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Module - 7 Lecture - 32 Introduction - II

Welcome everyone to the second lecture in Environmental Microbiology. This is the second part of the introduction.

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CONCEPTS COVERED	
 Microbial causes of death The basic unit of life – the cell 	
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We are going to look at microbial causes of death and the basic unit of life.

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Most of you know by now that the first time we think about microorganisms, it is because of disease and fatalities due to those diseases. So, what we call morbidity and mortality is one of the main reasons why we take an interest in many of the microorganisms or pathogens, as we call them. What we have over here in this slide are 4 graphs that are arranged by different nations, which are based on their income.

So, right at the top, in the upper left corner, we have the top 10 causes of death in low-middle income countries in 2016. After that, going clockwise, we have upper-middle income countries. Then we have high-income countries and low-income countries. Let us start with low-income countries. And you can see, out of the top 10 causes of death, we have lower respiratory infections, diarrheal diseases, HIV or AIDS, malaria, tuberculosis, birth complications, and birth asphyxia.

The red bars are communicable diseases, and most of these communicable diseases tend to be due to microbial organisms. So, if you look at the numbers, that is, I think that is 7 out of the 10 top causes of death in low-income countries are related to communicable diseases; and most of them, as I said, are related to pathogenic microorganisms. As the income level of the country increases, what happens?

You get better infrastructure, better health care, better education, all these factors contribute to less morbidity and less mortality for the society, for the population. So, if you look at lower-middle income countries, you can see, it has dropped; the number of red bars has dropped to 4 from 7. And these are lower, again, lower respiratory infections. Now, most of us know that lower respiratory infections are related to either fungi, bacteria or viruses.

And diarrheal diseases, most of them are related again to microbial infections. And birth complications may or may not be related to microorganisms. Tuberculosis is again due to a microorganism that causes tuberculosis. So, you can see that most of these diseases which are causing deaths in low-income and middle-income countries are all because of pathogenic microorganisms.

These are extremely important causes of death, which can be controlled with appropriate infrastructure, with appropriate medical care and health and awareness and education. So, you can see the difference. Low income, lower middle income, upper middle income; lower respiratory infections are the only category that is still there in the top 10 causes of death; and that is related again to fungal, bacterial and viral infections.

Then we come to high-income countries, and this is something that remains fixed in these highincome countries. So, it is much harder to control respiratory infections as compared to let us say, waterborne diseases, foodborne diseases that are all related to microbial pathogens. So, diarrheal diseases are very common, like I said, in low-income countries; and these are often related to contaminated food and water.

And when I say contaminated, I mean microbial contamination mainly by bacteria and protozoa. You can have worms as well, helminthic organisms. But these are some of the major pathogenic organisms, microorganisms that tend to cause morbidity and mortality in low-income countries where the infrastructure is lacking. So, when I say infrastructure is lacking, what I mean is clean water supply, good sanitation, good drainage systems; these are the things that help to avoid morbidity and mortality due to pathogenic microorganisms.

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So, that is, this data was from WHO. Now, let us take a closer look at what is happening in our own country. In our own country, I do not have too much data, but I have one set of data from 1993. And the top 10 causes of death arranged by gender is what is shown in this graph. So, the first one is circulatory system disorders. And that is followed by infectious and parasitic diseases.

Now, these infectious and parasitic diseases, that are the number 2 cause of death, and that is 18.3% for males and just a little bit higher than 15% for females. Now, most of these infectious and parasitic diseases are caused by microorganisms. The only other set of organisms that are part of this group are the helminthic organisms. So, you can see how important our understanding of microbiology is for better health care for the entire society.

