MODULE – 5 MICROBIAL GROWTH AND CONTROL

Lecture – 1: Growth of bacterial cultures, growth curve and measurement of microbial growth

Growth of bacterial cultures:

Growth is defined as an increase in cellular constituents which leads to a rise in cell number. As we are aware, microorganisms reproduce by binary fission or by budding. In order to study growth, normally one follows the changes in the total population number. The cells copy their DNA almost continuously and divide again and again by the process called binary fission. Binary fission which has been described earlier as the form of asexual reproduction in single-celled organisms by which one cell divides into two cells of the same size. Fortunately, few prokaryotic populations can sustain exponential growth for long. Environments are usually limiting in resources such as food and space. Prokaryotes also produce metabolic waste products that may eventually pollute the colony’s environment. Still, you can understand why certain bacteria can make you sick so soon after infection or why food can spoil so rapidly. Refrigeration retards food spoilage not because the cold kills the bacteria on food but because most microorganisms reproduce very slowly at such low temperatures.
**Binary fission:**

In the process of binary fission, the cell elongates and the DNA is replicated. Cell wall and plasma membrane begin to grow inward and cross-wall forms completely around the divided DNA. At the end the cell separates into two individual cells similar to the parent cell and contains all the contents a cell requires for its living including DNA (Figure 1).

Few bacterial species reproduce by budding. In this method, the cell forms a small initial growth, it enlarges and then it separates from the parent cell. Some filamentous bacteria like actinomycetes reproduce by producing chains of conidiospores and a few bacteria simply fragment.

![Fig. 1. Binary fission in bacteria](image)
Growth Curve:

The increase in cell number or growth in population is studied by analyzing the growth curve of a microbial culture. Bacteria can be grown or cultivated in a liquid medium in a closed system or also called as batch culture. In this method, no fresh medium is added and hence with time, nutrient concentration decreases and an increase in wastes is seen. As bacteria reproduce by binary fission, the growth can be plotted as the logarithm of the number of viable cells verses the time of incubation. The curve plotted shows four basic phases of growth; the lag, log, stationary, and death phase (Figure 2).

![Fig. 2. Growth curve of a typical bacterial cell](image)

**Lag Phase:** As the cells are introduced into the new medium, no immediate increase in cell number occurs. During this phase, the cells are undergoing a period of intense metabolic activity involving synthesis of enzymes and various other molecules required to divide in the coming phase. This phase can vary considerably in length depending on the nature of the medium and the microorganism. The medium may be different from the one the microorganism was growing in previously. The cells may be old and depleted of ATP, essential cofactors and ribosomes; these must be synthesized before growth can begin. So, the microorganism requires time to recover and young, vigorously growing cultures and fresh medium are to be used for the lag phase to be short.

**Log Phase:** In this phase the cell starts dividing in a logarithmic way and this is also called as exponential phase and the growth is balanced. Cellular reproduction is high during this period and the plot during this phase is a straight line. The cells are most active metabolically during this phase and the population is most uniform during this phase; therefore exponential phase cultures are usually used in biochemical and
physiological studies. But during this phase, the microorganisms may be particularly sensitive to adverse conditions. On the whole, in this phase the cells are growing and dividing and increasing in cell number. The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation/ n (n = number of generations). Hence, \( G = \frac{t}{n} \) is the equation from which calculations of generation time can be derived.

Exponential phase or log phase is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, unbalanced growth results. The individual cells may take slightly longer than others to go from lag phase to the log phase, and they do not all divided precisely together. If they divided together and the generation time is same, the number of cells in a culture would increase in a stair – step pattern, exactly doubling every 20 min or a particular time – a hypothetical situation called Synchronous growth. In an actual culture, each cell divides sometime during the 20 min generation time, with about 1/20 cells dividing each minute – a natural situation called nonsynchronous growth or asynchronous growth which appears as a smooth line, not as steps.

Organisms in a tube of culture medium can maintain logarithmic growth for only a limited time. As the number of organisms’ increases, nutrients are used up, metabolic wastes accumulate, living space may become limiting factor and aerobes suffer from oxygen depletion.

**Stationary Phase:** Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space". During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce
secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process. Starving bacteria frequently produce a variety of starvation proteins, which make the cell much more resistant to damage. They increase peptidoglycan cross-linking and cell wall strength. The Dps (DNA-binding proteins from starved cells) protein protects the DNA. Bacterial pathogens like *Salmonella typhymurium* become more virulent when starved.

**Death Phase:** Due to the conditions during the stationary phase, the death phase is seen as there is a decline in the number of viable cells. This phase also is like the log phase where the cell number is declining in a logarithmic way. The cell is said to be dead if it does not revive itself and reproduce when incubated again in a fresh medium. In this phase, the number of live cells decreases at a logarithmic rate, as indicated by the straight downward sloping diagonal line. The duration of this phase is as highly variable as the duration of log phase. Both depend primarily on the genetic characteristics of the organism.

**Mathematics of Growth:**

Microbial growth during the exponential phase is very important and of interest to microbiologists and the analysis applies to microorganisms dividing by binary fission. The time required by a cell to divide is called the generation time or doubling time. In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or $2^0, 2^1, 2^2, 2^3 \ldots \ldots \ldots 2^n$ (where $n = \text{the number of generations}$). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature. This might vary from organism to organism depending upon the environmental conditions etc. For example in *E.coli* the generation time is 20 min and hence after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. The population is doubling every generation; hence the increase in population is always $2^n$. 
where \( n \) is the number of generations. The resulting population increase is exponential or logarithmic.

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. The **generation time** is the time interval required for the cells (or population) to divide.

\[
G = \frac{t}{n}
\]

\( G \) (generation time) = (time, in minutes or hours)/\( n \)(number of generations)
\( t = \) time interval in hours or minutes

\( B = \) number of bacteria at the beginning of a time interval
\( b = \) number of bacteria at the end of the time interval
\( n = \) number of generations (number of times the cell population doubles during the time interval)

\[
b = B \times 2^n \quad \text{(This equation is an expression of growth by binary fission)}
\]

Solve for \( n \):

\[
\log b = \log B + n \log 2
\]

\[
n = \frac{\log b - \log B}{\log 2}
\]

\[
n = \frac{\log b - \log B}{.301}
\]

\( n = 3.3 \log b/B \)

\( G = t/n \)

Solve for \( G \)

\[
G = \frac{t}{3.3 \log b/B}
\]

**Example:** What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?

\[
G = \frac{t}{3.3 \log b/B}
\]

\[
G = \frac{240 \text{ minutes}}{3.3 \log 10^7/10^4}
\]

\( G = 24 \text{ minutes} \)
Measurement of Microbial Growth:

A number of techniques are available in order to measure growth of microbial populations. Either population number of mass may be calculated ad growth leads to increase in both.

Direct measurement of cell numbers:

Bacteria or microorganisms can be counted directly on the plate and also called as plate counting. Advantage of this method is that it measures the number of viable cells. Disadvantage is that, it is time consuming and expensive as one needs media and other conditions need to be maintained. Bacteria counted on plate counts are referred to as colony forming units as a single cell or a clump of bacterial cells can lead to a colony which contains many cells. The colonies when they are counted in plate count method are to be present sparsely for accurate counting as overcrowding can lead to incorrect counting. To solve this, one has to adapt the serial dilution method in order to get an accurate count.

Serial dilution and pour and spread plate: Supposing one has to accurately count the number of cells given in a solution, then serial dilution needs to be performed. A 1ml of the sample is taken and transferred to a tube containing 9ml of sterile water and this process can be repeated until we reach a considerable dilution (say $10^6$ to $10^7$). Once the original inoculum is diluted one needs to perform a pour plate or a spread plate technique in order to count the number of bacteria present in the diluted sample and then the original sample. In pour plate method the diluted sample is poured into the petriplate and then the medium which is at nearly 50°C is poured over the inoculum and mixed by gentle agitation. With this method, colonies grow within the nutrient agar as well as on the surface of the agar plate. As certain disadvantages are encountered in this method like heat sensitive microorganisms might not grow and also bacteria when they grow within the nutrient medium might not be useful for diagnostic purposes. In order to avoid these problems, spread plate method is mostly used (Fig. 3). A 0.1ml of the diluted sample is added to the surface of the nutrient medium and spread uniformly with the help of a glass
spreader and after incubation, the colonies can be counted and the concentration of the bacterial cells in the original sample is calculated as follows:

\[
\text{Number of bacteria/ml} = \text{Number of colonies on plate} \times \text{reciprocal of dilution of sample}
\]

**Membrane Filtration**: This method can be used in order to study if the quantity of the bacteria is very small as in aquatic samples like lakes, streams etc. Membranes with different pore sizes are used to trap different microorganisms. The sample is drawn through these special membrane filters and placed on an agar medium or on a pad soaked with liquid media. After incubation, the number of colonies can be counted and the number determined in the original sample. Selective media or differential media can be used for specific microorganisms. This is mostly used for analyzing aquatic samples.
**Microscopic count:** The Petroff-Hausser counting chamber or slide is easy, inexpensive and relatively quick method and also gives information about the size and morphology of the microorganisms. These specially designed slides have chamber of known depth with an etched grid on the chamber bottom (Fig.4).

\[
\text{Bacteria/mm}^3 = \frac{\text{bacteria/square} \times (25 \text{ squares})}{50}
\]

Bacteria can be counted by taking into account the chamber’s volume and any sample dilution. The disadvantage encountered in this method is that fairly large volume is required and also it is difficult to distinguish between living and dead cells. Microorganisms of larger sizes can be counted by using electronic counters such as coulter counter; where in the number of cells in a measured volume of liquid is counted. This method gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells.

