

MICROBIOLOGY LECTURE - (Sterilization and Disinfection)

Definition

- Sterilization is the process of killing or removing all viable organisms
- achieved by: physical & chemical

Disinfection

- is the process of removing or killing most but not all, viable organisms

Methods:

1. Chemical

- the substance is called a disinfectant it kills pathogens but may not kill viruses or spores

2. Physical process

- boiling or low pressure steam - reduces only the bioburden

Antiseptics:

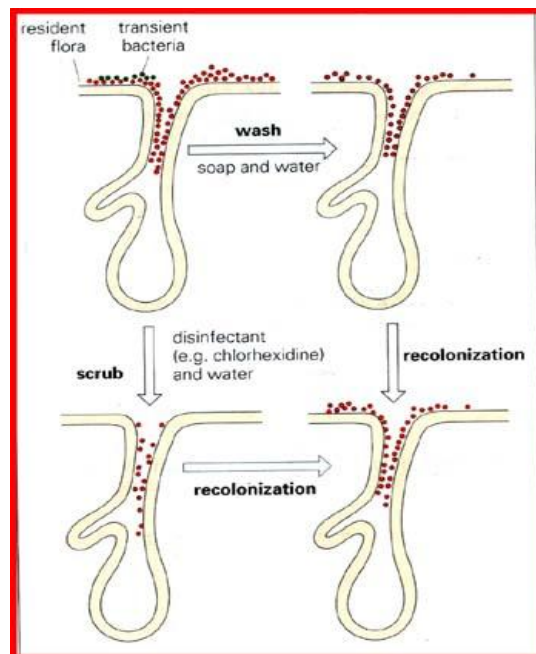
- A particular group of disinfectants - used to reduce the number of viable organisms in the skin.
- act differentially on organism and host tissue

1. Germicide

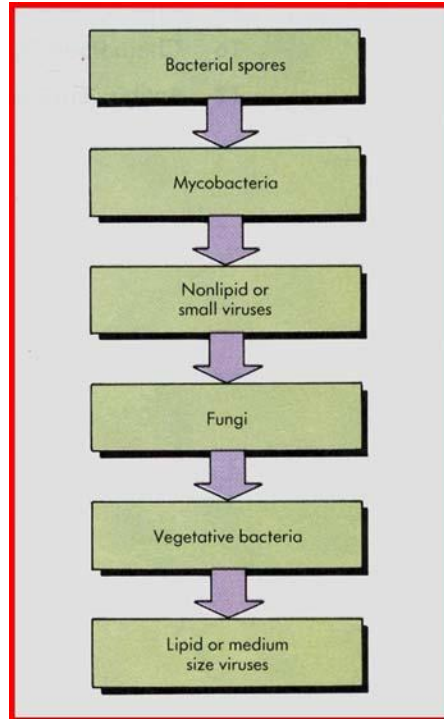
- chemical agent capable of killing microbes

2. Sporicide

- a germicide capable of killing bacterial spores



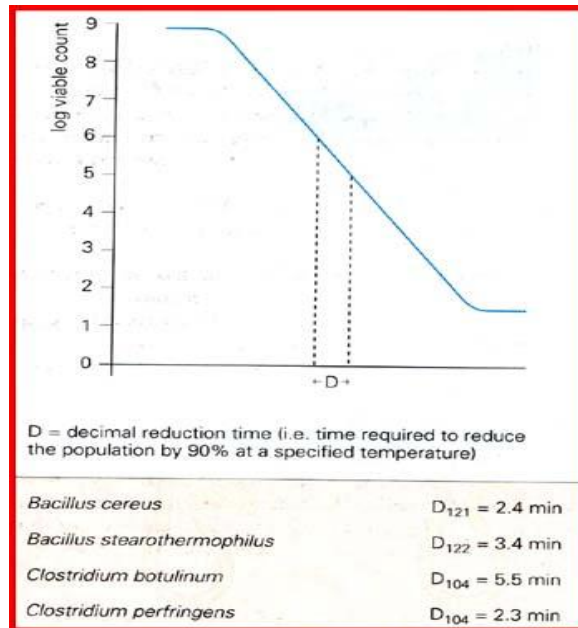
Antiseptics on normal skin flora



Microbes in decreasing order of resistance to germicidal chemicals

Pasteurization:

- reduces the total number of viable microbes in bulk fluids such as milk and fruit juices without destroying flavor and palatability.
- Decision whether to use Sterilization or Disinfection:
 - Cost
 - Damage involved
- Low bioburden is a prerequisite for cost-effective sterilization.
- **Uses**
 1. Prevention of hospital infection depends on
 - sterile equipments, instruments and dressings
 - isolation facilities
 - safe disposal of infected materials
 2. Microbiologists
 - production of sterile media and the laboratory activities
 3. Central to almost all areas of medical practice like surgery (from hand washing to needles and prosthesis)
- **Process:** The rate of killing of microorganisms depends upon the concentration of the killing agent and time of exposure.
 - $N = 1/CT$
 - N - number of survivors
 - C - concentration of agent
 - T - time of exposure to the agent



Mechanisms of action of antimicrobial agents:

1. Damage cell membrane
2. Denature proteins
3. Modify functional groups of proteins and nucleic acids
- Activity of a particular disinfectant may result from one or combination of pathways.

