Disinfection Terminology, Mode of Action and Resistance
Definitions

Selection of disinfectants

Mechanism of action (chemical and physical methods)

Mechanism of resistance

Role of biofilm
Decontamination

- The use of physical or chemical means to remove, inactivate, or destroy pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles.
- The surface or item is rendered safe for handling, use, or disposal.
- Comprises cleaning, disinfection or sterilization.
Cleaning

- The **physical removal of foreign material**, e.g., dust, soil, organic material such as blood, secretions, excretions and microorganisms.
- It **removes rather than kills** microorganisms.
- Uses water, detergents and mechanical action.
- The **most essential step** in reprocessing instruments and equipment.
Disinfection

• Any process used to reduce the number of pathogenic micro-organisms to the point where they no longer cause diseases
• Usually involves the removal of vegetative or non endo-spore forming pathogens
• Uses physical or chemical methods
• **Disinfectant**: An agent applied to inanimate objects.
• **Antiseptic**: A substance applied to living tissue.
• **Degerming**: Removal of most microbes in a limited area. Example: Alcohol swab on skin.
Sanitization

• Use of chemical agent on food-handling equipments to meet public health standards and minimize chances of disease transmission.

• Simply refers to thorough washing with only a detergent

• **Sanitizer**: chemical or physical agent used on food handling equipments to reduce bacterial numbers so as to meet public health standards.
Food preservation

• The process of treating and handling food to stop or slow down spoilage caused or accelerated by micro-organisms

• Involves:

1. Preventing the growth of bacteria, fungi, and other microorganisms

2. Retarding the oxidation of fats
Food preservation

• Common methods:
  1. Drying, freezing, vacuum packing
  2. Canning
  3. Preserving in syrup
  4. Sugar crystallisation
  5. Food irradiation
  6. Adding preservatives or inert gases such as carbon dioxide.
Disinfectant

- A chemical agent that destroys most pathogens but may not kill bacterial spores.
- Should only be used if heat treatment is impractical.
- The broad category of disinfection may be subdivided into high-level, intermediate-level, and low-level disinfection according to the anti-microbial activity of the disinfectant.
Low level disinfectant

• LLD is an agent that destroys all vegetative bacteria (except tubercle bacilli), lipid viruses, some nonlipid viruses, and some fungi, but not bacterial spores.

• Use QAC 0.4-1.6 %. 
Intermediate-level disinfectant

• ILD is an agent that destroys all vegetative bacteria, including tubercle bacilli, lipid enveloped and some nonlipid enveloped viruses, and fungus spores, but not bacterial spores.

• E.g., alcohol (ethyle, isopropyle) 70-95%, iodophore compounds (30-50 ppm/L), and phenolic compounds (0.4-5 %)
High-level disinfectant

- A chemical or physical agent or process that is capable of killing some bacterial spores when used in sufficient concentration, temperature, and under suitable conditions.

- E.g., moist heat (75-100 °C) for 30 min, gluteraldehyde 2%, hydrogen peroxide 3-25 %, peracetic acid (variable) and chlorine dioxide (100-1000 ppm free chlorine)
Spaulding classification

• A strategy developed by Dr. Earle H. Spaulding for reprocessing contaminated medical devices

• The system classifies devices as critical, semi-critical, or non critical based on the risk from contamination from a device to a patient.
Low risk (non critical) items

- Non critical items are items that come into contact with normal and intact skin or with the inanimate environment.
- Stethoscopes.
- Cleaning with a detergent and drying is usually adequate.
Intermediate risk (semi-critical) items

- Items that are in close contact with mucous membranes or with non-intact skin
- But do not penetrate the skin or enter sterile areas of the body.
- Cleaning followed by HLD is usually adequate.
- Respiratory equipment, flexible endoscopes, laryngoscopes, specula, endotracheal tubes, thermometers, and other similar instruments.
High risk (critical) items

- Items that penetrate sterile tissues such as body cavities and the vascular system.
- There is high risk of infection if such item is contaminated before penetrating the tissue.
- Cleaning followed by sterilization is required.
- High-level disinfection if sterilization is not possible,
- Surgical instruments, intra-uterine devices, vascular catheters, implants, etc.
Single Use Items

• They are prepackaged with the appropriate level of disinfection or sterilization and are disposed of after a single use.