![Fig. 4. Direct microscopic count of bacterial cells](image)

**Indirect methods of measurement of cell mass:**

Population growth leads to increase in the total cell mass, as well as in cell numbers. The following methods can be used.

**Turbidity:** As bacteria grow/multiply in a liquid medium, the medium becomes turbid (Fig. 5). Spectrophotometer is used in order to measure the turbidity. A beam of light is transmitted through a bacterial suspension to a light-sensitive detector. The fact that microbial cells scatter light striking them, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. The
extent of light scattering can be measured and is almost linearly related to bacterial concentration at low absorbance levels.

![Broth culture showing turbidity](image)

**Fig. 5. Broth culture showing turbidity**

**Dry weight:** This method is mostly used for filamentous bacteria and moulds. The microorganism is grown in liquid medium, filtered or centrifuged to remove extraneous material, and dried in an oven and then weighted. It is time consuming and hence not very sensitive.

**Continuous culture of Microorganisms:**

**Batch cultures:** Nutrient supplies are not renewed nor wastes removed.

**Continuous cultures:** continuous provision of nutrients and removal of wastes takes place. The population can be maintained in the exponential phase and at a constant biomass concentration for extended periods.

These can again be categorized into two types:

**Chemostat:** Where sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed.

**Turbidostat:** It has a photocell that measures the absorbance or turbidity of the cell culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density.

Used in food and industrial microbiology.
REFERENCES:

Text Books:


Reference Books:

MODULE – 5 MICROBIAL GROWTH AND CONTROL

Lecture – 2: Influence of Environmental factors on microbial growth

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. An understanding of these influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms. Prokaryotes are present or grow anywhere life can exist. The environments in which some prokaryotes grow would kill most other organisms. For example *Bacillus infernus* is able to live over 1.5 miles below the earth’s surface without O₂ and 60°C temperature. These microorganisms which can thrive and grow in such harsh conditions are often called *extremophiles*.

The major physical factors which affect microbial growth are solutes and water activity, pH, temperature, oxygen level, pressure and radiation.

**Solute and Water activity:** Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings. Microorganisms need to keep the osmotic concentration of their cytoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. In a hypertonic environment, the prokaryotes increase their internal osmotic concentration through the synthesis or uptake of choline, proline, glutamic acid and other amino acids. A few prokaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions. The enzymes of these bacteria are altered for the requirement of high salt concentrations for normal activity. Halophiles grow optimally in the presence of NaCl or other salts at a concentration above about 0.2M. These have extensively modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes. They require higher potassium levels for stability and activity. The plasma membrane of halophiles is also stabilized by high concentration of sodium ions.
Water activity ($a_w$) is the amount of water available to microorganisms and this can be reduced by interaction with solute molecules (osmotic effect). Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it’s $a_w$ is low. Microorganisms differ greatly in their ability to adapt to habitats with low water activity. In a low $a_w$ habitat, the microorganisms must expend extra effort to grow as it should maintain a high solute concentration to retain water. Such microorganisms are osmotolerant or can grow over wide range of water activity or osmotic concentration. Most of the microorganisms grow at $a_w=0.98$ or higher.

**pH:** It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$$\text{pH} = -\log [H^+] = \log (1/H^+)$$

The pH scale ranges from 1.0 to 14.0 and most microorganisms grow vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH values between 9.0 and 10. The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity. *Cyanidium caldarium* (algae) and archaeon *Sulfolobus acidocaldarius* are inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperature. Drastic changes/variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Prokaryotes die if the internal pH drops much below 5.0 to 5.5. External pH alterations also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. The microorganism needs to maintain a neutral cytoplasmic pH and for this the plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkalophiles maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. The antiport systems probably correct small variations in pH. In case of
too much acidity (below 5.5 to 6.0) \( S. \text{typhimurium} \) and \( E.\text{coli} \) synthesize an array of new proteins as part of what has been called as their acidic tolerance response. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. Microorganisms can change the pH of their own habitat by producing acidic or basic metabolic waste products. In order to maintain the pH, buffers are often included in the media to prevent growth inhibition. Phosphate is commonly used buffer and a good example of buffering agent. Peptides and amino acids in complex media also have a strong buffering effect.

**Temperature:** Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. Beyond a certain point of higher temperature, slow growth takes place and damages the microorganisms by denaturing enzymes, transport carriers and other proteins. The plasma membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don’t work rapidly. In summary, when organisms are above their optimum temperature, both the function and cell structure is affected at low temperature, function is affected. The cardinal temperatures vary greatly between microorganisms. Optimum usually range from 0°C to as high as 75°C, where as microbial growth occurs at temperature extending from -20°C to over 120°C. Archaen \textit{Geogemma barossii} grows anaerobically at 121°C. The major microbial groups differ from one another regarding their maximum growth temperature. Upper limit for protozoans is around 50°C, some algae and fungi can grow at temperatures as high as 55°C to 60°C.

**Did you know:** Prokaryotes have been found growing at or close of 100°C. Now thermophilic prokaryotes have been reported growing in surface of chimneys or black smokers located along rifts and ridges on the ocean floor that spew sulphide-rich superheated vent water with temperatures above 350°C. These microbes can grow and reproduce at or above 112°C. The proteins, membranes and nucleic acids of these prokaryotes are remarkably temperature stable and provide ideal subjects for studying the ways in which macromolecules and membranes are stabilized. Some thermostable enzymes from these organisms have important industrial and scientific uses. For ex., the
Taq polymerase from the thermophilic *Thermus aquaticus* is used extensively in the polymerase chain reaction.

Microorganisms are classified into five classes based on their temperature ranges for growth.

1. **Psychrophiles**: Microorganisms grow well at 0°C and the optimum growth temperature of 15°C or lower and maximum at around 20°C. These microorganisms are isolated from Arctic and Antarctic habitats. They have adapted to their environment in several ways. Their enzymes, transport systems and protein synthetic mechanisms function well at low temperatures. The cell membranes have high levels of unsaturated fatty acids and remain semifluid when cold. At higher than 20°C, the psychrophiles begin to leak cellular constituents because of cell membrane disruption. Microorganisms such as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium* belong to this group. The psychrophilic *Chlamydomonas nivalis* turns a snowfield or glacier pink with its bright red spores.

2. **Psychrotrophs or Facultative Psychrophiles**: In this group many species can grow at 0°C to 7°C, optimum between 20°C and 30°C. The spoilage of refrigerated foods is mainly caused by microorganisms belonging to this group.

3. **Mesophiles**: Growth optimum around 20°C to 40°C, minimum at 15°C to 20°C and maximum at 45°C or lower. Most of the organisms fall under or within this category including human pathogens.

4. **Thermophiles**: The microorganisms in this group can grow at temperature of 55°C or higher, minimum is usually around 45°C and growth optima at around 55°C to 65°C. Mostly prokaryotes and a few algae and fungi belong to this group. The habitats in which they grow include, composts, self-heating haystacks, hot water lines and hot springs. Microorganisms have more heat-stable enzymes and proteins synthesis systems, which function at high temperature. Heat stable proteins have high organized, hydrophobic interiors, more hydrogen bonds and other non-covalent bonds strengthen the structure. Amino acids like proline make the polypeptide chain less flexible and chaperones also aid in folding of proteins to stabilize them. DNA also is stabilized by specific histone like proteins. The
membrane lipids are also stable and tend to be more saturated, more branched and of higher molecular weight. Archaeal thermophiles have membrane lipids with ether linkages, which protect the lipids from hydrolysis at high temperatures.

