CHAPTER 18
CONTROL OF MICROBIAL GROWTH WITH DISINFECTANTS AND ANTISEPTICS

WHY IS THIS IMPORTANT?

- Infection control using disinfectants and antiseptics is essential to keep infections from spreading, particularly in hospitals.

OVERVIEW
IMPORTANT TERMINOLOGY

- Some treatments are used for both disinfection and antisepsis.
  - Disinfection is associated with inanimate objects.
  - Antisepsis is associated with human tissue and skin.

- Sterilization – the removal of all microbes
  - Sterilization does not destroy prions.
- Aseptic – an environment or procedure free from contamination

- Disinfection – the use of chemical or physical agents to kill or inhibit the growth of microorganisms
  - Heat
  - Alcohol
  - Ultraviolet radiation
  - Some disinfecting agents do not affect spores.
IMPORTANT TERMINOLOGY

- A chemical used on inanimate object is called a disinfectant.
- The same chemical used on skin and tissue is called an antiseptic.

IMPORTANT TERMINOLOGY

- De-germing – the removal of microbes from a surface by mechanical means
- Sanitization – disinfection of places or things used by the public
  - Used to reduce the number of pathogens to meet accepted public health standards

IMPORTANT TERMINOLOGY

- Sanitization is not sterilization but the same techniques can be used.
  - Steaming
  - High-pressure
  - High-temperature
  - Washing and scrubbing
IMPORTANT TERMINOLOGY

- Pasteurization – using heat to kill pathogens
  - Does not sterilize but is used to reduce number of pathogens
  - Also reduces the number of organisms that can cause spoilage

- Static – an agent that inhibits growth
  - In a bacteriostatic environment, numbers do not multiply but organisms are not dead.
- Cidal – an agent that kills

TARGETS FOR DISINFECTANTS AND ANTISEPTICS

- Bacteria are single-celled organisms with a simple anatomy.
- They have several targets:
  - Cell wall
  - Plasma membrane
    - Responsible for enclosing the cytoplasm
    - Involved with DNA replication
    - Involved in ATP Production
TARGETS FOR DISINFECTANTS AND ANTISEPTICS

- Bacteria are single-celled organisms with a simple anatomy.
- They have several targets:
  - Inhibition of protein synthesis
  - Alteration in protein structure

TARGETS: Cell Wall

- The cell wall maintains the integrity of the cell.
- Damaging the cell wall makes a cell susceptible to lysis.
- Several chemical agents can damage this barrier.

TARGETS: Plasma Membrane

- The plasma membrane is composed of a phospholipid bilayer.
- When this is disrupted, the cell loses its selective permeability.
- This leads to the death of the cell.
**TARGETS:**

**Plasma Membrane**

- Surfactants are very effective for disrupting the plasma membrane.
  - Made of polar molecules with hydrophobic and hydrophilic regions
  - They bind to and penetrate the phospholipid bilayer.
    - This causes openings to form.
  - They also affect virus envelopes.
    - Damage to the envelope causes the loss of capacity to infect.

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**TARGETS:**

**Plasma Membrane**

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**TARGETS:**

**Protein Structure and Function**

- Proteins are important molecules in the microbial cell.
- They have a three-dimensional shape directly related to the function.
  - A protein with a changed shape is called denatured.
  - Inhibition or elimination of function can result in cell death.
### TARGETS: Protein Structure and Function

- Denaturation involves breaking of hydrogen and other bonds.
  - These hold the three-dimensional shape.
  - When broken, the protein unfolds and is inactivated.
- Heat and strong solvents break hydrogen bonds.
  - This can result in total denaturation and coagulation.
- Metallic ions can inhibit enzymatic function blockading the active site.

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### TARGETS: Nucleic Acid Synthesis

- Nucleic acids are required for cell survival.
- Some agents can disturb this synthesis by binding irreversibly to DNA.
  - This prevents gene expression.
- Others are mutagenic and cause lethal mutations.

