

## Control of microbial growth

- History of microbial control
- Approaches to microbial control
- Selection of an antimicrobial procedure
- Heat
- Chemicals
- Filtration
- Radiation
- Preservation

## History of microbial control

- **Joseph Lister revolutionized surgery.** He developed effective methods that prevent surgical wound becoming infected.
- Initially he used **carbolic acid** to control wound infections and later developed the **methods of sterilizing instruments.**
- We know now that various microorganisms are responsible for many health problems and food spoilage; thus, **effective control of microorganisms** is needed on priority basis.
- We will discuss various methods of controlling microbial growth on **inanimate objects and some body surfaces.**
- Most of the methods discussed in this chapter are **non-selective** in that they **adversely effect all forms of life.**

## Approaches to control microbial growth

- **Physical methods:** include heat, irradiation, filtration and mechanical removal (washing).
- **Chemical methods:** use antimicrobial chemicals
- Some processes may use a **combination** of both **physical and chemical methods.**
- These methods are of **varying levels of effectiveness** against different organisms.
- The term **sterile** does not encompass **prions.**

**Note:** It is very difficult to destroy **prions** using standard sterilization procedures. **Combustion** is the basic sure method used to destroy prions.

**Table 5.1** Terms Commonly Used to Describe Antimicrobial Agents and Processes

Term	Definition
Antiseptic	A disinfectant that is non-toxic enough to be used on skin.
Aseptic technique	Use of specific methods to exclude contaminating microorganisms from an environment.
Bactericidal	Kills bacteria.
Bacteriostatic	Prevents the growth of, but does not kill, bacteria.
Decontamination	Treatment used to reduce the number of disease-causing microbes to a level that is considered safe to handle.
Degerm	Treatment used to decrease the number of microbes in an area.
Disinfectant	A chemical used to destroy many microorganisms and viruses.
Disinfection	A process that eliminates most or all disease-causing microorganisms and viruses on or in a product.

**Table 5.1** Terms Commonly Used to Describe Antimicrobial Agents and Processes

Fungicide	Kills fungi.
Cemicide	Kills microorganisms and inactivates viruses.
Pasteurization	A brief heat treatment used to reduce the number of spoilage organisms and to kill disease-causing microbes.
Preservation	The process of inhibiting the growth of microorganisms in products to delay spoilage.
antitize	To reduce the number of microorganisms to a level that meets public health standards; implies cleanliness as well.
terilant	A chemical used to destroy all microorganisms and viruses in a product, rendering it sterile.
Sterile	Completely free of all microorganisms and viruses; an absolute term.
Sterilization	The process of destroying or removing all microorganisms and viruses, through physical or chemical means.
Viricide	Inactivates viruses.

## Situational considerations

- Methods used for microbial control depend on the **situations and degree of control** required.
- Control measures required on routine basis in **daily life** may not be sufficient for situations such as **hospitals, microbiology laboratories, foods and food production laboratories.**
- **Daily life:** washing and scrubbing with soaps and detergents, cooking foods, cleaning surfaces and refrigeration.
- **Hospitals:** Danger of **nosocomial** infections (infections that are hospital acquired). In hospitals, **opportunistic infections** are a major concern. Thus strict and rigorous microbes control measures are required.
- **Microbiology labs:** routinely work with microbes so **rigorous microbes control methods and aseptic techniques** are needed.
- **Food and food production facilities:** Heat treatment, irradiation, use of chemicals which can prevent microbial growth, are some of the methods used routinely in food industry. **Surfaces should be kept free of microorganisms** to avoid contamination.



## Chemical destruction of microorganisms

- Germicidal chemicals can be used to **disinfect** and in some cases to **sterilize**.
- Unlike antibiotics, chemical disinfectants normally cause **irreversible chemical reactions** within the bacterial cell (e.g. coagulation and denaturation of proteins, solubilization of membranes, denaturation of DNA, etc.).
- Chemical disinfectants are generally **less reliable than heat** but are suitable to **disinfect large surfaces and heat sensitive items**.
- Some are **non-toxic** to the extent that they can be used as **antiseptics**.
- Some chemicals which are weakly germicidal but have a bacteriostatic action, can be used as **preservatives**.