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Rank	Cause of Death	Male	Female	Person
1	Cardiovascular diseases	20.3	16.9	18.8
2	COPD, asthma, other respiratory diseases	9.3	8.0	8.7
3	Diarrheal diseases	6.7	9.9	8.1
4	Perinatal conditions	6.4	6.2	6.3
5	Respiratory infections	5.4	7.1	6.2
6	Tuberculosis	7.1	4.7	6.0
7	Malignant and other neoplasms	5.4	6.0	5.7
8	Senility	4.0	6.5	5.1
9	Unintentional injuries: Other	5.2	4.5	
10	Symptoms signs and ill-defined conditions	4.6	5.0	

So, how have things changed from 1993 to, let us say 2003, in one decade? We now have a slightly different set of causes of death, and in that, we have 3 categories. So, we have diarrheal diseases. Now, you probably know that diarrheal diseases are one of the biggest causes of mortality in the age group of 0 to 1 and 0 to 5 years old. So, newborn children, especially the ones that do not have access to clean food, clean water, are the ones that are most at risk of diarrheal diseases.

So, these are the ones that are the highest morbidity and mortality cases. Then we come to respiratory infections and tuberculosis. So, these are number 5 and number 6. And it is very difficult to control respiratory infections. You know that you can control water, you can control food, but to control airborne diseases is very, very difficult. We are living through the COVID pandemic and we have realized how difficult it is to control such an infection.

So, these are basic diseases that are related to, like I said, morbidity and mortality. And we do understand that these types of microorganisms are something that we need to keep in mind and we need to make sure that we have some degree of understanding of their behavior and how they can affect us.

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Let us now come to something that you probably already know, but it is worth reviewing, and that is the unit of life. You have studied at some point in your school education that the unit of life is a cell. Now, we in microbiology, are going to restrict ourselves to unicellular organisms. And as I defined in the very first part, these unicellular organisms, unlike us, higher organisms, can live an independent existence.

They may be found in clusters, but they are entirely capable of living an independent existence without depending on any other organism or even their own; or even individuals of their species. So, this is our basic cell. It takes up nutrients. So, when I say cell over here, I am talking about a microbial cell. So, this microbial cell is going to absorb nutrients from its environment.

These nutrients can be organic or inorganic. And in the process, it will utilize these nutrients for creating what we call new biomass. So, this new biomass is going to be converted and the cell will be able to replicate. One single cell will replicate itself, become 2 cells; 2 will become 4, and 4 will become 8; and all of that, right. So, all this is the new biomass.

In the process of reproduction as well as growth, new cells will be created and some amount of waste will be generated. So, these excreted substances, may be fragments of biopolymers, they may be fragments of macromolecules, they may be fragments of DNA; whatever they are, they are going to be excreted back into the environment. So, this is our basic understanding.

This is a simplistic understanding of how a cell, how a microbial cell can survive in the environment. Now, different analogies can be used to describe a microbial cell or even any other cell. So, a cell can be compared to a biochemical reactor or a coding device. If I want to compare it to a biochemical reactor; you might say, why does it resemble a biochemical reactor?

The simple thing is that it is taking in chemicals. Just like a biochemical reactor, you have material that is added into the reactor; it is processed in the reactor; some of these chemical compounds will be transformed; new compounds will be generated, and that is the output from the reactor. That is our biochemical reactor. So, how is the cell similar? So, the cytoplasm of the cell is where new compounds are created from whatever is taken up from the environment. So, to that extent, it is a biochemical reactor. So, all the compounds that are required for the processes of growth and reproduction; remember, the biological objectives; every organism has only 2 objectives; one is to grow and survive, and the second is to reproduce. So, these are the 2 major objectives from a biological standpoint. So, the cytoplasm is where you have all the compounds for cell growth and function.

What is the nucleus doing? The nucleus contains the DNA, and this DNA is necessary for the cell to create copies of itself. Because reproduction means they are producing copies of themselves. So, the nucleus and nuclear region have, whether it is a bacteria or higher eukaryotes and so on; I will come to all those differences in a little bit. This is the DNA where the information required for replication and reproduction is stored.