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### TARGETS: Nucleic Acid Synthesis

- Radiation can interfere with DNA and RNA function.
  - Irradiation with gamma rays, ultraviolet radiation and X-rays causes mutations.
  - These can result in permanent inactivation of nucleic acids.
MICROBIAL DEATH

- There are special requirements to define microbial death.
- The most efficient is to determine whether an organism can reproduce.
  - Move it from the antimicrobial environment to fresh media.
  - If there is no growth, the organism is dead.
- Permanent loss of reproductive capability is the accepted definition of microbial death.
  - The death rate is logarithmic.

MICROBIAL DEATH: Factors Affecting Rate

- Several factors affect the rate of microbial death in a clinical setting:
  - Numbers
  - Duration of exposure
  - Temperature
  - Environment
  - Endospore formation

- Numbers – the greater the number of organisms, the longer it will take to kill
  - If large numbers of organisms are present, it takes time for the agent to reach them all.
- Duration of exposure can vary depending on:
  - Accessibility
  - Type of microbe
  - This is important to consider when using radiation treatment.
  - Temperature – the lower the temperature, the longer it will take to kill
MICROBIAL DEATH: Factors Affecting Rate

- The environment – particularly important in health care environments
  - Many pathogens are associated with organic materials.
    - Blood
    - Saliva
    - Bodily fluids
    - Fecal material
  - These inhibit accessibility of the antimicrobial agent to the organism.

MICROBIAL DEATH: Factors Affecting Rate

- Endospore formation – may be the most important factor
  - Spore-forming organisms are not susceptible to most chemical agents.
  - This is especially important for nosocomial infections.

METHODS FOR CONTROLLING MICROBIAL GROWTH

- There are three major methods for controlling microbial growth:
  - Chemical
  - Physical
  - Mechanical removal
CHEMICAL METHODS FOR CONTROLLING MICROBIAL GROWTH

- Many chemicals can kill microbes.
- Chemicals can also be harmful to humans.
- If they are to be useful as disinfectants, they must also be safe to use.

POTENCY OF DISINFECTANTS AND ANTISEPTICS

- Factors to consider when evaluating effectiveness are:
  - Time
  - Temperature
  - Concentration

- Death rate using a chemical agent is accelerated by increasing the temperature.
  - An increase of 10°C doubles the rate of chemical reaction.
- Changes in pH can increase or decrease an agent’s potency.
**POTENCY OF DISINFECTANTS AND ANTISEPTICS**

- For most chemical agents, increase in concentration = increase in its potency.
- This is not true for alcohol.
  - Increase in alcohol concentration actually hinders killing.
  - Alcohol must have some water associated with it.
    - This makes for better penetration and denaturation of proteins.

**EVALUATION OF DISINFECTANTS AND ANTISEPTICS**

- There is no completely satisfactory method for evaluating antimicrobial chemical agents.
- There are several tests including:
  - Comparison of the agent with phenol – the phenol coefficient
  - Disk method
  - Use dilution method

**EVALUATION METHODS: The Phenol Coefficient**

- Phenol was first used as a disinfectant by Joseph Lister in 1867.
- It is still considered the benchmark disinfectant that others are compared with.
- Comparison is reported as the phenol coefficient.
  - Phenol coefficient of 1.0 = same effectiveness as phenol
  - Greater than 1.0 = efficiency greater than phenol
  - Less than 1.0 = efficiency less than phenol
**EVALUATION METHODS:**

**The Disk Method**

- The disk method uses tiny disks of filter paper soaked in the agent.
- An agar plate is inoculated and the disks are placed at various positions.
- Inhibition of growth around the disk is called the zone of inhibition.
  - Sizes of the zones are not comparable.
  - Sizes may reflect differences in concentration and diffusion rates.
- This method cannot distinguish between microbicidal and microbistatic.

**EVALUATION METHODS:**

**The Use Dilution Method**

- The use dilution method is time-consuming.
- It can tell whether the agent is microbistatic or microbicidal.
- A series of solutions of different concentrations of the disinfectant are prepared.

