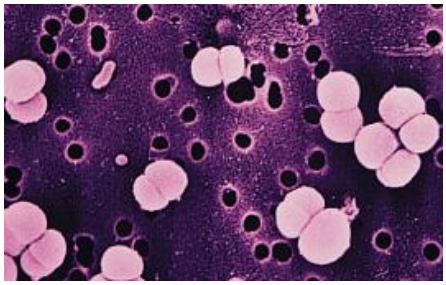
Chapter 7



The Control of Microbial Growth

1

Ch. 7 Objectives:

Students should be able to.....

1. Ch. 7: List **5 physical methods of controlling microbial growth**, and give an example of each.
   - *** Why is moist heat much more effective than dry air?
2. Describe how to measure the **effectiveness of a chemical disinfectant**.
3. List **6 different types of chemical disinfectants** and how they damage microbial cells.
   *** Distinguish between ***biocidal and biostatic*** treatments.
4. What are the two **most Resistant**, and the two **most Sensitive** microbial structures to antiseptic treatments? Why are these so?

2

The Control of Microbial Growth

- ***Sepsis*** **refers to microbial contamination.**
- ***Asepsis*** **is the absence of significant contamination.**
  - Aseptic surgery techniques prevent microbial contamination of wounds.

3

Terminology

1. ***Sterilization***: Removal of all microbial life.
2. ***Commercial Sterilization***: process kills *C. botulinum* endospores. (= the test organism!)
3. ***Disinfection***: Removal of pathogens.
   - ***Fomite*** = nonliving surface or inanimate object that may carry infectious microbes.
4. ***Antisepsis***: Removal of pathogens from living tissue. (Treatments = less harsh, to preserve tissue.)
5. ***Degerming***: Removal of microbes from a limited area.
6. ***Sanitization***: Lowering microbial counts on eating utensils.
7. ***Biocide/Germicide/Bacteriocide***: Kills microbes.
8. ***Germistasis/Bacteriostasis***: Inhibiting, not killing, microbes.

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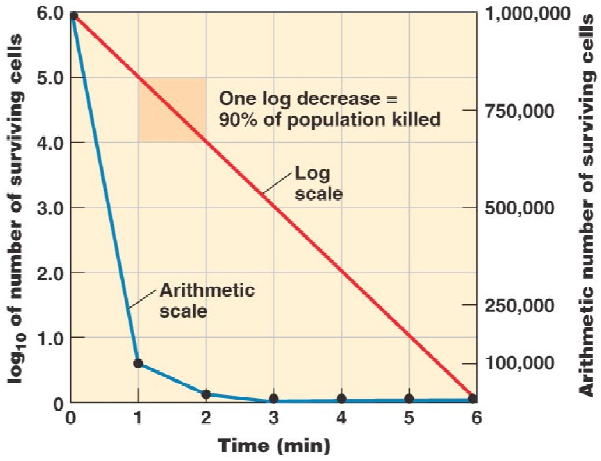
Microbial Death

- ***Bacterial populations die at a constant, logarithmic rate.***



(a) The data are plotted logarithmically (red line) and arithmetically (blue line).

Figure 7.1a

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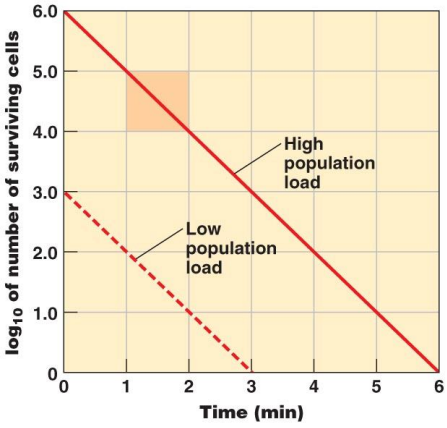
Effectiveness of antimicrobial treatment depends on:

1. **Number** of microbes.
2. **Environment**
   - (organic matter, pH, temperature, biofilms)
3. **Time** of exposure
4. **Microbial characteristics**



