Lec.4

Sterilization and disinfection

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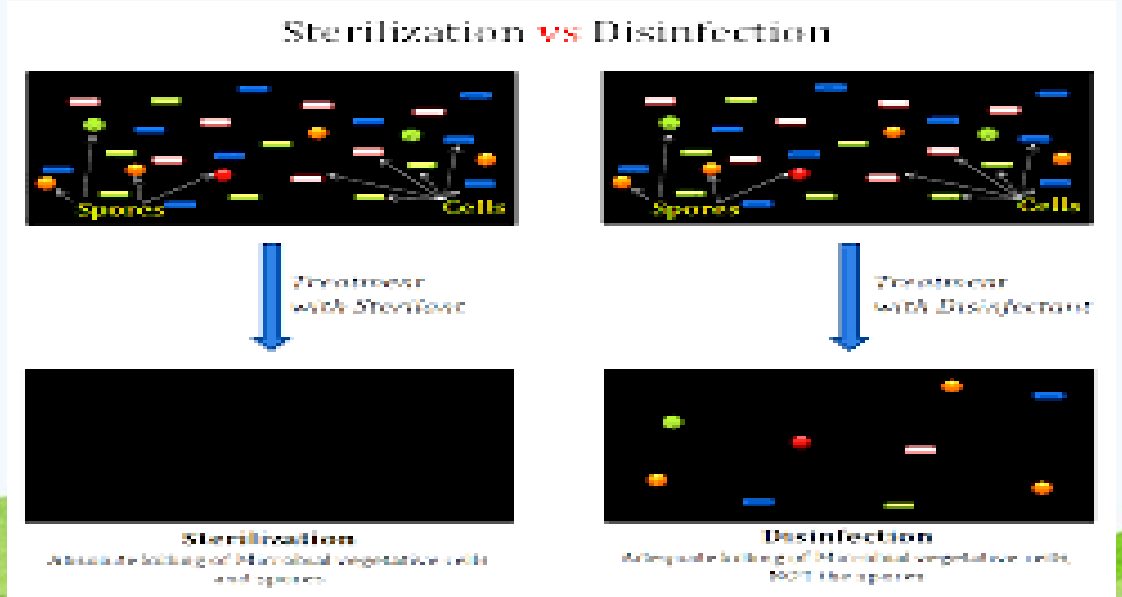
Sterilization: Sterilization is defined as the process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.

Disinfection: Disinfection is the killing, inhibition, or removal of microorganisms that may cause the disease.

Antiseptics: Antiseptics are chemical agents applied to the tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.



METHODS OF STERILIZATION AND DISINFECTION

A. Physical agents

B. Chemical agents

A. Physical Agents

1. Sunlight: Sunlight has an appreciable bactericidal activity. Its disinfectant action is primarily due to its content of ultraviolet rays.

2. Drying **:**Drying in air has a deleterious effect on many bacteria.

3. Heat **:**Heat is the most reliable and universally applicable method of sterilization and, wherever possible, should be methods of choice. Either dry or moist heat may be applied. Materials that may be damaged by heat can be sterilized at lower temperature, for longer periods or by repeated cycles.

Methods of sterilization and disinfection

A. Physical agents

1. Sunlight
2. Drying
3. Heat
   a. Dry heat
   b. Moist heat
4. Filtration
5. Radiation
6. Ultrasonic and sonic vibrations

B. Chemical agents

a. Agents that damage the cell membranes
   1. Surface-active disinfectants
   2. Phenolic compounds
   3. Alcohols

b. Agents that damage proteins
   1. Acids and alkalies
   2. Alcohols

c. Agents that modify functional groups of proteins and nucleic acids
   1. Heavy metals
   2. Oxidizing agents
   3. Dyes
   4. Alkylating agents

Mechanism of Action

Dry heat: The lethal effect of dry heat, or desssication in general, is usually due to protein denaturation, oxidative damage, and toxic effects of elevated levels of electrolytes.

Moist heat: It kills microorganisms by coagulation and denaturation of their enzymes and structural proteins.

a. Dry Heat Sterilization

1. **Red heat**: **Inoculating wires, loops and points** of forceps are sterilized by holding them almost vertically in a Bunsen flame until red hot.

2. **Flaming**: **Scalpel blades, glass slides, mouth** of culture tubes and bottles are exposed to a flame for a few seconds without heating them to become red hot.