**EVALUATION METHODS:**

**The Use Dilution Method**

- Cultures of the test organism are dried down on stainless steel cylinders.
  - Each cylinder is dipped for 10 minutes into one of the solutions.
  - The cylinders are removed and rinsed with water to remove any remaining chemical.
  - The cylinders are placed into a tube of growth medium.
  - This is incubated and observed for growth.
SELECTING AN ANTIMICROBIAL AGENT

- Some chemical agents are better for certain uses than others.
- Qualities to be considered include:
  - Is it reactive against all types of organisms without destroying tissue.
  - Is it effective in the presence of organic material.
  - Is it stable and, if possible, inexpensive.

ANTIMICROBIAL CHEMICAL AGENTS

- Chemicals that are used for disinfection and antisepsis are grouped into three types:
  - Those affecting proteins
  - Those affecting membranes
  - Those affecting viruses

ANTIMICROBIAL CHEMICAL AGENTS

- Proteins are denatured by destroying their three-dimensional shape.
  - If mild treatments are used and removed, the protein will refold.
  - Most antimicrobial chemical agents used in proper concentration will guarantee permanent denaturation of proteins.
ANTIMICROBIAL CHEMICAL AGENTS

Chemical agents control viral pathogens by inactivating the ability of the virus to infect or replicate.

This can be accomplished in two ways:
- Destroying the virion.
- Destroying replication or gene expression.

ANTIMICROBIAL CHEMICAL AGENTS

- Destroying the virion
  - Detergents, alcohols, and other denaturing agents can affect capsid proteins.
  - Viral envelopes are susceptible to agents that act on lipids.
- Destroying replication or gene expression
  - Alkylating agents e.g. ethylene oxide and nitrous acid, act as mutagens for viral nucleic acid.
  - This inhibits replication and proliferation.
TYPES OF CHEMICAL AGENTS

- Chemical agents are used more than physical means for disinfection, antisepsis, and preservation.
- Chemical agents affect cell walls, plasma membranes, proteins, or nucleic acids.
- Chemical agents destroy or inhibit growth of enveloped viruses, bacteria, fungi, and protozoans.
  - They are ineffective against protozoan cysts and bacterial endospores.

The effect of chemical agents varies with:
- Temperature
- Length of exposure
- Amount of contaminating organic material
- pH
- Concentration
- Stability

There are eight major categories of chemical agents:
- Phenol and phenolic compounds
- Alcohols
- Halogens
- Oxidizing agents
- Surfactants
- Heavy metals
- Aldehydes
- Gaseous agents
**PHENOL AND PHENOLIC COMPOUNDS**

- Phenolic compounds are derived from phenol.
  - Many have greater efficacy and fewer side effects than phenol.
- Phenols and phenolic compounds are low-level to intermediate-level disinfectants and antiseptics that:
  - Denature proteins
  - Disrupt the plasma membrane
  - Remain very effective in the presence of organic material
  - Remain active for prolonged periods
- They are commonly used as disinfectants in health care settings and laboratories.

**ALCOHOLS**

- Alcohols are bacteriocidal, fungicidal, and virucidal for enveloped viruses.
- They have no effect on fungal spores and bacterial endospores.
- They are intermediate-level disinfectants.
- Alcohol is often used to carry other antimicrobial chemicals.
  - This is referred to as a tincture.

**ALCOHOLS**

- Alcohol denatures proteins and disrupts the plasma membrane.
- It has the added benefit of being capable of evaporating.
- It is routinely used as a de-germing agent to prepare sites for injection.
HALOGENS

- Four members of this chemical family have antimicrobial activity:
  - Iodine
  - Chlorine
  - Bromine
  - Fluorine
- They are all intermediate-level antimicrobial chemical agents.

HALOGENS

- Halogens are effective against:
  - Bacterial and fungal cells
  - Fungal spores
  - Some bacterial endospores
  - Protozoan cysts
  - Many viruses

HALOGENS:

- Iodine is a well-known antiseptic.
- It is used medically as a tincture or as an iodophor.
  - Iodophors are longer lasting and non-irritating.
  - Betadine is an example of an iodophor.
    - It is routinely used to prepare skin for surgery.
    - It is also used to treat burns.
**HALOGENS:**

- **Chlorine**
  - Chlorine is found in drinking water, swimming pools, and waste-water.
  - It is major ingredient in disinfectants such as chlorine bleach.
  - It is used to disinfect kidney-dialysis equipment.

