BIOL 142 Chapter 8

Chapter 8: Controlling Microbial Growth *In Vitro*

35 slides. Burton's Microbiology 9th Edition — Chapter 8 (p. 121 — 139).

Definitions

- suffixes — **cide** and — **cidal**:
  - DESTRUCTION of a type of microbe.
  - Examples:
    - Bactericide
    - Fungicide
    - Virucide
    - "Germicide": **ethylene oxide**, **propylene oxide**, **aldehyde**.
- suffixes — **stasis** and — **static**:
  - INHIBITION but not complete destruction of a type of microbe.
  - Examples:
    - Bacteriostatic
    - Fungistatic
    - Virustatic
    - "Germistatic": **refrigeration**, **freezing**.

Definitions

- **Antisepsis**:
  - REDUCTION in the number of microorganisms and viruses, particularly potential pathogens, on living tissues.
  - Examples:
    - **Iodine**. **Alcohol**.
  - Antiseptics are frequently "Disinfectants" whose strength has been reduced to make them safe for living tissues.
- **Aseptic**:
  - refers to an environment or procedure FREE of pathogenic contaminants.
  - Examples:
    - **preparation of a surgical field**.
    - **handwashing (3 minute surgical scrub version)**.
    - **flame sterilization of laboratory equipment**.

Definitions

- **Degerming**:
  - **physical removal** of microbes by some sort of **mechanical** means.
  - Examples:
    - **the scrubbing part of Handwashing**
    - Alcohol **Swabbing** at the site of an injection.
- **Pasteurization**:
  - use of **HEAT** to destroy pathogens and reduce the number of spoilage microbes in foods and beverages.
  - heat treatment of **milk** and **fruit juice** is hot enough to *destroy most microbes but minimize the alteration of taste and destruction of nutrients*.

Definitions

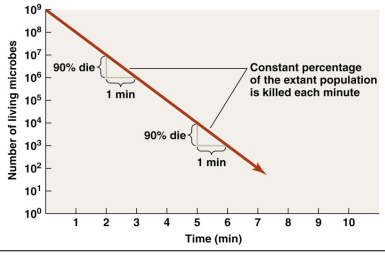
- **Sanitation**:
  - removal of pathogens from objects to meet PUBLIC HEALTH standards. (same as “disinfection”, just different location)
  - Examples:
    - washing dishes in **scalding water** in restaurants.
  - **standards** of sanitation **vary greatly** among governmental jurisdictions.
- **Disinfection**:
  - destruction of MOST microbes and viruses on NONLIVING tissue at HOME. (same as “sanitation”, just different location).
  - Examples:
    - **phenolics**
    - **alcohols**
    - **aldehydes**
    - **soaps**

Definitions

- **Sterilization**:
  - destruction of ALL microbes, viruses and bacterial endospores in or on an object.
  - Example:
    - preparation of **microbial culture media**
    - preparation of **canned food**
  - typically achieved by:
    - **steam under pressure**
    - **incineration**
    - **ethylene oxide gas**
  - term does not apply to prions (infectious proteins), because standard sterilization techniques do not destroy them.

Definitions

- **Microbial Death**:
  - the permanent loss of reproductive ability under ideal environmental conditions.
- **Microbial Death Rate**:
  - a way to evaluate the efficacy of an antimicrobial agent.
  - is constant over time for a particular microbe under a particular set of conditions.



Action of Antimicrobial Agents

- Alteration of cell walls & membranes:
  - cell wall maintains integrity of cell.
    - when damaged, the cell bursts because of osmotic effects.
  - cytoplasmic membrane controls passage of chemicals into and out of the cell.
    - when damaged, the cellular contents leak out.
  - in enveloped viruses, the envelope is a membrane made of proteins and phospholipids with the function of attaching a virus to its target cell.
    - when damaged, this interrupts viral replication.
    - thus, non-enveloped viruses have a greater tolerance of harsh environmental conditions and antimicrobial agents.

