Unit 3 Lecture 6

Introduction to Antimicrobials

An **Antimicrobial** is any method which interferes with the optimum growth of a microbe. Microbial death is defined as the inability of the organism to reproduce. **Sterility** is the complete destruction or removal of all microorganisms and viruses. Sterile is an absolute term. There is no “almost sterile.” Sterility requires all life forms to be eliminated from the various kingdoms of microorganisms including the vegetative state (those in active metabolism and reproduction) and those that are quite resistant to sterilization (*e.g.* endospores and cysts). Sterilization can be achieved by physical or chemical means. Microbial death is the permanent loss of reproductive ability of the microbe.

Disinfection is the complete destruction or removal of all vegetative pathogens and their toxins. It does not affect endospores. Therefore non-pathogenic life forms may survive. It does however require all vegetative forms of pathogens be eliminated. Disinfection can be achieved by physical or chemical means. A disinfectant is used on only on inanimate objects.

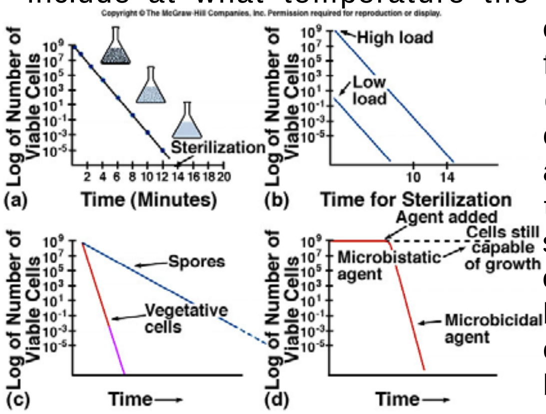
Aseptic techniques

Surgical asepsis is any barrier to exclude the admission of any viable organism. Medical **asepsis** is any barrier to exclude the admission of naturally communicable organisms. An **Antiseptic** is a chemical used to provide asepsis that is safe to use on body surfaces. An object or skin is **sanitized** when the mechanical destruction or removal of sufficient microbes has been performed to have a microbial population at a “safe level.” **Degermation** is the reduction of the number of microbes on the skin by scrubbing.

Word parts used with antimicrobials that assist in their definition.

- a-, an-: without
- anti-: against
- -cide, -cidal: killing of
- -stasis, -static: stop the increase of

Microbial control methods consist of three different methods: use of physical agents, which include heat and radiation, use of chemical agents; and mechanical removal devices (filtration). Factors that influence the action of antimicrobials depend on the procedure selected. Physical procedures depend on the method and the intensity used. More intense is always more effective. Chemical procedures also depend on the method used. The higher concentration is usually more effective but is not always the case. For example, 70-90% concentration is best for ethanol or isopropyl alcohol and not 100%. In some cases dilutions may stimulate growth. Other factors include at what temperature the chemical is used (higher is usually more effective), the pH of environment away from optimum, the time of application (longer is better), the number of organisms to be killed (large populations are harder or take longer to destroy), and the kinds of organisms present or the states they are in (Spores, vegetative, cysts, fungi, etc.). Additional factors may be whether the organism is Gram positive or Gram negative, what materials are to be treated, is the substance in an aqueous, viscous, or dry state. Are there soils present? And lastly, are solvents present that may interfere with organic matter.



Action Sites for Antimicrobials

Antimicrobials can be effective at various cellular sites. They can lyse the cell wall, dissolving it, or inhibition of new cell walls during reproduction. Detergents called surfactants lower the surface tension of the cell membrane altering the permeability of the cell causing a rupture of the membrane. Inside the cell, antimicrobials can produce metabolic inhibitors (poisons) that inhibit DNA, RNA, and ribosomal activity. They can modify protein structure and activity by denaturing proteins. They can inhibit oxidation/reduction reactions.

Physical Antimicrobials

Mechanical Methods: Mechanical removal functions to separate microbes from material containing them (it is neither -cidal or -static). Basically it is just scrubbing and rinsing of solid objects. Soaps and detergents aid the process as wetting agents. Soils inhibit the process.