**HALOGENS:**

- **Chloramines** are combinations of chlorine and ammonia.
  - Used in wound dressings, skin antiseptics, water supplies.
  - Less effective than chlorine as disinfectants/antiseptics.
  - Release their chlorine atoms more slowly therefore last longer.

**OXIDIZING AGENTS**

- Oxidizing agents are high-level disinfectants and antiseptics that prohibit bacterial metabolism.
  - They release hydroxyl radicals which kill anaerobic organisms.
  - They are very effective against infections of deep tissues.
    - They are routinely used in deep puncture wounds.
OXIDIZING AGENTS

The three most commonly used are:
- Hydrogen peroxide
- Ozone
- Peracetic acid
- Hydrogen peroxide is a common household antiseptic.

OXIDIZING AGENTS

- Ozone is a very reactive form of oxygen.
- It is generated when \( O_2 \) is exposed to electrical discharge.
- Some cities use ozone for water treatment.
  - It is expensive to produce.
  - It is difficult to maintain at the proper concentration.

OXIDIZING AGENTS

- Peracetic acid is the peroxide form of acetic acid and an extremely effective sporicide.
- It is used to sterilize surfaces and medical and food processing equipment.
  - It is not affected by organic contaminants.
  - It leaves no toxic residue.
SURFACTANTS

- There are two common surfactants:
  - Soap
  - Detergents

SURFACTANTS: Soaps

- One end of a soap molecule is ionic and is hydrophilic.
- The other end is a fatty acid and is hydrophobic.
  - This end breaks down oily deposits into tiny drops.
  - These mix with water and are washed away.
- Soaps are good de-germing agents but poor antimicrobial agents.
- They can be made more potent by adding antimicrobial triclosan.
- Detergents are positively charged organic surfactants that are more soluble in water than soap.
- The most popular are quaternary ammonium compounds (QUATS).

SURFACTANTS: Detergents – QUATS

- QUATS contain ammonium cations.
- They are low-level disinfectants/antiseptics.
- Their advantage is being odorless, tasteless, and harmless to humans.
- They are used in many industrial and medicinal applications including mouthwash.
**SURFACTANTS: Detergents – QUATS**

- QUATS disrupt the plasma membrane and are:
  - Bactericidal (especially for Gram-positive bacteria)
  - Fungicidal
  - Virucidal against enveloped viruses
- They are not useful for non-enveloped viruses, mycobacteria, or bacterial endospores.
- They are inhibited by the presence of organic contaminants.

**HEAVY METALS**

- The ions of heavy metals are inherently antimicrobial.
- Heavy metals include:
  - Arsenic
  - Zinc
  - Mercury
  - Silver
  - Copper

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**HEAVY METALS**

- Mercury and silver were formerly used in clinical situations.
- Mercury has been abandoned.
- Silver is still occasionally used in:
  - Surgical dressings
  - Burn creams
  - Catheters
HEAVY METALS

- The mechanism of action is through protein denaturation.
- Heavy metals are low-level bacteriostatic agents.

ALDEHYDES

- Aldehydes are compounds containing a terminal –CHO group.
- Two highly reactive aldehydes are used as antimicrobials.
  - Glutaraldehyde – used in liquid form
  - Formaldehyde – used in both liquid form and gaseous form
- Aldehydes cross-link to organic functional groups.
- These reactions denature proteins and inactivate nucleic acids.