Action of Antimicrobial Agents

- damage to proteins & nucleic acids:
  - protein function depends on its 3-D shape.
    - extreme heat or certain chemicals can **denature** proteins.
  - chemicals, radiation, and heat can alter or destroy nucleic acids:
    - produces fatal mutants.
    - halts protein synthesis through action on RNA.

The Selection of Microbial Control Agents

- Ideally, agents should be:
  - **inexpensive**
  - **fast-acting**
  - **stable during storage**
  - **capable of controlling 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.
    - microbial control based on the site of a medical procedure.
      - penetrating versus non-penetrating medical instruments and supplies.

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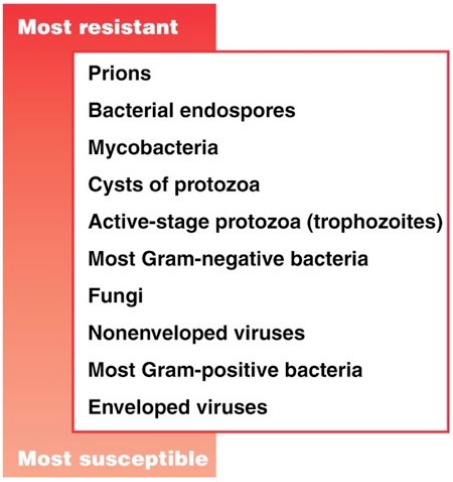
Relative Susceptibilities of Microbes to Antimicrobial Agents



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Physical Methods of Microbial Control: Moist Heat

- **Moist Heat**:
  - used to disinfect, sanitize, and sterilize.
  - denatures proteins & destroys cytoplasmic membranes.
  - more effect than dry heat.
  - methods of microbial control using moist heat:
    - **boiling**
    - **autoclaving**
    - **pasteurization**
    - **ultrahigh-temperature sterilization**

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Moist Heat Method: Boiling

- kills vegetative cells of:
  - bacteria
  - fungi
  - protozoan trophozoites
  - most viruses
- Boiling Time is critical:
  - different elevations (altitudes) require different boiling times.
- Things that can survive boiling:
  - endospores
  - protozoan cysts
  - some viruses

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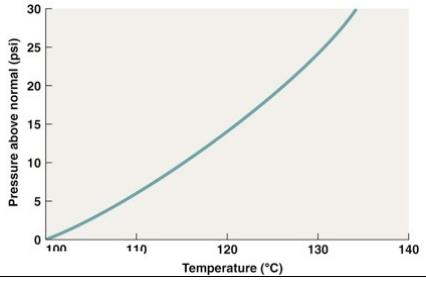
Moist Heat Method: Autoclaving

- pressure applied to boiling water prevents steam from escaping
- boiling temperature increases as pressure increases.
- large single volumes & solids take longer to autoclave.
  - 1 liter in 1 container takes longer than 1 liter in a bunch of vials.
- autoclave conditions:
  - **121ºC** at **15 psi** for **15 minutes**.



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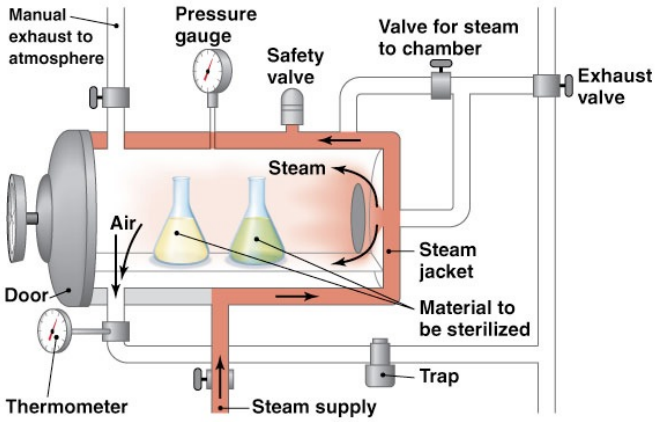
Moist Heat Method: Autoclaving



