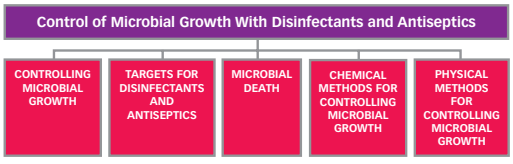
CHAPTER 18
CONTROL OF MICROBIAL GROWTH WITH DISINFECTANTS AND ANTISEPTICS

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

IMPORTANT TERMINOLOGY

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

IMPORTANT TERMINOLOGY

- 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

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

IMPORTANT TERMINOLOGY

- Degerming – 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

IMPORTANT TERMINOLOGY

- -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 for chemical agents:
  - Cell wall
  - Plasma membrane
  - Protein structure and function
  - Nucleic Acids

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

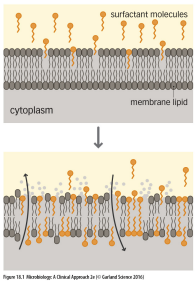
TARGETS: Plasma Membrane

- 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

TARGETS: Plasma Membrane



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

TARGETS: Nucleic Acids

- 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

TARGETS: Nucleic Acids

- 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 of microbes
  - Duration of exposure
  - Temperature
  - Environment
  - Endospore formation

MICROBIAL DEATH: Factors Affecting Rate

- 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

POTENCY OF DISINFECTANTS AND ANTISEPTICS

- 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:
  - Phenol coefficient
  - Disk diffusion 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 Diffusion 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 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

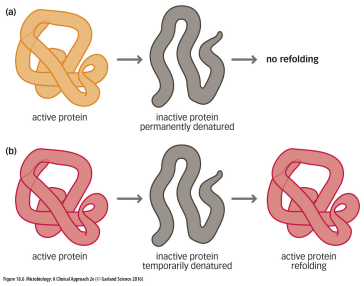
SELECTING AN ANTIMICROBIAL AGENT

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

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



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

TYPES OF CHEMICAL AGENTS

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

TYPES OF CHEMICAL AGENTS

- 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 evaporating
- It is routinely used as a degerming 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

- 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 wastewater treatment
- It is major ingredient in disinfectants such as chlorine bleach
- It is used to disinfect kidney dialysis equipment

HALOGENS: Chlorine

- 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

- Hydrogen peroxide is a common antiseptic
- Bacterial catalase can break it down but the amount of peroxide used overwhelms the amount of catalase produced by bacteria

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 degerming 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

SURFACTANTS: Detergents – QUATS

- QUATS (quaternary ammonium compounds) 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:
  - Bacteriocidal (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

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
  - Exhausts
  - 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

GASEOUS AGENTS

- 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

HEAT

- 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

HEAT

- 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

REFRIGERATION, FREEZING, AND FREEZE-DRYING

- Freezing can preserve food
  - It does not sterilize
  - It slows metabolic rate
  - There is no microbial 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

RADIATION

- 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

RADIATION

- There are two types of radiation:
  - 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

RADIATION: Ultraviolet Radiation

- 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