3. **Incineration**: **This is an efficient method for** the sterilization and disposal of contaminated materials at a high temperature. Such as pathological waste materials, surgical dressings, contaminated material, animal carcasses and other clinical waste.

4. **Hot air oven**: **Hot air oven is the most widely** used method of sterilization by dry heat. It is used to process materials which can withstand high temperatures for length of time needed for sterilization by dry heat, but which are likely to be affected by contact with steam. Hot air oven is electrically heated, with heating **. It should be fitted with a fan** to provide forced air circulation throughout the oven chamber, a temperature indicator, a control thermostat and timer, open mesh shelving and adequate wall insulation.

b. Moist Heat Sterilization : Moist heat is divided into three forms:

A. At temperature below 100°C
B. At a temperature of 100°C
C. At temperature above 100°C

A. **At temperature below 100°C: It includes:**

1. **Pasteurization of milk: Disinfection by** moist heat at temperature below 100°C is termed **pasteurization. Milk can be** pasteurized in two ways. The temperature is **63°C for 30 minutes (holder method) or 72°C for 15–20 ,and the seconds (flash method) followed by rapid cooling to 13°C or lower.** All non-sporing pathogens such as *mycobacteria, brucellae and salmonellae* are destroyed by these processes. ***Coxiella burnetii is relatively heat resistant and may*** survive the holder method.

2. **Vaccine preparatior: Vaccines prepared** from non-sporing bacteria may be inactivated in a water bath at 60°C for one hour.

3. **Inspissation: Media such as Lowenstein-** Jensen and Loeffler's serum are rendered sterile by heating at 80-85°C for half-an hour on three successive days **(fractional sterilization). This process is called inspissation and instrument used is called *inspissator*.**

4. **Water bath: Washing or rinsing laundry** or utensils in water bath at 70-80°C for few minutes will kill most non-sporing microorganisms present.

5. **Low temperature steam formaldehyde (LTSF) sterilization: In this method**, steam at sub-atmospheric pressure at the temperature of 75°C with formaldehyde vapor is used. The efficacy of LTSF sterilizers is tested by using ***Bacillus stearothermophilus as biological control.***

B. At temperature of 100°C

1.Boiling: Boiling at 100°C for 10–30 minutes :kills all vegetative spores and some bacterial spores.

2. Steam at atmospheric pressure at 100°C for 90 minutes This can be provided by the traditional **Koch and Arnold steamer (or by the** multipurpose autoclave).
They are exposed to steam at atmospheric pressure for 90 minutes
One single exposure to steam for 90 minutes ensures complete sterilization.

3. Tyndallization: An exposure of steam at 100°C for 20 minutes on three successive days is called **tyndallization or intermittent sterilization. This is a fractional method of** sterilization. The instrument commonly used is Koch and Arnold steamer.

C. At temperature above 100°C

Steam under pressure: Steam above 100°C or saturated steam is a more efficient sterilizing agent than hot air. **Autoclave** Autoclaving is the process of sterilization by saturated steam under high pressure with 121°C **.Uses of autoclave :**

1.For sterilizing culture media and other laboratory supplies, aqueous solutions, rubber material, dressing materials, gowns, dressing, linen, gloves, instruments and pharmaceutical products.

2. For all materials that are water-containing, permeable or wettable and not liable to be damaged by the process.

3. Particularly useful for materials which cannot withstand the higher temperature of hot air oven.

4. Filtration

Filtration is the principal method used in the laboratory for the sterilization of heat labile materials, e.g. sera, solutions of sugars or antibiotics used for the preparation of culture media.