5. **Hyperthermophiles:** Few microorganisms can grow at 96°C or above and have maximum at 100°C; and growth optima between 80°C and about 113°C. *Pyrococcus* and *Pyrdictium occultum* are examples of marine hyperthermophiles found in hot floors of the sea floor.

**Oxygen Concentration:** An aerobe is an organism able to grow in the presence of atmospheric O$_2$ and the ones that grow in its absence is an **anaerobe**. Organisms which completely are dependent on atmospheric O$_2$ for growth are **obligate aerobes**, and it serves as the terminal electron acceptor for the electron transport chain in aerobic respiration and employs it in the synthesis of sterols and unsaturated fatty acids. Organisms which do not require O$_2$ for growth but do grow better in its presence are called **facultative anaerobes**. **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O$_2$ and grow equally well whether it is present or not. **Obligate anaerobes** like *Bacteroides*, *Fusobacterium*, *Clostridium pasteurianum*, *Methanococcus*, *Neocallimastix*, do not tolerate O$_2$ at all and die in its presence. Aerotolerant and obligate anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for the purpose. **Microaerophiles** are those organisms that are damaged by the normal atmospheric levels of O$_2$ (20%) and require O$_2$ levels between the range of 2% to 16% for growth. The nature of bacterial O$_2$ responses can be readily determined by growing the bacteria in culture tubes filled with a solid culture medium or a special medium like thioglycollate broth, which contains a reducing agent to lower O$_2$ levels (Fig. 6). Aerobic microorganisms are cultured, either the culture vessel is shaken to aerate the medium or sterile air is pumped. Anaerobic microorganisms require special anaerobic media containing reducing agents such as thioglycollate or cysteine may be used. Removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas is also preferred. CO$_2$ and nitrogen is added to the chamber since many anaerobes require a small amount of CO$_2$ for best growth. The technique in which gas pak jar is used can be used.
Prokaryotes and protozoa are found arranged among all the 5 types of microorganisms. Fungi are normally aerobic, but species particularly among yeasts, are facultative anaerobes. Algae are almost always obligate aerobes. The different relationships with O₂ appear due to several factors, including the inactivation of proteins and the effect of toxic O₂ derivatives. Enzymes can be inactivated when sensitive groups like sulphydryls are oxidised. A notable example is the nitrogen fixation enzymes nitrogenise which is very O₂ sensitive.

Oxygen accepts electrons and is readily reduced because its two outer orbital electrons are unpaired. The reduction products such as superoxide radical, hydrogen peroxide and hydroxyl radical can be resulted by flavoproteins, several other cell constituents and radiation.

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- \\
O_2^- + e^- + 2H^+ & \rightarrow H_2O_2 \\
H_2O_2 + e^- + H^+ & \rightarrow H_2O + OH^- 
\end{align*}
\]

These are extremely toxic because they are powerful oxidizing agents and rapidly destroy cellular constituents. As microorganisms can be killed, they need to protect themselves from such oxygen products. Microorganisms possess enzymes that afford protection against toxic O₂ products. Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase (SOD) and catalase, which catalyze the destruction of superoxide radical and hydrogen peroxide respectively. Peroxidase also can be used to destroy hydrogen peroxide.

\[
\begin{align*}
2O_2^- + 2H^+ & \rightarrow O_2 + H_2O_2 \text{ (Superoxide dismutase)} \\
2H_2O_2 & \rightarrow 2H_2O + O_2 \text{ (Catalase)} \\
H_2O_2 + NADH + H^+ & \rightarrow 2H_2O + NAD^+ \text{ (Peroxidase)} 
\end{align*}
\]

Aerotolerant microorganisms may lack catalase but almost always have superoxide dismutase. Strict anaerobes lack either of the enzymes or have them in very low concentrations and therefore cannot tolerate O₂. Aerobic microorganisms can be grown in an aerated medium which is aerated by shaking the vessel or sterile air is pumped. But for anaerobes, O₂ must be excluded from the medium. Reducing agents such
as thioglycollate or cysteine can be used to flush out O₂. Nitrogen gas can also be used to eliminate O₂ with a vacuum pump, and sometimes CO₂ and nitrogen is added to the chamber, as anaerobes require a small amount of CO₂ for best growth and also gas pack jar can also be used.

Pressure: Most microorganisms always are subjected to pressure of 10 atmospheres (atm). The hydrostatic pressure can reach to 600 to 1100 atm in the deep sea with temperature about 2°C to 3°C. Organisms can survive and adapt at these extreme conditions and many are barotolerant, increased pressure does adversely affect them but not as much as it does to nontolerant bacteria. The barophillic organisms are those growing in the guts of deep sea invertebrates such as amphipods and holothurians and grow more rapidly at high pressures. These bacteria may play an important role in nutrient recycling in the deep sea. Bacterial genera of *Photobacteria, Shewanella, Colwellia* are barophiles. Some members of the Archaea are thermophiles for example *Pyrococcus spp.*, *Methanococcus janaschii*.

![Fig. 6. Oxygen requirements in bacteria](image)

**Pressure:**

Most organisms on land or on the surface of water is always subjected to a pressure of 1 atm. The hydrostatic pressure can reach 600 to 1100 atm in the deep sea. Despite these extremes, bacteria survive and adapt. Many are barotolerant. Some bacteria in the gut of deep sea invertebrates such as amphipods and holothurians are truly barophilic and grow more rapidly at high pressures (Ex. *Photobacterium, shewanella, Colwellia*).
Radiation:

Electromagnetic radiation of various types bombards our world. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases – gamma rays and X rays are much more energetic than visible light or infrared waves (Fig. 7). Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation, infrared rays and radio waves. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun as visible light. Many forms of electromagnetic radiation are very harmful to microorganisms. Ionizing radiation, radiation of very short wavelength or high energy can cause atoms to lose electrons or ionize. The two major forms of ionizing radiation, X rays which are artificially produced and gamma rays which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations, higher levels are directly lethal. Some prokaryotes like *Deinococcus radiodurans* and bacterial endospores are resistant and can cause a variety of changes in cells like; it breaks hydrogen bonds, oxidises double bonds, destroys ring structures and polymerizes some molecules. Oxygen enhances these destructive effects, probably through the generation of hydroxyl radicals (OH). Destruction of DNA is the most important cause of death of microorganisms. Ultraviolet radiation kills all kinds of microorganisms due to its short wavelength (approximately 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. Formation of thymine dimmers in DNA is the primary mechanism of UV damage; these dimmers inhibit DNA replication and function. This damage is repaired by photo reactivation, where blue light is used by a photo reactivating enzyme (photolyase) to split the thymine dimmers. Dark reactivation, where a short sequence containing the thymine dimmers can also be excised and replaced in the absence of light. Damage can also be repaired by the recA protein in recombination repair and SOS repair.
Visible light – immensely beneficial because it is the source of energy for photosynthesis. Visible light when present in sufficient intensity can damage or kill microbial cells. Pigments called photosensitizers and O$_2$ are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophyll, cytochromes and flavins which absorb light energy, become excited or activated and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O$_2$ generating singlet oxygen ($'O_2$).

\[
P (\text{light}) \rightarrow P(\text{activated})
\]

\[
P(\text{activated}) + O_2 \rightarrow P + 'O_2
\]

Singlet oxygen is very reactive, powerful oxidizing agent that will quickly destroy a cell. Many microorganisms that are airborne or live on exposed surface use carotenoid pigments for protection against photooxidation. Carotenoids effectively quench singlet oxygen that is absorbing energy from singlet oxygen and convert it back into the unexcited ground state.

Microbial Growth in Natural Environments:

Microorganisms in a particular location are exposed to many overlapping gradients of nutrients and various other environmental factors. Microorganisms will grow in ‘microenvironments’ until an environmental or nutritional factor limits growth. Liebig’s law of minimum states that the total biomass of an organism will be determined by the nutrient present in the lowest concentration relative to the organisms requirements. Shelford’s law of tolerance states that there are limits to environmental factors, below and above which a microorganism cannot survive and grow, regardless of the nutrient supply. Each organism has a specific temperature, pH, oxygen level and hydrostatic pressure to
The growth of a microorganism depends on both the nutrient supply and its tolerance of the environmental conditions. In response to low nutrient levels (oligotrophic environments) and intense competition, many microorganisms become more competitive in nutrient capture and exploitation of available resources. Morphology of the organism can change in order to increase its surface area and ability to absorb nutrients. Microorganisms can also undergo step by step shut down of metabolism except for housekeeping genes. Natural substances can also directly inhibit microbial growth and reproduction in low-nutrient environments. These agents include phenolics, tannins, ammonia, ethylene etc. This may be a means by which microorganisms avoid expending limited energy resources until an adequate supply of nutrients becomes available.