(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

Figure 7.1b

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** Actions of Microbial Control Agents **

1. **Alteration of Membrane permeability.**
   a) Leakage
   b) Dissolution of PL bilayer
2. **Damage to Proteins.**
   a) Destruction
   b) Denaturation
3. **Damage to Nucleic Acids.**
   a) Chemical alterations
   b) Destruction or mutations

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7.1) Physical Methods of Microbial Control

A. Heat:

- ***Thermal death point (TDP)***: Lowest temperature at which ALL cells in a culture are killed in ***10 min***.
- ***Thermal death time (TDT):*** Time to **kill ALL cells** in a culture (at a given temperature!).
- **Decimal reduction time (DRT):** Minutes to kill 90% of a population at a given temperature.
  - *Drop population by an order of magnitude!!*

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A. Moist Heat

- denatures proteins

1. *Autoclave:* Steam under pressure
  - Much higher temps reached at > 1 atm

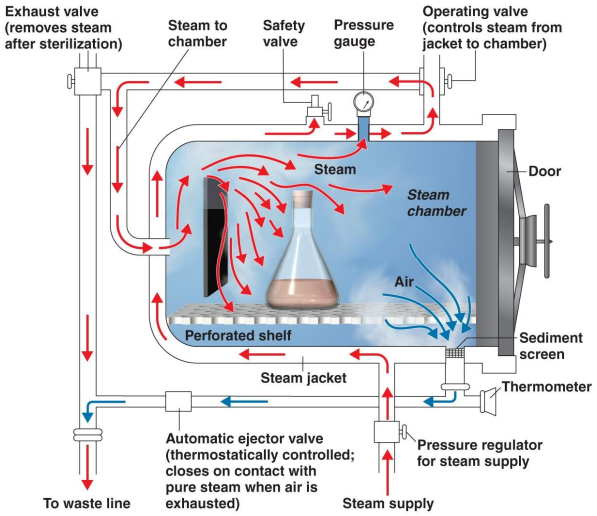

Figure 7.2

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Moist Heat: (#2)

2. *Pasteurization* reduces spoilage organisms and pathogens.

- Equivalent treatments:
  - **63°C for 30 min.**
  - High-temperature short-time: **72°C for 15 sec.**
  - Ultra-high-temperature: **140°C for <1 sec.**
  - ***Thermoduric*** organisms survive (& endospores).

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B. Dry Heat

- ***Dry Heat*** **sterilization kills by *oxidation*.**
  - Flaming
  - Incineration
  - Hot-air sterilization
    - Not as effective as Autoclave (moist heat, high press.)

	Hot-air	Autoclave
Equivalent treatments	170˚C, 2 hr	121˚C, 15 min

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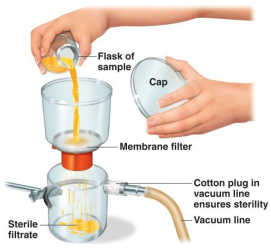
Other Physical Methods of Microbial Control



C. **Filtration** removes microbes.

D. **Low temperature** inhibits microbial growth.

1) Refrigeration
2) Deep freezing
3) Lyophilization

E. **High pressure** denatures proteins.

F. **Desiccation** prevents metabolism.

G. **Osmotic pressure** causes plasmolysis.

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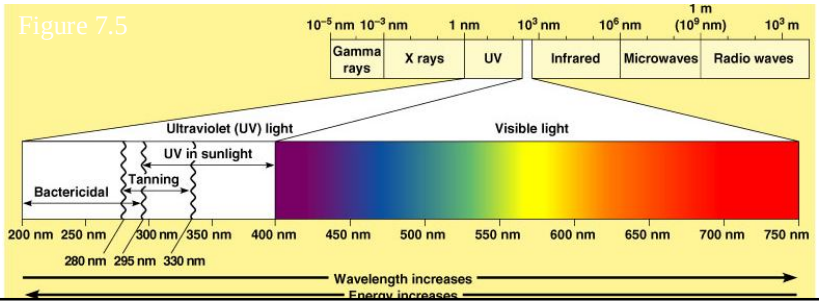
Physical Methods of Microbial Control