Uses:-

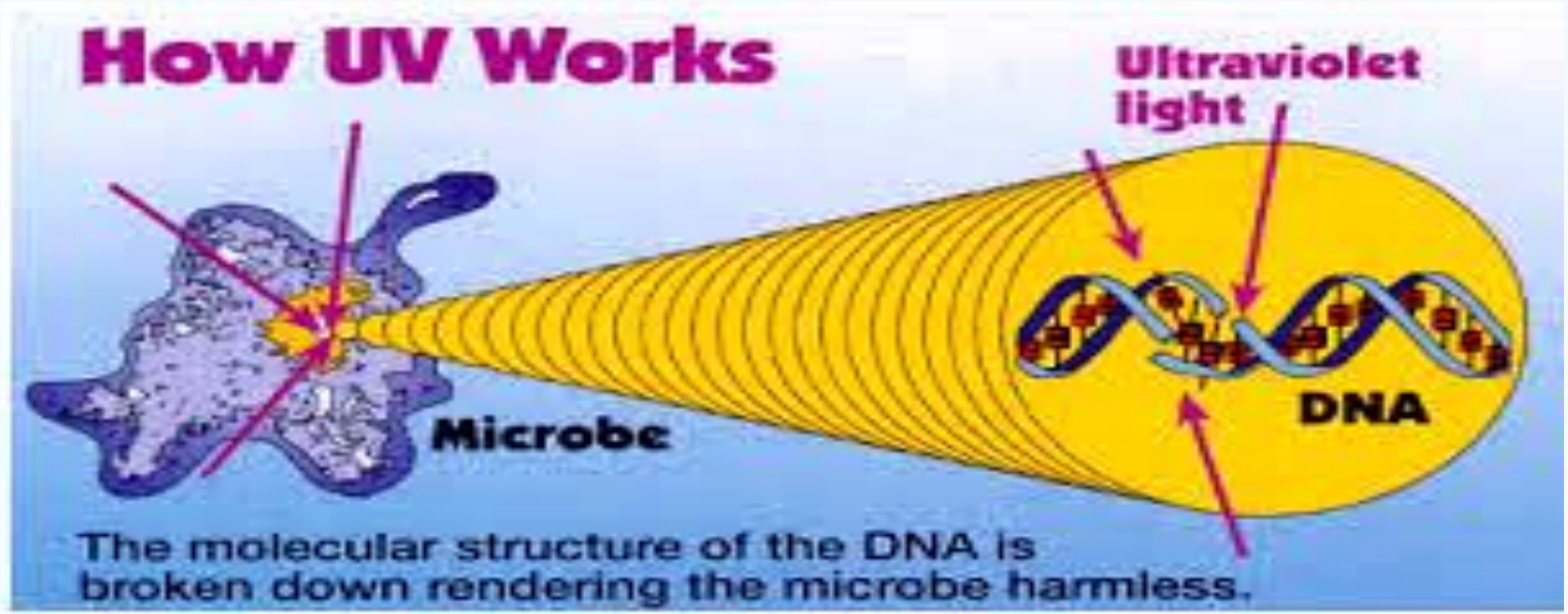
1. **Heat-sensitive solutions: For sterilization of** pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics and other heat-sensitive solutions.
2. **For separation of bacteriophages and bacterial toxins from bacteria.**
3. **Isolation of organisms which are scanty in fluids.**
4. **Concentration of bacteria from liquids, e.g.** in testing water samples for *cholera vibrios* or typhoid bacilli.
5. **For virus isolation.**

5. Radiation Two types of radiations are used:

1. **Non-ionizing radiations: Infrared and** ultraviolet rays are of non-ionizing type
The effectiveness of UV light as a lethal and mutagenic agent is closely correlated with its wavelength. The most effective bactericidal wavelength is in the 240–280 nm range, with the optimum being about 260 nm, the wavelength most effectively absorbed by DNA and this interferes with DNA replication.

Microbial sensitivity to UV radiation:
i. Bacterial spores are generally more resistant to UV light than are vegetative cells.
ii. Viruses are also inactivated.
iii. To disinfect drinking water.
iv. **Disinfection of enclosed areas such** as entryways, hospital wards, operating theatres, laboratories and in ventilated safety cabinets in which dangerous microorganisms are being handled.

2. **Ionizing radiations: These include X-rays, g**(gamma) rays and cosmic rays. These have very high penetrative power and are highly lethal to all cells including bacteria. Ionizing radiations damage the DNA by various mechanisms.


How UV Works
Ultraviolet light
Microbe
DNA
The molecular structure of the DNA is broken down rendering the microbe harmless.

B. Chemical Agents

Germicidal chemicals can be used to disinfect and, in some cases, sterilize.

Characteristics of a Disinfectant

An ideal antiseptic or disinfectant should:

1. Have a wide spectrum of activity and must be effective against a wide variety of infectious agents (Gram-positive and gram-negative bacteria, acid-fast ; bacteria, bacterial endospores, fungi, and viruses)
2. Be active at high dilutions and in the presence of organic matter
3. Be effective in acid as well as alkaline media
4. Have speedy action
5. Have high penetrating power
6. Be stable
7. Be compatible with other antiseptics and disinfectants
8. Not corrode metals
9. Not cause local irritation or sensitization
10. Not interfere with healing
11. Not be toxic if absorbed into circulation
12. Be cheap and easily available
13. Be safe and easy to use.