So, this is what we call the genetic code. And without this genetic code, the cell cannot reproduce. So, the species survival, and basically, the survival of the species depends on the reproduction of the individuals. And therefore, it is also a coding device, because it is that code that has to be passed on from one generation to the other.

Is it an open system or a closed system? The answer is - it is an open system. An open system is defined by the fact that it takes up material as well as energy from the environment and puts out energy as well as a mass into the environment. So, by definition, it is an open system that is separated from the environment, simply by a cell membrane. A cell wall is not necessarily present in all cells. I will come to that again in a little bit.

Are these systems in a state of equilibrium with the environment? Are they in steady state? Are they unsteady or non-steady state? Are they static systems? Are they dynamic systems? By definition again, all living organisms, as long as they are alive are dynamic systems. They are not at equilibrium. Most of them are not at equilibrium, because they are constantly taking up material from the environment and spewing out toxic material into the environment.

So, there can be situations where the cell may be dormant; and you may want to consider that as a steady-state. You may consider endospores to be static. But these are descriptions that I have marked them with question marks because they depend on the nature of the situation. It depends on the environmental conditions. So, we are going to concentrate, like I said, on unicellular organisms, microorganisms, that have an independent self-contained existence. And that is the limitation of whatever we are going to do. There are multicellular higher organisms that have cells that are interdependent. They are differentiated into tissues, organs that have specific functions. If you remove a cell from your skin or any other part of your body and give it all the nutrients that it needs, it is still not going to reproduce, because it is dependent on other cells for all its functions.

So, every cell in our body is; because we are multicellular organisms, we are not microorganisms, we are multicellular organisms, and every cell in our body is differentiated and it has specific functions, and it is interdependent on other cells and tissue and organs within the body. So, we are not going to go there. We are going to restrict ourselves to unicellular microorganisms.

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Characteristics of living cells

- Metabolism: cell is an open system takes nutrients from the environment, transforms it into biomass and energy and the output is waste excreted into the environment
- Reproduction: cells reproduce themselves based on their genetic code
- Differentiation: cells can adapt to new environmental conditions such as changing into spores to survive in hostile conditions
- Communication: chemicals released or taken up by cells are used to signal changes in the environment, etc.
- Movement: Cells can move in response to light, chemicals, etc.
- Evolution: Cells modify themselves to adapt to new conditions resulting in new species over long periods of time

How do we define whether a cell is living or nonliving? So, the textbook that I am referring to here, has 6 different characteristics that are considered to define a living cell. The first thing is metabolism; is the cell capable of deriving both mass as well as energy for itself. Is it dependent on others or is it capable of taking up nutrients from the environment, transforming it into biomass and energy, and excreting what is not required?

So, the first thing is metabolism. The second thing is reproduction. The cells have to be able to reproduce themselves based on their genetic code. The third is differentiation. The cells need to adapt themselves to new environmental conditions. And will they be able to survive hostile conditions? So, let us say, the environment; let me give you a simple example. Most microorganisms require a lot of water in their environment to survive.

Now, supposing you have a change in the situation and there is very little water in the environment; will these microorganisms cease to exist? Will they die? The answer is, some of

them can change form; they can transform themselves into what are called endospores or spores. These spores have been found to be able to survive tens of hundreds of thousands of years. There are reports in the literature, where people have claimed that spores can survive even thousands of years. So, these are methods that the cell has for adapting itself to new environmental conditions.

Can they communicate with each other? The answer to that is yes. They release chemicals into the environment, and these chemicals can be used as signals for other cells of the same species to understand what is going on.

So, I will show you some examples a little bit later in subsequent topics, where certain bacterial cells will gravitate towards or rather move towards greater oxygen levels or greater substrate levels, meaning food levels, greater light levels, and so on. So, this communication and movement are related to whatever is required by that species of bacteria; it may be light, it may be chemicals, it may be oxygen, whatever it is.

