LECTURE PRESENTATIONS
For BROCK BIOLOGY OF MICROORGANISMS, THIRTEENTH EDITION
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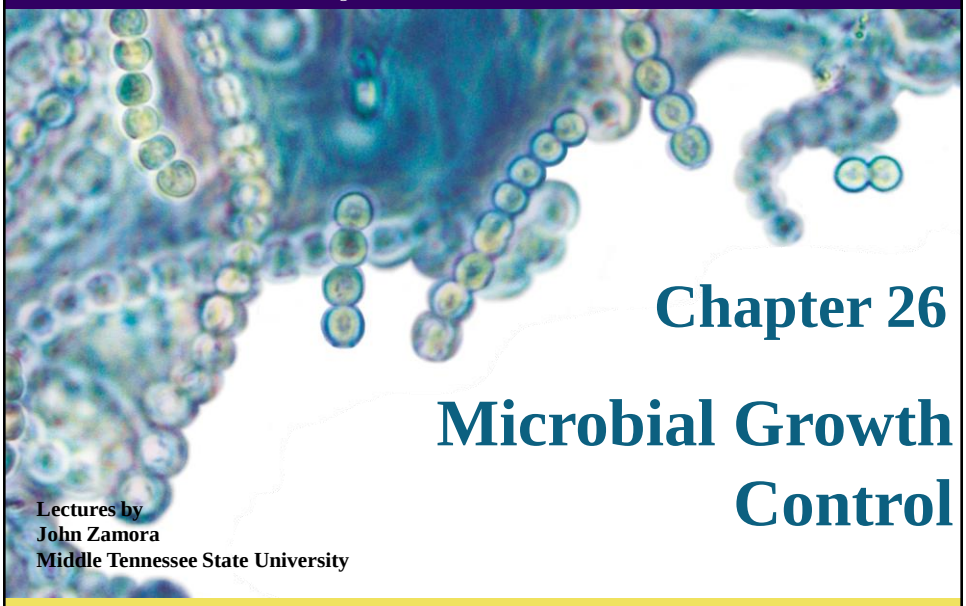

Chapter 26

Microbial Growth Control

Lectures by
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Microbial Growth Control

- *Sterilization*
  - The killing or removal of all viable organisms within a growth medium
- *Inhibition*
  - Effectively limiting microbial growth
- *Decontamination*
  - The treatment of an object to make it safe to handle
- *Disinfection*
  - Directly targets the removal of all pathogens, not necessarily all microorganisms

Microbial Growth Control

- *Disinfectant*
  - Specialized chemical or physical agents called disinfectants can kill microorganisms or inhibit microbial growth.
  - e.g. chlorine gas and sodium hypochlorite solution are used to disinfect drinking water.

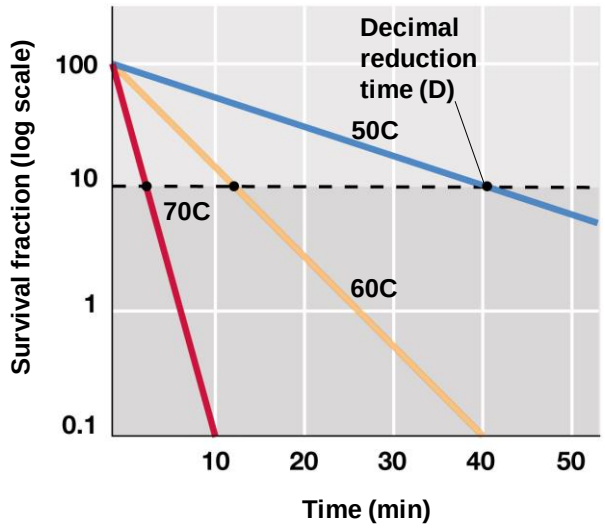
I. Physical Antimicrobial Control

- 26.1 Heat Sterilization
- 26.2 Radiation Sterilization
- 26.3 Filter Sterilization

