with hepatitis E, after 10 to 100 virus particles, this is ridiculous.

Now, they are ridiculously low. Now, this is important to mention that across the country, every now and then, we have jaundice outbreaks. So, we have individual incidences of jaundice and then, we have gen jaundice outbreaks and one of the most severe jaundice outbreak that we had in recent years, was in Shimla, in the winter 2015 and 2016 where thousands of people got jaundice.

And then, it was tapped and found out that it is hepatitis E, more information was then released, saying that Shimla has had such jaundice outbreaks before and earlier it was

hepatitis A, now hepatitis E nothing of it, even if little amount of hepatitis E virus or A virus enters the water distribution system, people will get infected and when they poop, their poop will have more amount of virus because now, the virus has replicated many - many times more in their bodies and then, because of poor sewage treatment plant and because of poor sewage management practices, more people are likely to get infected and then, these viruses may also find their way through water distribution system and then, the next thing we know, we have an epidemic of hepatitis A or hepatitis E.

Here is the thing, because it is a viral disease, for most of them we do not have real cure. We have ways to inhibit or slow down the infection, we have ways to treat the symptoms, but we do not have cure. So, people who are already immune compromised, they suffer a lot when it comes to hepatitis A and hepatitis E, it is endemic in many regions in India. Now, let us look at astrovirus. It is single standard RNA virus and it is a leading cause of gastroenteritis.

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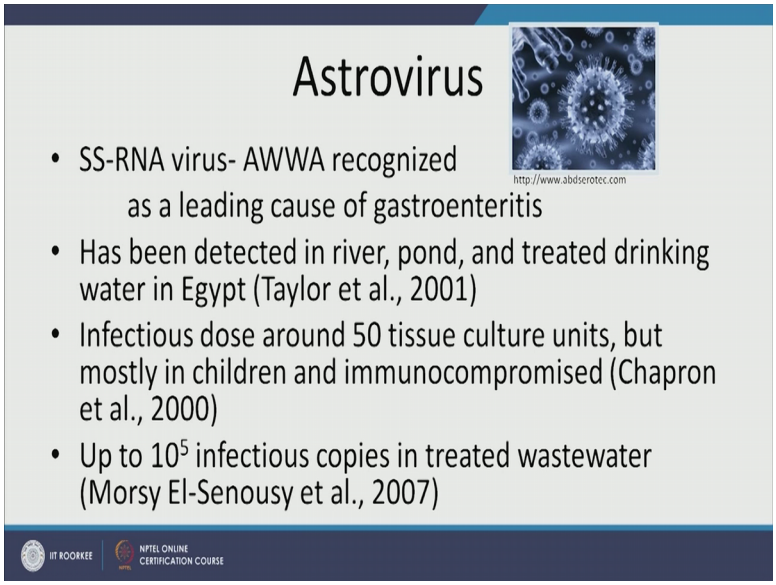

It has been detected not only in Egypt, in elsewhere too and its infectious doses are around 50 tissue culture units, but it usually infects people who have poor immune system and they are very prevalent in wastewater. So, you know, for these viruses in these pathogens, there is always a background level concentration, that you can find in wastewater because someone or somewhere is carrying these pathogens in our community, it is not just sufficient amount to be infectious.

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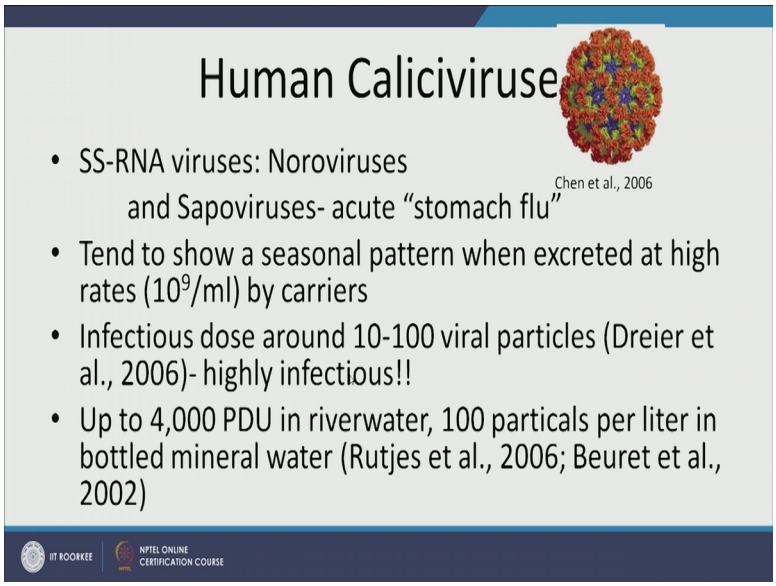

