

Chapter 7

Control of MOs by Physical and Chemical Agents

- (1). ***Destroy Pathogens and Prevent Transmission***
- (2). ***Reduce or eliminate MOs responsible for contamination of water, food, and other etc.***

Sterilization: (latin: sterilis) – unable to produce offspring - barren

- *Process where all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat.*
- *Totally free of viable mos, spores and other infectious agents.*
- *Sterilant*: *sterilization by a chemical agent.*

Disinfection:

- *The killing, inhibition, or removal of most may cause disease.*
- *Goal: destroy potential pathogens*
- *Reduces the total microbial population*
- *Disinfectants: agents, (usually chemicals)*
Carry out disinfection, used on inanimate objects.

(a disinfectant does not necessarily sterilize an object bc viable spores and few mos may remain).

Sanitization:

- *The microbial population is reduced to safety levels for the public health standards.*
- *The inanimate object is cleaned and partially disinfected.*
- *Example: eating utensils in restaurants.*

Antisepsis:

- *Prevention of infection or sepsis and accomplished with antiseptics*
- ***Antiseptics***: *chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth.*
-- Reduce the total microbial population
--(Not a toxic as disinfectants bc can not destroy too much host tissue)

Suffix Denotation of Antimicrobial agents:

- ***Cide***: *substances that kill (Latin for cida-kill)*
- ***Germicide***: *kills pathogens (and nonpathogens) but not necessarily endospores.*
Disinfectants or antiseptic can be effective against a specific Group: bactericide, fungicide, algicide or viricide.
- ***Static***: *do not kill, but prevent growth, if removed growth will resume.*
- *Bacteriostatic or Fungistatic.*

Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

Antimicrobial agent

- *An agent that kills mos or inhibits their growth*

Six Factors:

1. *Population Size*
2. *Population Composition*
 - *Mos differ in susceptibility*
 - *Endospores are more resistant than the vegetative form*
 - *Younger cells are destroyed quicker than older cells*
 - *Mycobacterium tuberculosis is more resistant than most bacteria*
3. *Concentration of microbial antimicrobial agent*
 - *Most often, the more concentrated a chemical agent, the more rapidly mos are destroyed.*
 - *Sometimes agent is more effective at lower concentrations: 70% ethanol is more effective than 95% ethanol, because its activity is more enhanced by the presence of water.*
4. *Duration of Exposure:*
 - *The longer the population is exposed to the a microbial agent, the more organisms are killed. To achieve sterilization, exposure should reduced population to 10^{-6} or less.*
5. *Temperature:*
 - *An increase in temperature at which the chemical acts often enhances its activity.*
6. *Local environment:*
 - *Population of mos will either be protect or aid in its destruction.*
 - *Organic matter can protect mos against heating and chemical disinfectants.*
 - *Biofilms: organic matter in a surface biofilm will protect the mos.*
 - *biofilm and mos are usually hard to remove.*
 - ***Example: clean, then disinfect or sterilize.***
 - Syringes and medical and dental equipment*
Should be cleaned then sterilized, bc presence of too much organic matter can increase the risk of pathogens by protecting the pathogen.
 - Drinking Water: the more organic matter, the more chlorine needed.*

Four Most Frequently Employed Physical Methods/Agents in Control of Mos:

1. *Heat*

- *Either moist or dry, heat is still one of the most popular methods used to destroy mos.*
- *Moist heat: kills viruses, bacteria, and fungi*
- *Exposure to boiling water, 10 minutes – destroys vegetative cells and eukaryotic spores.*
- *Boiling water (100 °C or 212 °F) is not hot enough to destroy bacterial endospores (which may survive hours of boiling)*
- *Boiling can be used for disinfection, but not sterilizing.*

Thermal Death Time (TDT):

- *shortest time needed to kill all organisms in a microbial suspension at a specific temperature under defined conditions.*

Decimal Reduction Time (D value):

- *The time required to kill 90% of the mos or spores in a sample at specific temperature.*
- *D value is written with a subscript, indicating the temperature for which it applies.*
- *More precise*
- *Used to estimate the relative resistance of a mo to difference temp. through calculating the Z value.*

Z Value:

- *The increase in the temp. required to reduce D to 1/10 its value*

F Value:

- *Time in minutes at a specific temperature needed to kill a population of cells or spores.*
- *Usually 250 °C or 121.1 °C)*

Steam Sterilization:

- *Moist heat sterilization – carried out at temp above 100 °C to destroy bacterial endospores*
- *Autoclave: Saturated Steam under pressure.*
 - *Developed in 1884 by Chamberland*
 - *Water is boiled to produce steam which is released into the jacket and into the autoclave's chamber.*
 - *Air is initially in chamber is forced out, until the chamber is filled with saturated steam and outlets are closed.*
 - *Hot saturated steam will fill the chamber until it reaches 121 °C and 15 pounds of pressure.*
(saturated steam destroys all vegetative cells and endospores within 10 to 12 minutes - 15 minutes is the standard time to provide a margin of safety).
 - *Larger volumes will require longer autoclave times.*

Moist heat kills (thought) by:

- Degrading nucleic acids
- Denaturing enzymes and other essential proteins.
- May disrupt Cell membrane.

Bacterial endospores are killed only if kept at 121 °C for 10-12 minutes.

To ensure items have been autoclaved:

- Biological indicator
Culture tube containing sterile ampule of medium and a paper strip covered with *Bacillus stearothermophilus* or *Clostridium PA3679*, after autoclaving, ampule broken, and incubated for several days. (much longer process)
- Autoclave tape with an indicator strip that turns a different color or spell out the word autoclave.

Pasteurization:

- Controlled heating well below boiling.
- Does not sterilize a beverage, but does kill any pathogen present and drastically slows spoilage by reducing the level of nonpathogenic spoilage mos.
- Developed by Pasteur from wine spoilage study
- Observed wine, found bacteria responsible for lactic acid and acetic acid fermentation.
- Brief heat at 55 to 60 °C destroyed the mos and preserved wine for longer periods.
- German Chemists V. H. and F. Soxhlet adapted technique for preserving milk and reducing milk transmissible disease.
- 1889 introduced into the US
- Milk, beer, and etc are pasteurized.

Milk Pasteurized:

Two Ways

1. 63 °C for 30 minutes
2.
 - **Flash Pasteurization** (high temperature short term (HTST))
Large quantities of milk at 72 °C for 15 seconds, then rapid cooling.
 - **Ultrahigh-temperature Sterilization (UHT)**
 - Dairy Industry
 - Milk and Milk products are treated at 140 to 150 °C for 1 to 3 seconds.
 - Does not require refrigeration
 - Example: small coffee creamers

Dry Heat Sterilization:

- Sterilization in the absence of water.

- Oven heated at 160 to 170 °C for 2 to 3 hours.
- **Microbial Death results from:**
 - Oxidation of cell constituents and denaturation of proteins.
- DH is less effective than MH
Clostridium botulinum spores are killed in 5 minutes in MH and 2 hours after dry heating.

2. **Low Temperatures**

- Used by inhibiting growth and reproduction by freezing and refrigeration.
- Freezing at -20 °C or lower stops microbial growth bc of the low temperature and the absence of liquid water.
- Some mos will be killed by ice crystal disruption of the cell membrane.
- Freezing is a very good method for long term storage of microbial samples.
- Refrigeration greatly slows microbial growth, but does not halt it completely.
- Most pathogens are mesophilic and do not grow well at temp above 4 °C.
- Refrigerated items may be ruined by growth of psychrophilic and psychrotrophic mos, especially if water is present.
- Good for short term storage.

3. **Filtration**

- Reduces the microbial population in solutions that are heat-sensitive and sometimes can be used to sterilize solutions and media.
- Does not directly destroy mos, rather removes them.

Two types of filters:

1. **Depth filters**

- Fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter.
- Solution with mos are sucked (filtered) through this layer under vacuum and microbial cells are removed by entrapment and adsorption to the surface of the of filter materials.

2. **Membrane filters**

- Circular and porous membrane
- A little over 0.1mm thick
- Made of cellulose acetate ,or other synthetic material
- There are many different sizes, but 0.2um in diameter are used for the removal of most vegetative cells, but not viruses.
- Membrane filters remove mos by screening them out much as a sieve separate large particles from small ones.
- Have replaced depth filters.
- Used to: sterilize [pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat-sensitive solutions.

Air Filtration:

- Air can be sterilized by filtration
- Example: surgical masks and cotton plugs on culture vessels.
- **Laminar flow biological safety cabinets:**
Employs **high-efficiency particulate air filters** which remove 99.97% of 0.3 μm particles.
 - These cabinets force air through HEPA filters, then project a vertical curtain of sterile air across the cabinet opening.
 - Used to prevent contamination of the room.
 - Used when working with *Mycobacterium tuberculosis*, tumor viruses, and recombinant DNA.