And then finally, we come to evolution. Are these cells capable of evolving and adapting? One of the things that we have already seen is adaptation to new environmental conditions. Now, if the change in environmental conditions is permanent, then these cells will modify themselves for those new conditions; and perhaps a new species will result from those conditions. So, these cells are capable of modifying themselves, adapting themselves to new conditions; and that will result in new species over a long period of time.

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As I said, what is the biological objective for all organisms? Reproduction and growth of the cell are the 2 main biological objectives. Now, how will those objectives be achieved? The first thing is replication of the genetic material, which is the DNA. You also need an ample amount

of energy. Where is the energy going to come from? So, the nutrients that are absorbed from the environment are going to serve two purposes.

They will provide mass as well as energy for the microorganism to create new biomass. Remember that simply replicating the DNA is not enough. We need copies of the DNA as well as new biomass. So, all this is required and you need to create new macromolecules, new carbohydrates, proteins, fats, nucleic acids; all these compounds which form the entirety of any given cell, have to be reproduced.

So, reproduction and growth require both those functions, the biochemical reactor functions as well as the coding functions. So, when I was talking about the biochemical reactor function; so, when we say it is a chemical machine or a chemical reactor, chemical transformation is occurring inside the cell. How are these chemical reactions happening? Enzymes act as catalysts for these biochemical reactions.

Most of these biochemical reactions are impossible without enzymes as catalysts. So, whether they are catabolic or anabolic; catabolic means breaking down and anabolic means building up. And so, you have polymers being broken down to monomers, which is catabolic; and then monomers being used to build new polymers, and that is anabolic. So, that is what we have over here.

And energy is generated, not in all of these reactions, but many of these reactions. So, we are going to look throughout the remaining part of the course, we are going to look at how different species of bacteria have found ways and means of generating energy by combining several different chemicals or compounds under different conditions. And that allows them to generate ATP. ATP as you know is the energy currency of all living cells.

So, that is the chemical reactor or the biochemical reactor part of the analogy. And then we come to cell functions as coding devices. What is the code? The code is the genetic code. So, you have the DNA; it has a particular code; that code has to be replicated in exact form for the new cells to form and continue to survive. If there is any damage to the DNA code, it may or may not survive.

That organism may or may not; when I say survival, it may or may not be in a position to reproduce, and so on. So, the first thing is that the DNA has to be stored, processed, and replicated. That is the first requirement. The second requirement is: you have a single parent cell; I am talking about bacteria; the simplest example. You have a single-parent cell. And as it absorbs nutrients from the environment, it will split apart into what we call 2 daughter cells. So, the initial DNA has to be copied. 2 copies have to be formed; they have to be exact, and the 2 daughter cells are going to have the same genetic code. So, that is essential for the species

to reproduce or to continue. Each strand of the DNA; you know that the DNA is a doublestranded molecule, it is a helical structure, it has a double-strand, and each strand of the DNA is going to serve as a template for generating RNA.

Now, there are 3 types of RNA. There is a messenger RNA, a transfer RNA, and a ribosomal RNA. Now, these 3 different types of RNA are going to do different parts of the process. I have given references to figures in the textbook. You can refer to them. They are very simple, neat sketches of how the different types of RNA are involved in what we call the transcription process.

So, a single strand of the DNA is going to serve as a template for generating RNA. This RNA is then going to serve as a template for producing specific proteins as well as enzymes. And that process is called translation. So, we have the reproduction of the DNA; then the DNA goes to RNA in the process of transcription and RNA goes to proteins in the process of translation. **(Refer Slide Time: 24:01)**



Now, let us take a little quick look at the chemical structure of DNA as well as RNA because these are the nucleic acids that are the basis of the genetic code and how it is reproduced in subsequent generations of a given species. So, we have 5 different nitrogenous bases. We have 2 categories of bases, pyrimidine, and purine. You have cytosine, thymine, and uracil. Cytosine is found in DNA as well as RNA. It is shown by the single letter C.