Now, next we have human calicivirus against another single stranded RNA virus. It is called norovirus, it causes really bad diarrhea. So, stomach flu it is called and it tends to show a seasonal pattern, when excreted at high rates by carriers. Infectious dose is about 10 to power 100 viral particles, same as hepatitis A and E and thus, you know it is highly infectious even in developed countries, people suffer from stomach flu, it is not pleasant. Alright!.

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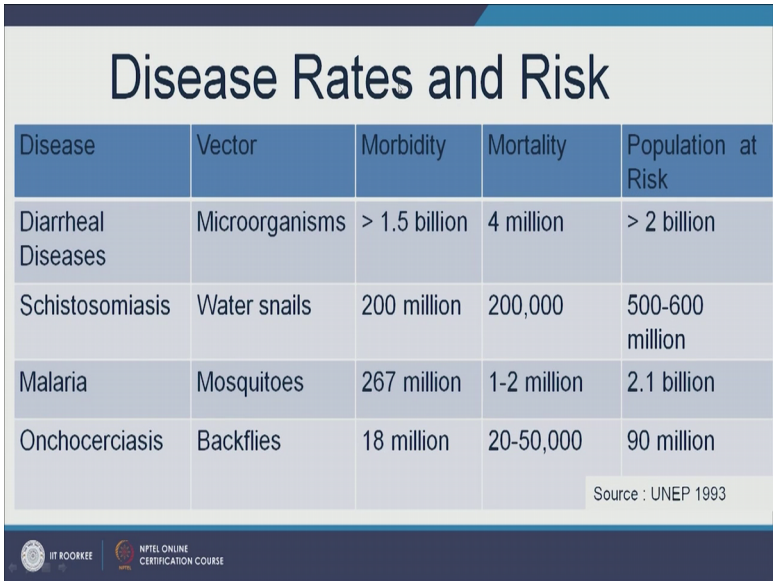

Now, let us look at disease rates in risk. Diarrheal diseases they have, not here in the units here, billion million thousand. So, we know the diarrheal diseases are more of an issue than these schistosomiasis, even more than malaria, even more than disease caused by black flies, definitely more than them, but it also has higher population, that is at risk.

But the important thing is that, these numbers are extremely high. Every single human life is important and most of us cannot even imagine what these numbers are, but I hope this gives you an idea of how much work needs to be done, when it comes to water treatment and ensuring the portability of water by the time it reaches the consumer.

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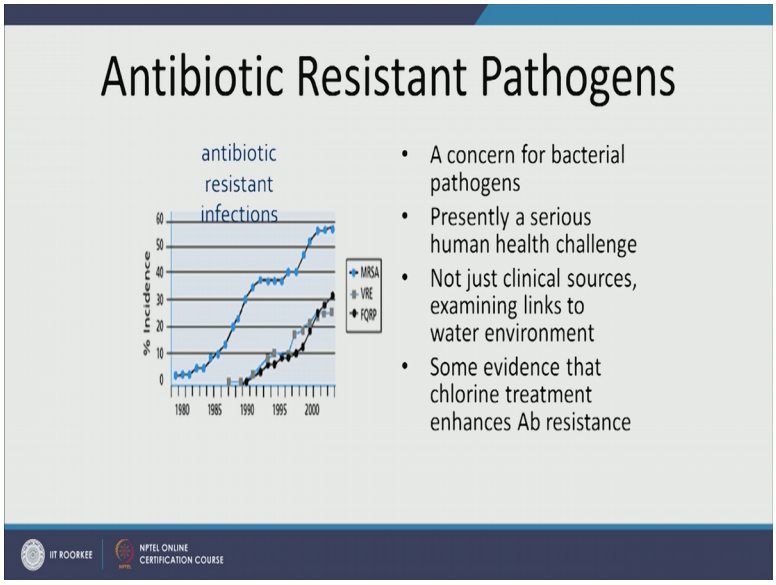