26.1 Heat Sterilization

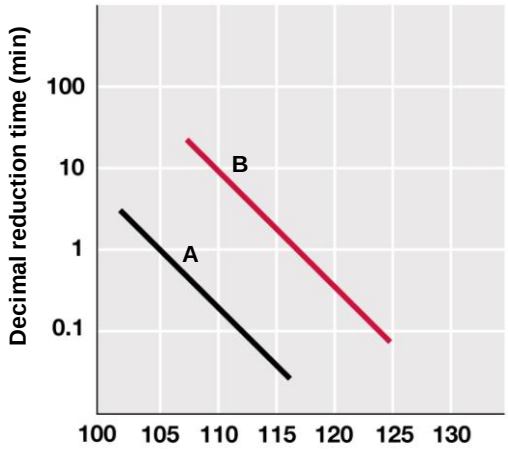
- *Heat sterilization* is the most widely used method of controlling microbial growth (Figure 26.1)
  - High temperatures *denature* macromolecules
  - Amount of time required to reduce viability tenfold is called the *decimal reduction time* (Figure 26.2)
- Some bacteria produce resistant cells called *endospores*
  - Can survive heat that would rapidly kill vegetative cells

Figure 26.1 The effect of temperature over time on the viability of a mesophilic bacterium



The decimal reduction time, D, is the time at which only 10% of the original population of organisms remains viable at a given temperature.
For 70°C, D = 3 min; for 60°C, D = 12 min; for 50°C, D = 42 min.

Figure 26.2 The relationship between temperature and the rate of killing in mesophiles and thermophiles

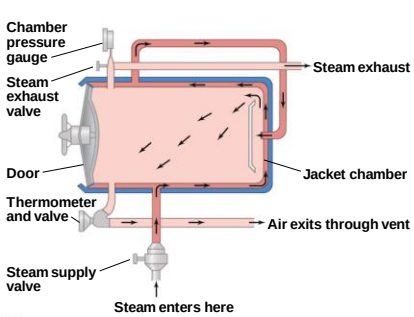


Data were obtained for decimal reduction times, D, at several different temperatures. For organism A, a typical mesophile, exposure to 110°C for less than 20 sec resulted in a decimal reduction, while for organism B, a thermophile, 10 min was required to achieve a decimal reduction.

26.1 Heat Sterilization

- The *autoclave* is a sealed device that uses steam under pressure (Figure 26.3)
  - Allows temperature of water to get above 100°C
  - Not the pressure that kills things, but the high temperature
- *Pasteurization* is the process of using precisely controlled heat to reduce the microbial load in heat-sensitive liquids
  - Does not kill all organisms, so it is different than sterilization

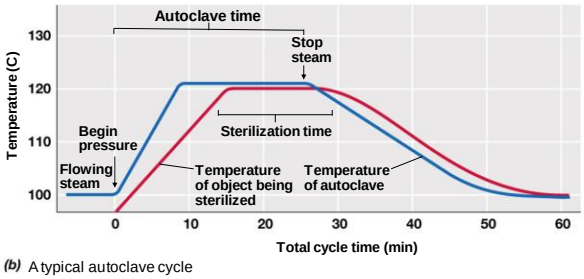
Figure 26.3 The autoclave and moist heat sterilization



(a) The flow of steam through an autoclave

The pressure-lock door and the automatic cycle controls on the right panel. The steam inlet and exhaust fittings are on the right side of the autoclave.

(c) A modern research autoclave



The temperature of the object rises and falls more slowly than the temperature of the autoclave. The temperature of the object must reach the target temperature and be held for 10–15 minutes to ensure sterility, regardless of the temperature and time recorded in the autoclave.

(b) A typical autoclave cycle

26.2 Radiation Sterilization

- Microwaves, UV, X-rays, gamma rays, and electrons can reduce microbial growth
- UV has sufficient energy to cause modifications and breaks in DNA
  - UV is useful for decontamination of surfaces (Figure 26.4)
  - Cannot penetrate solid, opaque, or light-absorbing surfaces