Thymine is shown by the letter T. It is found only in DNA. Uracil is found only in RNA. We have 2 purine bases with 2 rings. Adenine is also found in ATP; it is present in both DNA and RNA and ATP, which is different. And then we have guanine, which is shown by the letter G. And that is also found in both DNA and RNA.

So, I have given you the molecular weights for each of the bases over here. So, you can calculate the molecular weight of either a nucleotide or a nucleoside. We will come back to this issue later. So, these are the letters C, T, U, A, and G, that refer to the individual bases. So, you might say that the genetic code of all living organisms on the planet, as far as we know, is based on these 5 letters. So, the alphabet that nature is using is not very diverse.

In fact, you might say, it has just 4 letters, because the DNA has 4 letters and the RNA has 4 letters. So, that is how limited it is. And the enormous diversity that you see around you, is based on just these 5 nitrogenous bases. So, all of life is based on just these 5.

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As I have mentioned in the previous slide that we know how the DNA is part of our genetic code and how the DNA provides a template for the RNA, 3 types of RNAs to be formed. And these RNAs are going to be coding for the proteins, and the proteins are the ones that do all the real work. So, how does all this happen? And what happens when, let us say, either the DNA or any of the subsequent molecules; if any of them gets damaged, what happens?

Now, nature has built-in defenses, and it does not allow just one thing to be made in only one way. So, there is what is called degeneracy in the code. And let us take an example here. So, each codon is made of 3 nucleotides. So, in the messenger RNA, we have a whole sequence of nucleotides. And each sequence of 3 nucleotides is called a codon. So, if we look at this rough sketch over here, this is an mRNA strand and each segment is a codon.

So, these are 3 nucleotides in the sequence. Now, each of these 3 nucleotides has a specific sequence, which is encoding for a specific amino acid. Now, we have 4 possible nitrogenous bases in both the DNA as well as the RNA, there is one that is different in both cases. So, we

have, in the case of the codons, we have 64 possible codons. Now, these 64 possibilities are going to be translated into only 20 amino acids.

So, what that means is that nature has built-in degeneracy. So, from a human point of view, we would normally think of this as inefficiency or what; but what that does from a natural perspective is that it allows some amount of change, it allows the species to continue replication and reproduction and so on, despite mutations in the DNA. So, that is the importance of the degeneracy of the genetic code, you might say.

So, these codons are written like I said, in their base sequence, in the mRNA, just like it is shown over here. And each set of 3 codes for an amino acid; because we have 20 amino acids and we have 64 possible codons, so, we have several codons translating for the same amino acid. So, leucine, which is an amino acid has 6 codons, alanine has 4 codons, and you can refer to the textbook for more examples of that.

And that is how, despite mutations, despite damage to the DNA, the species can continue to replicate its DNA. It can continue to produce proteins, despite any possible damage. So, that is something that helps the species to continue and survive.

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So, let us take another look at various types of microbes that we are going to be looking at. And you can see microbes all around you. You can see microorganisms. They are ubiquitous in the environment, which means, no matter where you go, where you are, whether it is too hot, where it is too cold, in your normal environment, you will find any number of microorganisms.

So, what I am going to show you in this slide and the next few slides are various examples of different types of microorganisms. So, since I work in the area of water, here are some

examples of tap water and wastewater. So, you can see, in the wastewater example, it is far more clear. You can see some somewhat round-shaped microorganisms. So, you can see these are coccus, but they have a slightly elongated shape.