Now let us talk about antibiotic resistant pathogens. Now, we know the pathogens and I told you the list of pathogens which is not exhaustive, but it is a good starting point, especially when it comes to developing country like India and it is very relevant here. Now, imagine these pathogens were deadly enough to begin with, and who have enough contribution to morbidity and mortality, now acquire the capability to not respond to drugs. So, I have some disease, let us say typhoid, caused by salmonella typhi and I am giving it medicines, but it is not responding, because it is resistant to the antibiotics now.

And this is quite common in India now. If you remember, now the doctors are prescribing different antibiotics and very soon, they will run out of them. So, the reason they are responding to their prescribed different antibiotics is, because people know that

the diseases are no longer responding to it, for example, let us look at tuberculosis. (TB) caused by a particular kind of micro bacterium, a bacteria.

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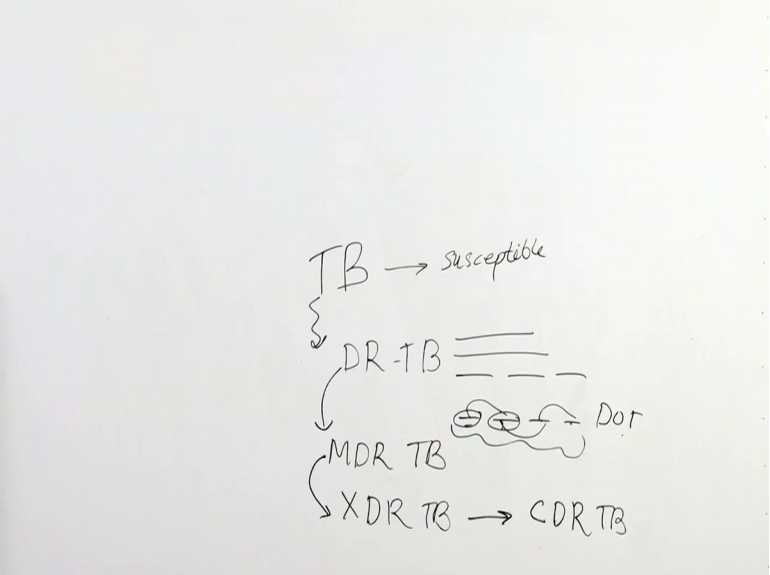

Now, long time ago when I was a kid, I had tuberculosis. Many people in our community had this, including me but we all were very fortunate to live in a good place, with a good government hospital. We all completed our treatment and we became fine. You know, we are healthy.

So, our tuberculosis at that time was susceptible, to exposure of antibiotics. After few years, because many people were not completing the tuberculosis treatment and for many other reasons, we will talk about the reasons later when we talk about antibiotic resistance in detail; tuberculosis started developing drug resistance. So, they developed what is called drug resistant tuberculosis. So, people found out that there were patients, who were not responding to certain antibiotics.

So they, let us say, they did not respond to the first line of antibiotics and they started giving them another antibiotic and eventually, they started giving them multiple antibiotics together, because the drug was inefficient in killing the mycobacterium tuberculosis, when it was just used once and then people developed a dot program for treating antibiotics and in this dot program, they skipped from one antibiotic to another.