ALDEHYDES: Glutaraldehyde

- Research laboratories use 2% solutions of glutaraldehyde.
  - This effectively kills bacteria, viruses, and fungi.
  - Treatment for 10 minutes will disinfect most objects.
  - Treatment for 10 hours will sterilize.
ALDEHYDES:

Formaldehyde

• Health care workers use a 37% formaldehyde solution – formalin.
• It is used to disinfect:
  • Isolation rooms
  • Exhuasts
  • Cabinets
  • Surgical instruments
  • Dialysis machines
• Formaldehyde is an irritant for mucous membranes and is also carcinogenic.

GASEOUS AGENTS

• Many items cannot be sterilized with heat or chemicals.
• They can be sterilized using highly reactive antimicrobial and sporicidal gases.
  • Ethylene oxide
  • Propylene oxide
  • β-propiolactone

• Gases rapidly penetrate and diffuse into any space.
• Over time, they can denature proteins and DNA.
• They kill everything they come in contact with and cause no damage to inanimate objects.
GASEOUS AGENTS

- Ethylene oxide is the most frequently used gaseous agent.
- It is used in hospitals and dentists’ offices for sterilizing instruments and equipment.

GASEOUS AGENTS

- Gaseous agents have several disadvantages:
  - They are explosive, poisonous, and potentially carcinogenic.
  - Disinfection with gaseous agents takes considerable time.
  - There is a need for continuous cleanup.

PHYSICAL METHODS FOR CONTROLLING MICROBIAL GROWTH

- Physical methods for controlling microbial growth include:
  - Drying
  - Heating
  - Cold
  - Filtration
  - Osmotic pressure
  - Radiation
HEAT

• Heat is usually lethal to most pathogenic microbes.
• Two types of heat can be used:
  • Moist heat – from hot water, boiling water, or steam
  • Dry heat – from hot air with low moisture e.g. from ovens

HEAT

• Hot air ovens are used for glassware, metallic instruments, powders, and oils.
• Temperatures between 150-180°C for two to four hours ensure the destruction of spores as well as vegetative cells.
• Exposure to very high temperature dry heat reduces microbes to ash and gases.

HEAT

• Dry heat dehydrates microbial cells.
  • The absence of water inhibits metabolism.
  • Protein denaturation takes a long time.
HEAT

Moist heat can be as effective as dry heat in a much shorter time at lower temperature.
- It quickly denatures proteins which halts microbe metabolism and causes death.

Adequate sterilization with heat depends on:
- Temperature
- Length of time
- Higher temperatures require shorter treatment times.
- Thermal death time (TDT) is the shortest length of time needed to kill all organisms at a specific temperature.
- Thermal death point (TDP) is the lowest temperature needed to kill all organisms in 10 minutes.

There are three ways of using moist heat:
- Pressurized steam
- Boiling
- Pasteurization
HEAT: Pressurized Steam

- Pressurized steam gives the highest temperature.
  - This results from increased pressure.
  - An example is an autoclave.
- Sterilization occurs when steam condenses to liquid water on the object.
  - Hot water gradually raises the object's temperature.
- Autoclaves are superior for sterilizing heat-resistant materials.
- They are not useful for substances that repel water.

HEAT: Boiling

- Boiling is easy but does not kill heat-resistant cells.
  - It is effective for disinfection but not sterilization.
- Boiling for 30 minutes kills most non spore-forming pathogens.

HEAT: Pasteurization

- Pasteurization is used to reduce microbial load.
  - It destroys pathogens.
  - It preserves flavor and nutritive value in foods.
  - It does not sterilize.
HEAT: Pasteurization

- Pasteurization is accomplished in two ways:
  - Flash method – temperature of 71.6°C for 15 seconds
  - Batch method – temperature of 63-66°C for 30 minutes
- It does not affect endospores, non-pathogenic lactobacilli, micrococci, or yeasts

REFRIGERATION, FREEZING, AND FREEZE-DRYING

- Cold temperatures retard the growth of microorganisms.
  - They slow the rate of enzymatic reactions.
  - They do not kill.
- Refrigeration is used to delay the spoilage of food.
  - Bacteria and molds will continue to grow.
  - It is useful only for a limited period.