• Gloves, needles, syringes, and tongue depressors.
Disposal Items:
- Dispose

Non-Critical Items:
- Clean
- Dry & Store

Semi-Critical Items:
- Clean
- High-level disinfection or sterilization
- Dry & Store

Critical Items:
- Clean
- Sterilization
- Dry & Store
Sterilization

• Any process that **kills or eliminates all forms of transmissible agents including spores** from a surface, equipment, article, or biological culture medium

• Any item classified as critical recommended to be cleaned and sterilized in between use

• Uses either physical or chemical processes

• **Sterilant**: An agent that destroys all forms of microbes to achieve sterilization
Note:

- Unlike sterilization, disinfection is not sporicidal.
- A few disinfectants will kill spores with prolonged exposure times (3–12 hours); these are called chemical sterilants.
- At similar concentrations but with shorter exposure periods, these same disinfectants will kill all microorganisms except large numbers of bacterial spores; they are called high-level disinfectants.
• **Bacteriostatic**: prevents growth of bacteria.

• **Germicide**: A chemical agent that **kills** certain microorganisms, particularly pathogenic organisms ("germs"). It includes both antiseptics and disinfectants.

• **Bactericide**: An agent that kills bacteria, most do not kill endospores.

• **Viricide, Fungicide, Sporicide** ………
• **Biocide**: A chemical or physical agent that kills all microorganisms, pathogenic and nonpathogenic.

• **Biologic indicator (BI)**: A standardized preparation of *bacterial spores* on or in a carrier used to demonstrate whether sterilizing conditions have been met.
Pasteurization

A process developed by Louis Pasteur of heating milk or other liquids to reduce or to significantly kill the number of pathogenic and spoilage organisms.

Methods:
- Classical: 63 °C for 30 minutes
- High Temperature Short Time: 72 °C for 15 seconds
- Ultra High Temperature: 140 °C for < 1 second

Also termed as “heat disinfection”.

Prevents transmission of milk-borne diseases caused by: *Salmonella, Campylobacter, Listeria, Mycobacteria*
• **Pyrogens**: Fever producing agents e.g., endotoxins of Gram negative bacteria

**Antisepsis**

• Use of chemical agent on skin or other living tissue
• To eliminate microorganisms
• No sporicidal action
• E.g., alcohol and iodophores

**Asepsis**: means to prevent contamination
Selection of Disinfectants

- Based on the function the disinfectant expected to perform
- Ideally, a disinfectant should be broad spectrum, non-irritating, non-toxic, non-corrosive and inexpensive.
- Selection decisions should include effectiveness against the potential pathogenic agent, safety to people, impact on equipment, the environment, and expense.
- Disinfectant effectiveness depends on many factors. These include:
1. Prior cleaning of the object
2. Type of contaminating microorganism.
3. Degree of contamination.
4. Amount of proteinaceous material present.
5. Presence of organic matter and other compounds such as soaps may neutralize some disinfectants
6. Presence of biofilms
7. Chemical nature of disinfectant (the mode of action)
8. Concentration and quantity of disinfectant.
9. Contact time and temperature (depend on the degree of contamination and organic matter load).

11. Application temperature, pH and interactions with other compounds.

12. Toxicity to the environment and relative safety to people that may be exposed.

13. Cost
Mode of Action

- Damage to cell wall
- Alteration of membrane function
- Damage to proteins
- Damage to nucleic acids
Damage to Cell Wall

Effects on bacteria and fungi:
- Blockage of cell wall synthesis
- Degradation of cell wall components
- Reduction of its stability and integrity

Ex: Penicillin, detergents, alcohols
Effects on microbes including enveloped viruses:

- Bind and penetrate membrane lipids
- Loss of selective permeability resulting in leakage of cytoplasmic contents.

Ex: Surfactants - surface active agents.
Cell wall damage
Irreversible binding to microbial DNA results in:

- Cessation of transcription and translation
- Mutations

Ex: Formaldehyde and ionizing radiation
Effects on Proteins

- Blockage of enzyme active sites prevents binding of substrate.
- Protein denaturation.

Example: Heat, acids, alcohols, phenolics, and metallic ions.
Effects on Proteins