H. Radiation damages DNA

- ***Ionizing radiation*** (X rays, gamma rays, electron beams) → •OH
- ***Nonionizing radiation*** (UV)
- (***Microwaves*** kill by heat; not especially antimicrobial)



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7.2) Chemical Methods of Microbial Control

- **Principles of effective disinfection**
  - **Factors for consideration:**
    1. **Concentration of disinfectant**
    2. **Organic matter**
    3. **pH**
    4. **Time**

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A. Chemical Methods of Microbial Control: Evaluation

- **Evaluating a Disinfectant:**

1. Use-dilution test:

a) Metal rings dipped in test bacteria are dried.

b) Dried cultures placed in disinfectant for 10 min at 20°C.

c) Rings transferred to culture media to determine whether bacteria survived treatment.

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Evaluating a Disinfectant:

2. Disk-diffusion method:

– ***Zone of Inhibition/ZOI*** = diameter of clearing / sensitivity in mm.

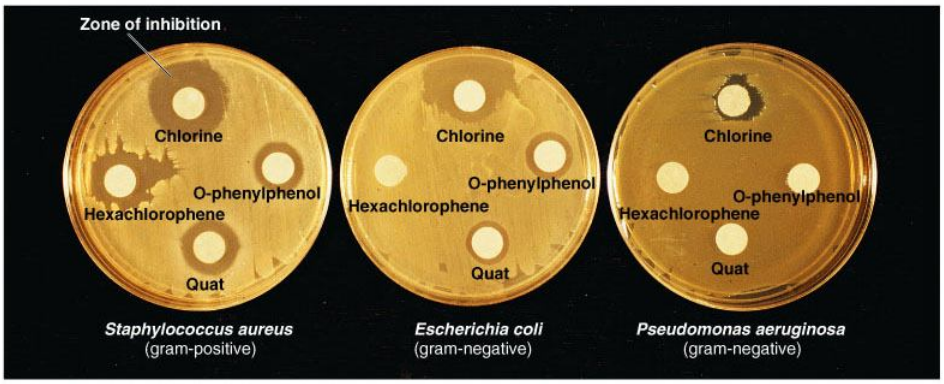
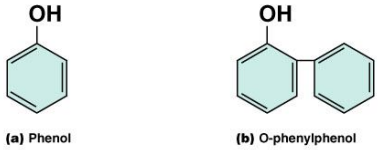


Figure 7.6 16

B. Types of Disinfectants

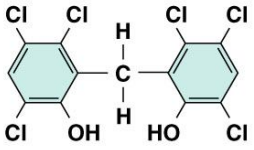
1. Phenol:

- *Denature proteins.*
  - ❖ ***"chaotropic agents"***
- *Disrupt plasma membranes.*



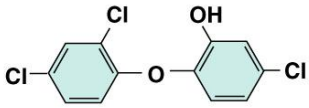
(a) Phenol (b) O-phenylphenol

a) Phenolics: Lysol



(c) Hexachlorophene (a bisphenol)

b) Bisphenols: Hexachlorophene, Triclosan

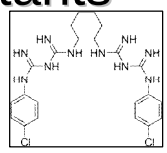


(d) Triclosan (a bisphenol)

Figure 7.7

Types of Disinfectants

2. Biguanides: Chlorhexidine.



- *Disrupt plasma membranes.*

3. Halogens: Iodine, Chlorine.

- *Oxidizing agents.*
- **Bleach** is hypochlorous acid (HOCl).
- **Iodophores** = betadine (organic combo w/ I).