So, if they are microbes that are resistant to this, hopefully they will not be resistant to this and when we start this treatment, they will die out and those who are resistant to it, will die here and those who are resistant to it, will die here. Eventually, the patient will be cured, that is the hope. So, from drug resistance, as a result of the playing around with so many antibiotics, not just during the treatment, but also by contaminating our environment, we develop multi - drug resistant and tuberculosis.

So, for example, why did we have dot program, because we found out that tuberculosis was multi drug resistant. So, not all tuberculosis bacteria in a patient will be susceptible to all of these or any one of these antibiotics. Then we came across extremely drug resistant tuberculosis. So, you know, even if a microbe is resistant, it is resisting it as sufficiently or extremely high dose of these drugs might be able to kill the bacteria.

But now, we are getting into extreme drug resistance and there are instances, where people believe, now we have completely drug resistant tuberculosis which does not resist to any drug, the incidences are far low and there is just some, I give it over, whether we should declare, we have got this or not complete drug resistance, but this is an example to show you how within less than 3 decades, tuberculosis has gone from drug resistant to extreme drug resistant tuberculosis.

So, what happens when pathogens acquire drug resistance, especially a problem for bacterial pathogens, it is a serious human health challenge? We are going back to post antibiotic era and here is the thing; we have studies that show us that it is not just dependent on the hospital, you know, giving drugs and making people resistant, making pathogens resistant, it is not the only reason. In fact, environment, especially aquatic environment and water environment has a huge role to play and some evidence is showing and there is a recent paper that I will give you as part of your homework, but Dr. April Goo has shown that chlorine treatment enhances antibiotic resistance.

So, notice here, this is MRSA, which is methicillin resistant staphylococcus auras and then, we have the vancomycin resistance enterococci. Now, here is the thing that vancomycin, is a very important drug. It is the last line of drug, if a microbe is not very good for human being either. So, we do not give vancomycin until the microbe has stopped responding to all other treatments, even this resistance has been growing, even vancomycin resistance has been growing. Now here is how they can do these.

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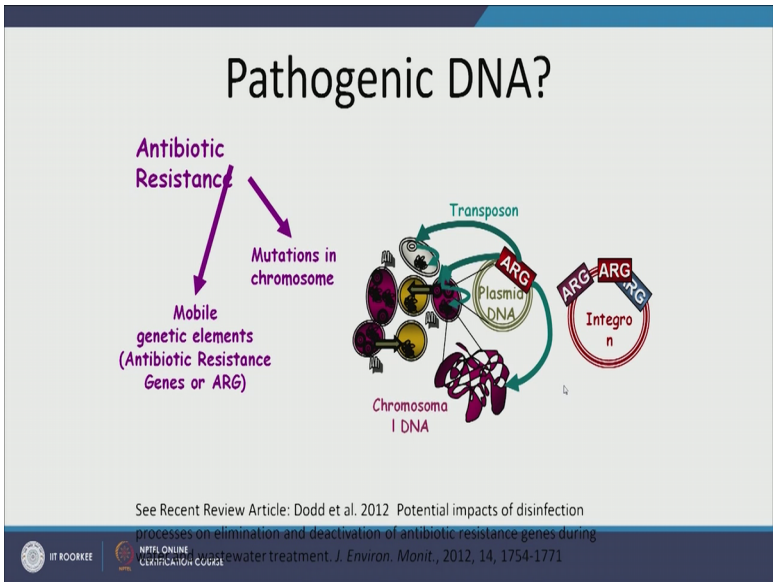

How does antibiotic resistance operate, how do microbes become antibiotic resistant? So, this slide is titled pathogenic DNA. Now, here is a thing, people are noticing that antibiotic resistance is not necessarily a characteristic of the microbe, but it is an acquired characteristic - something they can share, something they can inherit. So, they can undergo horizontal and vertical transfer of this capability.

Now, if you remember in the first few lectures, I talked about DNA and showed how DNA encodes for the capabilities of microbe, what protein micro can express, what job it can do or not depends on its DNA. So, antibiotic resistance is an example of pathogenic DNA. Either because there is a mutation in chromosome and then in presence of antibiotic, that could be mutation. Now, if a farm finds that, “Oh! Well I can survive antibiotic resistant because I have this mutation and then it completes all the susceptible microbes and now, we have antibiotic resistant pathogen. This is one way. Then, there are other ways that it can transfer horizontally.