Growth of natural prokaryotic populations outside the laboratory can be determined by counting the number of viable microorganisms present. A viable microorganism is one that is able to grow actively, resulting in the formation of a colony or visible turbidity in liquid medium. John R. Postgate of University of Sussex in England was one of the first to note that microorganisms stressed by survival in natural habitats were sensitive to secondary stresses. Such stresses can produce viable microorganisms that have lost the ability to grow on media normally used for their cultivation. To determine the growth potential of such microorganism, Postgate micro viability assay, in which microorganisms are cultured in the thin agar film under a cover slip. The ability of a cell to change its morphology, even if it does not grow beyond the single stage indicates that the microorganism does show life signs.

The situation in natural environments with mixed populations is much more complex. Often only 1 to 10% of observable cells are able to form colonies. The microbiologist is attempting to grow microorganisms that perhaps never have been cultured or characterized. At present, molecular techniques involving PCR amplification and small subunit ribosomal RNA analysis are increasingly used to analyze diversity of uncultured microbial population.
**Quorum sensing:**

Bacteria can communicate with one another and behave cooperatively. Quorum sensing or autoinduction is a phenomenon in which bacteria monitor their own population density through sensing the levels of signal molecules, sometimes called autoinducers because they can stimulate the cell that releases them. The concentration of these signal molecules increases along with the bacterial population until it rises to a specific threshold and signals the bacteria that the population density has reached a critical level or quorum. The bacteria then begin expressing sets of quorum-dependent genes. Quorum sensing has been found among both gram-negative and gram-positive bacteria. Quorum sensing was first discovered in gram-negative bacteria and is best understood in these microorganisms. The most common signals in gram-negative bacteria are acyl-homoserine lactones (HSLs). These diffuse into the target cell and once they reach a sufficiently high level, acyl HSLs bind to specific receptor proteins and trigger conformational change, they bind to target sites on the DNA and stimulate transcription of quorum sensitive genes. The genes needed to synthesize acyl HSLs is also produced frequently, thus amplifying the effect by the production and release of more auto inducer molecules (Fig. 8).

Examples in gram negative bacteria are 1) bioluminescence production by *Vibrio fisheri* 2) *Pseudomonas aerogionosa* synthesis and release of virulence factors 3) Conjugal transfer of genetic material by *Agrobacterium tumefaciens* and 4) antibiotic production by *Erwinia carotovora* and *Pseudomonas aureofaciens*. Gram positive bacteria also regulate activities by quorum sensing often using an oligopeptide pheromone signals. Good examples are mating in *Enterococcus faecalis*, competence induction in *Streptococcus pneumonia*, and stimulation of sporulation by *Bacillus subtilis*, and production of many toxins and other virulence factors by *Staphylococcus aureus*. Another important function of quorum sensing is to promote the formation of mature biofilms by the pathogen, *Pseudomonas aeroginosa*, as it may play a role in cystic fibrosis. Quorum sensing is an example of what might be called multicellular behaviour in that many individual cells communicate and coordinate their activities to act as a unit. Other examples of such complex behaviour are pattern formation in colonies and fruit body formation in the Myxobacteria.
Fig. 8. Quorum sensing

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MODULE – 5 MICROBIAL GROWTH AND CONTROL

Lecture 3: The use of physical methods in Microbial control

Although microorganisms are beneficial and necessary or human well-being, microbial activities have undesirable consequences such as food spoilage and disease. To minimize their destructive effects, it is essential to kill a wide variety of microorganisms or inhibit their growth. The goal is twofold, to destroy pathogens and prevent their transmission and to reduce or eliminate microorganisms responsible for the contamination of water, food and other substances.

Sometimes it is necessary to eliminate the microorganisms completely from an object, whereas sometimes only partial destruction may be required in other situations. The control of microbial populations on inanimate objects, like eating utensils, surgical instruments is of considerable importance. Sterilization – is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat. When sterilization is achieved by a chemical agent, the chemical is called a sterilant. Disinfection – is the killing, inhibition or removal of microorganisms that may cause disease. Disinfectants are agents, usually chemical used to carry out disinfection and does not necessarily sterilise an object because viable spores and few microorganisms may remain. Sanitization is closely related to disinfection. It is sometimes necessary to control microorganisms on living tissue with chemical agents. Antisepsis – is the prevention of infection or sepsis and is accomplished with antisepsics. These chemical agents are applied to living tissue and they prevent infection by killing or inhibiting pathogen growth or they reduce the total microbial population. Substances that kill organisms often have the suffix – cide, germicide – kills pathogens but not necessary endospores. A disinfectant or antiseptic can be effective against a specific group and may be called a bactericide, fungicide, algicide and viricide. Other chemicals do not kill, but they do prevent growth, and if these are removed, growth will resume. Their names end in – static like, bacteriostatic and fungistatic.
**Conditions influencing the effectiveness of antimicrobial agent activity:**

The efficiency of an antimicrobial agent is not simple and is affected by some factors as stated below.

**Population size:** A larger microbial population requires a longer time to die than a smaller one as an equal fraction of it is killed during each interval.

**Population composition:** The nature of the organisms being treated as they differ markedly in susceptibility varies the effectiveness of an agent. For example, younger cells are usually more readily destroyed than mature organisms and bacterial endospores are much more resistant to most antimicrobial agents than are vegetative forms. Mycobacterium tuberculosis for example is very much resistant to most of the antimicrobial agents than most other bacteria. Hence, the composition of population also plays a major role in the effectiveness of antimicrobial agents.

**Concentration of an agent:** Often, but not always, the more concentrated a chemical agent or intense a physical agent, the more rapidly microorganisms are destroyed. This is not always true and over a short range, a small increase in concentration leads to an exponential rise in effectiveness, but beyond a certain point, the increase may not raise the killing. Sometimes an agent is more effective at lower concentrations. For example, 70% ethanol is more effective than 95% because its activity is enhanced in the presence of water.

**Duration of exposure:** The more the exposure duration, the more organisms are killed. Hence, this also form a vital effectiveness criterion for the antimicrobial agents.

**Temperature:** An increase in temperature at which the agent acts also often enhances its activity. Frequently, a lower concentration of disinfectant or sterilizing agent can be used at a higher temperature.

**Local environment:** The local environment in which the population is being controlled also plays a major role that may offer protection or aid in its destruction. For example, because heat kills more readily at an acid pH, acid foods and beverages such as fruits and tomatoes are easier to pasteurize. The organic matter also can protect microorganisms against heating and chemical disinfectants. Hence, for example when city’s water supply has a high content of organic matter, more chlorine must be added to disinfect the water.
Use of physical methods in microbial control:

Heat:

Heating is still one of the most popular ways to destroy microorganisms. Fire and boiling water have been used since the time of Greeks for sterilization and disinfection. Either moist heat or dry heat may be applied. Moist heat kills viruses, bacteria and fungi. Exposure to boiling water for 10 min is sufficient to kill or destroy vegetative cells and eukaryotic spores, but not enough to kill or destroy bacterial endospores, hence boiling does not sterilize but can be used for disinfection of drinking water and objects not harmed by water. In order to destroy bacterial endospores, moist heat sterilization must be carried out at temperatures above 100 °C and this requires the use of saturated steam under pressure. This can be carried out with an autoclave (Chamberland, 1884). Water is boiled to produce steam, which is released through the jacket and into the autoclave’s chamber. Hot, saturated steam enters the chamber and the desired temperature and pressure, usually 121°C and 15 pounds is reached (Fig. 9). At this temperature saturated steam destroys all vegetative cells and endospores. Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.

![Fig. 9. View of an autoclave](image)

Pasteurization is a process where many substances such as milk, are treated with controlled heating at temperatures well below boiling (in honour of its developer Louis Pasteur). Milk, beer and many other beverages are now pasteurized. Pasteur examined the spoiled wine and detected the presence of microorganisms like bacteria which were responsible for the production of lactic acid and acetic acid fermentations which resulted in the spoilage of wine. He then discovered that brief heating at 55 to 60°C would destroy...
these microbes and preserve wine for long periods. Hence, pasteurization does not sterilize a beverage or milk but kills any pathogens present and slows spoilage by reducing the level of non-pathogenic spoilage microbes. Milk in older methods of pasteurization was held at 63°C for 30 min. Now, mostly two methods are used, flash pasteurization or high temperature short-term (HTST) pasteurization, which consists of quick heating to about 72°C for 15 sec and then rapid cooling. The other method used in dairy industry is ultrahigh-temperature (UHT) sterilization, where milk and milk products are heated at 140 to 150°C for 1 to 3 sec. The products pasteurized by this method needs no refrigeration and can be stored at room temperature for about 2 months.

