STERILIZATION AND DISINFECTION

DR A. R. OJEWUYI
Definitions

• Sterilization: A process that results in the destruction or elimination all forms of microbial life. It is an absolute term.

• Disinfection: A process that results in the destruction or elimination of many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

• Germicide: An agent that can kill microorganisms, particularly pathogenic organisms.
Definitions

• Antiseptics: Germicides applied to living tissue and skin
• Disinfectants: Germicides applied only to inanimate objects.
• Cleaning: The removal of visible soiling organic or inorganic material from objects and surfaces, by manual or mechanical means through the use of water with detergents or enzymatic products.
Definitions

• Decontamination is a procedure that removes pathogenic microorganisms from objects so that they are safe to handle, use, or discard.
Determining the Appropriate Action: Sterilization or Disinfection?

• Earle H. Spaulding helped by coming up with Spaulding’s Classification:
  – Critical items
  – Semi-critical items
  – Non-critical items
Critical Items:

- Objects that enter sterile tissue or the vascular system. There is a high risk of infection if such items are contaminated with any microorganism, including bacterial spores. They must be absolutely sterile because any microbial contamination could result in disease transmission.
- Examples are: surgical instruments, cardiac and urinary catheters, implants, and ultrasound probes used in sterile body cavities.
- Minimum requirement is sterilization
Semi-Critical Items

• Objects that come in contact with mucous membranes or non-intact skin, should be free of all microorganisms, although small numbers of bacterial spores may safely be present.

• Examples are respiratory therapy and anesthesia equipment, some endoscopes, laryngoscope blades, esophageal manometry probes, endocavitary probes, anorectal manometry catheters, and diaphragm fitting rings.

• Minimum requirement is high-level disinfection.
Non-Critical Items

- Objects that come in contact with intact skin but not mucous membranes. There is virtually no documented risk of transmitting infectious agents to patients through these objects when they are used as noncritical items because intact skin acts as an effective barrier to most microorganisms.

- Examples are bedpans, blood pressure cuffs, crutches, bed rails, bedside tables, furniture, and floors. These items (e.g., bedside tables, bed rails) could contribute to transmission of infection by contaminating the hands of health care workers or medical equipment that will subsequently come in contact with patients.

- Minimum requirement is **low-level disinfection**
Classification of Disinfectants Based on Levels of Disinfection

- **High-level disinfectants**: Eliminate all microorganisms in or on an instrument, except for small numbers of bacterial spores. At higher concentrations or longer exposure times these may be sterilants.

- **Intermediate-level disinfectants**: might be cidal for mycobacteria, vegetative bacteria, most viruses, and most fungi but do not necessarily kill bacterial spores.

- **Low-level disinfectants**: kill most vegetative bacteria, some fungi, and some viruses.
Factors Affecting the Efficacy of Disinfection and Sterilization

- Number and location of microorganisms
- Nature of microorganisms
- Concentration and Potency of Disinfectants
- Physical and Chemical Factors: pH, temperature
- Presence of organic and Inorganic matter
- Duration of Exposure
- Biofilms
Prions*
(CJD, BSE)
\[\downarrow\]
Coccidia
(Cryptosporidium)
\[\downarrow\]
Spores
(Bacillus, C. difficile)
\[\downarrow\]
Mycobacteria
(M. tuberculosis, M. avium)
\[\downarrow\]
Cysts
(Giardia)
\[\downarrow\]
Small non-enveloped viruses
(Polio virus)
\[\downarrow\]
Trophozoites
(Acanthamoeba)
\[\downarrow\]
Gram-negative bacteria (non-sporulating)
(Pseudomonas, Providencia)
\[\downarrow\]
Fungi
(Candida, Aspergillus)
\[\downarrow\]
Large non-enveloped viruses
(Enteroviruses, Adenovirus)
\[\downarrow\]
Gram-positive bacteria
(S. aureus, Enterococcus)
\[\downarrow\]
Lipid enveloped viruses
(HIV, HBV)