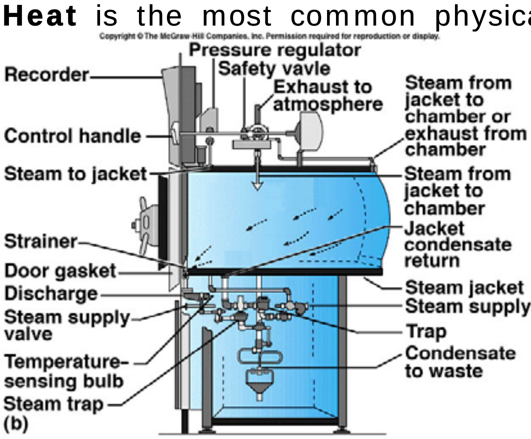
Centrifugation is used only for liquid materials. Centrifugal force acts like gravity in separating organisms from liquid. **Sedimentation** is also used only for fluids. Suspended organisms settle due to gravity. Neither method sterilizes. They only reduce the microbial load.

Filtration is the using of thin membrane filters. Removal of organisms occurs by passing liquids or gas through filters that have various size pores. Smaller pore sizes filter large amounts of microbes. In the laboratory filtration is used to prepare media that cannot withstand heat. Some common types used in microbiology are Millipore filters (cellulose acetate), Seitz filters (asbestos fibers), Chamberland-Pasteur filters (porcelain), Berkefeld filters (diatomaceous earth), Corning glass filters (sintered glass), and HEPA (high efficiency particulate air). Special types of filters are available and required for removing viruses.

Energy emissions or **[Radiations]** can be –cidal. Ionizing radiation functions by attacking nucleic acids causing mutations. Ultraviolet light acts in the same manner but has limited energy so there is limited penetration. Direct exposure is required to kill and it is effective on air borne organisms and on surfaces as a disinfectant. There is potential damage to human skin and eyes with UV light.

Beta rays are of medium energy and provide good penetration and safety. Beta radiation is used for sterilization of small, disposable items and foods. Gamma rays and X-rays are high energy and provide good penetration but hazards do exist. It is used for sterilization of larger, disposables only. Ultrasonic emissions are –cidal. It shatters cell structure (action site) and is used in cleaning glassware, instruments, etc. Microwaves produce heat with some (?) killing capacity. Ineffective energy emissions such as, visible light, radio waves, and alpha rays have low energy so penetration is limited. They have no practical value as energy converted to heat.

Heat is the most common physical antimicrobial method. Moist heat is –cidal. It coagulates cellular proteins. Steam under pressure is called **autoclaving**. It is effective, efficient, and economical for most materials, especially those products or materials that are not rendered useless by heating. It kills endospores when done correctly. Time and temperature relationship to sterilize is dependent on the material to be sterilized. Fifteen pounds pressure at 121°C for 15 minutes is very effective and used most often in the laboratory when making media. Infectious waste may require 132°C for 30-60 minutes. Raising pressure will not significantly reduce exposure time and may harm item being autoclaved.



Free steam (100°C) is used for treating solid surfaces. In a single exposure it kills vegetative forms but spores survive. In a multiple exposure process, "**Tyndallization**" or fractional sterilization, there is a triple exposure and incubation process. What is hoped to happen is that endospores will revert to the vegetative state then be killed with the next application of free steam. Boiling is used for liquids or solids immersed in liquid. Ten to fifteen minutes kills all vegetative forms but five and one-half hours are required for some endospores. Boiling of water is the norm for outbreaks of community water supply contamination.

Pasteurization is used for liquids. It is only a “sanitizing” process. The temperature kills pathogens likely to be present; however non-pathogens are reduced to “safe” level. Different levels of time and temperature are used; 63°C for 30 min.; 72°C for 15 seconds, 134°C for 1 second (ultra high temperature [UHT] pasteurization); and 140°C for 1 second UHT sterilization.

Dry heat is –cidal to the organisms. Incineration is used for disposables. We flame our inoculating loops. It is the most effective and cost effective way to kill all microorganisms. However, in choosing incineration, one must remember that is destroys everything. Sterilizing ovens kill by dehydration. They are used for solid objects. Timing varies due to temperature. If at 160°C, a two hour minimum exposure is required. But if at 171°C, only one hour exposure is required.

Low temperatures are static. They will not sterilize. Low temperatures do slow metabolism preventing overgrowth. However, one must remember that psychrophilic organisms can multiply in refrigerated foods, spoiling them.

Freezing temperatures slows growth to the extreme. Enzyme systems are slowed down by cold temperatures. In a moist environment slow freezing may provide some killing by the formation of ice crystals. Quick freezing is used in the lab to store microbes. Lyophilization is freeze drying and is used for preservation of dehydrated life forms (Microbes, sperm, embryos, etc.)

Desiccation is also static. Growth is prevented in absence of sufficient water at normal temperature. It is most commonly used in food preservation.