Dry heat sterilization can also be used on many objects in the absence of water. The items to be sterilized are placed in an oven at 160 to 170 °C for 2 to 3 hours. Oxidation of cell constituents and denaturation of proteins results in the death of microbes. Most laboratories sterilize glass Petri dishes and pipettes with dry heat. This method though is not suitable for heat sensitive materials like many plastic and rubber items.

Low temperatures

Another convenient method to inhibit the growth and reproduction of microorganisms is to use lower temperatures like freezing or refrigeration. Mostly this method of control is used in food microbiology. Freezing items at -20°C or lower stops microbial growth because of the absence of liquid water, and the ice crystal disruption of cell membranes at this temperature. This method is also used for long term storage of microbial samples in the laboratories at -30 to -70 °C in the form of glycerol stocks. This method of control at low temperatures slows microbial growth and reproduction, but does not halt it completely. Fortunately, most pathogens, are mesophilic and do not grow well at low temperatures around 4°C. Thus refrigeration is a good technique only for short-term storage of food and other items.

Filtration:

In order to sterilize solutions which is heat sensitive, filtration is an excellent way to reduce the microbial population. The filters simply remove the microbes instead of killing them. Depth filters consists of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution is passed through the filter which is sucked through this layer under vacuum and microbial cells are
removed. The material used mostly is unglazed porcelain, asbestos or other similar materials. Membrane filters are also used and have replaced depth filters in recent times. These filters are made up of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, and other synthetic materials. These filters vary in size with pore sizes mostly of 0.2 to 0.5 µm in diameter and used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1ml to many litres. These filters are mostly used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics and other heat sensitive solutions.

The other way this method is used is in the laminar flow biological safety cabinets where the air is sterilized by filtration. These cabinets contain high-efficiency particulate air (HEPA) filters, which remove 99.97% of 0.3µm particles. The safety cabinets are most useful as the culturing of any organisms requires contamination free air to reduce the growth of other undesired organisms or for the preparation of media, examining tissue cultures etc (Fig. 10).

![Image of Biological safety cabinet]

**Radiation:**

We have discussed about the effects of radiation on the growth of microorganisms earlier. The radiations like ultraviolet and ionizing can be used for sterilizing objects. **Ultraviolet radiation** around 260 nm is quite lethal but does not penetrate glass, dirt films, water and other substances very effectively. UV radiation is used as a sterilizing agent only in a few specific situations, like UV lamps are placed on the ceilings of rooms or in biological safety cabinets to sterilize air and other exposed surfaces. Commercial UV units are available for water treatment. Pathogens and microorganisms are destroyed when a thin layer of water is passed under the lamps (water purifiers).
Ionizing radiation penetrates deep into objects and is an excellent sterilizing agent. It destroys bacterial endospores and vegetative cells of both prokaryotic and eukaryotic origin but not against viruses. Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures and plastic disposable supplies such as syringes, and Petri dishes.

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MODULE – 5 MICROBIAL GROWTH AND CONTROL

Lecture 4: The use of chemical methods in Microbial control

Use of Chemical agents in microbial control:

The chemical agents are mostly employed in disinfection and antisepsis. The proper use of these agents is essential to laboratory and hospital safety. Factors such as the kinds of microorganisms potentially present the concentration and nature of the disinfectant to be used and the length of treatment should be considered. Many disinfectants are available and each has its own advantages and disadvantages, but ideally the disinfectant must be effective against a wide variety of infectious agents, at high dilutions and in the presence of organic matter and should not be toxic to people or corrosive for common materials. The disinfectant must be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces.

Phenols:

In 1867 Joseph Lister employed it to reduce the risk of infection during operations and phenol was the first widely used antiseptic and disinfectant. Today phenol and phenolics such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals. Lysol is made of a mixture of phenolics which is commercially available disinfectant. They act by denaturing proteins and disrupting cell membranes. Phenolics are tuberculocidal and effective in the presence of organic material and remain active on surfaces long after application. However, they do have a disagreeable odour and can cause skin irritation. Hexachlorophene has been one of the most popular antiseptics because once applied it persists on the skin and reduces skin bacteria for long periods.

Alcohols:

Alcohols are the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal. Ethanol and isopropanol are the two most popular alcohol germicides. They act by denaturing proteins and possibly by dissolving membrane lipids. Small instruments like thermometers can be disinfected by soaking them for 10 to 15 min in alcohol solutions. A 70% ethanol is more effective than 95% as water is needed for proteins to coagulate.
Halogens:

Halogens exist as diatomic molecules in the free state and form salt like compounds with sodium and most other metals. Iodine and chlorine are the most important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. Spores can be destroyed at higher concentrations. Iodine is often applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Skin scars result and sometimes iodine allergies can result. In today’s date, brands like Wescodyne for skin and laboratory disinfection and Betadine for wounds is being used as iodine is complexed with an organic carrier to form iodophor; and these are mostly used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfection.

Chlorine is mostly used as a disinfectant for municipal water supplies and swimming pools and also employed in dairy and food industry. It may be applied as chlorine gas, sodium hypochloride or calcium hypochloride, all of which yield hypochlorous acid (HClO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water. Ozone sometimes has been used successfully as an alternative to chlorination in Europe and Canada. Small amounts of drinking water can be disinfected with halazone tablets. It slowly releases chloride when added to water and disinfects it in about half an hour.

Heavy metals:

Heavy metals such as mercury, silver, arsenic, zinc and copper were used as germicides and these have been most recently superseded by other less toxic and more effective germicides. A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea but now erythromycin is used instead of silver nitrate because it is effective against Chlamydia as well as Neisseria. Silver sulfadiazine is used on burns. Copper sulphate is an effective algicide in lakes and swimming pools. The action of these heavy metals is mostly on the proteins, and they combine often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.
**Quaternary ammonium compounds**

**Detergents** are organic molecules that serve as wetting agents and emulsifiers and are amphipathic in nature and hence solubilize otherwise insoluble residues and are very effective cleansing agents and are different from soaps, which are derived from fats. Only cationic detergents are effective disinfectants characterized by positively charged quaternary nitrogen and a long hydrophobic aliphatic chain. They disrupt microbial membranes and may also denature proteins. Mostly used as disinfectants for food utensils and small instruments and as skin antiseptics. Several brands are on the market. Zephiran contains benzalkonium chloride and Ceepryn, cetlypyridinium chloride.

**Aldehydes:**

Formaldehyde and glutaraldehyde are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by cross-linking and alkylating molecules. Formaldehyde is usually dissolved in water or alcohol before use. A 2% buffered solution of glutaraldehyde is an effective disinfectant and is mostly used to disinfect hospital and laboratory equipments. These are mostly sporicidal and can be used as chemical sterilants.

**Sterilizing gases:**

Gases may also be used as sterilizing agents in order to sterilize many heat-sensitive items such as disposable Petri dishes and many syringes, heart-lung machine components, sutures etc. Ethylene oxide gas is used for this purpose as it readily penetrates packing materials, even plastic wraps and is both microbicidal and sporicidal and kills by combining with cell proteins. Batapropiolactone (BPL) is occasionally sued as a sterilizing gas in the liquid form to sterilize vaccines and sera. Recently vapour-phase hydrogen peroxide has been used to decontaminate biological safety cabinets.

**Hydrogen peroxide:**

\(\text{H}_2\text{O}_2\)effects are direct and indirect actions of \(\text{O}_2\) as it forms hydroxyl free radical which is highly toxic and reactive to cell. As an antiseptic, 3% \(\text{H}_2\text{O}_2\) serves a variety of needs including skin and wound cleansing, bedsore care and mouth washing. It is especially useful in treating infection by anaerobic bacteria because of the lethal effects of \(\text{O}_2\) on these forms. When it is applied to a wound, the enzyme catalase in the tissue decomposes the \(\text{H}_2\text{O}_2\) into water and free \(\text{O}_2\). The \(\text{O}_2\) causes the wound tissues to bubble and the bubbling removes microorganisms mechanically. Also, the sudden release of \(\text{O}_2\)
brings about chemical changes in certain microorganisms, and these changes lead to microbial death.

**Acids and alkalis:**
Conditions of very low or high pH can destroy or inhibit microbial cells; but they are limited in application due to their corrosive, caustic and hazardous nature. Aqueous solutions of ammonium hydroxide remain a common component of detergents, cleansers and deodorizers. Organic acids are widely used in food preservation because they prevent spore germination and bacterial and fungal growth. Acetic acid (in the form of vinegar) is a pickling agent that inhibits bacterial growth; propionic acid is commonly incorporated into breads and cakes to retard moulds, benzoic acid and sorbic acids are added to beverages, syrups etc to inhibit yeasts.