So, horizontally is like let us say that, this has antibiotic resistant in it then, it can transfer this to other microbes and they can also acquire antibiotic resistant genes or ARG and this transfer happens., usually by mobile genetic elements and if you remember, we talked about plasmid, which is extra chromosomal a native material and they are very easy to transfer from one bacteria to another, and we have already talked about horizontal gene transfer. So, this slide should remind you of that. So, integron, plasmid and

transposon all of this play very important role, they have mobile genetic elements, they play important role in transmitting and transferring antibiotic resistance.

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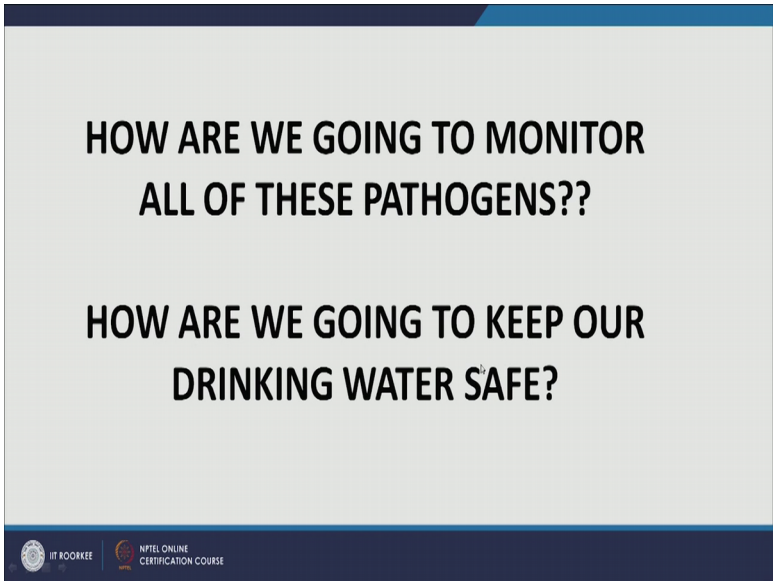

So this is a very important question before us. So, we need to monitor these pathogens and we need to keep our drinking water safe. So, once we have monitored the pathogens we not only now need to know what kind of pathogen it is, but we also need to know if the pathogen is antibiotic resistant or drug resistant and if it is, then we need to have different kinds of treatment methods.

So, that not only we kill ourselves, but we also destroy the pathogenic DNA or the genetic material that gives a microbe the capability to resist antibiotics because if we do not, we might kill the pathogen, but the genetic material might still be lying around, for some other microbe to come and pick it up, because here is the thing with horizontal gene transfer, not only microbes can pick up genetic material from a live microbe, but they can also pick up stray genetic material. Let us just say, in their environment.

So, this is a very important challenge for humankind right now; how are we going to keep our drinking water safe and today, at the conclusion this is lecture, I want to introduce you to the 3 mechanisms that we use for monitoring pathogens in drinking water. The usual mechanism is that; let us use an indicator microbe. All these pathogens are very hard to handle and they all, when we try to test for them, one of the way, means is that let us culture them, let us grow them and if they grow, then we know they were

present, yeah! Because spontaneous generation does not happen, but growing them is very tricky and also it is very dangerous, because we can, the laboratory personnel can get exposed to the pathogens.

So, what we do is, we use indicator microorganisms, we grow them. These microorganisms tell us that a fecal matter is present or not. If a fecal matter is present in the water, then we do not want to drink it, because pathogens might be present. So, the way we monitor right now these pathogens is, we look for this fecal matter indicators.

