Sterilization and disinfection.

Sterilization: is the killing or removal of *all* microorganisms, including bacterial spores, which are highly resistant.

Disinfection: is the killing of many, but not all, microorganisms. For adequate disinfection, pathogens must be killed, but some organisms and bacterial spores may survive.

Chemicals used to kill microorganisms on the surface of skin and mucous membranes are called **antiseptics**.

Bacteriostatic: means that the agent prevents the growth of bacteria .

Bactericidal: means that it kills bacteria.

Advantage of Sterilization:

1- Prevent transmission of disease.
2- Prevent contamination and growth of undesirable bacteria.
3- Prevent spoilage of material by microorganism.

Methods of Sterilization: there are 2 methods 1- Chemical methods
2- Physical methods

Chemical methods: Chemicals vary greatly in their ability to kill microorganisms.

Chemical agents act primarily by one of the three mechanisms:

(1) disruption of the lipid-containing cell membrane.
(2) modification of proteins.
(3) modification of DNA.

1- DISRUPTION OF CELL MEMBRANES

Alcohol: is widely used to clean the skin before immunization or venipuncture. It acts mainly by disorganizing the lipid structure in membranes, but it denatures proteins as well. Ethanol requires the presence of water for maximal activity (i.e., it is far more effective at 70% than at 100%). Seventy percent ethanol is often used as an antiseptic to clean the skin prior to venipuncture.

Detergents: are "surface-active" agents composed of a long-chain, lipid-soluble, hydrophobic portion and a polar hydrophilic group, which can be a cation, an anion, or a nonionic group. These surfactants interact with the lipid in the cell membrane through their hydrophobic chain and with the surrounding water through their polar group and thus disrupt the membrane.

Phenols: was the first disinfectant used in the operating room (by Lister in the 1860s), but it is rarely used as a disinfectant today because it is too caustic. **Chlorhexidine:** is a chlorinated phenol that is widely used as a hand disinfectant prior to surgery ("surgical scrub") and in the cleansing of wounds.

Hexachlorophene: which is a biphenol with six chlorine atoms, is used in germicidal soaps, but concern over possible neurotoxicity has limited its use.

2- MODIFICATION OF PROTEINS

Chlorine: is used as a disinfectant to purify the water supply and to treat swimming pools. It is also the active component of hypochlorite , which is used as a disinfectant in the home and in hospitals. Chlorine is a powerful oxidizing agent that kills by cross-linking essential sulfhydryl groups in enzymes to form the inactive disulfide.

Iodine: is the most effective skin antiseptic used in medical practice and should be used prior to obtaining a blood culture and installing intravenous catheters because contamination with skin flora such as *Staphylococcus epidermidis* can be a problem. Iodine, like chlorine, is an oxidant that inactivates sulfhydryl-containing enzymes.

Iodine is supplied in two forms:

(1) Tincture of iodine (2% solution of iodine and potassium iodide in ethanol) is used to prepare the skin prior to blood culture. Because tincture of iodine can be irritating to the skin, it should be removed with alcohol.

(2) Iodophors are complexes of iodine with detergents that are frequently used to prepare the skin prior to surgery because they are less irritating than tincture of iodine.

Heavy Metals: Mercury and **silver** have the greatest antibacterial activity of the heavy metals and are the most widely used in medicine. They act by binding to sulfhydryl groups, thereby blocking enzymatic activity.

Silver sulfadiazine: is used to prevent infection of burn wounds.

Hydrogen Peroxide: is used as an antiseptic to clean wounds and to disinfect contact lenses. Its effectiveness is limited by the organism's ability to produce catalase, an enzyme that degrades H2O2. **Hydrogen peroxide** is an oxidizing agent that attacks sulfhydryl groups, thereby inhibiting enzymatic activity.

Formaldehyde & Glutaraldehyde:

Formaldehyde, which is available as a 37% solution in water (formalin), denatures proteins and nucleic acids.

Glutaraldehyde, which has two reactive aldehyde groups, is 10 times more effective than formaldehyde and is less toxic. In hospitals, it is used to sterilize respiratory therapy equipment, endoscopes, and hemodialysis equipment.