The best known disinfectant screening test is the **phenol coefficient test** in which the potency of a disinfectant is compared with that of phenol. A series of dilutions of phenol and the experimental disinfectant are inoculated with the test bacteria *Salmonella typhi* and *Staphylococcus aureus*, then placed in a 20 or 37°C bath. These inoculated disinfectant tubes are next subcultured to regular fresh medium at 5 min interval, and the subcultures incubated for two or more days. The highest dilutions that kill the bacteria after a 10 min exposure, but not after 5 min, are used to calculate the phenol coefficient. The reciprocal of the appropriate test disinfectant dilution is divided by that for phenol to obtain the coefficient. Suppose that the phenol dilution was 1/90 and maximum effective dilution for disinfectant X was 1/450. The phenol coefficient of X would be 5. The higher the coefficient value, the more effective the disinfectant under these test conditions. A value greater than 1 means that the disinfectant is more effective than phenol.
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MODULE – 5 MICROBIALGROWTH AND CONTROL

Lecture 5: Antimicrobial Chemotherapy

Microorganisms can grow on and within other organisms which leads to the development of disease, disability and death of an organism. Thus the control or destruction of microorganisms residing within the bodies of humans and other animals is of great importance. Modern medicine is dependent on chemotherapeutic agents, chemical agents that are used to treat diseases. Most of them are antibiotics, microbial products or their derivatives that can kill susceptible microbes or inhibit their growth.

The development of chemotherapy began with the work of German physician Paul Ehrlich, who reasoned that a chemical with selective toxicity that would kill pathogens and not human cells might be effective in treating disease. In 1904 he found that the dye trypan red was active against the trypanosome that causes African sleeping sickness and could be used therapeutically. Arsphenamine was active against syphilis. Dogmagk discovered that Protonoid red, a dye for staining leather, was nontoxic for animals and completely protected mice against pathogenic streptococci and staphylococci. Dogmagk has actually discovered sulphonamides or sulfa drugs and for this discovery he received Nobel Prize in 1939. The first antibiotic to be used therapeutically was penicillin. Stephan Fleming was the first to discover penicillin from the fungus Penicillium notatum. Later other scientists, Flare and Chain carried out the work further and the three scientists were awarded with Nobel Prize in 1945 for the discovery and production of penicillin. After the discovery of Penicillin, scientists became interested in other compounds produced by microbes which could be used as antibiotics. Selman Waksman announced in 1944 that he and his associates has found a new antibiotic, streptomycin, produced by the actinomycete Streptomyces griseus. He received the Nobel Prize in 1952 and his success led to a worldwide search for other antibiotic-producing microbes. Microorganisms producing chloramphenicol, neomycin, Terramycin and tetracycline were isolated by 1953. This led to the discovery of powerful drugs and has transformed modern medicine and greatly alleviated human suffering.
General characteristics and mechanisms of action of antimicrobial agents:

A successful chemotherapeutic agent must have selective toxicity. The degree of selective toxicity may be expressed in terms of 1) the therapeutic dose, the drug level required for treatment of a particular infection and 2) the toxic dose, the drug level at which the agent becomes too toxic for the host. The therapeutic index is the ratio of the toxic dose to the therapeutic dose. The larger the TI, the better the chemotherapeutic agent. A drug that disrupts a microbial function not found in eukaryotic animal cells has a greater selective toxicity and a higher therapeutic index. Example, therapeutic index of penicillin is high as the action of penicillin is that it inhibits the peptidoglycan synthesis of bacterial cell walls, and as eukaryotic cells do not contain cell walls, the effect is minimum. The drug which has a low therapeutic index can cause undesirable effects on the host, called as side effects as the drug may inhibit the same process in host cells or damages the host in other ways. Hence, chemotherapeutic agents must be administered with great care.

Drugs can range in their effectiveness. Many are narrow-spectrum drugs- that are; they are effective only against a limited variety of pathogens, while the broad-spectrum drugs attack many different kinds of pathogens. Drugs can be classified according to the microbial group they act against; antibacterial, antifungal, antiprotozoan and antiviral. Chemotherapeutic agents can be synthesized by microorganisms or manufactured by chemical procedures independent of microbial activity. Most of the agents are natural or synthesized by bacteria or fungi (Table 1). In contrast, several important agents are completely synthetic (Sulphonamides, trimethoprim, chloramphenicol, ciprofloxacin, isoniazid and dapsone). While some are semi synthetic, natural antibiotics that have been chemically modified by the addition of extra groups to make them less susceptible to inactivation by pathogens. Ampicillin, carbenicillin, and methicillin are good examples.
Table 1. Microbial sources of some antibiotics

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Streptomyces</em> spp.</td>
<td>Amphotericin B</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol (also synthetic)</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
</tr>
<tr>
<td></td>
<td>Kanamycin</td>
</tr>
<tr>
<td></td>
<td>Neomycin</td>
</tr>
<tr>
<td></td>
<td>Nystatin</td>
</tr>
<tr>
<td></td>
<td>Rifampin</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td></td>
<td>Tetracyclines</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
</tr>
<tr>
<td><em>Micromonospora</em> spp.</td>
<td>Gentamicin</td>
</tr>
<tr>
<td></td>
<td>Bacitracin</td>
</tr>
<tr>
<td><strong>Bacillus</strong> spp.</td>
<td>Bacitracin</td>
</tr>
<tr>
<td></td>
<td>Polymyxins</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em> spp.</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
</tr>
<tr>
<td><em>Cephalosporium</em> spp.</td>
<td>Cephalosporins</td>
</tr>
</tbody>
</table>

Mechanism of action:

An antimicrobial agent’s adverse effect on cells is known as its mode of action. They have a range of cellular targets (Table 2). They fall in the following categories:

1. Cell wall synthesis inhibition
2. Protein synthesis inhibition
3. Nucleic acid synthesis inhibition
4. Cell membrane disruption
5. Metabolic antagonism

The most selective antibiotics are those that interfere with the synthesis of bacterial cell walls (e.g., penicillin, cephalosporins, vancomycin and bacitracin). These drugs have a high therapeutic index because bacterial cell walls have a unique structure not found in eukaryotic cells. The next selective antibiotics are those that inhibit protein synthesis by binding to the prokaryotic ribosome. These drugs can discriminate between the prokaryotic and eukaryotic ribosomes and hence their therapeutic index is high too. Some drugs bind to the 30S while others bind to the 50S ribosomal subunit. Several different mechanisms can be affected: aminoacyl t-RNA binding, peptide bond...
formation, mRNA reading and translocation (e.g., streptomycin, gentamicin, spectinomycin, clindamycin, chloramphenicol, tetracycline, erythromycin etc). The drugs that inhibit nucleic acid synthesis or damage cell membranes are often not as selectively toxic as other antibiotics, the reason being that prokaryotes and eukaryotes do not differ much with respect to nucleic acid synthetic mechanisms or the cell membrane structure (e.g., quinolones and polymyxins). The last category of drugs act as antimetabolites: they block the functioning of metabolic pathways by competitively inhibiting the use of metabolites by key enzymes. Sulfonamides and several other drugs inhibit folic acid metabolism (e.g., sulphanilamide, sulfamethoxazole and sulfaacetamide). Most bacterial pathogens synthesize their own folic acid and are therefore susceptible to inhibitors of folate metabolism. Humans cannot synthesize folic acid and must obtain it from in their diet and hence does not affect the host and has high therapeutic index.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Wall Synthesis Inhibition</strong></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>Inhibit transpeptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall peptidoglycan. Activate cell wall lytic enzymes.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td></td>
</tr>
<tr>
<td>Methicillin</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Binds directly to the D-Ala-D-Ala terminus and inhibits transpeptidation</td>
</tr>
<tr>
<td></td>
<td>Inhibits cell wall synthesis by interfering with action of the lipid carrier that transports wall precursors across the plasma membrane</td>
</tr>
<tr>
<td><strong>Protein Synthesis Inhibition</strong></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Binds with the 30S subunit of the bacterial ribosome to inhibit protein synthesis and causes misreading of mRNA.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Binds to the 50S ribosomal subunit and blocks peptide bond formation through inhibition of peptidyl transferase.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Bind to the 30S ribosomal subunit and interfere with aminoacyl-tRNA binding</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Bind to the 50S ribosomal subunit and inhibit peptide chain elongation</td>
</tr>
<tr>
<td>Erythromycin and clindamycin</td>
<td></td>
</tr>
</tbody>
</table>
### Nucleic Acid Synthesis Inhibition