(a) Native State

(b) Complete Denaturation

(c) Different Shape

(d) Blocked Active Site

Active site can no longer accept the substrate, and the enzyme is inactive.
<table>
<thead>
<tr>
<th>Target</th>
<th>Antiseptic or disinfectant</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell envelope: cell wall, outer membrane)</td>
<td>Glutaraldehyde</td>
<td>Cross-linking with functional group of proteins</td>
</tr>
<tr>
<td>Cytoplasmic membrane</td>
<td>QACs</td>
<td>Denature proteins and disrupt cell membrane</td>
</tr>
<tr>
<td></td>
<td>Chlorhexidine</td>
<td>Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm</td>
</tr>
<tr>
<td></td>
<td>Alcohols</td>
<td>▪ Dissolve membrane lipids, denatures proteins.</td>
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<td></td>
<td>Phenols</td>
<td>Leakage; some cause uncoupling</td>
</tr>
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<tr>
<td>Cross-linking of macro-molecules</td>
<td>Formaldehyde</td>
<td>Cross-linking with functional group of proteins, RNA, and DNA</td>
</tr>
<tr>
<td>DNA intercalation</td>
<td>Acridines</td>
<td>Intercalation between two layers of base pairs in DNA</td>
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<tr>
<td>Interaction with thiol groups</td>
<td>Silver compounds</td>
<td>Denaturation of membrane-bound enzymes</td>
</tr>
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<tr>
<td>Effects on DNA</td>
<td>Halogens</td>
<td>Inhibition of DNA synthesis</td>
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<td></td>
<td>Hydrogen peroxide, silver ions</td>
<td>DNA strand breakage</td>
</tr>
<tr>
<td>Oxidizing agents</td>
<td>Halogens</td>
<td>Oxidation of thiol groups</td>
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<tr>
<td></td>
<td>Peroxygens</td>
<td>Hydrogen peroxide: formation of free OH radicals, which oxidize thiol groups in enzymes and proteins</td>
</tr>
<tr>
<td></td>
<td>Ethylene oxide</td>
<td>Denature proteins and affect functional groups of DNA</td>
</tr>
<tr>
<td>Target</td>
<td>Physical method</td>
<td>Mechanism of action</td>
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</tbody>
</table>
| Effects on Nucleic Acids | Ionizing radiation | • Cessation of transcription and translation  
                                                                • Mutations             |
| Effects on Proteins | Moist heat      | – Coagulation of proteins                               |
|                  |                 | – Denaturation of proteins                           |
|                  | Dry heat        | – Dehydration                                          |
|                  |                 | – Denaturation                                          |
|                  |                 | – Oxidation (burning to ashes)                          |
|                 | Filtration      | Removes microbes and spores from liquids and air        |
Different types of microorganisms vary in their response to antiseptics and disinfectants in view of their different cellular structure, composition, and physiology.
<table>
<thead>
<tr>
<th>Types of organisms</th>
<th>Level</th>
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</thead>
<tbody>
<tr>
<td>Prions (e.g., Creutzfeldt-Jakob Disease)</td>
<td>- Sodium Hydroxide soap for one hour</td>
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<tr>
<td></td>
<td>- 18 min prevacuum steam sterilization</td>
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<td></td>
<td>(134-137C)</td>
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<tr>
<td>Bacterial spores (e.g. Clostridium teteni, Clostridium</td>
<td>Sterilization</td>
</tr>
<tr>
<td>difficile)</td>
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<tr>
<td>Coccidia (Cryptosporidium)</td>
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<tr>
<td>Some spores generated by spore forming bacteria</td>
<td>High Disinfection</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Intermediate Disinfection</td>
</tr>
<tr>
<td>Nonlipid or small viruses (polio, coxsackie)</td>
<td>Low Disinfection</td>
</tr>
<tr>
<td>Fungi (e.g., Aspergillus, Candida)</td>
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<tr>
<td>Vegetative bacteria (S. aureus, P. aeruginosa)</td>
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<tr>
<td>Lipid viruses (HIV, HBV, HCV, herpes, myxoviruses)</td>
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Microbial resistance to antiseptics and disinfectants

- It can be classified as
  1. **Intrinsic** a natural property of an organism
  2. **Acquired** by mutation or acquisition of plasmids or transposons
Intrinsic Resistance Mechanisms

- By 2 mechanisms:
  1. Permeability barrier:
     - Reduced uptake
     - Depends on the nature and composition of outer layers of the organism
  2. Degradation of a compound by enzymes (less common)
Role of biofilm

• The association of microorganisms with solid surfaces
• Reduced sensitivity may be due to:
  1. Reduced access of a disinfectant to the cells:
     – nature of antiseptic/disinfectant,
     – binding capacity of glycocalyx toward antiseptic or disinfectant
     – rate of growth of microcolony relative to diffusion rate of chemical inhibitor
2. Chemical interaction between the disinfectant and the biofilm itself,
3. Modulation of the microenvironment
4. Nutrient limitation
   • Reduced growth rate
   • Production of degradative enzymes (and neutralizing chemicals)
5. Genetic exchange between cells in a biofilm
Acquired Resistance Mechanisms

- Arise by either mutation or the acquisition of genetic material in the form of plasmids or transposons.

- **Mechanisms of plasmids mediated resistance**
  1. Inactivation
  2. Efflux
  3. Decreased uptake
  4. Cell surface alterations (outer membrane proteins)
Mutational resistance to antiseptics and disinfectants

- The mechanism is nonspecific and involves:
  1. Alterations in the cell envelopes (Outer membrane modification involve changes in fatty acid profiles and outer membrane proteins).
  2. Efflux mechanism
  3. MDR systems: in which an operon or gene is associated with changes in antiseptic or disinfectant susceptibility.
THANKS