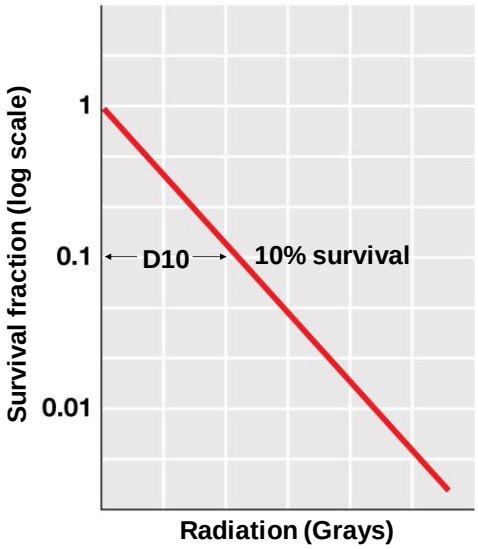
Figure 26.4 A laminar flow hood

An UV light source prevents contamination of the hood when it is not in use. When in use, air is drawn into the cabinet through a HEPA filter. The filtered air inside the cabinet is exhausted out of the cabinet, preventing contamination of the inside of the hood. The cabinet provides a contaminant-free workspace for microbial and tissue culture manipulations.

26.2 Radiation Sterilization

- *Ionizing radiation*
  - Electromagnetic radiation that produce ions and other reactive molecules
  - Generates electrons, hydroxyl radicals, and hydride radicals
  - Some microorganisms are more resistant to radiation than others
    - Amount of energy required to reduce viability tenfold is analogous to *D* value (Figure 26.5)

Figure 26.5 Relationship between the survival fraction and the radiation dose of a microorganism



The D10, or decimal reduction dose, can be interpolated from the data as shown.

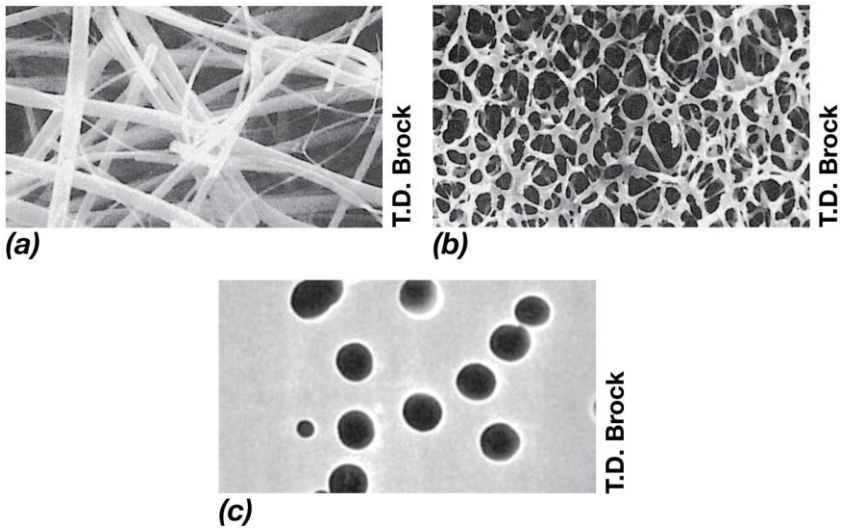
26.2 Radiation Sterilization

- Sources of radiation include cathode ray tubes, X-rays, and radioactive nuclides
- Radiation is used for sterilization in the medical field and food industry
  - Radiation is approved by the WHO and is used in the USA for decontamination of foods particularly susceptible to microbial contamination
    - Hamburger, chicken, spices may all be irradiated

26.3 Filter Sterilization

- Filtration avoids the use of heat on sensitive liquids and gases
  - Pores of filter are too small for organisms to pass through
  - Pores allow liquid or gas to pass through
- *Depth filters*
  - *HEPA* filters (Figure 26.6a)
- *Membrane filters*
  - Function more like a sieve (Figure 26.6b and c)