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>Binds to EF-G and blocks translocation</td>
</tr>
<tr>
<td>and other quinolones</td>
<td>Inhibit bacterial DNA gyrase and thus interfere with DNA replication, transcription, and other activities involving DNA.</td>
</tr>
<tr>
<td>Rifampin.</td>
<td>Blocks RNA synthesis by binding to and inhibiting the DNA-dependent RNA polymerase</td>
</tr>
</tbody>
</table>

### Cell Membrane Disruption

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin B.</td>
<td>Binds to the plasma membrane and disrupts its structure and permeability properties</td>
</tr>
</tbody>
</table>

### Metabolic Antagonism

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>Inhibit folic acid synthesis by competition with $p$-aminobenzoic acid.</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Blocks tetrahydrofolate synthesis through inhibition of the enzyme dihydrofolate reductase.</td>
</tr>
<tr>
<td>Dapsone.</td>
<td>Interferes with folic acid synthesis</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>May disrupt pyridoxal or NAD metabolism and functioning. Inhibits the synthesis of the mycolic acid “cord factor”</td>
</tr>
</tbody>
</table>

### Factors influencing the effectiveness of antimicrobial drugs:

A complex array of factors influences the effectiveness of drugs. **First**, the drug must actually be able to reach the site of infection. The mode of administration plays an important role. A drug such as penicillin G is not suitable for oral administration because it is relatively unstable in stomach acid, while gentamicin and other aminoglycosides are not well absorbed from the intestinal tract and must be injected intramuscularly or given intravenously. Nonoral routes of administration often are called **parenteral routes**.  
**Second**, the pathogen must be susceptible to the drug. Actively growing and dividing cells are susceptible rather than dormant ones. Penicillins and other drugs like cephalosporins affect pathogens only if they are actively growing and dividing as they inhibit cell wall synthesis and do not harm mycoplasmas, which lack cell walls. **Third**,
the chemotherapeutic agent must exceed the pathogen’s MIC value if it is going to be effective. Finally, the most concerning is that chemotherapy has been rendered less effective and much more complex by spread of drug resistance plasmids.

**Antibacterial drugs:**

**Sulfonamides or Sulfa drugs**

These are also **structural analogues**, those molecules which are structurally similar to metabolic intermediates. These compete with metabolites in metabolic processes because of their similarity, but are just different enough so that they cannot function normally in cellular metabolism. The first antimetabolites to be used successfully were the sulphonamides or sulfa drugs which are structurally related to sulphanilamide, an analogue of p-aminobenzoic acid, which is used in the synthesis of the cofactor folic acid. When this enters a bacterial cell, it competes with PABA for the active site of an enzyme involved in folic acid synthesis, and the folate concentration decreases. The decline in folic acid is detrimental to bacteria because folic acid is essential to the synthesis of purines and pyrimidines, the bases used in the construction of DNA, RNA and other important cell constituents. This leads to cessation of bacterial growth and death of the pathogen. Sulfonamides are selectively toxic for many pathogens because these bacteria manufacture their own folic acid and cannot effectively take up the cofactor. In contrast, humans cannot synthesize folic acid and must obtain it in the diet; therefore sulphonamides will not affect the host.

**Quinolones:**

Are synthetic drugs that contain the 4-quinolone ring. These are effective when administered orally and the first quinolone, nalidixic acid was synthesized in 1962. Other quinolones belonging to fluoroquinolones has been produced (ciprofloxacin, norfloxacin and ofloxacin). They act by inhibiting the bacterial DNA gyrase or topoisomerase II, probably by binding to the DNA gyrase complex. This enzyme introduces negative twists in DNA and helps separate its strands. DNA gyrase inhibition disrupts DNA replication and repair, transcription, bacterial chromosome separation during division, and other cell processes involving DNA. These drugs are broad-spectrum and are highly effective against enteric bacteria such as *E.coli* and *Klebsiella pneumonia*. Other gram-negative pathogens like *Haemophilus, Neisseria, and Pseudomonas aeruginosa* are also susceptible. Gram negative bacteria include *Staphylococcus aureus, Streptococcus*
*pyogenes* and *Mycobacterium tuberculosis*. Currently they are mostly used in treating urinary tract infections, sexually transmitted diseases caused by *Neisseria* and *Chlamydia*, gastrointestinal infections, respiratory tract infections, skin infections and osteomyelitis.

**Penicillins:**

Penicillin G or benzylpenicillin was the first antibiotic to be used in medicine. Most penicillins are derivatives of 6-aminopenicillanic acid and differ from one another only with respect to the side chain attached to its amino group. The most crucial feature of the molecule is the β-lactam ring, which appears to be essential for activity. Penicillinase, the enzyme synthesized by many penicillin resistant bacteria, destroys penicillin activity by hydrolyzing a bond in this ring. Their structures do resemble that of the terminal D-alanyl D-analine found on the peptide side chain of the peptidoglycan subunit. It has been proposed that penicillins inhibit the enzyme catalyzing the transpeptidation reaction because of their structural similarity, which would block the synthesis of a complete, fully cross-linked peptidoglycan and lead to osmotic lysis. An increasing number of bacteria are penicillin resistant. Penicillinase –resistant penicillins such as methicillin, nafcillin, and oxacillin are frequently employed against these bacterial pathogens. Penicillin G is highly active against most gram-positive bacteria, low against gram negative; but destroyed by acid and penicillinase. Penicillin V is more acid resistant than penicillin G. Ampicillin is against gram positive and gram negative bacteria, but is acid stable. Carbenicillin is active against gram-negative bacteria like *Pseudomonas* and *Proteus*, is acid stable, not well absorbed b small intestine. Although penicillins are the least toxic of the antibiotics, about 1 to 5% of the adults in the USA are allergic to them. Occasionally a person will die of a violent allergic response; therefore patients should be questioned about penicillin allergies before treatment is begun. Cephalosporins are similar to penicillins and are given to people with penicillin allergy.

**Cephalosporins:**

Originally isolated from the fungus *Cephalosporium* in 1948 and their β-lactam ring structure is very similar to that of penicillins and resembles them in inhibiting cell wall synthesis or the transpeptidation reaction during peptidoglycan synthesis. They are broad spectrum drugs frequently given to patients with penicillin allergies. Most
cephalosporins (including cephalothin, cefoxitin, ceftriaxone and cefoperazone) are administered parenterally.

**Tetracyclines:**

These are a family of antibiotics with a common four-ring structure to which a variety of side chains are attached. Naturally produced tetracyclines, oxytetracycline and chlorotetracycline are produced by some species of the actinomycete genus *Streptomyces*; others are semi synthetic drugs. These antibiotics inhibit protein synthesis by combining with the small (30S) subunit of the ribosome and inhibiting the binding of aminoacyl-tRNA molecules to the ribosomal A site. These are broad spectrum antibiotics active against gram-negative, gram-positive bacteria, rickettsias, Chlamydia.

**Aminoglycoside antibiotics:**

Streptomycin, kanamycin, neomycin and tobramycin are synthesized by *Streptomyces*, whereas gentamicin comes from a related bacterium, *Micromonospora purpurea*. Aminoglycosides bind to the small ribosomal subunit and interfere with protein synthesis and also cause misreading of the genetic message carried by mRNA. These antibiotics are most active gram-negative pathogens, but they are quite toxic and can cause deafness, renal damage, loss of damage, nausea and allergic responses.

**Erythromycin (Macrolides):**

Erythromycin is synthesized by *Streptomyces erythraeus* and is usually bacteriostatic, broad spectrum and binds with the 23S rRNA of the 50S ribosomal subunit to inhibit peptide chain elongation during protein synthesis. The other macrolides, Clindamycin is effective against a variety of bacteria including *Staphylococci* and anaerobes such as *Bacteroides*. Azithromycin is particularly effective against *Chlamydia trachomatis*.

**Vancomycin:**

Vancomycin is produced by *Streptomyces orientalis* and blocks peptidoglycan synthesis by inhibiting the transpeptidation step that cross-links adjacent peptidoglycan strands. It is affective against Staphylococcus and some members of the genera *Clostridium, Bacillus, Streptococcus* and *Enterococcus*. 
**Chloramphenicol:**

Mostly made by chemical synthesis and was previously being produced from cultures of *Streptomyces venezuelae*. It binds to 23S rRNA on the 50S ribosomal subunit and inhibits the peptidyl transferase and is bacteriostatic. It is a broad spectrum antibiotic but is quite toxic and the common side effect is a temporary or permanent depression of bone marrow function, leading to aplastic anemia and a decreased number of blood leukocytes.