4. Radiation

- **Ionizing radiation:** radiation of very short wavelengths or high energy.
 1. X-rays - artificially produced
 2. Gamma Rays - emitted during radioisotope decay
 - Ionizing radiation is an excellent sterilizing agent and penetrates deep into the object.
 - Destroys bacterial endospores and vegetative cells, both eukaryotic and prokaryotic, but not always effective against viruses.
- **Ultraviolet (UV) radiation:** kills most bc of its short wavelength (10 to 400nm)
 - The most lethal form has a wavelength of 260nm, wavelength most effectively absorbed by DNA
 - Mechanism of Damage: formation of thymine dimers in DNA. Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function.
 - Although UV at 260nm is lethal, it **does not penetrate** glass, dirt films, water, and other substances, therefore it can be used as a sterilizing agent only in a few specific situations.
 - UV radiation burns the skin and damages the eyes

Use of Chemical Agents in Control:

Phenolics:

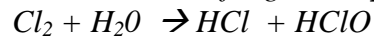
- First widely used antiseptic and disinfectant
- Lister (1867) used to reduce the risk of infections during operations.
- Phenol and phenolic (phenol derivatives) are used in hospitals and laboratories as disinfectants.
- Cresols, xylenols, and etc.
- Act by denaturing proteins and disrupting cell membranes.

Alcohols:

- Among the most widely used disinfectants and antiseptics
- Bacterial and fungicidal , but not sporicidal
- Some lipid-containing viruses are destroyed.
- Two most important alcohol germicides are: ethanol and isopropanol (70-80%)
- Act by denaturing proteins and possibly by dissolving membrane lipids.
- 10 to 15 minutes soaking is sufficient to disinfect thermometers and small instruments.

Halogens:

- Any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table.
- Exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals.
- Iodine and chlorine are important antimicrobial agents
 - **Iodine:** skin antiseptic and kills by oxidizing cell constituents and iodinating cell protein.
 - Higher concentrations kill spores
 - **Chlorine:** disinfectant for water supplies and swimming pools, also used in dairy and food industry.
 - May be used as a chlorine gas, sodium hypochlorite, or calcium hypochlorite all yielding hypochlorous acid (HClO) and then atmospheric oxygen.
 - Damages by the oxidation of cellular materials and destruction of vegetative bacteria and fungi, **Not spores.**



Heavy Metals:

- Mercury, silver, arsenic, zinc, and copper were used as germicides.
- Now there are other less toxic and more effective germicides.
- Many are bacteriostatic than bactericidal
- A 1% Silver nitrate is still used in the eyes of infants to prevent ophthalmic gonorrhea)
- Silver sulfadiazine is used on burns
- Copper sulfate is an effective algicide in lakes and swimming pools.

Quaternary Ammonium Compounds:

- **Detergents:** organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends
 - Detergents solubilize otherwise insoluble residues and are very effective cleaning agents.
 - Different than soap which is derived from lipids.
 - Anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants.

- *Most popular disinfectants are quaternary ammonium compounds that have a positive charged quaternary nitrogen and a long hydrophobic aliphatic chain.*
- *Function to disrupt microbial membranes and may also denature proteins.*

Aldehydes:

- *Common – formaldehyde and glutaraldehyde – are highly reactive molecules that combine with nucleic acids and proteins and inactivate them.*
- *Sporicidal and can be used as chemical sterilants.*
- *Glutaraldehyde an effective disinfectant in hospitals and laboratory equipment*

Sterilizing Gases:

- ***Ethylene Oxide (EtO)***
- *Used on plastic petri dishes and syringes (heat sensitive, heart-lung machines components, sutures, and catheters).*
- *Both a microbial and sporicidal*
- *Kills by combining with cell proteins.*
- *Effective sterilizing agent bc rapidly penetrates materials*

Evaluation of Antimicrobial agent Effectiveness:

Phenol Coefficient test:

- *Best known disinfectant screening test*
- *Where 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 water bath.*
- *The inoculated disinfectant tubes are next subcultures to fresh medium at 5 minute intervals and the subcultures are incubated for two or more days.*
- *The highest dilution that kills the bacteria after 10 minutes exposure, but not after 5 minutes are used to calculate the phenol coefficient.*
- *The reciprocal of the appropriate test disinfectant dilution is divided by that of phenol to obtain the coefficient.*
- *The higher the phenol coefficient value, the more effective the disinfectant.*
- *A value greater than 1 means that the disinfectant is more effective than phenol.*