Factors affecting efficacy:

1. Physical environment
2. Presence of moisture
3. Temperature and Ph
4. Concentration of the agent
5. Hardness of water
6. Bioburden and the object
7. Mature and state of microbes in bioburden
8. Ability of microbes to inactivate the chemical agent

Techniques for Sterilization

A. Heat - preferred choice

- ease of use
- controllability
- cost
- efficiency
- Types:
 1. Dry heat
 - Incineration
 - use of bunsen burner

- hot air oven (one hour)
- 2. **Moist heat under pressure**
 - autoclave - most effective for sterilization (121°C, 15 mins.)
 - pressure aids in penetration
 - derived from thermal death curves for pathogens like Clostridium
 - sterilization of surgical instruments, dressing and heat resistant pharmaceuticals
- 3. **Boiling Water**
 - for a few minutes can be used as a rapid emergency measure to disinfect instruments
 - kills vegetative bacteria but not all spores
- 4. **Pasteurization**
 - done at 62.8 - 65.6 °C for 30 minutes
 - used for fluids
 - reduce the number of bacteria
 - eliminate pathogen present in small numbers
 - improve shelf-life of milk
 - **Flash pasteurization**
 - 71.7°C for 15 secs.
 - after the process, the fluid should be kept at a temperature below 10°C to minimize, subsequent bacterial growth.

B. Irradiation

- **Gamma irradiation** - used for sterilizing large batches of small volume items such as:
 1. Needles, syringes, catheters, gloves
 2. Vaccines
 3. To prevent food spoilage
- capital cost is high but process is 100% efficient
- killing mechanism involves production of free radicals that break the bonds in DNA
- sporicidal at higher doses (4.5 mega rads)

C. Filtration

- used to produce particles and pyrogen-free fluid.
- composed of nitrocellulose
- work by electrostatic attraction and physical pore size
- purify drinking water
- to recover very small number of organism from large volumes of fluid
- can be used for quantitating bacteria in fluids

D. Chemical agents (liquid or gas phase)

1. Alkylating agents

a. Gases

i.

ethylene oxide (toxic and explosive; prosthetic heart valves)

ii. **formaldehyde** (extremely unpleasant odor; and irritant to mucous membrane decontaminate rooms and exhaust protective cabinets)

b. Liquid

i. **Glutaraldehyde** - disinfects heat-sensitive articles (endoscopes and inanimate surfaces)

2. Oxidizing agents

a. Hydrogen peroxide

- 3 - 6% kills most bacteria
- 10-25% kills all including spores
- (disinfects plastic implants, contact lenses and surgical prosthesis)

b.

Halogens - I₂ or Cl₂ containing:

- **Iodine** compounds
 - most effective, virtually effective among all organisms including spore-formers and mycobacteria.
 - effective in acid pH because more free I₂ is liberated
 - acts more rapidly than other halogens and quaternary NH₄ compounds
 - effectivity reduced by serum, feces, body fluids, Na thiosulfate and NH₃

Povidone iodine used to disinfect metal surfaces and tissues.

ii. **Chlorine** compounds

- used extensively
- 3 forms available
 - Cl₂
 - HOCl (hypochlorous acid)
 - OCl (hypochlorite)
- good germicide activity, though spores are resistant to it.

2. Phenolic compounds

- rarely used as disinfectant nowadays
- historical interest it was used as comparative standard for assessing the activity of germicidal compounds

- **Phenol coefficient** - ratio of germicidal activity by a test compound compared to that by a specified concentration of phenol
- sporocidal at T approaching 100°C but not at room temperature
- effective on resistant mycobacteria
- Activity potentiated by halogenation and aromatic group into their nucleus
- **Hexachlorophene**

4. Quarternary Ammonium compounds

- organic groups covalently linked to nitrogen (Benzalkonium chloride and Cetyl pyridinium chloride. Bacteriostatic at low concentrations, bactericidal at high. Not effective against Pseudomonas, Mycobacteria and Trichophyton)

5. Alcohols

- germicidal activity increases with increasing chain length (5-8 carbons)
- most commonly used - ethanol and isopropanol
- no activity against spores
- activity is more active in the presence of water hence 70% alcohol is more active than 95% alcohol.
- common disinfectants used for skin surfaces
- extremely effective when followed by treatment with iodophor

6. Heavy metals

- soluble salts of Hg, arsenic, silver and other heavy metals
- by forming mercaptides with sulfhydryl groups of cysteine residue
- mercurials - merthiolate, mercurochrome
- silver compounds - irritant and caustic effect; silver nitrate (prophylaxis); sulfadiazine cream; wound, colloidal silver compounds used in ophthalmology

E. Others - freezing, lysis, desiccation, ultrasonication, use of electrical discharges