**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Has been replaced by newer methods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