Ethylene Oxide: Ethylene oxide gas is used extensively in hospitals for the sterilization of heat sensitive materials such as surgical instruments and plastics. It kills by alkylating both proteins and nucleic acids.

Acids & Alkalis: Strong acids and alkalis kill by denaturing proteins. Although most bacteria are susceptible, it is important to note that *Mycobacterium tuberculosis* and other mycobacteria are relatively resistant to 2% NaOH, which is used in the clinical laboratory to liquefy sputum prior to culturing the organism. Weak acids, such as benzoic, propionic, and citric acids, are frequently used as food preservatives because they are bacteriostatic.

3- MODIFICATION OF NUCLEIC ACIDS: A variety of dyes not only stain microorganisms, but also inhibit their growth.

A- crystal violet (gentian violet): which is used as a skin antiseptic. Its action is based on binding of the positively charged dye molecule to the negatively charged phosphate groups of the nucleic acids.

B-Malachite green: a triphenylamine dye like crystal violet, is a component of Löwenstein-Jensen′s medium, which is used to grow *M. tuberculosis*. The dye inhibits the growth of unwanted organisms in the sputum during the 6-week incubation period.

PHYSICAL AGENTS:-

The physical agents act either by imparting energy in the form of heat or radiation or by removing organisms through filtration.

HEAT: Heat energy can be applied in three ways:

1- moist heat (either boiling or autoclaving) 2-dry heat 3-pasteurization.

heat kills by denaturing proteins, but membrane damage and enzymatic cleavage of DNA may also be involved. Moist heat sterilizes at a lower temperature than dry heat, because water aids in the disruption of noncovalent bonds (e.g., hydrogen bonds).

Moist heat sterilization, usually **autoclaving,** is the most frequently used method of sterilization. Because bacterial **spores are resistant to boiling** (100°C at sea level), they must be exposed to a higher temperature; this cannot be achieved unless the pressure is increased. For this purpose, an autoclave chamber is used in which steam, at a pressure of 15 lb/in2, reaches a temperature of 121°C and is held at that temperature for 15 to 20 minutes. This kills even the highly heatresistant spores of *Clostridium botulinum*, the cause of botulism, with a margin of safety.

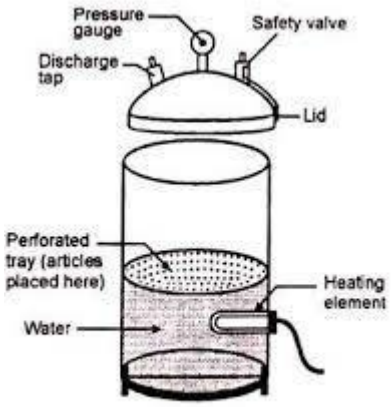

Fig. 1. Autoclave

Sterilization by dry heat, on the other hand, requires temperatures in the range of 180°C for 2 hours. This process is used primarily for glassware.

Pasteurization: is used primarily for milk, consists of heating the milk to 62°C for 30 minutes followed by rapid cooling. (“Flash” pasteurization at 72°C for 15 seconds is often used.) This is sufficient to kill the vegetative cells of the milk borne pathogens (e.g., *Mycobacterium bovis, Salmonella, Streptococcus, Listeria*, and *Brucella*).

RADIATION: The two types of radiation used to kill microorganisms are **ultraviolet (UV) light and X-rays**. The greatest antimicrobial activity of UV light occurs at 250

to 260 nm, which is the wavelength region of maximum absorption by the purine and pyrimidine bases of DNA.

X-rays have higher energy and penetrating power than UV radiation and kill mainly by the production of free radicals (e.g., production of hydroxyl radicals by the hydrolysis of water). X-rays are used in medicine for sterilization of heat-sensitive items, such as sutures and surgical gloves, and plastic items, such as syringes.

FILTRATION: is the preferred method of sterilizing certain solutions (e.g., those with heat-sensitive components). In the past, solutions for intravenous use were autoclaved, but heat-resistant endotoxin in the cell walls of the dead gram-negative bacteria caused fever in recipients of the solutions. Therefore, solutions are now filtered to make them **pyrogen-free** prior to autoclaving. The most commonly used filter is composed of nitrocellulose and has a pore size of 0.22 μm. This size will retain all bacteria and spores.