Figure 26.6 Microbiological filters

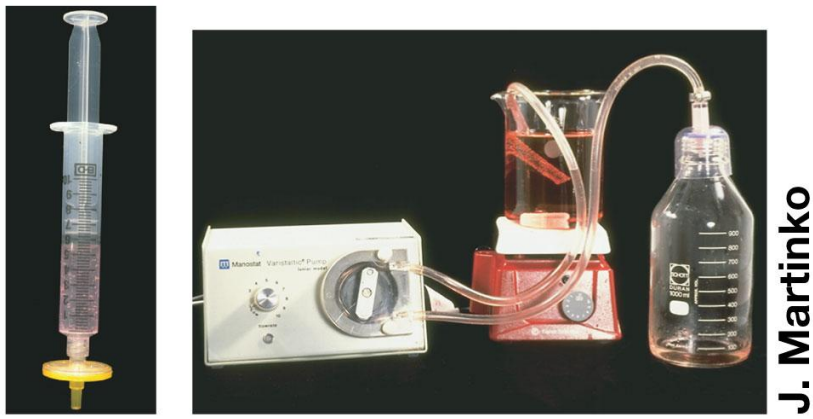


Scanning electron micrograph showing the structure of (a) a depth filter, (b) a conventional membrane filter, and (c) a nucleopore filter

26.3 Filter Sterilization

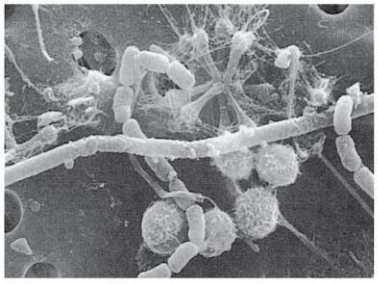
- *Membrane filters* (cont'd)
  - Filtration can be accomplished by syringe, pump, or vacuum (Figure 26.7)
  - A type of membrane filter is the nucleation track (nucleopore) filter (Figure 26.8)

Figure 26.7 Membrane filters



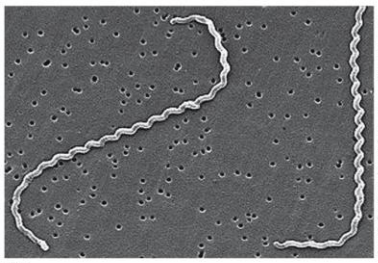
Disposable, presterilized, and assembled membrane filter units. Left: a filter system designed for small volumes. Right: a filter system designed for larger volumes

Figure 26.8 Scanning electron micrographs of bacteria trapped on nucleopore membrane filters



(a)

(a) Aquatic bacteria and algae. The pore size is 5 µm.



(b)

(b) Leptospira interrogans. The bacterium is about 0.1 µm in diameter and up to 20 µm in length. The pore size of the filter is 0.2 µm.

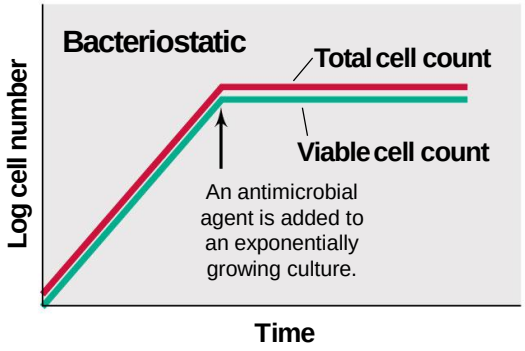
II. Chemical Antimicrobial Control

- 26.4 Chemical Growth Control
- 26.5 Chemical Antimicrobial Agents for External Use

26.4 Chemical Growth Control

- Antimicrobial agents can be classified as *bacteriostatic*, *bacteriocidal,* and *bacteriolytic* (Figure 26.9)

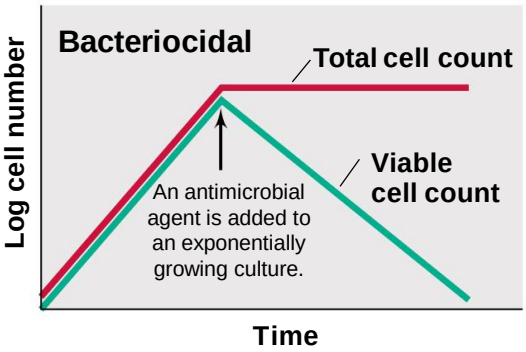
Figure 26.9a Bacteriostatic, bacteriocidal, and bacteriolytic antimicrobial agents



(a) The turbidity of each culture, coupled with viable plate counts, establishes the relationship between viable and total cell counts.

- Bacteriostatic agents are frequently inhibitors of protein synthesis and act by binding to ribosomes.
- If the concentration of the agent is lowered, the agent is released from the ribosome and growth resumes.
- Many antibiotics work by this mechanism.

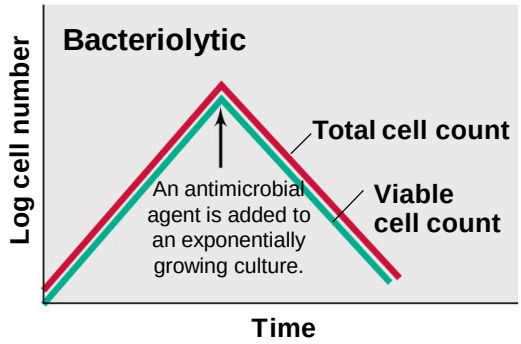
Figure 26.9b Bacteriostatic, bacteriocidal, and bacteriolytic antimicrobial agents



(b) The turbidity of each culture, coupled with viable plate counts, establishes the relationship between viable and total cell counts.

- Bacteriocidal agents bind tightly to their cellular targets, are not removed by dilution, and kill the cell.
- The dead cells, however, are not destroyed, and total cell numbers, reflected by the turbidity of the culture, remain constant .

Figure 26.9c Bacteriostatic, bacteriocidal, and bacteriolytic antimicrobial agents



(c) The turbidity of each culture, coupled with viable plate counts, establishes the relationship between viable and total cell counts.

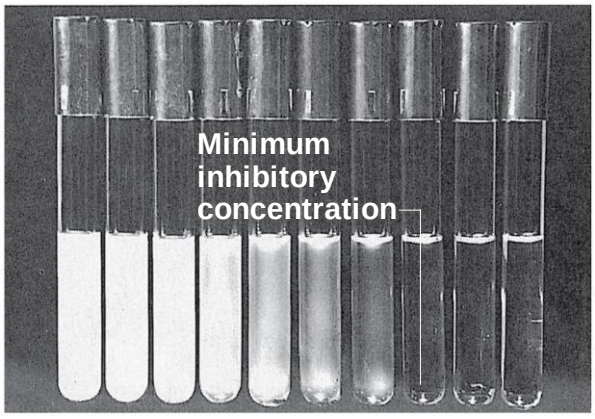
- Some -cidal agents are also -lytic agents, killing by cell lysis and release of cytoplasmic contents.
- Lysis decreases the viable cell number and also the total cell number, shown by a decrease in culture turbidity.

- Bacteriolytic agents include antibiotics that inhibit cell wall synthesis, such as penicillin, and chemicals such as detergents that rupture the cytoplasmic membrane.

26.4 Chemical Growth Control

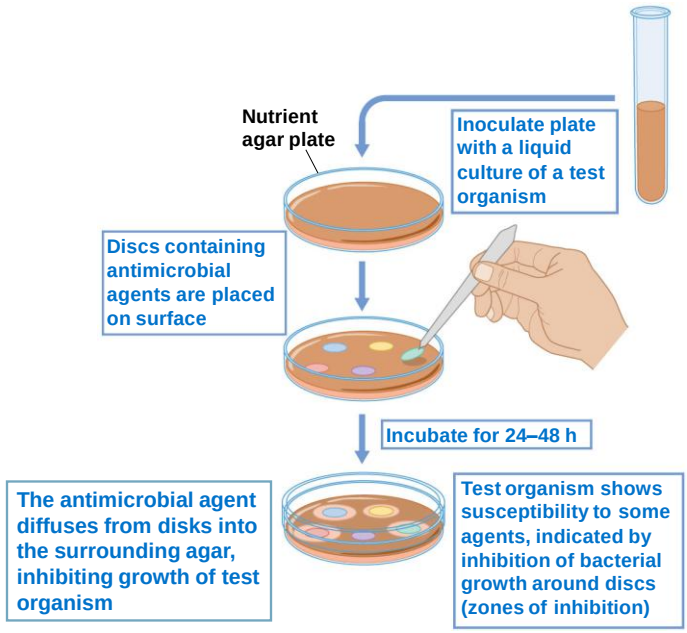
- *Minimum inhibitory concentration (MIC)* is the smallest amount of an agent needed to inhibit growth of a microorganism (Figure 26.10)
  - Varies with the organism used, inoculum size, temp, pH, etc.
- *Disc diffusion assay*
  - Antimicrobial agent added to filter paper disc
  - MIC is reached at some distance
    - *Zone of inhibition*
      - Area of no growth around disc

Figure 26.10 Antimicrobial agent susceptibility assay using dilution methods



The assay defines the minimum inhibitory concentration (MIC). A series of increasing concentrations of antimicrobial agent is prepared in the culture medium. Each tube is inoculated with a specific concentration of a test organism, followed by a defined incubation period. Growth, measured as turbidity, occurs in those tubes with antimicrobial agent concentrations below the MIC.