So, there are microbes that are present only in the gut of humans and certain other animals and we look for these microbes, instead of looking for the pathogens. So, one of the ways we do is, we grow them here, on our membrane and typically this is filtered drinking water. It is placed on an EOC in ethylene blue medium; it is selective for only lactose fermenting bacteria which are coli forms. So, coli forms, the colony forming bacteria and these are the ones that grow in our gut, we incubate them at 37 C and this dark, shiny appearance will tell us that coli forms are present and then we can count them.

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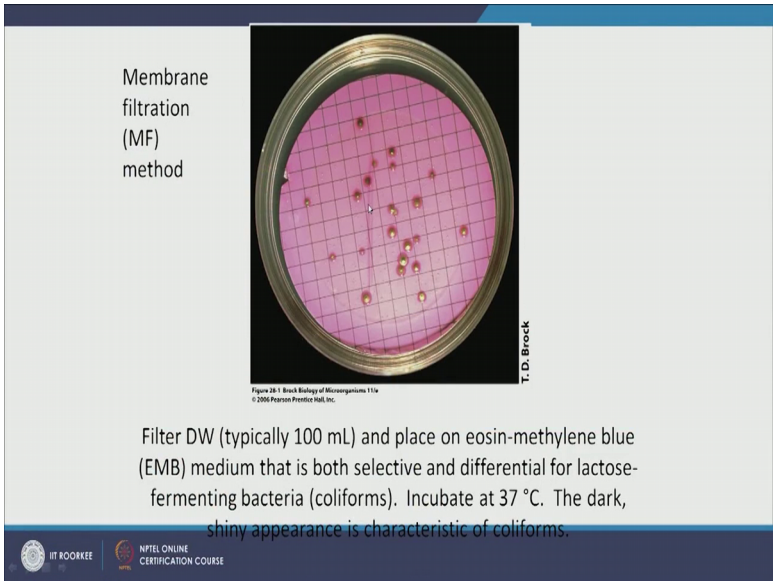

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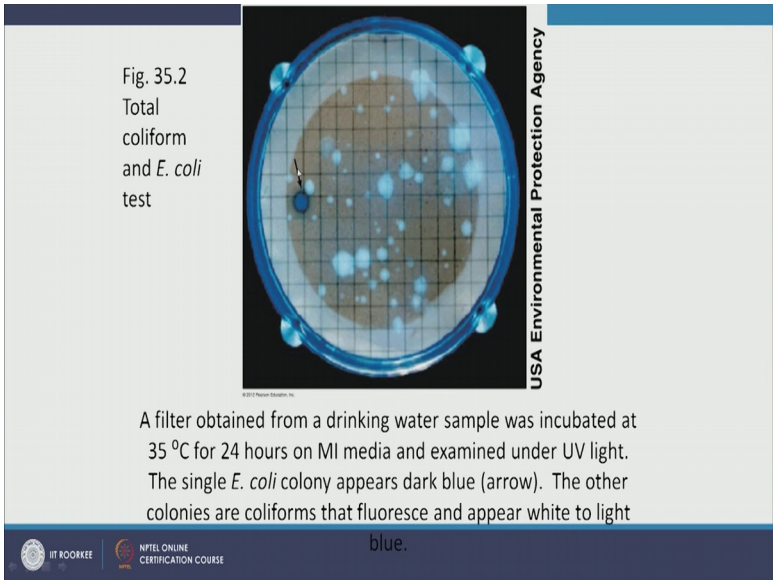

The other is, how we want to separate total coli forms. So, coli forms are tiny microbes from colonies - colony forming microbes. Most of them are gut microbes, but not all of them. So, if I want to find out how much of it is fecal coli form and how much of it is total coli form, because some coli form could be naturally found in soil, sediments and other environments. So, for that what we can do is, this here on MI media and all these light - white color colonies, actually we are seeing this under UV lamp.

So, the same thing here, the same plate here, now we are seeing under UV lamp and the one,, that shows white we know these are our coli forms and then, I am not sure how clearly you can see this, but there is a dark blue colony here, this is E coli. So, we now know how much fecal coli forms are present and how much total coli forms are present.