**Drug Resistance:**

The most serious threat to the successful treatment of microbial diseases is the spread of drug-resistant pathogens. There are different ways in which bacteria develop resistant to drugs. A particular type of resistance mechanism is not confined to a single class of drugs. Two bacteria may use different resistance mechanisms to withstand the same drug and resistant mutants arise spontaneously and the mutants are not created directly by exposure to a drug. One mechanism of gaining resistance by pathogens is by preventing entrance of the drug. Penicillin G is ineffective towards gram negative bacteria as it cannot penetrate the envelopes outer membrane. Mycobacteria resist drugs because of the high content of mycolic acids in a complex lipid layer outside their peptidoglycan which is just impermeable to most drugs. A decrease in permeability can lead to sulfonamide resistance too. The second mechanism of resistance is to pump the drugs out of the cell after it has entered. Translocases present on the plasma membrane of pathogens, often called efflux pumps expel the drugs. These are also called as multidrug resistance pumps as these are relatively nonspecific and can pump many different drugs. Many are drug/proton antiporters – where a proton enters the cell and the drug leaves (*E. coli, Pseudomonas aeruginosa, Mycobacterium smegmatis and staphylococcus aureus*). The third mechanism is to inactivate the drugs through chemical modifications. Best known example is the hydrolysis of the β-lactam ring of much penicillin by the enzyme penicillinase. Sometimes, groups can also be added to the drugs in order to inactivate them. For example, chloramphenicol contains two hydroxyl groups that can be acetylated in a reaction catalyzed by the enzyme chloramphenicol acyltransferase with acetyl CoA as the donor, which catalyze the acetylating of amino groups.
Basically, the chemotherapeutic agent’s acts on a specific target and when the target enzyme or organelle is modified, resistance arises as it is no longer susceptible to the drug. For example, erythromycin and chloramphenicol have the affinity towards the ribosomes and this can be decreased by a change in the 23S rRNA to which they bind. The antibiotic binding can also be reduced as in the case of Enterococci, which become resistant to vancomycin by changing the terminal D-alanine-D-alanine in their peptidoglycan to D-alanine-D-lactate. In sulfonamide resistant bacteria the enzyme that uses p-aminobenzoic acid during folic acid synthesis often has a much lower affinity for sulfonamides. Mycobacterium tuberculosis has become resistant to the drug rifampin due mutations that alter the β subunit of its RNA polymerase and the drug cannot bind to it and block the initiation of transcription.

**Origin and transmission of drug resistance:**

Bacterial chromosomes and plasmids harbour the genes for drug resistance. Spontaneous mutations in the bacterial chromosome, although they do not occur very often, will make bacteria drug resistant. These mutations, sometimes results in a change in the drug receptor; therefore the antibiotic cannot bind and inhibit. Host resistance mechanisms help in destroying the mutants, however, when a patient is being treated extensively with antibiotics, some resistant mutants may survive and flourish. Frequently, a bacterial pathogen is drug resistant because it has a plasmid bearing one or more resistance genes; such plasmids are called R plasmids (resistance plasmids). These genes often code for enzymes that destroy or modify drugs; for example, the hydrolysis of penicillin or the acetylation of chloramphenicol as discussed in the previous section and many others. These R plasmids can also be rapidly transferred from one bacterial cell which possesses it to another through normal gene exchange processes such as conjugation, transduction and transformation. A single plasmid may contain resistance genes for several drugs, and hence a pathogen population can become resistant to several antibiotics simultaneously.

Antibiotic resistance genes are also located on genetic elements other than plasmids. Many composite transposons contain genes for antibiotic resistance both in gram-negative as well as gram-positive bacteria. Few examples and their resistance markers are Tn5 (kanamycin, bleomycin, streptomycin), Tn9 (Chloramphenicol), Tn10 (tetracycline), Tn21 (Streptomycin, spectinomycin, sulfonamide), Tn551 (erythromycin)
and Tn4001 (gentamicin, tobramycin, kanamycin). These genes on composite transposons can move rapidly between plasmids and through a bacterial population very rapidly.

Extensive drug treatment can also lead to the development and spread of antibiotic-resistant strains because the antibiotic destroys normal, susceptible bacteria that would usually compete with drug-resistant pathogens leading to a super infection. This is a significant problem because of the existence of multiple-drug resistant bacteria. In order to discourage the emergence of drug resistance, several strategies can be employed like; the drug can be given in a high enough concentration to destroy susceptible bacteria and most spontaneous mutants that might arise during treatment. Two different drugs can be administered sometimes, with a hope that each drug will prevent the emergence of resistance to the other. Finally, broad-spectrum drugs, should be used only when definitely necessary. Another approach is to search for new antibiotics that microorganism have never encountered. Structure based or rational drug design is a third option. Pharmaceutical companies are now looking for drugs to treat diseases like AIDS, cancer and the common cold by using the bioinformatics tools. Sequencing and analysis of pathogen genomes almost certainly will be useful in identifying new targets for antimicrobial drugs. In recent times, bacteriophages are being used to treat many bacterial infections also called Phage therapy. Bandages are saturated with phage solutions, phage mixtures are administered with orally, and phage preparations are given intravenously to treat *Staphylococcus* infections.
### Antifungal drugs:

<table>
<thead>
<tr>
<th>Agent</th>
<th>Common Method of Administration*</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole, Miconazole</td>
<td>O, T, IV</td>
<td>Skin irritation, Severe itching, nausea, fever, thrombophlebitis</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>IV</td>
<td>Fever, chills, nausea, vomiting, anemia, kidney damage, blindness</td>
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<tr>
<td>Nystatin</td>
<td>T</td>
<td>Mild headaches, nerve inflammations, gastrointestinal disturbances</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>T, O</td>
<td>Less toxic than many fungal agents</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>O</td>
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Fungal cells are similar to human cells than the bacterial cells and hence treatment of fungal infections generally has been less successful than that of bacterial infections. Many drugs are quite toxic for humans and they have a detoxification system that modifies many antibiotics, probably by hydroxylation. Despite their low therapeutic index, a few drugs are useful in treating major fungal diseases. Fungal infections are often subdivided into infections of two types, superficial mycoses and systemic mycoses. Several drugs are used to treat superficial mycoses like dermatophyte infections such as athlete’s foot (Clotrimazole, miconazole, ketoconazole), and oral and vaginal candidiasis (Nystatin). They are thought to disrupt fungal membrane permeability and inhibit sterol synthesis. Griseofulvin is given orally to treat chronic dermatophyte infections. It is thought to disrupt the mitotic spindle and inhibit cell division; it also may inhibit protein and nucleic acid synthesis.

Systemic infections are very difficult to control and can be fatal. Amphotericin B is commonly used and it binds to the sterols in fungal membranes, disrupting membrane permeability and causing leakage of cell constituents. It is used only for life threatening infections. Fluconazole is used in the treatment of candidiasis, cryptococcal meningitis and coccidioidal menginitis. Just like antibacterial drugs, overuse of antifungal drugs...
leads to an increase in drug resistance. For example, *Candida* infections are becoming more frequent and drug resistant.

**Antiviral drugs:**

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<thead>
<tr>
<th>Table 4. Antiviral Drugs</th>
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<tbody>
<tr>
<td><strong>Drug</strong></td>
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<tr>
<td><strong>Antiviral Drugs That Inhibit Nucleic Acid Synthesis</strong></td>
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<tr>
<td>Ribavirin</td>
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<tr>
<td>Adenosine arabinoside</td>
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<td></td>
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<tr>
<td><strong>Nucleoside analogs</strong></td>
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<tr>
<td>Representative (see also Figure 10.7): Azidothymidine (AZT)</td>
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<tr>
<td><strong>Antiviral Drugs That Inhibit Viral Proteins</strong></td>
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<tr>
<td>Protease Inhibitors</td>
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</table>

Most antiviral drugs disrupt either critical stages in the virus life cycle or the synthesis of virus-specific nucleic acids. **Amantadine** and rimantadine can be used to prevent influenza A infections. Amantadine blocks the penetration and uncoating of influenza virus particles. **Acyclovir**, is also used in the treatment of herpes infections. Research on anti-HIV drugs has been particularly active. Many of the first drugs to be developed were reverse transcriptase inhibitors such as **azidothymidine (AZT)**. These interfere with reverse transcriptase activity and therefore block HIV reproduction. More recently HIV protease inhibitors have been developed. These mimic the peptide bond that is normally attacked by the protease. Probably the most publicized antiviral agents are **interferons**. These small proteins, produced by the host, inhibit virus replication and may be clinically useful in the treatment of influenza, hepatitis, herpes and colds.
REFERENCES:

Text Books:


Reference Books: