**Chapter 9**

**Basic Principles of Microbial Control**

* **Action of Antimicrobial Agents**
  + Alteration of cell walls and membranes
    - Cell wall maintains integrity of cell
      * When disrupted, cannot prevent cell from bursting due to osmotic effects
    - Cytoplasmic membrane contains cytoplasm and controls passage of chemicals into and out of cell
      * When damaged, cellular contents leak out
    - Viral envelope responsible for attachment of virus to target cell
      * Damage to envelope interrupts viral replication
    - Nonenveloped viruses have greater tolerance of harsh conditions
  + Damage to proteins and nucleic acids
    - Protein function depends on 3-D shape
      * Extreme heat or certain chemicals denature proteins
    - Chemicals, radiation, and heat can alter or destroy nucleic acids
      * Can produce fatal mutants
      * Can halt protein synthesis through action on RNA

**Selection of Microbial Agents**

Ideal:

* + Inexpensive
  + Fast-acting
  + Stable during storage
  + Capable of controlling all microbial growth while being harmless to humans, animals, and objects

**Factors Affecting the Efficacy of Antimicrobial Methods**

* + Site to be treated
    - Harsh chemicals and extreme heat cannot be used on humans, animals, and fragile objects
    - Method and level of microbial control based on site of medical procedure
  + Relative susceptibility of microorganisms
    - Effectiveness of germicides classified as high, intermediate, or low
      * High-level kill all pathogens, including endospores
      * Intermediate-level kill fungal spores, protozoan cysts, viruses, and pathogenic bacteria

Low-level kill vegetative bacteria, fungi, protozoa, and some viruses

**Methods for Evaluating Disinfectants and Antiseptics**

* + Phenol coefficient
    - Evaluating the efficacy of disinfectants and antiseptics by determining an agent’s ability to control microbes as a ratio to that of phenol
    - Greater than 1.0 indicates that agent is more effective than phenol

Has been replaced by newer methods

**Physical Methods of Microbial Control**

* **Heat-Related Methods**
  + Effects of high temperatures
    - Denaturation of proteins
    - Interference with integrity of cytoplasmic membrane and cell walls
    - Disruption of structure and function of nucleic acids
  + Thermal death point – lowest temperature that kills all cells in broth in 10 minutes
  + Thermal death time – time to sterilize volume of liquid at set temperature
  + Moist heat
    - Used to disinfect, sanitize, and sterilize
    - Kills by denaturing proteins and destroying cytoplasmic membranes
    - More effective than dry heat; water better conductor of heat than air
    - Methods of microbial control using moist heat
      * Boiling
        + Kills vegetative cells of bacteria and fungi, protozoan trophozoites, and most viruses within 10 minutes at sea level
        + Temperature cannot exceed 100ºC at sea level; steam carries some heat away
        + Boiling time is critical
        + Water boils at lower temperatures at higher elevations; requires longer boiling time
        + Endospores, protozoan cysts, and some viruses can survive boiling
      * Autoclaving
        + Pressure applied to boiling water prevents steam from escaping
        + Boiling temperature increases as pressure increases
        + Autoclave conditions – 121ºC, 15 psi, 15 minutes
      * Pasteurization
        + Pasteur’s method
        + Today, also used for milk, ice cream, yogurt, and fruit juices
        + 140ºC for 1 second, then rapid cooling
        + Treated liquids can be stored at room temperature
        + Not sterilization; heat-tolerant and heat-loving microbes survive
        + These do not cause spoilage prior to consumption
        + These are generally not pathogenic
        + Milk

Batch method – 30 minutes at 63ºC

Flash pasteurization – 72ºC for 15 seconds

Ultrahigh-temperature pasteurization – 134ºC for 1 second

* + - * Ultrahigh-temperature sterilization
        + 140ºC for 1 second, then rapid cooling
        + Treated liquids can be stored at room temperature
  + Dry heat
    - Used for materials that cannot be sterilized with or are damaged by moist heat
    - Denatures proteins and oxidizes metabolic and structural chemicals
    - Requires higher temperatures for longer time than moist heat
    - Incineration – ultimate means of sterilization
* **Refrigeration and Freezing**
  + Decrease microbial metabolism, growth, and reproduction
    - Chemical reactions occur slower at low temperatures
    - Liquid water not available
  + Psychrophilic microbes can multiply in refrigerated foods
  + Refrigeration halts growth of most pathogens
  + Slow freezing more effective than quick freezing
  + Organisms vary in susceptibility to freezing
* **Dessication and Lyophilization** 
  + Drying inhibits growth due to removal of water; only microbiostatic
  + Lyophilization used for long = term preservation of microbial cultures
    - Prevents formation of damaging ice crystals
* **Osmotic Pressure**
  + High concentrations of salt or sugar in foods to inhibit growth
  + Cells in a hypertonic solution of salt or sugar lose water; cell desiccates
  + Fungi have greater ability than bacteria to survive hypertonic environments
* **Radiation**
  + Ionizing radiation
    - Wavelengths shorter than 1 nm – electron beams, gamma rays, and X rays
    - Ejects electrons from atoms to create ions
    - Ions disrupt hydrogen bonding, oxidize double covalent bonds, and create hydroxide ions; hydroxide ions denature other molecules (DNA)
    - Electron beams – effective at killing but do not penetrate well
    - Gamma rays – penetrate well but require hours to kill microbes
    - X rays require too much time to be practical for growth control
  + Nonionizing radiation
    - Wavelengths greater than 1 nm
    - Excites electrons and causes them to make new covalent bonds
      * Affects 3-D structure of proteins and nucleic acids
    - UV light causes pyrimidine dimers in DNA
    - UV light does not penetrate well
    - Suitable for disinfecting air, transparent fluids, and surfaces of objects

**Chemical Methods of Microbial Control**

* **Phenol and Phenolics** 
  + Intermediate- to low-level disinfectants
  + Denature proteins and disrupt cell membranes
  + Effective in presence of organic matter and remain active for prolonged time
  + Commonly used in health care settings, labs, and homes (Lysol, triclosan)
  + Have disagreeable odor and possible side effects
* **Alcohols**
  + Intermediate-level disinfectants
  + Denature proteins and disrupt cytoplasmic membranes
  + Evaporate rapidly – both advantageous and disadvantageous
  + Swabbing of skin with 70% ethanol prior to injection
* **Halogens**
  + Intermediate-level antimicrobial chemicals
  + Believed that they damage enzymes via oxidation or by denaturing them
  + Iodine tablets, iodophores (Betadine®), chlorine treatment of drinking water, bleach, chloramines in wound dressings, and bromine disinfection of hot tubs
* **Oxidizing Agents**
  + Peroxides, ozone, and peracetic acid kill by oxidation of microbial enzymes
  + High-level disinfectants and antiseptics
  + Hydrogen peroxide can disinfect and sterilize surfaces of objects
    - Catalase neutralizes; not useful for treating open wounds
  + Ozone treatment of drinking water
  + Peracetic acid – effective sporocide used to sterilize equipment
* **Surfactants**
  + “Surface active” chemicals that reduce surface tension of solvents to make them more effective at dissolving solutes
  + Soaps and detergents
    - Soaps have hydrophilic and hydrophobic ends; good degerming agents but not antimicrobial
    - Detergents are positively charged organic surfactants
  + Quats – colorless, tasteless, harmless to humans, and antimicrobial; ideal for many medical and industrial application
    - Low-level disinfectants
* **Heavy Metals**
  + Ions are antimicrobial because they alter the 3-D shape of proteins, inhibiting or eliminating their function
  + Low-level bacteriostatic and fungistatic agents
  + 1% silver nitrate to prevent blindness caused by *N. gonorrhoeae*
  + Thimerosal used to preserve vaccines
  + Copper controls algal growth in reservoirs, fish tanks, swimming pools, and water storage tanks; interferes with chlorophyll
* **Aldehydes** 
  + Compounds containing terminal –CHO groups
  + Cross-link with amino, hydroxyl, sulfhydryl, and carboxyl groups to denature proteins and inactivate nucleic acids
  + Glutaraldehyde both disinfects (short exposure) and sterilizes (long exposure)
  + Formalin used in embalming and disinfection of rooms and instruments
* **Gaseous Agents**
  + Ethylene oxide, propylene oxide, and beta-propiolactone used in closed chambers to sterilize items
  + Denature proteins and DNA by cross-linking functional groups
  + Used in hospitals and dental offices
  + Can be hazardous to people, often highly explosive, extremely poisonous, and potentially carcinogenic
* **Enzymes**
  + Antimicrobial enzymes act against microorganisms
  + Human tears contain lysozyme, which digests the peptidoglycan cell wall of bacteria
  + Scientists are looking for ways to use enzymes to control microbes in the environment
    - Lysozyme used to reduce the number of bacteria in cheese
    - Prionzyme can remove prions on medical instruments
* **Antimicrobials**
  + Antibiotics, semi-synthetic, and synthetic chemicals
  + Typically used for treatment of disease
  + Some used for antimicrobial control outside the body

**Development of Resistant Microbes**

* Little evidence that extensive use of products containing antiseptic and disinfecting chemicals adds to human or animal health
* The use of such products promotes the development of resistant microbes