**Table 5.2 Using Heat to Control Microorganisms**

Method	Characteristics and Typical Uses
Boiling	Fast, reliable, and inexpensive method to destroy most microbes. Unreliable for killing endospores. Can be used to treat drinking water.
Pasteurization	Significantly decreases the numbers of heat-sensitive microorganisms, including spoilage microorganisms and pathogens (except sporeformers). Milk and juices are routinely pasteurized.
Pressurized steam	Achieves temperatures above 100°C, which is necessary to destroy endospores. Used to sterilize surgical instruments and other items that can be penetrated by steam. Also used to produce canned foods.
Dry heat	High temperatures for long lengths of time can be used to sterilize items. Flame is used to sterilize bacteriological loops. Ovens are used to sterilize glassware and items that are not readily penetrated by steam.

## Potency of germicidal chemicals

Disinfectants are grouped according to their potency

**Table 5.3 Usual Pattern of Antimicrobial Activity of Germicides**

Category of Germicide	Bacteria			Fungi	Viruses	
	Endospores	Mycobacteria	Others	All	Nonlipid	Lipid
Sterilant	yes	yes	yes	yes	yes	yes
High Level	some	yes	yes	yes	yes	yes
Intermediate Level	no	varies	yes	yes	varies	yes
Low Level	no	no	varies	varies	no	varies

Intermediate-level and low-level disinfectants are also called **general purpose disinfectants**.

## Factors that regulate chemical germicides selection

- **Toxicity:** by nature, germicides are somewhat toxic to humans and the environment.
- **Activity in the presence of organic matter (active or inactive):** Hypochlorite is readily inactivated by organic matter, however, phenolics can tolerate the presence of some organic matter.
- **Compatibility with material to be sterilized:** some items can not tolerate liquid disinfectants so use gaseous alternatives.
- **Residue:** Some leave a residue which is **toxic or corrosive**. Sterilized items must be rinsed with **sterile water**.
- **Cost/Availability:** Some are less expensive and readily available e.g. hypochlorite. Some are expensive and are not readily available e.g. Ethylene oxide.
- **Storage and stability:** Phenolics and iodophores are available as concentrated stock solutions. But some have limited shelf life.
- **Environmental risk:** some retain their antimicrobial activity after use and can interfere with sewage treatment system. They should be neutralized before disposal.

## Chemical disinfectants

- **Alcohols:**
  - Aqueous solutions of 60-80% ethanol/isopropanol rapidly kill vegetative bacteria and fungi.
  - They do not reliably destroy endospores and naked viruses.
  - Alcohols probably coagulate enzymes and proteins and damage lipid membranes.
  - Proteins are **more soluble and denature more easily in alcohol mixed with water** and that is why aqueous solutions (70% ethanol) are more effective than pure alcohols.
- **Aldehydes:** include both **Glutaraldehyde** and **Formaldehyde**
- **Glutaraldehyde:**
  - A 2% solution of alkaline glutaraldehyde is one of the most widely used liquid sterilants for treating heat sensitive medical items.
  - It destroys all forms of microbial life including endospores and viruses.

## Chemical disinfectants cont'd

- **Formaldehyde:** It is used as a gas or an aqueous 37% solution called **formalin**.
  - It is an extremely effective germicide which kill all forms of life within minutes.
  - It is used to kill bacteria and inactivates viruses for use as a **vaccines**.
  - It is also used to preserve **biological specimens**.
- **Chlorhexidine:**
  - extensively used in **antiseptic products** (skin creams, disinfectants and mouth wash).
  - It has low toxicity and can kill a wide range of microbes including vegetative bacteria, fungi and some enveloped viruses by reacting with proteins.
- **Ethylene oxide:**
  - is an extremely useful **gaseous sterilizing agent** and destroy all microbes including endospores and viruses.
  - A special chamber is used to sterilize items with ethylene oxide. It is carcinogenic in nature.

## Chemical disinfectants cont'd

- **Halogens:** include **chlorine and iodine** and are thought to work by oxidizing proteins and other cell components.
- **Chlorine:**
  - destroy all types of microorganisms and viruses.
  - Chlorine releasing compounds such as **sodium hypochlorite** are used to disinfect waste liquids, swimming pool water, instruments and surfaces and, at much lower concentration, to disinfect drinking water.
  - Properly chlorinated drinking water contains ~ **0.5ppm chlorine**. **Household bleach is 5.25% sodium hypochlorite**.
- **Iodine:**
  - unlike chlorine, does not reliably kill endospores.
  - **Tincture of iodine** or **iodophores** are used as disinfectants or antiseptics.
  - Some *Pseudomonas* species survive in the concentrated solutions of iodophores.