FIG. 1. Descending order of resistance to antiseptics and disinfectants. The asterisk indicates that the conclusions are not yet universally agreed upon.
Cleaning

- Cleaning is the removal of foreign material such as soil, blood, fluids, tissue or faeces from objects and is normally accomplished using water with detergents or enzymatic products.
- Thorough cleaning is required before high-level disinfection and sterilization because inorganic and organic materials that remain on the surfaces of instruments interfere with the effectiveness of these processes.
- Also, if soiled materials dry or bake onto the instruments, the removal process becomes more difficult and the disinfection or sterilization process, less effective or ineffective.
- Surgical instruments should be pre-soaked or rinsed to prevent drying of blood and to soften or remove blood from the instruments.
- Cleaning may be done manually or mechanically using automated systems.
Methods of Sterilization and Disinfection

• Physical Methods

• Chemical Methods

  Use of different classes of chemical agents which may be liquid or gaseous
## Methods of Sterilization and Disinfection

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Physical Agents

• **Heat** – Most reliable method, It causes
  - Protein denaturation & coagulation
  - Hydrolysis & breakdown of protein
  - Oxidative damage
  - Toxic effects of elevated electrolyte levels
• **Dry heat**

  o **Flaming** – Wire loop and forceps tips over Bunsen flame till red hot
  
  o **Incineration** – For disposables

  o **Hot air oven** – 160° C for 1 hour (glassware, forceps, scalpels, swabs, dusting powder, )

  o **Tyndallization** – Repeated heating at temperatures 90 – 100° C for 15 min on 3 successive days allowing the material or substance to cool to atmospheric temperature in between.
• Moist heat
  ▪ Pasteurization of milk at temperature 63° C for 30 minutes.
  ▪ Boiling at a temperature of 100° C 10 – 30 mins after immersing in water.
  ▪ Steam under pressure (Autoclave) – 121° C at 15psi for 15-30 mins.
  ▪ Moist heat destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins.
  ▪ The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed pre-vacuum sterilizer.

Sterilization control – Spores of *Bacillus stereothermophilus* (now *Geobacillus stearothermophilus*)

Chemical indicators, autoclave tapes
• **Filtration**

Remove bacteria from heat labile liquids – sera, sugars/ antibiotics for culture media

Types of filters – Candle filters, asbestos filters, sintered glass filters, membrane filters

HEPA (High-efficiency particulate air)- filters
• **Radiation**

- Non-ionizing
  - Infra red - syringes, catheters
  - UV Rays - entry ways, TB labs

Low penetrating power

- Ionizing (Gamma rays, X Rays, Cosmic rays) cold sterilization,
  For plastics, glass, swabs, catheters, animal feed, cardboard, oils, greases, fabrics, metal fabrics.

- Ultrasonic & Sonic vibrations
  Used in dentistry but not very common.
Chemical Agents

• Criteria for an ideal chemical agent –
  • Have wide spectrum of activity
  • Be active in the presence of organic matter
  • Be effective in acid and alkaline media
  • Be stable, have speedy action & high penetrative power
  • Be compatible with other antiseptics and disinfectants
  • Not corrode metals OR cause local irritation/sensitization
  • Not interfere with healing
  • Not be toxic if absorbed into circulation
  • Be cheap, safe, easy to use & easily available
  • No agent has all of this
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Alcohols

Ethyl alcohol (ethanol, alcohol), and Isopropyl alcohol (isopropanol, propan-2-ol) are the most widely used. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Increased efficacy in the presence of water. 70-90%, not absolute

**Mechanism of action:** They cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis
Aldehydes

- Glutaraldehyde: An important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment.
- Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses.
- Glutaraldehyde is more active at alkaline than at acidic pHs.

- Mechanism of action: Cross-linking of proteins in cell envelope and elsewhere in the cell
Aldehydes

- Formaldehyde (methanal, CH₂O): is a monoaldehyde that exists as a freely water-soluble gas. Formaldehyde solution (formalin) is an aqueous solution containing methanol to delay polymerization.
- Used as a disinfectant and sterilant in liquid or in combination with low-temperature steam. Formaldehyde is bactericidal, sporicidal, and virucidal, but it works more slowly than glutaraldehyde.

