Practical Principles of Microbial Physiology // 4th Stage **Lab 7**
Effect of Disinfectants and Antiseptics on microbial growth **3 pages**

Terminology

Disinfection: is the killing, inhibition or removal of most disease-producing organisms on the inanimate objects such as binges and surgical apparatus

Disinfectants: strong chemical agents used to carry out disinfection on the inanimate objects.

Antisepsis: a process involves chemical destruction of vegetative pathogens on animate surfaces such as skin, mucous membrane, and wounds

Antiseptics: chemical agents with sufficiently low toxicity, applied to the surface of living tissue to kill or inhibit pathogen growth both *in vitro* and *in vivo*.

Types of chemical agents

There are eight major categories of chemical agents:

- **Phenol and phenolic compounds**
- **Alcohols**: Ethanol, Isopropanol
- **Halogens**: Iodine, Chlorine, Bromine, Fluorine
- **Oxidizing agents**: Hydrogen peroxide, Ozone, Peracetic acid
- **Surfactants**: Soap, Detergents
- **Heavy metals**: Arsenic, Zinc, Mercury, Silver, Copper
- **Aldehydes**: Formaldehyde, Glutaraldehyde
- **Gaseous agents**: Ethylene oxide, Propylene oxide, β- propiolactone

Mechanisms of action of chemical agents

Different Chemical agents have different mechanisms of action, these mechanisms include:

- **Attack proteins:**
  - Oxidize, hydrolyze, or bind to proteins.
  - Change 3D structure, usually irreversibly.
- **Dissolve membranes or damages cell walls:**
  - Leaky membranes mean vitamins and metabolites are escape.
  - Cell wall destroyed, loss of osmotic protection.
- **Damage to nucleic acids:**
  - DNA denatured or broken; cell cannot replicate.
  - RNA molecules in ribosomes affected.

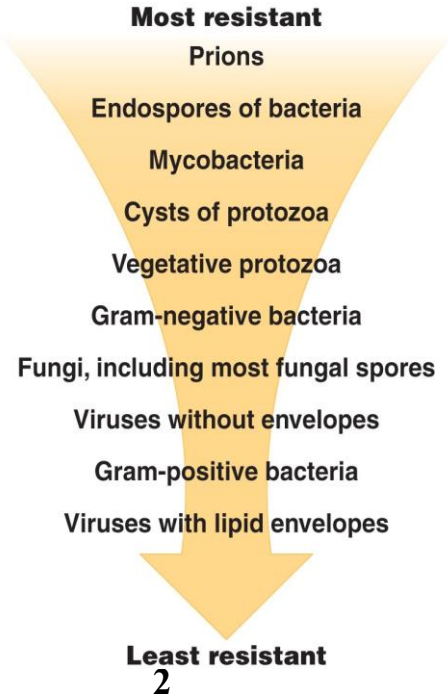
Conditions influencing the effectiveness of chemical agent activity

1. **Population size**: larger populations take longer to kill than smaller populations.
2. **Population composition**: microorganisms differ markedly in their sensitivity to various agents.
3. **Concentration or intensity of the chemical agent**: higher concentrations or intensities are generally more efficient.
4. **Duration of exposure**: the longer the exposure, the greater the number of organisms killed.
5. **Temperature**: a higher temperature will usually (but not always) increase the effectiveness of chemical agent.
6. **Local environment**: environmental factors, such as pH, viscosity can profoundly influence the effectiveness of a particular chemical agent.

Properties of good antiseptic / disinfectant

- Rapid acting and effective, even in the presence of organic material (like blood, vomit, feces).
- Active against all pathogens.
- Effective at room temperature.
- Non-staining and good odor.
- Penetrate materials without damaging them.
- Easy to prepare and stable over time.
- Inexpensive and easy to apply.
- The ideal antiseptic has to have similar properties as an ideal disinfectant. But the primary importance for antiseptics is the selective toxicity which means toxicity to microorganisms but not to human cells.

Microbial Characteristics



Aim

To measure the relative effectiveness of different types of antiseptics and disinfectants against microorganisms

Materials

- Nutrient agar plates.
- Broth cultures of *Escherichia coli* and *Staphylococcus aureus*.
- Beaker containing sterile disks of filter paper
- Forceps
- Chemical agents (5% for each of them)
  - Antiseptics: Hydrogen Peroxide, Iodine Solution, Mercury Chlorite, Ethanol
  - Disinfectants: Sodium Hypochlorite, Copper Sulfate

Procedure

1. Label the bottom of nutrient agar plates with name of bacteria and chemical agent.
2. Swab entire surface of nutrient agar plates with:
   - *E. coli*
   - *Staph. aureus*
   - Any other microorganisms
3. Pick up sterile disk with lightly flamed forceps then dip it in to a beaker of the chemical agent and place it in specific position of inoculated nutrient agar plate.
4. Incubate all the plates at 37° C for 24 - 48 hours.

Results

Observations and Interpretations

After incubation time, examine your plates for zones of inhibition, measuring the width of each zone of inhibition using a metric ruler then record your results in following table.

Microorganism	**Inhibition zone (mm)**					
	Hydrogen peroxide	**Iodine solution**	**Mercury chlorite**	**Ethanol**	**Sodium hypochlorite**	**Copper sulfate**
Escherichia coli						
Staph. aureus						
Bacillus subtilis						