Osmotic pressure: Hypertonic solutions are static. A high solute concentration around microbe causes plasmolysis or removal of water from protoplasm. It is used in food preservation. Hypotonic solutions or a lower solute concentration than inside microbe causes plasmoptysis which is the addition of water to protoplasm. It will kill the organism if the cell bursts, but there is no practical use for this method.

Cavitation is the use of sound waves to sterilize. It may be used in dental offices.

Chemical Antimicrobials

Chemical Antimicrobials

The factors to consider in choosing a chemical antimicrobial (used as disinfectants, antiseptics, sterilants, preservatives, sanitizers, degermers) are the microbial population (Bacterial, viral, fungal, etc.) involved, the presence of significant morphological features, the number of microbes in the population, and their pathogenicity. Another factor to consider is the Phenol Coefficient (P.C.) which compares effectiveness of a chemical to phenol under standard conditions. It is expressed as a ratio. Values above 1 are more effective than phenol whereas values below 1 are less effective than phenol. Additional factors to consider in choosing a chemical antimicrobial are its stability, availability, solubility, pH, cost, conditions for use, time allotted, temperature restrictions, material requiring treatment, and size or area requiring treatment.

One third to one-half of all antimicrobial chemicals currently being used are Halogens (iodine, fluorine, chlorine, bromine). They are intermediate level disinfectants. Chlorine is used in water purification of drinking water, swimming pools and in waste water treatment. Chloramines and hypochlorites are sanitizing bleaches. Antiseptics (used on human tissue) include “Tincture” of iodine, Iodophors, Povidone-iodine, and Betadine.

Phenol (carbolic acid) is a disinfectant. An example is Cresols (Lysol). Antiseptic phenols include Resorcinol, hexylresorcinols, and Hexachlorophene (Septisol). Chlorhexidine (Hibiclens) is used as an obstetric antiseptic. Phenolics are intermediate-to-low level disinfectants that denature proteins and disrupt cell membranes in some organisms.

Alcohols are both disinfectants and antiseptics. Seventy to 90% alcohol is used to degerm skin because the denaturization of proteins requires water. Ethyl alcohol, Isopropyl alcohol, propylene glycol and all used in the clinical setting.

Soaps and detergents (**surfactants**)

Anionic soaps and detergents are most commonly found in the household. The active part of molecule is an anion that has low -cidal properties (sanitizers). Cationic detergents have a cation as an active part of molecule. They have good cidal properties in proper dilutions. They cause leakages in cell membranes. Examples of note ate Quaternary ammonium compounds (“Quats”), Zephiran, and Ceepryn

Heavy metal antiseptics have been around for many years. Organic mercury compounds such as Merthiolate, Mercurochrome are poor antiseptics. Silver compounds such as silver nitrate has been used in salves for burns and in lotions and eye drops (Formerly used in Crede’s method to prevent gonorrhea in newborns). Examples of products include Organics like Silvol, Argyrol, Neosilvol.

Aldehydes are reducing agents. Formaldehyde (37% solution is “formalin”) is a preservative used in embalming, vaccines, and tissue preservation. Glutaraldehyde (Cidex) is a milder chemical that acts against wide variety of microbes. It is often used to clean respiratory therapy equipment.

Gases are used to sterilize heat labile materials or large spaces. Ethylene oxide is lethal to all forms of microbes, the most penetrating of gas sterilants, but leaves an irritating residue. Beta-propiolactone is also lethal to all forms of microbes and next best in penetrating microorganisms. Formaldehyde and glutaraldehyde gas are lethal to all forms of microbes, but have poor penetrating ability and are irritating.

Acids and Bases can be used as disinfectants because; organisms have an optimum temperature at which they can replicate. Organic acids used include acetic, benzoic, and salicylic acid. Bases include lye, (NaOH or KOH), lime ($Ca(OH)_2$) and household ammonia.

Oxidizing agents

Oxygen kills anaerobes by interfering with their metabolic pathway. Hydrogen peroxide is very effective in killing anaerobes. Other oxidizing agents include potassium permanganate (surface antiseptic) and sodium perborate (as a mouthwash ingredient).

Characteristics of an “ideal” disinfectant

1. “-Cidal” to all pathogens in any form
2. Rapid action
3. Non-corrosive
4. Safe for non-pathogenic life forms
5. Deeply penetrating
6. Odorless
7. Non-staining/non-residual
8. Water soluble
9. Easily applied
10. Inexpensive & easily obtained
11. Non-toxic to human or animal tissue