Mechanism of action: Cross-linking of proteins, RNA, and DNA
Aldehydes

- Ortho-Phththalaldehyde. (OPA) is a newer disinfectant that has potent bactericidal and sporicidal activity and has been suggested as a replacement for glutaraldehyde in endoscope disinfection. OPA is an aromatic compound with two aldehyde groups.
- Mechanism of action is similar to that of glutaraldehyde
Anilides

• Antiseptics, but they are rarely used in the clinic. Triclocarban (TCC; 3,4,49-triclorocarbanilide) is the most extensively studied in this series and is used mostly in consumer soaps and deodorants. TCC is particularly active against gram-positive bacteria but significantly less active against gram-negative bacteria and fungi and lacks appreciable substantivity (persistency) for the skin.

• The anilides are thought to act by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane, leading to cell death.
Biguanides

• Chlorhexidine probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products, also as a disinfectant and preservative.

• This is due to its broad-spectrum efficacy, substantivity for the skin, and low irritation.

• Mechanism of action: Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm.
Halides/Halogen Releasing Compounds

• Chlorine- and iodine-based compounds are the most significant microbicidal halogens used in the clinic and have been traditionally used for both antiseptic and disinfectant purposes.
Halides/Halogen Releasing Compounds

- Chlorine-releasing agents (CRAs): The most important types of CRAs are sodium hypochlorite, chlorine dioxide, and the N-chloro compounds such as sodium dichloroisocyanurate (NaDCC).
- Sodium hypochlorite solutions are widely used for hard-surface disinfection (household bleach) and can be used for disinfecting spillages of blood containing HIV or HBV.
- Hypochlorous acid is the active moiety responsible for bacterial inactivation by CRAs.
- CRAs at higher concentrations are sporicidal.
- Mechanism of action: Oxidation of thiol groups to disulfides, sulfoxides, or disulfoxides and inhibition of DNA synthesis.
Halides/Halogen Releasing Compounds

• Iodine and Iodophors: Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal
• Aqueous or alcoholic (tincture) solutions of iodine are used as antiseptics, they are associated with irritation and excessive staining and are generally unstable
• These problems were overcome by the development of iodophors (“iodine carriers” or “iodine-releasing agents”); such as povidone-iodine and poloxamer-iodine in both antiseptics and disinfectants. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active “free” iodine.
• Mechanism of action: Similar to chlorine
Silver compounds

• The most important silver compound in use is silver sulfadiazine.

• Mechanism of action: Reacts with membrane-bound enzymes (interaction with thiol groups) inactivates them.
Peroxygens

• Hydrogen peroxide (H₂O₂): is a widely used biocide for disinfection, sterilization, and antisepsis. It is a clear, colourless liquid that is commercially available in a variety of concentrations ranging from 3 to 90%.

• H₂O₂ is considered environmentally friendly, because it can rapidly degrade into water and oxygen.

• It can be used in the gaseous phase as a sterilant.

• Mechanism of action: Produces hydroxyl free radicals (•OH) which acts as an oxidant by attacking essential cell components, including lipids, proteins, and DNA. Exposed sulfhydryl groups and double-bonds are particularly targeted.
Peroxygens

- Peracetic acid (PAA) (CH₃COOOH) is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations (0.3%).
- PAA also decomposes to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases, unlike H₂O₂ and remaining active in the presence of organic loads.
- Its main application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and hemodialyzers, but it is also used as an environmental surface sterilant.
- Mechanism of action: Produces hydroxyl free radicals (•OH) which attack essential cell components, including lipids, proteins, and DNA. Exposed sulfhydryl groups and double-bonds are particularly targeted.
Phenolic-type Antimicrobial Agents

• Long been used for their antiseptic, disinfectant, or preservative properties, depending on the compound.
• Mechanism of action: They have membrane-active properties which induce progressive leakage of intracellular constituents.
Quaternary Ammonium Compounds

- Surface-active agents (surfactants) have two regions: a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Surfactants are classified into cationic, anionic, non-ionic.
- Quaternary ammonium compounds (QACs), are cationic agents, and are useful antiseptics and disinfectants. They are sometimes known as cationic detergents. E.g. Centrimide.
- Have limited activity.
- Healthcare–associated infections have been reported from contaminated quaternary ammonium compounds used to disinfect patient-care supplies or equipment, such as cystoscopes or cardiac catheters.

