Control of Microorganisms

Methods used to control the growth of microorganisms and their transmission of infectious disease involve stopping the growth of the microorganism for a period of time, reducing the number of microorganisms to a safe level, or destroying the microorganisms. The degree of effectiveness depends on the number of microorganisms, the type of microorganism, their physiological state, such as the stage of growth or formation of endospores, and the environment in which they are growing (glassware, instruments, tissue, food).

Terms Related to Destruction of Microorganisms

1. sterilization - the destruction of all microorganisms, including endospores, on an object or in a material.

2. disinfection - the destruction of pathogens, but not endospores, on an object or in a material. The number of pathogens is reduced or growth is inhibited to a level that does not produce disease.

3. antisepsis - chemical disinfection of the skin, mucosal membranes, or other living tissues.

4. germicide ("cide" = kill) - a chemical agent that rapidly kills microorganisms.
   Specific germicides include:

   a. sporicide - kills spores
   b. bactericide - kills bacteria
   c. viricide - kills viruses
   d. fungicide - kills fungi

Terms Related to Suppression of Microorganisms

1. asepsis ("without infection") - the absence of pathogens from an object or area. Aseptic techniques prevent the entry of pathogens into the body.

There are two types of asepsis:

a. surgical asepsis - techniques designed to exclude all microorganisms. It prevents infectious agents from reaching a wound. Prevention begins before the patient enters the operating room and includes removal of hair and cleansing and disinfecting the skin with an antiseptic that removes skin oils and microorganisms from the area of the skin to be opened. Substances include Betadine, Isodine, Ioprep, and Surgidine.

b. medical asepsis - techniques designed to exclude microorganisms associated with communicable diseases. It includes dust control, hand-washing, use of individualized equipment and instruments, waste disposal, care of instruments, syringes, needles, thermometers, and dressings.

2. sanitization - the reduction or removal of pathogens on inanimate objects by chemical or mechanical cleansing.

3. bacteriostasis -("static" = halt) - bacterial growth and multiplication are inhibited, but the bacteria are not killed.

Terms for Destruction or Suppression of Microorganisms

1. antimicrobial - agents that destroy or suppress any microorganism.

2. antibiotic - a product formed by microorganisms that destroy or suppress microorganisms.

Physical Agents

1. Heat

Heat is the most common, inexpensive, simplest, reliable, and effective method used to destroy microorganisms. Heat denatures the proteins and enzymes of the microorganisms. Most pathogens will be destroyed at temperatures between 50° and 70°C for a duration of 10 minutes, except endospores which may survive 1 to 2 hours at 100°C.

The heat used in sterilization is either moist or dry heat.

a. moist heat

(1). boiling - Bacteria, fungi, and many viruses are destroyed by boiling at 100°C for 10 to 30 minutes. Some viruses and endospores may require boiling for up to 20 hours. Sterilization is accomplished by denaturing the proteins and enzymes of the microorganisms.

(2). steam under pressure - Water is heated under pressure which raises the temperature above 100°C which denatures proteins and amino acids. The most common device used is an autoclave for sterilizing surgical bandages, instruments, media, and contaminated material. At a pressure of 15 pounds/square inch and a temperature of 121°C for 15 to 20 minutes it will destroy microorganisms and endospores.

(3). pasteurization - It is mainly used in the food and dairy industries and it involves raising the temperature high enough to destroy pathogens or inhibit their growth without affecting the quality of the product.

b. dry heat

Dry heat penetrates substances more slowly than moist heat.

(1). incineration - The burning of disposable substances in a chamber.

(2). direct flaming - Sterilization of inoculating loops, needles, and rims of test tubes with a bunsen burner. Wire heated to a red glow is 100% effective.

(3). hot-air oven - Sterilization of glassware, test tubes, petri dishes, instruments, syringes, and needles requires higher temperatures for a longer period of time than other methods. Most endospores will be destroyed at 160-165°C for a period of 2 hours.

2. cold

The effect of low temperature on microorganisms depends on the type of microorganism and the intensity of the application. Temperature in a refrigerator ranges from 0-8°C and has a bacteriostatic effect which reduces the metabolic rate of most organisms so that they cannot reproduce or synthesize toxins.

Freezing at -20°C kills most bacteria, but some may survive in a frozen state.

3. drying or desiccation

To grow and multiple, microorganisms require water. The removal of water by evaporation or freeze-drying (solid to gas) inhibits growth and reproduction of microorganisms by inhibiting enzymes. They may be viable for years so when water is made available they resume growth and reproduction.