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Moist Heat Method: Pasteurization

- used for milk, ice cream, yogurt, and fruit juice.
- NOT a method of sterilization.
  - heat-tolerant microbes can survive!!!
- Pasteurization of milk:
  - Historical (**Batch**) Method: 60ºC for 30 minutes.
  - **Flash** Method: 72ºC for 15 minutes.
  - Ultrahigh-temperature pasteurization: 135ºC for 1 second.
  - Ultrahigh-temperature sterilization: 140ºC for 1-3 seconds.
    - treated liquids can be stored at room temperature.

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Physical Methods of Microbial Control: Dry Heat

- **Dry Heat**:
  - used for materials that cannot be sterilized with moist heat.
  - denatures proteins and oxidizes metabolic and structural chemicals.
  - requires higher temperates and longer heat times than moist heat methods.
  - **incineration** is the ultimate means of sterilization.
    - heating loops in the bunsen burner incinerates microbes.

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Physical Methods of Microbial Control: Refrigeration & Freezing

- decreases microbial metabolism, growth & reproduction.
  - chemical reactions occur more slowly at low temperatures.
  - liquid water not available for many needed reactions.
- **Psychrophilic** microbes can still grow.
- **Refrigeration** halts the growth of most pathogens.
- **Slow Freezing** is more effective at inhibiting microbial metabolism than quick freezing because the ice crystals have time to form and puncture microbial cell membranes
- organisms vary in susceptibility to freezing.

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Physical Methods of Microbial Control: Desiccation

- **Desiccation**:
  - is **drying**, and has been used for thousands of years to preserve foods such as fruit, peas, beans, grain, nuts and yeast.
  - it works by removing the liquid water needed for metabolism.
  - can inhibit the spread of most pathogens.
  - some molds can still grow, which need little water.

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Physical Methods of Microbial Control: Lyophilization

- **Lyophilization**:
  - is a technique **combining freezing & drying**, to preserve microbes and other cells for years.
    - this process starts with a instant freeze of a culture using liquid nitrogen or frozen carbon dioxide (dry ice)
    - then a vacuum removes frozen water through a process called sublimation, in which the water is transformed directly from a solid to a gas (skipping the liquid state).
  - this process prevents the formation of the ice crystals that can damage cell membranes and kill cells.

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Physical Methods of Microbial Control: Filtration

- **Filtration**:
  - is the passage of a liquid or gas through a sieve designed to trap particles — in this case, really small stuff such as cells, bacteria, spores and viruses.
  - a vacuum can be used to pull the fluids through the filter.
  - in the late 1800s, filters were able to trap cells, but the pores were still too large to trap viruses, and at the time they were named "**filterable viruses**", and today we just call them **viruses**.
  - today, we have filters with pores small enough to trap viruses as well.
  - **good method for sterilizing heat sensitive materials:**
    - eye meds, antibiotics, vaccines, liquid vitamins, enzymes and culture media.

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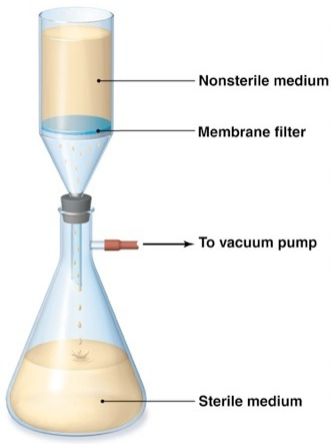
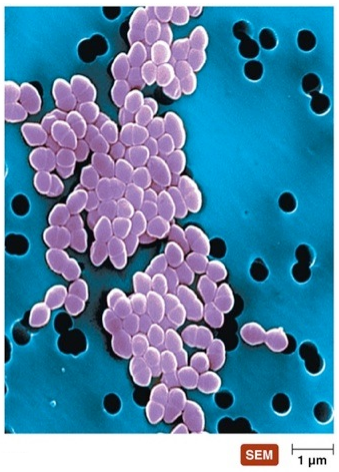
Physical Methods of Microbial Control: Filtration





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Physical Methods of Microbial Control: Osmotic Pressure

- **Osmotic Pressure**:
  - high concentrations of salt or sugar in foods to inhibit growth.
  - cells in **hypertonic solutions** of salts or sugar will lose water to that solution.
  - fungi have a greater ability than bacteria to survive hypertonic environments.