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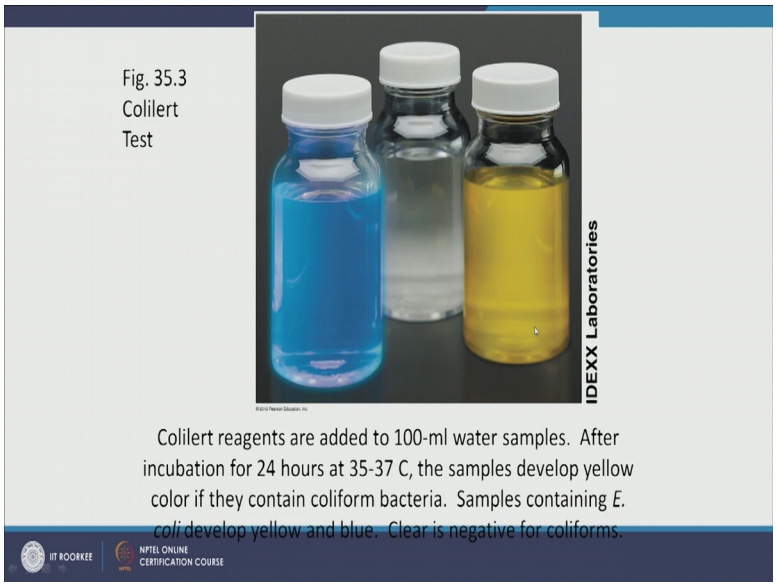

The other is collier test. Now, collier test is also used in India. So, what we do is, we add collier which has some food and in some regions, we add them in water and then, we incubate them at 35 – 37 C for one day and depending on the change in color, we can tell what kind of bacteria it is. So, if it is clear, nothing changes we know there are no coli forms. If they develop yellow, we know there are coli forms and if they have fecal coli forms, it will turn blue.

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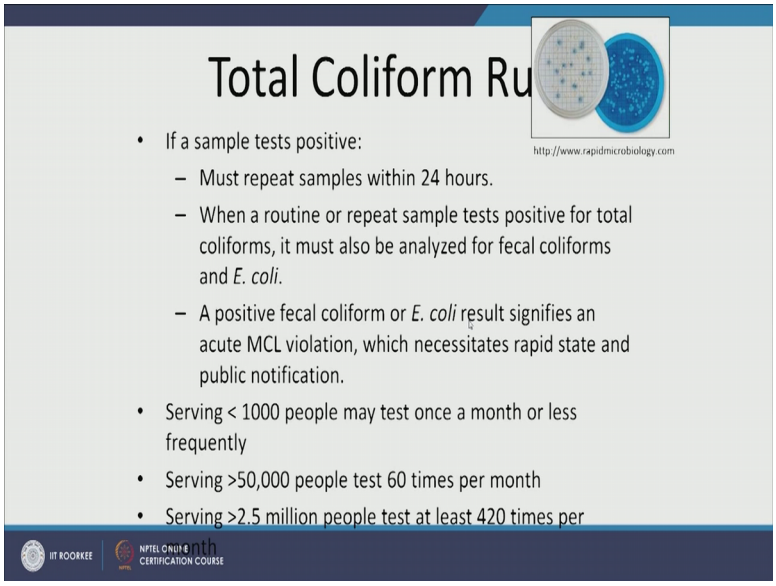

So, before I go ahead and talk about total coli form rules, look here. It is very important to understand that total coli form is only an indicator organism. But it is very easy to do and very safe practice, relative to in comparison to other techniques which actually directly detect the pathogens.

Now, if we have a sample that tests positive for these, we should repeat them within 24 to make sure it is not a false positive and then when, they test consistently positive for total coli form, then we test them for fecal coli form and if they are testing, if they have fecal coli form then, the water is not safe for drinking and then, we have certain rules about how many times these tests should be done.