4. ultra-violet radiation and ionizing radiation (x-rays and gamma rays)

Both types of radiation damage the DNA of microorganisms and denatures their proteins. U-V radiation cannot penetrate materials such as glass, clothing, dirt, paper, or pus and is used to kill microorganisms on surfaces. Germicidal lamps in operating rooms, nurseries, and communicable disease wards reduce the number of bacteria in the air, but they do not sterilize.

5. filtration - Filtration is the passage of material or liquids through a filter containing small pores that retain microorganisms. Membrane filters of cellulose acetate are the most common, but gauze, cotton, and paper serve as filters for air. Vaccines that require the presence of live viruses, such as polio, are passed through filters which prevent bacteria from going through the pores. Air filters are used in operating rooms to lower the number of airborne microorganisms.

Chemical Agents

Chemical agents are used to control the growth of microorganisms on inanimate objects and on living tissue. Most chemical agents reduce the number of microorganisms (disinfect), but do not achieve sterility. Selection of a disinfectant depends on the mode of action, concentration necessary, the type of microorganism, the number of microorganisms present, the type of material to be disinfected, temperature, and pH.

Disinfectants are classified based on their chemical structure and activity.

1. phenols (carbolic acid)

Phenol and derivatives called phenolics disrupt the plasma membrane, denature proteins, and inactivate enzymes. Phenol is rarely used any more because it causes skin irritation and has a disagreeable odor.

Common phenolics are cresols such as that found in Lysol, and hexachlorophene which is used as an antiseptic.

2. alcohols

Ethanol and isopropanol are widely used as skin antiseptics, but they are not effective against enveloped viruses or bacterial endospores. They effectively destroy bacteria and fungi by disrupting the lipids in the plasma membrane resulting in lysis, and denatures proteins.

3. surface-active agents (surfactants)

They include soaps and detergents that lower the surface-tension of liquid molecules and make microorganisms accessible to other agents. The pH of soaps is usually alkaline which destroys some bacteria. Detergents are more effective against gram-positive than gram-negative bacteria and they disrupt the plasma membrane.

4. halogens

The major halogens are iodine and chlorine. Iodine is an effective antiseptic for the skin and superficial wounds. It destroys many kinds of bacteria, some endospores, fungi, and some viruses. Iodine inhibits protein function in the microorganism. Chlorine is used as a disinfectant and it is a strong oxidizing agent which affects functions of the microorganisms's enzymes.

5. ions of heavy metals

The major metal ions are mercury and silver, and they function in denaturing proteins and enzymes. Both are used in combination with other substances as antiseptics.

6. chlorhexidine

Chlorhexidine is used as an antiseptic on skin and mucosal membranes and functions in disruption of the plasma membrane of gram-positive and gram-negative bacteria resulting in lysis.

7. alkylating agents

Alkylating agents disrupt the structure of proteins and nucleic acids. Formaldehyde (.2 - .4%) is used to inactivate viruses and toxins used in vaccines. It also destroys spores in bacteria and fungi. Glutaraldehyde is less irritating and more effective than formaldehyde. It kills many microorganisms, viruses, and endospores. Ethylene oxide is a gas used in a closed chamber to sterilize materials. It kills all bacteria, including endospores. Betapropiolactone kills spores in concentrations not much more than required for killing cells. The effect is rapid and it disappears in a few hours. It is used to sterilize bone, cartilage, and artery grafts.

Antimicrobial Chemotherapy

Chemotherapy is the use of chemical substances to treat various aspects of disease.

Chemotherapeutic agents (drugs) are any chemical substances used in medical practice to treat disease.

Antimicrobial agents are a special group of chemotherapeutic agents used to treat infection caused by microorganisms. Many are produced by microorganisms and are specifically called antibiotics. Some antimicrobial agents are synthetic drugs which are produced in a laboratory. Others may be semisynthetic drugs which are synthesized by chemically modifying a substance from a microorganism.

Properties of Antimicrobial Agents

1. solubility in body fluids - They must dissolve in body fluids to be transported and reach the area of infection or microorganisms.

2. selective toxicity - They must harm the microorganisms without causing significant damage to the host.

3. spectrum of activity - The range of different microorganisms on which an antimicrobial agent acts is its spectrum of activity.

There are two major types of spectrum activity:

a. broad-spectrum activity - Agents are effective against a great number and types of microorganisms, including gram-positive and gram-negative bacteria. They are useful when an infection is caused by an unidentified microorganism and it increases the chances that the microorganism will be susceptible to the drug.

A disadvantage is that many normal flora of the host are destroyed. Normal flora compete with and help destroy pathogens and other microorganisms. Some normal flora may flourish when the competition is diminished and they become opportunistic pathogens.

b. narrow-spectrum activity - Agents are effective against only a small number or type of microorganism. They reduce the chance of normal flora being destroyed and decrease the chance the microorganisms will develop drug resistance.