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Physical Methods of Microbial Control: Radiation

- **Ionizing Radiation**:
  - wavelengths are shorter than 1 nm.
  - ejects electrons from atoms to create ions.
  - ions will do the following:
    - disrupt hydrogen bonding
    - oxidize double covalent bonds
    - create hydroxide ions which will denature other molecules such as DNA.
  - Electron Beams:
    - effective at killing but do not penetrate well.
    - used to sterilize spices, meats, Sutures, Gloves, Syringes
  - Gamma Rays:
    - penetrate well but require hours to kill microbes.
    - used to sterilize spices, meats, fresh fruit and vegetables.

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Physical Methods of Microbial Control: Radiation

- Non-Ionizing Radiation:
  - wavelengths are greater than 1 nm.
  - excites electrons, causing them to make new covalent bonds.
    - affects the 3-D structure of proteins and nucleic acids.
  - UV light at 260 nm causes pyrimidine dimers in DNA
  - UV light does not penetrate well.
  - UV light used to disinfecting:
    - air
    - transparent fluids
    - surfaces of objects such as barber's shears and operating tables.
    - sewage water (cuts down on the amount of chlorine needed).

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Physical Methods of Microbial Control: Radiation

The circular **radura symbol** is used in the United States to label irradiated foods.

Non-irradiated Irradiated

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Chemical Methods of Microbial Control: Phenol & Phenolics

- **Phenol** & **Phenolics**:
  - intermediate to low level disinfectants.
  - denatures proteins & disrupts cell membranes.
  - effective in the presence of organic matter.
  - remain active for prolonged time.
  - commonly used in:
    - health care settings
    - labs
    - home
  - have a **disagreeable odor** & possible side effects
    - **rashes**
  - Example:
    - active ingredient in **Lysol**

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Chemical Methods of Microbial Control: Alcohols

- **Alcohols**:
  - intermediate level disinfectants
  - denatures proteins & disrupts cytoplasmic membranes.
  - more effective than soap in removing bacteria from hands !!!
  - Rubbing Alcohol (Isopropranol) is slightly superior to Drinking Alcohol (Ethanol).
  - 100% alcohol is not effective as the denaturation of proteins requires water — this is why you see isopropranol sold in 70% and 90% concentrations.
  - may evaporate before antimicrobial action takes place.
  - Example:
    - swabbing of skin with 70% ethanol prior to needle injection.

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Chemical Methods of Microbial Control: Halogens

- Halogens:
  - intermediate level antimicrobial chemicals
  - hypothesized to damage enzymes via oxidation or by denaturation.
  - Examples:
    - **iodine tablets** to purify water
      - some protozoan cysts can still survive (you take your chances)!
    - **Betadine** (an iodophor)
    - **Chlorine** treatments to public drinking water and pools
    - **Bleach**
    - chloramines (like chlorine but lasts longer)
    - bromine disinfection of hot tubs, pools, cooling towers