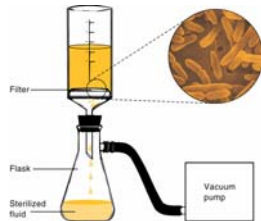
Table 5.4 Chemicals Used in Sterilization and Disinfection, and Preservation of Non-food Substances

Chemical (examples)	Characteristics	Uses
<b>Metals</b> (silver)	Most metal compounds are too toxic to be used medically.	Silver sulfadiazine is used in topical dressings to prevent infection of burns. Silver nitrate drops can be used to prevent eye infections caused by <i>Neisseria gonorrhoeae</i> in newborns. Some metal compounds are used to prevent microbial growth in industrial processes.
<b>Ozone</b>	This unstable molecular form of oxygen readily breaks down.	Used to disinfect drinking water and wastewater.
<b>Peroxygens</b> (hydrogen peroxide and peracetic acid)	Readily biodegradable and less toxic than traditional alternatives. The effectiveness of hydrogen peroxide as an antiseptic is limited because the enzyme catalase breaks it down. Peracetic acid is a more potent germicide than is hydrogen peroxide.	Hydrogen peroxide is used to sterilize containers for aseptically packaged juices and milk. Peracetic acid is widely used to disinfect and sterilize medical devices.
<b>Phenolic Compounds</b> (triclosan and hexachlorophene) <b>Phenolic compounds are active ingredients in lysol.</b>	Wide range of activity, reasonable cost, remains effective in the presence of detergents and organic contaminants, leaves an active antimicrobial residue.	Triclosan is used in a variety of personal care products, including toothpastes, lotions, and deodorant soaps. Hexachlorophene is highly effective against <i>Staphylococcus aureus</i> , but its use is limited because it can cause neurological damage.
<b>Quaternary Ammonium Compounds</b> (benzalkonium chloride and cetylpyridinium chloride)	Non-toxic, enough to be used on food preparation surfaces. Inactivated by anionic soaps and detergents.	Widely used to disinfect inanimate objects and to preserve non-food substances. <b><i>Pseudomonas</i> resist the effects and can grow in them.</b>

## Removal of microbes by filtration

### Filtration of fluids

- Filtration is commonly used to sterilize heat-sensitive fluids (e.g. culture media, human blood products, etc.)
- **Two types** of filters are in general use:
  - **Depth filters**, which have an ill-defined pore size, but trap bacteria based on flowing through a complex matrix (e.g. Nylon wool). They **can not** reliably remove viruses.



### Filtration of air:

- **Membrane filters** are created out of polymers, and contain pores of a defined size. Depending on size, may clear bacteria only or bacteria and viruses.
- special filters called high-efficiency particulate air (**HEPA**) filters remove from air almost all microorganisms.
- They are used in **specialized hospital rooms, biological safety cabinets and laminar flow hoods**.

## Using Irradiation to destroy microbes and viruses

- Short wavelength electromagnetic radiation such as **microwaves, gamma rays** and **ultraviolet light** are valuable tools for microbial control.
- **Gamma irradiation** is used extensively to sterilize **heat sensitive materials** including medical equipments, disposable items and drugs.
- **Food, fruits and vegetables** can also be either sterilized or pasteurized with gamma irradiation depending on the doses used.
- Endospores are resistant to both gamma and ultraviolet irradiation.
- Radiation kills by
  - A) **Generating heat** (microwaves)
  - B) **Generation of reactive free radicals** (like superoxide and hydroxyl) by **gamma rays**. This results in direct damage to DNA and proteins.
  - C) **Direct damage to DNA (UV radiation)**. Used to limit bacterial growth in culture hoods and rooms etc.

## Preservation of perishable products

Preventing or slowing the growth of microbes extends the shelf life and can be achieved using various methods

1. **Use of preservative chemicals**
  - Non-food items use germicides i.e. formaldehyde in shampoo.
  - Food items use weak acids (i.e. benzoic, sorbic and propionic acids.)
  - Nitrates/nitrites are typically used to preserve meats (**inhibit the germination of endospores and growth of *Clostridium botulinum***).
2. **Low-temperature**
  - Slows enzymatic reactions
  - Freezing forms ice crystals that damage microbial cells
3. **Reducing water availability**
  - **Adding salt or sugar:** high solute environment causes plasmolysis and prevent growth of microbes). However, *Staphylococcus aureus* (food poisoning organism) can grow under very high salt concentrations.
  - **Drying food (dessication, lyophilization):** however, **drying does not kill microbes**. Number of salmonellosis cases are sometime reported when dried eggs or their products are used.

## Next Lecture

- Read chapter 21