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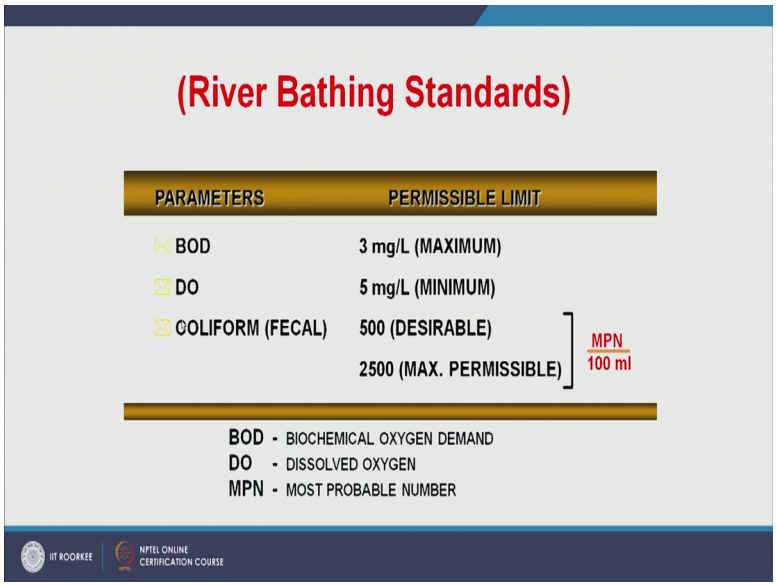

Now, these are some river bathing standards we have. Look here, BODDO we will talk about them later, but look at coli form. Fecal coli form for bathing in river, we want up to 500 not more than that MPN per 100 ml, but if there are more than that, then we do not penalize anybody and total coli form, that we want are up to 2,500 this is maximum permissible. So, we want a coli form, fecal coli form to lie around 500 but definitely, not exceed to 2,500.

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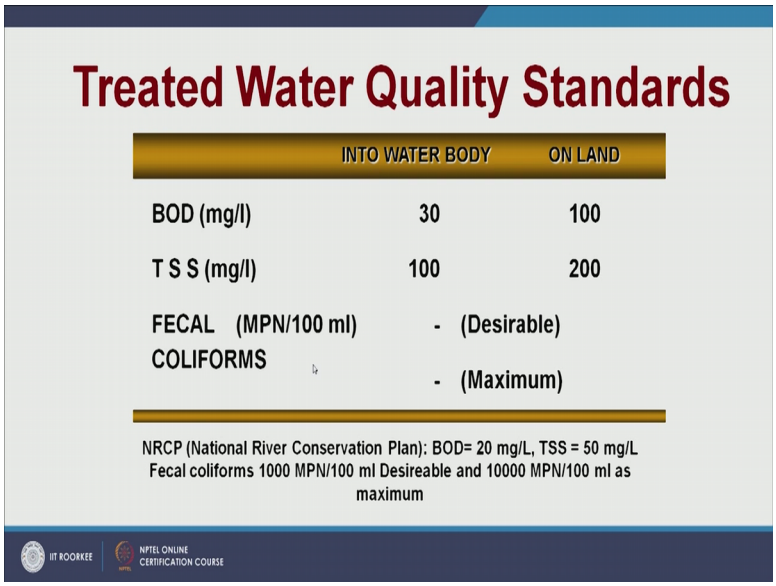

Now, these are treated water quality standards. We want that our fecal coli forms are 0, nothing none is allowed. Alright students, so, this is all for today and in the next lecture we will go ahead and we will talk more about drinking water microbiology, the challenges that we have and how are we trying to handle them in our country. Now, before I bid adieu in this lecture, I do want to mention that, please note, not all bacteria are pathogenic, a very small portion of them are pathogenic.

Even without pathogens, usual drinking water has lots of microbes in earth and the purpose of disinfection is not to kill all the microbes, some of them might actually be helpful for us. The purpose is to only kill the pathogens, but we do not have any technology to do this selective disinfection and thus, we make do with complete disinfection, using chlorine and other disinfecting agents, but there are many - many challenges and I will talk about them in the next lecture.

Thank you very much.