Mechanism of action: Generalized membrane damage involving phospholipid bilayers.
Vapor-Phase Sterilants

- Heat-sensitive medical devices and surgical supplies can be effectively sterilized by liquid sterilants (in particular glutaraldehyde, PAA, and hydrogen peroxide) or by vapor-phase sterilization systems.
- The most widely used active agents in these “cold” systems are ethylene oxide, formaldehyde and, more recently developed, hydrogen peroxide and PAA.
Vapor-Phase Sterilants

- Ethylene oxide and formaldehyde are both broad spectrum alkylating agents. However, their activity is dependent on active concentration, temperature, duration of exposure, and relative humidity.
- As alkylating agents, they attack proteins, nucleic acids, and other organic compounds; both are particularly reactive with sulfhydryl and other enzyme-reactive groups.
- Ethylene oxide gas has the disadvantages of being mutagenic and explosive but is not generally harsh on sensitive equipment, and toxic residuals from the sterilization procedure can be routinely eliminated by correct aeration.
- *B. atrophaeus* is the recommended biologic indicator for use with ethylene oxide gas.
Vapor-Phase Sterilants

- Vapor-phase hydrogen peroxide and PAA are considered more active (as oxidants) at lower concentrations than in the liquid form. Both active agents are used in combination with gas plasma in low-temperature sterilization systems.
- Their main advantages over other vapor-phase systems include low toxicity, rapid action, and activity at lower temperature; the disadvantages include limited penetrability and applications.
Vapor-Phase Sterilants

Hydrogen Peroxide Gas Plasma

• Gas plasmas have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas).

• Gas plasmas are generated in an enclosed chamber under deep vacuum; radiofrequency or microwave energy is used to excite the gas (i.e., hydrogen peroxide) molecules and produce charged particles, many of which are in the form of free radicals (e.g., hydroxyl and hydroperoxyl).

• The biologic indicator used with this system is *B. stearothermophilus* spores.
Flash Sterilization

• “Flash” steam sterilization was originally defined by Underwood and Perkins as sterilization of an unwrapped object at 132° C (270° F) for 3 minutes at 27 to 28 pounds (12.3 to 12.7 kg) of pressure in a gravity displacement sterilizer.

• Higher heat, shorter time for urgently needed surgical materials
Prions

For sterilizing critical devices: clean the device and sterilize it by one of four methods:

1. Immerse in a solution of 40 g NaOH in 1 L of water for 1 hour; remove and rinse in water, then transfer to an open pan and put in autoclave [121°C gravity displacement or 134°C porous or prevacuum sterilizer] for 1 hour.

2. Immerse instruments in 40 g of NaOH in 1 L of water for 1 hour and heat in a gravity displacement sterilizer at 121°C for 30 minutes. (However, the combination of sodium hydroxide and steam sterilization may be deleterious to surgical instruments, sterilizers, as well as harmful to sterilizer operators who would be breathing vaporized chemicals unless engineering controls or use of personal protective equipment prevents exposure.

3. Place in autoclave at 134°C for 18 minutes in a pre-vacuum sterilizer.

4. Place in autoclave at 132°C (270°F) for 1 hour in a gravity displacement sterilizer.
Control of sterilization

Controls or Indicator could be:

• Mechanical – Gauge, printouts
• Chemical – Bowie dick tapes, Brownes tubes
• Biologic – Spores of *B. stearothermophilus*
  spores of *B. atrophaeus*
THANK YOU