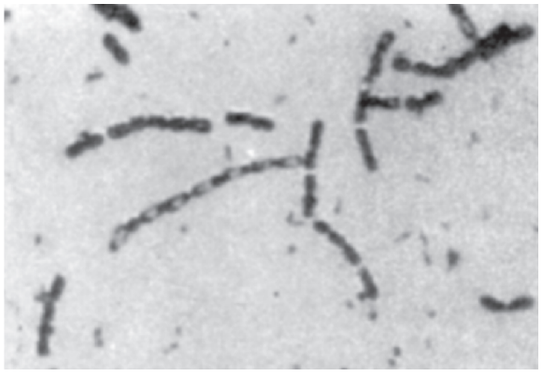

Fig. 2.10: Gram-stained smear of *Bacillus subtilis* showing Gram-positive bacilli in chains with spores which appear as unstained areas within the bacilli (×1000)

Cultural characteristics

L-forms are difficult to grow and usually require a medium that is solidified with agar as well as having the right osmotic strength. L-forms are produced more readily with penicillin than with lysozyme.

Colonies of L-forms of bacteria on agar medium show a characteristic '**fried-egg**' appearance with a dark thick centre, where many of the organisms embed themselves and grow within the agar, and a lighter periphery consisting of organisms lying on the surface of the agar. In liquid medium they grow in the form of clumps. Some L-forms are capable of reverting to normal bacillary forms upon removal of the inducing stimulus. Other L-forms are, however, stable and never revert. Presence of residual peptidoglycan is essential for reversion. It acts as a primer in its own biosynthesis.

Key Points

- Prokaryotes such as ***bacteria are simple cells*** with no internal membranes or organelles.
- ***Eukaryotes have a nucleus and organelles*** such as mitochondria, and complex ***internal membranes*** (e.g. fungi and human cells).
- ***Structures external*** to cell wall of bacteria are ***flagella, pili*** or ***fimbriae, capsule*** and ***slime layer***.
- Flagella are used for movement, pili for adhesion, and capsules protect the bacteria from antibacterial agents such as lytic enzymes and inhibit phagocytosis thus contributing to the virulence of bacteria.
- ***Bacterial cytoplasm contains*** chromosomal nuclear material, ribosomes, mesosomes and inclusions/storage granules.
- ***Sporulation*** is a response to starvation in ***Bacillus*** spp. and ***Clostridium*** spp.

Important Questions

1. Differentiate between prokaryotes and eukaryotes in a tabulated form.
2. Draw a labelled diagram of a bacterial cell.
3. Describe the cell wall of bacteria.
4. Write short notes on:
   (a) Bacterial cell wall (b) Bacterial capsule (c) Bacterial spore

Multiple Choice Questions

1. Which of the following bacteria is cell wall deficient?
   (a) *Mycoplasma*
   (b) *Treponema*
   (c) *Staphylococcus*
   (d) *Klebsiella*
2. Which of the following bacterial structures is/are involved in attachment to cell surface?
   (a) Flagella
   (b) Fimbria
   (c) Capsule
   (d) Mesosomes
3. When determining distances and sizes, the smallest unit of measure is:
   (a) Centimetre
   (b) Millimetre
   (c) Micrometre
   (d) Nanometre
4. Which of the following are eukaryotes?
   (a) Fungi
   (b) Bacteria
   (c) Chlamydiae
   (d) Mycoplasmas
5. What function does a condenser serve in light microscope?
   (a) Focuses the light onto our eyes
   (b) Focuses the light rays on the sample
   (c) Increases light intensity
   (d) Reduces glare
6. Fimbriae present on the outer surface of bacteria are used for:
   (a) Identification of bacteria
   (b) Bacterial motility
   (c) Adherence to surfaces
   (d) Antibiotic resistance

Answers

1. a **2.** b **3.** d **4.** a **5.** b **6.** c

moving the plate for about 10 seconds. Allow the agar to set and incubate at 37°C for 48 hours. After incubation, colonies will be seen well distributed throughout the depth of the medium and can be enumerated using colony counters. Count the colonies in three plates containing 50–500 colonies/plate. Multiply the average number/plate by the dilution factor to obtain the viable count/ml in the original suspension.

Shake culture

It is made by melting nutrient agar in a test tube, cooling it to 45°C and inoculating it while molten from a liquid medium with a drop from a capillary pipette. Withdraw the pipette, replace the cap or plug and discard the pipette into disinfectant. Mix the contents of the tube by rotation between the palms of the hands before the agar solidifies and incubate it at 37°C for 24 hours and look for the growth of the organisms.

Liquid culture

Liquid cultures in tubes, bottles or flasks may be inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes. Large inocula can be employed in liquid cultures and hence this method is adopted for blood culture and for sterility tests, where the concentration of bacteria in inocula are expected to be small. Liquid cultures are also preferred when large yields are desired.

Aerobic culture

For cultivation of aerobes the incubation is done in an incubator under normal atmospheric condition. The temperature of incubation for most of the human pathogenic bacteria is 37°C. For cultivation of many fungi incubation of the inoculated media should be carried out at 25–28°C. To prevent drying of the medium when prolonged incubation is necessary, as in the cultivation of the tubercle bacilli, screw-capped bottles should be used instead of test tubes or plates.

Culture in an atmosphere with added carbon dioxide

Some organisms, such as capnophilic streptococci, require extra CO_2 in the air in which they are grown and others, such as the pneumococcus and gonococcus, grow better in air supplemented with 5–10% CO_2. For this CO_2 jars are used. The required amount of air is withdrawn with a vacuum pump and replaced with CO_2 from a cylinder. CO_2 incubators which provide a predetermined and regulated amount of CO_2 in a suitably humid atmosphere are commercially available. Screw caps on containers of liquid media must not be tight and should preferably be replaced by a closure that allows entry of CO_2.

Culture in microaerophilic atmosphere

Microorganisms like *Actinomyces israelii* are micro-aerophilic. This is done by an evacuation replacement method with 5% O_2, 10% CO_2 and 85% N_2.

Anaerobic culture

A variety of methods are available for the culture of anaerobic organisms in the clinical laboratory. Exclusion of oxygen from the medium is the simplest method, and is effected by growing the organisms within the culture medium such as freshly steamed liquid media and deep nutrient agar with 0.5% glucose and minimal shaking and solidified rapidly by placing the tube in cold water. Liquid media soon become aerobic unless a reducing agent such as glucose 0.5–1.0%, ascorbic acid 0.1%, cysteine 0.1%, sodium thioglycollate 0.1%, or particles of meat in cooked meat broth are added. Liquid media should be prereduced by holding in a boiling water bath for 10 minutes to drive off dissolved oxygen, then quickly cooled to 37°C just before use.

Cooked meat broth, CMB (original medium known as 'Robertson's bullock-heart medium') has a special place in anaerobic bacteriology; and thioglycollate broth and its modifications are also very useful. CMB is suitable for growing anaerobes in air and also for the preservation of stock cultures of aerobic organisms. The inoculum is introduced deep in the medium in contact with the meat. Meat particles are placed in 30 ml bottles to a depth of about 2.5 cm and covered with about 15 ml broth.

Anaerobes have special nutritional requirements for vitamin K, haemin and yeast extract, and all primary isolation media for anaerobes should contain these three ingredients.

Anaerobic jars

When an oxygen-free or anaerobic atmosphere is required for obtaining surface growths of anaerobes, anaerobic jars provide the method of choice. The most reliable and widely used anaerobic jar is the McIntosh-Fildes' anaerobic jar. It is a cylindrical vessel made of glass or metal with a metal lid which is held firmly in place by a clamp (Fig. 3.3). The lid has two tubes with taps, one acting as gas inlet and the other as the outlet. On its undersurface, it carries a gauze sachet carrying alumina pellets coated with palladium. It acts as a room temperature catalyst for the conversion of hydrogen and oxygen into water. It acts as a catalyst, as long as the sachet is kept dry.

Inoculated culture plates are placed inside the jar and the lid clamped tight. The outlet tube is connected to a vacuum pump and the air inside is evacuated. The outlet tap is then

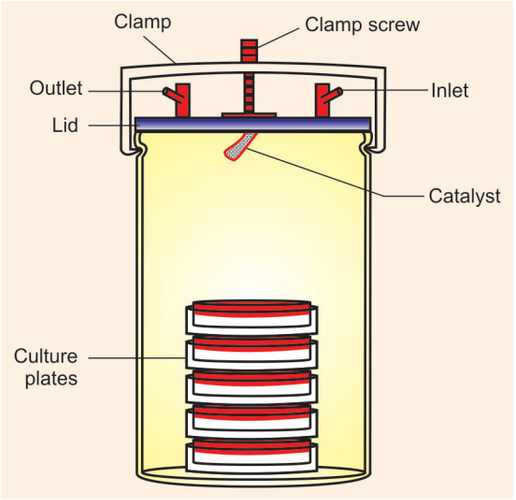

Fig. 3.3: Anaerobic jar

Chapter

5

Sterilization, Disinfection and Biomedical Waste Management

STERILIZATION AND DISINFECTION

Sterilization is defined as the process by which an article, a surface or a medium is freed of all microorganisms including viruses, bacteria, their spores and fungi, both pathogenic and non-pathogenic.

Disinfection is a process of destruction or removal of organisms capable of giving rise to infection. Disinfectants are capable of killing vegetative bacteria, fungi, viruses and rarely bacterial spores.

Antisepsis is the destruction or inhibition of micro-organisms in living tissues thereby limiting or preventing the harmful effects of infection. A disinfectant that is applied to living tissue is referred to as an **antiseptic**.

Various agents used in sterilization and disinfection may be divided into:

- **Physical agents**
  - Sunlight
  - Drying
  - Heat
  - Filtration
  - Radiation
- **Chemical agents**
  - Phenols and cresols
  - Halogens
  - Aldehydes
  - Alcohols
  - Dyes
  - Vapour-phase disinfectants
  - Surface active disinfectants

PHYSICAL AGENTS

Sunlight

Sunlight possesses ultraviolet rays which along with heat rays are responsible for appreciable germicidal activity. These rays, however, cannot penetrate through glass, i.e. window-panes. This is one of the natural methods of sterilization of water in tanks, rivers and lakes.

Drying

Water constitutes 80% of the weight of the bacteria and is also essential for the growth of bacteria. Therefore, drying has deleterious effect on many bacteria. However, spores are unaffected by drying.

Heat

Heat is the most reliable, certain and rapid method of sterilization. It can be easily controlled and unlike chemical disinfection, leaves no potentially harmful residue. Unless the material to be sterilized is heat-sensitive, this method should be preferred.

There are two types of heat—dry heat and wet heat.

- **Dry heat** is believed to kill microorganisms by causing destructive oxidation of essential cell constituents. Dry heat at 100°C for 60 minutes and 115°C for 60 minutes can kill all vegetative bacteria and fungal spores, respectively. Bacterial spores can be killed by dry heat at 160°C for 1 hour or 180°C for 20 minutes. On the whole dry heat is less efficient sterilization process than moist heat.
- **Moist heat** causes denaturation and coagulation of proteins. When steam condenses on cooler surface, it releases its latent heat and raises the temperature of its surface. If spores are present, steam condenses on them and increases their water content leading to hydrolysis and breakdown of bacterial proteins.

Sterilization by dry heat

Red heat

Inoculating wires and *loops*, *points of forceps* and *spatulas* are sterilized by holding them almost vertical in a Bunsen burner flame until red hot (Table 5.1).

Flaming

Scalpel blades, *needles*, *mouths of culture tubes* and *bottles*, *glass slides* and *coverslips* are sterilized by passing the article through the Bunsen flame without allowing them to become red hot.

Category	Type of waste	Type of bag or container to be used	Treatment and disposal options
White (trans-lucent)	**Waste sharps including metals:** Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded and contaminated metal sharps.	Puncture-proof, leak-proof, tamper-proof containers	Autoclaving or dry heat sterilization followed by shredding or mutilation or encapsulation in metal container or cement concrete; combination of shredding-cum-autoclaving; and sent for final disposal to iron foundries (having consent to operate from the State Pollution Control Boards or Pollution Control Committees) or sanitary landfill or designated concrete waste sharp pit.
Blue	(a) **Glassware:** Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes. (b) **Metallic body implants**	Cardboard boxes with blue-coloured marking	Disinfection (by soaking the washed glass waste after cleaning with detergent and sodium hypochlorite treatment) or through autoclaving or microwaving or hydroclaving and then sent for recycling.

* Disposal by deep burial is permitted only in rural or remote areas where there is no access to common biomedical waste treatment facility. This will be carried out with prior approval from the prescribed authority and as per the standards specified in Schedule-III. The deep burial facility shall be located as per the provisions and guidelines issued by Central Pollution Control Board from time to time.

Note:

1. Microbiological waste and all other clinical laboratory waste shall be pre-treated by sterilization before packing and sending to the common biomedical waste treatment facility.
2. Mutilation or shredding must be to an extent to prevent unauthorized reuse.
3. Autoclaving of biomedical waste shall be done at a temperature of not less than 121°C and pressure of 15 pounds per square inch for not less than 60 minutes.

Key Points

- ***Sterilization*** is a process that ***kills*** or ***removes all*** organisms (and their spores) in a material or an object.
- ***Disinfection*** is a process that ***kills*** or ***removes pathogenic organisms*** in a material or an object.
- ***Antisepsis*** is the application of a chemical agent externally on a ***live surface*** (skin or mucosa) to destroy organisms or to inhibit their growth (all antiseptics are disinfectants but not vice versa).
- **Sterilization** is usually achieved by ***moist heat*** (steam under pressure in an autoclave; most popular), ***dry heat*** (hot air oven) or gaseous chemicals.
- **Hospital waste** should be ***segregated at source***, since 85% of the waste is non-hazardous and can be disposed of easily into the municipal bin; waste should be segregated in bags of different colours to facilitate appropriate treatment and disposal.

Important Questions

1. Define the terms sterilization, disinfection and antisepsis. Name various agents used for sterilization and discuss the role of hot air oven in sterilization.
2. Discuss the role of moist heat in sterilization.
3. Write short notes on:
   (a) Hot air oven
   (b) Autoclave
   (c) Sterilization by filtration
   (d) Sterilization by radiation
4. Name different types of hospital wastes and discuss in detail the methods of disposal of hospital waste.

Multiple Choice Questions

1. Destruction or inhibition of microorganisms in living tissues is known as:
   (a) Sterilization
   (b) Disinfection
   (c) Antisepsis
   (d) None of the above

2. Which of the following disinfectants has little activity against viruses?
   (a) Phenols
   (b) Sodium hypochlorite
   (c) Iodine
   (d) Formaldehyde