4. nonallergenic - The drug should not cause an allergic reaction, hypersensitivity, or anaphylactic shock in the host.

5. stability - Toxicity should not be altered by food, other drugs, or conditions of the host and the concentration in the blood and tissue fluids should be stable over a period of hours (degraded and excreted slowly).

6. resistance - Few microorganisms should be resistant or have the ability to develop resistance to the effects of the drug. Two drugs with different modes of action on the microorganism may be administered. The drugs do not interfere with each other, have an additive or synergistic effect and reduce the likelihood of resistance.

Most antimicrobial agents are antibacterial agents that are bactericidal (killing) or bacteriostatic (growth-inhibiting) while having minimal effects on host cells. Antimicrobial agents may be categorized by their mode of action.

There are five modes of action:

1. inhibition of cell wall synthesis - Peptidoglycan synthesis in the cell wall of growing bacteria is affected. The cell wall is weakened and lysis occurs. Human cells are not affected because they do not have cell walls or peptidoglycan in their plasma membrane. Drugs such as vancomycin and bacitracin interfere with the synthesis of peptidoglycan chains. Penicillin and cephalosporin prevent the linking between the peptidoglycan chains.

2. disruption of the plasma membrane - The selective permeability of the plasma membrane is affected and important substances used in metabolic activities leave the bacterial cell. The drugs used are selectively toxic. Polymyxin B causes damage by attaching to the phospholipids of the membrane. It allows dissolved substances to pass freely into and out of the cell eventually resulting in lysis. Antifungal drugs, such as nystatin, amphotericin B, and miconazole, combine with sterols in the fungal membrane causing disruption and leakage of cytoplasmic contents.

3. inhibition of protein synthesis - Antimicrobial drugs affect the bacterial ribosome which is made up of a large and small protein subunit. When ribosomal function is disturbed, the synthesis of protein may slow down so that normal growth cannot occur or protein synthesis may stop. Ribosomal function may be affected in different ways. Chloramphenicol and erythromycin react with the large ribosomal subunit and prevent peptide bonds from forming between the amino acids. Streptomycin, neomycin, kanamycin, and gentamicin bond with the small subunit and cause the genetic code on mRNA to be improperly read and inhibit initiation of protein synthesis by preventing the ribosome from moving. Tetracycline bonds with the small subunit and interferes with the attachment of the tRNA carrying the amino acid to the ribosome.

4. inhibition of nucleic acid synthesis - DNA replication and the copying of the information from DNA by mRNA is affected (transcription). Quinolones (ciprofloxacin) are synthetic drugs that block the enzyme which unwinds the DNA double helix during preparation for replication. Rifampin is a semisynthetic drug that inhibits the enzyme RNA polymerase which is necessary for transcription.

5. inhibition of synthesis of essential metabolites - The synthesis of folic acid (B vitamin), which is needed to make the nitrogenous bases of DNA is inhibited. Sulfonamides (sulfa drugs) are antimetabolites which function as competitive inhibitors of an enzyme in the pathway that forms folic acid. Sulfonamides resemble the substrate para-aminobenzoic acid (PABA). The enzyme that normally converts PABA to folic acid combines with the sulfonamide instead. The combination prevents folic acid synthesis and stops the growth of the microorganism.

Antiviral Agents

Chemical agents that inhibit the growth of viruses are not as common as antibiotics. A few inhibit a wide variety of viruses, while most are restricted to a single genus or group of viruses.

There are four modes of action:

1. antiviral proteins synthesized by cells infected with a virus - Interferons are small proteins that are produced in most cells when they are infected by a virus. Once produced, interferon prevents the subsequent infection of noninfected cells.

2. interfere with viral DNA synthesis - Most antiviral drugs are structural analogues of the normal purines and pyrimidines that form nucleic acids. Because they are structurally similar to the normal nucleotides, they prevent viral reproduction by inhibiting DNA synthesis. Iodoxuridine, Vidarabine, and Acyclovir are used to treat herpesvirus eye infections, herpesvirus encephalitis, cytomegalovirus infections in newborns, and herpes zoster.

3. acted on by viral enzymes or that block the activity of viral enzymes - Retrovirus replication requires that the viral RNA first be transcribed into DNA. The viral enzyme that catalyzes this step is reverse transcriptase. Retroviruses cause leukemias and AIDS. Azidothymidine (AZT) inhibits the action of reverse transcriptase.

4. prevent the release of the viral nucleic acid into the cytoplasm of the infected cell - Amantadine prevents the release of viral RNA into the cytoplasm and is effective against influenza virus type A. Ribavirin inhibits nucleic acid synthesis by interfering with the formation of guanine nucleotides.