So, these are all from wastewater. In tap water, it is not very clear where the microorganisms are. There is a lot of organic matter and you can see some regularly shaped microorganisms. Then, these are water, like I said, water and wastewater samples. I have some more examples. (Refer Slide Time: 30:43)



Here is a stained biofilm. Now, biofilm is: when bacteria or other microorganisms, grow attached to surfaces. So, when they grow attached to surfaces, you will find that they produce a sticky layer. And very often when you touch a surface, especially rocks that are underwater; when you touch fixtures, water fixtures which are in contact with water, very often you will find that there is a slimy feeling when you touch these surfaces. That slimy layer is generally a microbial layer or a biofilm. And your teeth are another example of biofilms growing inside the mouth. If you do not bathe regularly and all, you will have biofilms growing on other parts of the body as well. So, they are everywhere. Then you have stained biofilm. So, here you have a stained biofilm. And this particular biofilm has been stained with a fluorescent dye. These dyes will fluoresce green if the cell membrane is intact. So, you can see that if the cell is intact, it will fluoresce green. And the ones that have damaged cell membranes, are no longer living; and therefore they are fluorescing red. So, we use this kind of technique in fluorescence microscopy sometimes to differentiate between living cells and dead cells.

Here you have another example of microbial flora on the human tongue. Your own mouth is a huge reservoir of all kinds of bacteria growing in the mouth. And those of you who may have experienced it, if you forget to brush or something, you know that sticky feeling in the mouth,

that is exactly excessive microbial growth. So, this is a consortium of bacteria that have been colored. Different species that are growing on the human tongue; grow on the teeth, they grow on the tongue, they grow on the cheeks, inner cheeks, and all of that. So, here, the species, the different species have been falsely colored, just to differentiate them, and you can see the diversity, just on the human tongue. Then, this is marine water. So, you can see a large number of examples of different types of microorganisms; and larger ones as well as smaller ones.

So, you have cyanobacteria, you have algae, you have diatoms. All these things exist in any water sample in nature. So, if you take a sample from a river, a pond, marine water, sea, freshwater; no matter where you go, you pick a sample; even ice. You might say ice does not have microorganisms. It will have microorganisms. I will show you some photos of that in later lectures.

So, I also want to point out that microorganisms exist in the environment, they also exist within the body. Our ability to digest food is partly dependent on the presence of microorganisms within our bodies. And we know about probiotics, right. Yogurt or Dahi is considered a probiotic because it provides beneficial microorganisms which are helpful in digesting lactose, which is milk sugar.

So, it is not just outside the body that is full of microorganisms, but it is also within the body that we have a large number of microorganisms. So, they exist in the gastrointestinal tract; they exist in the mouth; they exist in other organs of the body, the skin, hair, ears, and so on. I have already mentioned that they exist in soil, air, ice, deep ocean water, sediment, even boiling water.

We normally think that boiling water is sterile water. Under normal conditions, which is what we are dealing with, that would be true. But if you go to, let us say, a hot water spring where the temperature is higher than 100°, and if you imagine that, oh! no microbes can survive over there; wrong again. You will find microbes that have adapted themselves to boiling water temperatures.

They are not the kind of bacteria that will exist in tap water. They are a different species of bacteria that have adapted themselves and remain viable in those conditions.

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(Video Starts: 35:35) What I want to highlight to you is that microbes are present in every possible environment on this planet, there is no part of the planet that is not colonized by these microbial communities. Some species of bacteria are capable of surviving anywhere, everywhere. And the same microorganisms are not necessarily going to be able to survive in a different environment.

So, these are the things that are important to keep in mind. Here, I have examples of microalgae. Microalgae are the ones that grow as unicellular algal cells. You can have multicellular algae as well. This is a Raceway Pond. It is a recent development in microbiology that algal biorefineries are being set up to create what is called oil. And this is algal oil or biodiesel of some kind. So, these are microalgae-based refineries. These are photobioreactors for cultivating microalgae. And these oils have been developed from that. So, these are relatively recent developments.

Here you can see a video of a paramecium feeding on bacteria. So, the bacteria are very small. These are the specks. And the large organism which is crossing the screen over and over again is a paramecium or protozoa. So, these are all microorganisms and they are all heterotrophs in this case. But you can have others as well. (Video Ends: 37:09)

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Okay, that brings me to the end of this particular lecture and we will continue with another topic in the next lecture.