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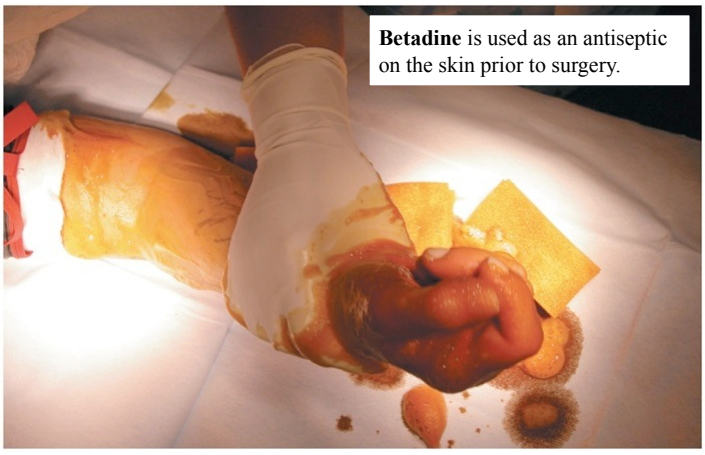
Chemical Methods of Microbial Control: Halogens



Betadine is used as an antiseptic on the skin prior to surgery.

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Chemical Methods of Microbial Control: Oxidizing Agents

- **Oxidizing Agents**:
  - high level disinfectants & antiseptics.
  - **kills by oxidation of microbial enzymes.**
  - Examples:
    - Peroxides such as **Hydrogen Peroxide** which can disinfect and sterilize surfaces but is not useful for treating open wounds because of its catalase activity that will also damage your cells.
    - **Ozone** (**O_3**) is a reactive form of oxygen that is generated when Oxygen (O_2) is subjected to an electrical discharge — is noted as that "fresh smell" after a thunderstorm. It is more expensive and more effective than chlorine.
    - **Peracetic Acid** is used to sterilize food processors and leaves no toxic residue... it is capable of dissolving some metals.

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Chemical Methods of Microbial Control: Surfactants

- **Surfactants**:
  - are "surface active" chemicals which reduce the surface tension of solvents such as water by *decreasing the attraction among the individual molecules.*
  - Examples:
    - **Soaps**:
      - have hydrophilic & hydrophobic ends.
      - good mechanical degerming agents but NOT antimicrobial.
      - if a soap is "*advertised as antiseptic*" it is because it typically contains other antimicrobial chemicals.
    - **Detergents**:
      - are positively charged organic surfactants.
    - **Quaternary Ammonia Compounds (Quats)**:
      - low level disinfectants
      - ideal for many medical and industrial applications
      - Example: **Cepacol mouthwash**

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Chemical Methods of Microbial Control: Heavy Metals

- **Heavy Metals**:
  - low level bacteriostatic & fungistatic agents.
  - heavy metal ions will denature proteins.
  - Examples:
    - 1% **silver nitrate** eye drops used to prevent blindness caused by *N. gonorrhoeae*.
      - Was commonly applied (and in some places still is) to a newborns eyes just after a vaginal birth if the mom has the infection.
    - **Thimerosal** (contains mercury) used to preserve vaccines.
      - was falsely linked as a cause of Autism.
      - controversy resulted in removing thimerosal from all childhood vaccinations, but they are still used in some adult vaccinations.
    - **Copper** to control algal growth
      - it interferes with chlorophyll.

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Chemical Methods of Microbial Control: Aldehydes

- **Aldehydes**:
  - compounds containing terminal —CHO groups.
  - cross-link functional groups to denature proteins and inactivate nucleic acids.
  - Examples:
    - **Glutaraldehyde**: 10 minutes will disinfect but 10 hours will sterilize. 2% solutions used to wipe down hospital rooms.
    - **Formalin** used in embalming and disinfection of rooms and instruments. It is very irritating to the mucous membranes and carcinogenic.

Chemical Methods of Microbial Control: Gaseous Agents

- **Gaseous Agents**:
  - denatures proteins and DNA by cross-linking functional groups.
  - gases such as **ethylene oxide** are used in closed chambers to sterilize items.
  - used in hospitals & dental offices to sterilize:
    - sutures
    - plastic lab materials
    - mattresses & pillows
    - electronic equipment
  - disadvantages:
    - extremely poisonous & hazardous to people
    - highly explosive
    - potentially carcinogenic