Unit 4: Sterilization, Disinfection, & Antimicrobial Therapy
(Chapters 12 & 13)

**Aseptic Principles**

- **Sterilization**: the killing or removal of all microbes in a material or on an object
- **Disinfection**: the reduction of the number of pathogenic microbes to the point where they pose no danger of disease
  - **Disinfectant**: a chemical agent used on inanimate objects to destroy microbes; most do not kill spores
  - **Antiseptic**: a chemical agent that can be safely used externally on living tissue to destroy or inhibit the growth of microbes

**Sanitization**: thorough washing with soap or detergent to reduce bacterial numbers that meets public health standards
- **Sanitizer**: a chemical agent typically used on food handling and eating utensils to reduce bacterial numbers that meets public health standards

**Brain Check…**

1. Which would you prefer when eating out: sanitization or disinfection?

2. What is the difference between a disinfectant and an antiseptic?

**Terms to Know p. 340**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilization</td>
<td>The killing or removal of all microorganisms in a material or an object.</td>
</tr>
<tr>
<td>Disinfection</td>
<td>The reduction of the number of pathogenic microorganisms to the point where they pose no danger of disease.</td>
</tr>
<tr>
<td>Antiseptic</td>
<td>A chemical agent that can safely be used externally on living tissue to destroy or inhibit the growth of microbes.</td>
</tr>
<tr>
<td>Disinfectant</td>
<td>A chemical agent used on inanimate objects to destroy microorganisms. Most disinfectants do not kill spores.</td>
</tr>
<tr>
<td>Sanitizer</td>
<td>A chemical agent typically used on food-handling equipment and eating utensils to reduce bacterial numbers so as to meet public health standards. Sanitization may simply refer to thorough washing with only soap or detergent.</td>
</tr>
<tr>
<td>Bacteriostatic agent</td>
<td>An agent that inhibits the growth of bacteria.</td>
</tr>
<tr>
<td>Germicide</td>
<td>An agent that kills bacteria. Most such agents effectively kill certain microorganisms, but only inhibit the growth of others.</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>An agent that kills bacteria. Most such agents do not kill spores.</td>
</tr>
<tr>
<td>Virus</td>
<td>An agent that infects viruses.</td>
</tr>
<tr>
<td>Fungicide</td>
<td>An agent that kills fungi.</td>
</tr>
<tr>
<td>Sporicide</td>
<td>An agent that kills bacterial endospores or fungal spores.</td>
</tr>
</tbody>
</table>

**Controlling Microbial Growth Principles**

1. **Proportional** death rates: based on the logarithmic growth of microbes, the percentage of microbes that die in a minute will continue to follow that same percentage each minute after that (30% die in a min, 30% of the remaining microbes die the second min, & so on)

2. **Time required for sterilization**: the fewer organisms present, the shorter the time needed to achieve sterility
  - Role of organic matter: tissue debris & blood are important to remove because their presence impairs the effectiveness of many chemical agents

3. **Microbial susceptibilities**: microbes differ in their susceptibility to antimicrobial agents
Chemical Antimicrobials
- **Bacteriocidal**: bacterial killers
- **Bacteriostatic**: bacterial growth inhibitors
- Effects on potency (or effectiveness)
  1. **Time**: how long are the microbes exposed to the antimicrobial agent
  2. **Temp**: increasing the temp accelerates the chemical reaction and therefore the potency of a chemical agent
  3. **pH**: changing the pH can increase the antimicrobial's ability to penetrate a cell
  4. **Concentration**: increasing the concentration can increase the effects of most antimicrobial agents

Evaluating Effectiveness
- Phenol Coefficient
- **Kirby-Bauer test**: uses small disks of filter paper soaked in a chemical agent that are placed on a cultured plate; look for the **zone of inhibition**, a clear area around the disk showing that it killed the bacteria
- Use Dilution Assay

Disinfectant Selection Criteria
- Fast-acting
- Stable around organics
- Non-toxic
- Wide spectrum
- Non-damaging to materials
- Easy to prepare
- Stable
- Readily available
- Inexpensive
- No offensive odor

Modes (Mechanisms) of Action
* Recall: cell membranes are made of proteins & lipids
- Effects on Proteins: when proteins are **denatured**, their hydrogen bonds are disrupted and the functional shape of the protein is destroyed
- Effects on Membranes (lipids): **surfactants** are substances that make insoluble lipids soluble in water so they can be washed away
  - Ex. alcohols & detergents
- Viruses: have to be inactivated or rendered permanently incapable of infecting or replicating in cells; some agents that work on bacteria may not work on them because they can remain infective even after their proteins have been denatured

Classes of Disinfectant & Modes of Action
1. **Soaps and Detergents**: can kill many species of Streptococcus, Micrococcus, Neisseria, and destroy influenza viruses; use is usually followed up by an application of 70% alcohol solution;
   - Read the Public Health article on p. 345
2. **Acids/Alkalis**: denature proteins by lowering or raising pH; typically used as food preservatives
   - Ex. Lactic and propionic acid retard mold growth in breads
3. **Heavy Metals**: Se, Cu, Hg, & Ag; denature proteins; silver nitrate & merthiolate
4. **Halogen**: oxidize cell components; hypochlorous acid in drinking water and pools with the addition of copper sulfate for algal growth
**Classes of Disinfectant & Modes of Action**

5. **Alcohols**: when mixed with water, they denature proteins (ethyl alcohol)

6. **Phenolics**: disrupt cell membranes, denature proteins, & inactivate enzymes; used on skin, medical instruments, dishes, & furniture (Lysol)

7. **Oxidizers**: disrupt proteins (hydrogen peroxide)

8. **Alkylating Agents**: disrupt proteins & nucleic acids so they aren’t used if they may affect human cells (formaldehyde)

---

**Brain Check…**

1. How does time have an effect on the potency of an antimicrobial agent? What about pH?

2. What class of disinfectants are commonly used for water?

3. Why is denaturing nucleic acids so damaging? (think about the function of DNA)

---

**Physical Agents - Heat**

- **Thermal Death Point**: temp that kills all the bacteria in a 24 hr old culture at pH 7 in 10 min
- **Thermal Death Time**: time required to kill all of the bacteria in a culture at a specific temp
- **Decimal Reduction Time**: length of time needed to kill 90% of the microbes in a given population at a specific temp
  - A food processor needs to know the thermal death time for the most resistant organism likely to be in the food

**Physical Agents - Heat**

- **Dry Heat**: sterilizes oils & powders
  - Uses an oven (171°C for an hr)
  - Open flame (avoid floating ash!!)

- **Moist Heat**: sterilizes vegetative (active) cells & inactivates viruses
  - Uses water (>100°C+ for an hr+)
  - Can kill endospores!!
  - Ex. autoclave

- **Pasteurization**: kills microbes, but doesn’t sterilize (hence, milk and milk products are only good for a certain # of days)

---

**Use of Low Temperatures**

- **Cold temp**: retards the growth of microbes, but doesn’t sterilize foods, etc.
- **Refrigeration**: prevents food spoilage at 5°C
- **Freezing**: preserves both foods at -20°C & microbes at -180°C in liquid nitrogen
- **Drying**: absence of water inhibits enzyme action (keep toilet seats and bathroom fixtures dry)
- **Freeze Drying**: drying a material from a frozen state; used for long-term preservation of microbes

**Irradiation**

- **Ultraviolet light**: kills by damaging DNA (absorbed by DNA bases) & proteins; bacteria have DNA repair mechanisms; water treatment use

- **Ionizing radiation**: additionally produce peroxides; used to sterilize plastic lab & medical equipment and pharmaceutical products; in Europe, milk and other foods (meats & seafood) are irradiated for sterility

- **Microwave radiation**: can sterilize media
Additional Disinfection Methods

- **Sonication**: disruption of cells by using sound waves; can cause cavitation (formation of a partial vacuum in a liquid... cytoplasm is mainly water); causes bacteria to disintegrate; doesn't sterilize
- **Filtration**: passage of material through a filter or straining device; used to sterilize materials that could be damaged by heat sterilization (ingredients, drugs, etc.)
  - Air: HEPA filters in operating rooms, burn units, & lab hoods
  - Water: removal of microbes & particles from public water supplies and sewage treatment plants
- **Osmotic Control**: high conc. of solutes to create hypertonic solutions, plasmolysis (the loss of water in cells with cell walls)

Brain Check...

1. What type of heat should be used to kill an endospore?
2. Why is heat preferred to cold when it comes to sterilization?
3. Colder temperatures are ____ while hotter temperatures are ____.
   a. bacteriocidal; bacteriostatic
   b. bacteriostatic; bacteriocidal

Antimicrobial Chemotherapy

- **Chemotherapy**: the use of chemical agents to kill pathogenic organisms without harming the host; in microbiology, antimicrobial agents refer to a special group of chemotherapeutic agents used to treat diseases caused by microbes.
  - **Antibiotics**: antimicrobial agent produced by microorganisms
  - **Synthetics**: made in labs; sometimes by chemically modifying a substance from a microorganism
  - **Semi-synthetics**: made partly by lab synthesis & by microorganisms

Antimicrobial Drug Properties

1. **Selective Toxicity**: has to harm the microbes without causing significant damage to the host
   - Toxic dosage level (causes host damage) vs. therapeutic dosage level (eliminates the pathogen)
   - Chemotherapeutic index: measure of how toxic an agent is to the body relative to its toxicity for an infectious agent
     - Easy for bacterial treatments that inhibit cell wall synthesis, etc since we are different
     - Tough for parasitic worm treatments since they are animals too

Antimicrobial Drug Properties

2. **Activity Spectrum**: range of different microbes that a antimicrobial agent works against; broad vs. narrow spectrum

Modes of Action: Drug Target Sites fig 13.2 on p. 368

- Rifamycin works against RNA only
- Antimetabolites: affect the use of cell metabolites so that a cell can’t carry out necessary metabolic functions
Drug Side Effects

- Toxicity
- Allergy

- Microflora disruption: broad-spectrum antimicrobial agents can cause this problem because they harm normal microflora
  - Can lead to superinfections: infections caused by new pathogens when the defensive ability of normal microflora is destroyed

Drug Resistance

- Acquisition: follows genetic changes due to natural selection
  - Spontaneous mutations occur in bacterial populations at about 1:10 million-10 billion organisms
  - Mutants then go on to make progeny…
  - Antibiotics don’t induce mutations, but can create favorable environments

- Chromosomal resistance: due to mutations in chromosomal DNA and usually are effective to only a single type of antibiotic

Drug Resistance

- Plasmid resistance: happens outside the chromosome
  - Can carry 6-7 genes that each confer resistance to a different antibiotic
  - Plasmids can be transferred from one bacteria to another via transduction (transfer from a bacteriophage) or conjugation

Top 3 Mechanisms of Resistance

1. Altered target: affects bacterial ribosomes, causing the protein produced or target to be modified & the agent can’t bind to the target anymore
   - Ex. Resistance to erythromycin & rifampin

2. Altered membrane permeability: blocks an agent from entering the bacterial cell
   - Ex. Resistance to tetracycline, but the presence of penicillin can help with this

3. Novel enzyme activity: can destroy or inactivate the agent
   - Ex. Resistance to penicillin

Brain Check…

1. What is an advantage to using a broad-spectrum antibiotic? A disadvantage?

2. Why is plasmid resistance bad?

3. What is the difference between a therapeutic dosage level and a toxic dosage level?
Resistance Considerations

- Drug developments: we have backups against infections such as 1st line, 2nd line, and 3rd line drugs, but some drugs have already reached the 8th and 10th lines

1. Cross resistance: resistance to 2 or more similar agents by a common mechanisms (like enzyme activity)

2. Limiting drug resistance: development of resistance when medication is discontinued before all pathogens are killed

3. Synergism: treating with 2 antibiotics simultaneously so that they can exert an additive effect

Determining Microbial Drug Sensitivity

- Disk diffusion: Kirby-Bauer method is still the most popular

- Automated Methods: more efficient and less expensive; allow doctors to choose an appropriate antibiotic early in the infection versus prescribing a broad-spectrum antibiotic while waiting for lab results; as short as 3-6 hours

Ideal Drug Attributes

- Solubility
- Selective Toxicity
- Stable toxicity level
- Non-allergenic
- Tissue concentration stable
- Resistance acquired slowly
- Long shelf life
- Reasonable cost

Why is Antimicrobial Resistance so Bad?

1. It kills
2. Hampers the control of infectious diseases
3. Increases the costs of health care
4. Jeopardizes health care gains to society
5. Potential to threaten health security, and damage trade and economies

Factors that Influence Drug Resistance

- Leading factor is the over-prescription by physicians of antimicrobials, even in the absence of appropriate indications.
- In many developing countries, problems typically arise because antimicrobial agents are readily available and can be purchased as a commodity without the advice or prescription of a physician or other trained health care provider.
- Human behavior like self-medication and noncompliance with recommended treatments (forgetting to take medication, prematurely discontinue the medication as they begin to feel better or if they cannot afford a full course of therapy.

Controlling Drug Resistance

1. Slow the development of resistant bacteria and prevent the spread of resistant infections
2. Expand data reporting in assistance with surveillance efforts to combat resistance
3. Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria
4. Improve international collaboration and capacities for antibiotic resistance prevention, surveillance, control and antibiotic research and development
Antifungals – Cell Membrane Target

- Fungal infections are tough to treat since these are eukaryotic cells; can cause toxic side effects; needed because of resistant strains developing and an increase in immunosuppressed patients (AIDS)
  
- Imidazoles
  
- Tolnaftate (Tinactin)
  
- Terbinafine (Lamisil)

Antivirals

- Recent development because it’s hard to find agents that can act on viruses within cells without harming the host cell, inhibit some phase of viral replication, but they don’t destroy the viruses
  
- Interferons: natural defense because these are antiviral proteins made by infected cells and when released, they induce neighboring cells to make them too
  
- Immunoenhancers: agents that stimulate the immune system against infections

Antiprotozoan Drugs

- Protozoans are single-celled, pathogenic protists (eukaryotic). Protozoal infections occur throughout the world and are a major cause of morbidity and mortality in some regions such as Africa and South-East Asia; 3 main types of infections: malaria, giardiasis, & trichomoniasis
  
- The actions of antiprotozoal drugs against the infections are complex and are not fully understood. Some of them may interfere with reproduction of or damage protozoal DNA to limit the spread of an infection
  
- 2 main groups: antimalarial drugs and miscellaneous antiprotozoals. In addition to their use as antiprotozoals, some of them such as metronidazole and doxycycline are also used for treating bacterial infections

Antihelminthic Drugs

- Anthelmintics are agents used to eradicate intestinal worms (helminthes) from the body (tapeworms, roundworms and flukes are classified as helminthes). Anthelmintics are effective in eradicating worms, but proper hygiene is necessary to prevent re-infection. Washing hands properly before meals and after visiting the toilet is essential... and you have to treat the entire family!!!