4. Alcohols: Ethanol, Isopropanol.

- *Denature proteins, dissolve lipids.*

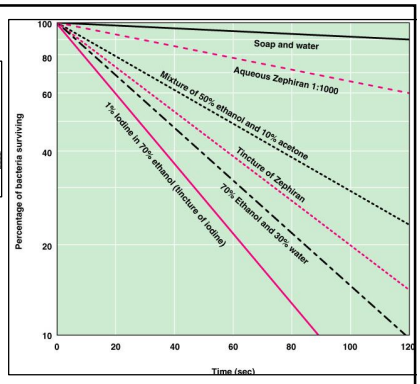


TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes***

Concentration of Ethanol (%)	Time (sec) 10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.

Types of Disinfectants

5. Heavy Metals: Ag, Hg, Cu, Zn

- ***Oligodynamic action:***
  - ***Very small amounts exert antimicrobial activity!***
- *Denature proteins.*
  - Highly cationic.
  - Large, attractive surfaces.

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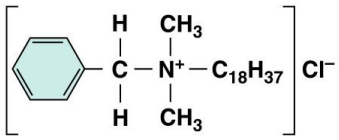
Types of Disinfectants

6. Surface-Active Agents or *"Surfactants":*

- **Detergents**
- **Emulsifiers**



Benzalkonium chloride

COMPOUND	ACTIVITY
Soap	Degerming
Acid-**anionic detergents**	Sanitizing
Quarternary ammonium compounds ("**Quats**") -- Cationic detergents	Bactericidal, Denature proteins, disrupt plasma membrane

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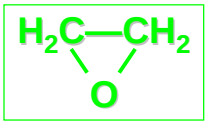
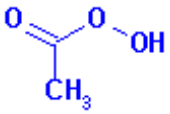
Types of Disinfectants

7. **Chemical Food Preservatives:**
   - a) ***Organic Acids***
     - *Inhibit metabolism.*
     - Sorbic acid, benzoic acid, calcium propionate
     - Control molds and bacteria in foods and cosmetics
   - b) ***Nitrite*** prevents endospore germination
   - c) ***Antibiotics***: Nisin and natamycin prevent spoilage of cheese
8. **Aldehydes:**
   - *Inactivate proteins by covalently cross-linking* with reactive functional groups **(–NH_2, –OH, –COOH, —SH).**
   - **Glutaraldehyde, formaldehyde**
9. **Gaseous Sterilants:**
   - *Denature proteins.*
   - **Ethylene oxide**



10. **Peroxygens:**
    - *Oxidizing agents.*
    - **O_3, H_2O_2, peracetic acid**



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Microbial Characteristics and Microbial Control

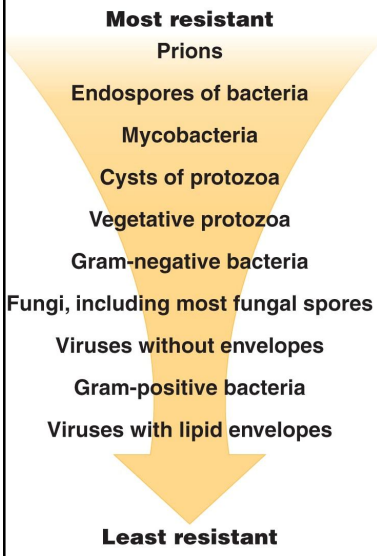

Figure 7.11

Acellular & Cellular Microbes:

1. **Prion 3D prot. Strx** (acellular)
   - **Refolds easily**
   - **Must be incinerated!**
2. **Endospore resistance**
   - & Protistan cysts
3. **Gram– :** LPS/OM porins
4. **Unenveloped viruses** (acell.)

❖ ***All above have relatively enhanced resistance to chemical biocides.***

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Microbial Characteristics and Microbial Control

Chemical agent	Effectiveness against:	
	Endospores	*Mycobacteria*
Phenolics	Poor	Good
Quats	None	None
Chlorines	Fair	Fair
Alcohols & Iodine	Poor	Good
Glutaraldehyde	Fair	Good

*** Microbial control methods, especially biocides, are not equally effective against all microbes. ****