- Freezing can preserve food.
  - It does not sterilize.
  - It slows metabolic rate.
  - There is no growth or spoilage.
REFRIGERATION, FREEZING, AND FREEZE-DRYING

- Freezing can also be used to preserve microorganisms.
  - Organisms to be preserved are frozen in glycerol.
  - This prevents the formation of ice crystals.

REFRIGERATION, FREEZING, AND FREEZE-DRYING

- Freeze-drying (lyophilization) preserves cells by removal of water.
  - Organisms are frozen in liquid nitrogen and subjected to high vacuum.
  - Containers are then sealed under vacuum.
  - Organisms are viable in this state for years.
  - It is used for long-term storage.
  - Addition of water restarts the growth process.

FILTRATION

- Filtration is useful for sterilizing liquids.
  - It involves passing the liquid through membrane filters.
    - Pores in the filter are too small to allow for the passage of microorganisms.
    - Filters are made with specific pore sizes.
Filtration

- Filters can be used for:
  - Growth media
  - Drugs
  - Vitamins
  - Some commercial food preparation
- Filtration is used to sample and test water samples for fecal coliform contamination.

Filtration

- Filters can also purify air.
  - High-efficiency particulate air filters are called HEPA filters.
  - These are seen in operating rooms, burn units, clean rooms of laboratories.
  - They are used in laboratory facilities such as the Centers for Disease Control (CDC) to keep organisms from escaping.
    - Filters are soaked in formalin before disposal.

Osmotic Pressure

- Osmotic pressure has been used in food preservation for many decades.
- High concentrations of salt or sugar or other substances are used in food preservation because:
  - It creates a hypertonic medium.
  - It draws water from the organisms.
  - It leads to plasmolysis and death.
RADIATION

- Radiation is energy emitted from atomic activities.
  - It is dispersed at high velocity.
- The cell’s molecules absorb some of the energy which changes DNA structure.

There are two types of radiation:

- Ionizing radiation – causes electrons to be ejected from the atoms
  - Includes gamma rays, X-rays, and high-speed electron beams
  - DNA is very sensitive to this type of radiation.
  - When DNA is exposed to this type this causes the breakdown of chromosomes.

- Non-ionizing radiation – excites atoms but does not ionize them
  - Best seen with ultraviolet radiation
  - It leads to abnormal bonds within molecules such as the formation of thymine dimmers.
RADIATION: Ionizing Radiation

- All ionizing radiation can penetrate liquids and most solid materials.
  - Gamma rays are the most penetrating.
- Flour, meat, fruits, and vegetables are routinely irradiated to kill microorganisms, parasites, and insects.

RADIATION: Ionizing Radiation

- Sterilization of medical products by ionizing radiation is rapidly expanding and now includes:
  - Drugs
  - Vaccines
  - Plastics
  - Syringes
  - Gloves
  - Tissue used in grafting
  - Heart valves
- The main drawback is the potential radiation poisoning of the operators.

RADIATION: Ultraviolet Radiation

- Ultraviolet radiation disrupts cells by generating free radicals and is a powerful killer of:
  - Fungal cells
  - Spores
  - Bacterial cells
  - Protozoans
  - Viruses
- It is used for disinfection but not sterilization.
- It is used in germicidal lamps in hospital rooms, operating rooms, food preparation areas, and dental offices.
RADIATION: Ultraviolet Radiation

- Using UV radiation can be effective in reducing post-operative infection by:
  - Preventing droplet transmission
  - Curtailing growth of microorganisms in food
  - Inhibiting growth of organisms in water, vaccines, drugs, plasma, and tissues used for transplantation

The major disadvantages are:

- Poor penetration
- Damaging effects seen over long exposure to human tissues
- Retinal damage
- Cancer
- Skin wrinkling

HAND WASHING

- Hand washing is one of the most important historical discoveries in medicine.
- The simple act of washing your hands can inhibit the spread of pathogens.
- The effectiveness of hand washing is related to:
  - The type of soap used
  - The time taken
- Hospitals use bacteriocidal soaps because they are very effective at preventing pathogen transmission.
- Household soap can be effective if enough time is taken to do the job thoroughly.