Figure 26.11 Antimicrobial agent susceptibility assay using diffusion methods



26.5 Chemical Antimicrobial Agents for External Use

- These antimicrobial agents can be divided into two categories
  - Products used to control microorganisms in commercial and industrial applications
    - Examples: chemicals in foods, air-conditioning cooling towers, textile and paper products, fuel tanks
  - Products designed to prevent growth of human pathogens in inanimate environments and on external body surfaces
    - *Sterilants*, *disinfectants*, *sanitizers,* and *antiseptics*

26.5 Chemical Antimicrobial Agents for External Use

- *Sterilants:* Chemical sterilants, also called sterilizers or sporicides, destroy all forms of microbial life, including endospores.
  - Chemical sterilants are used in situations where it is impractical to use heat or radiation for decontamination or sterilization.
  - Liquid sterilants such as a sodium hypochlorite solution or amylphenol are used for instruments that cannot withstand high temperatures or gas.

26.5 Chemical Antimicrobial Agents for External Use

- Hospitals and laboratories, must be able to decontaminate and sterilize heat-sensitive materials, such as;
  - thermometers
  - lensed instruments
  - polyethylene tubing and
  - reusable medical equipment.

26.5 Chemical Antimicrobial Agents for External Use

- *Disinfectants*: are chemicals that kill microorganisms, but not necessarily endospores and are used on inanimate objects.
  - Disinfectants such as ethanol and cationic detergents are used to disinfect floors, tables, bench tops, walls, etc. and important for infection control in hospitals.
  - General disinfectants are used in households, swimming pools and water purification systems

26.5 Chemical Antimicrobial Agents for External Use

- *Sanitizers*: are agents that reduce, but may not eliminate, microbial numbers to levels considered to be safe.
  - Food contact sanitizers are widely used in the food industry to treat surfaces such as mixing and cooking equipment, dishes and utensils.
  - Non–food contact sanitizers are used to treat surfaces such as counters, floors, walls, carpets, and laundry.

26.5 Chemical Antimicrobial Agents for External Use

- *Antiseptics* and *germicides* are chemical agents that kill or inhibit growth of microorganisms and that are nontoxic enough to be applied to living tissues.
  - Most of the compounds in this category are used for handwashing or for treating surface wounds.
  - Certain antiseptics are also effective disinfectants; they are effective antimicrobial agents when applied to inanimate surfaces.

Figure 2 Handwashing

Handwashing is the easiest and one of the most important interventions to prevent pathogen spread in healthcare, home and laboratory settings. This handwash station is in a clinical laboratory.

26.5 Chemical Antimicrobial Agents for External Use

- Ethanol, is categorized as an antiseptic, but can also be a disinfectant.
- This depends on the concentration of ethanol used and the exposure time, with disinfection generally requiring higher ethanol concentrations and exposure times of several minutes.

26.5 Chemical Antimicrobial Agents for External Use

- Antimicrobial Efficacy
  - Several factors affect the efficacy of chemical antimicrobial agents.
  - For example, many disinfectants are neutralized by organic material.
  - These materials reduce effective disinfectant concentrations and microbial killing capacity.

26.5 Chemical Antimicrobial Agents for External Use

- Antimicrobial Efficacy (cont'd)
  - Pathogens are often encased in particles or grow in large numbers as biofilms, covering surfaces of tissue or medical devices with several layers of microbial cells.
  - Biofilms may slow or even completely prevent penetration of antimicrobial agents, reducing or negating their effectiveness.

26.5 Chemical Antimicrobial Agents for External Use

- Antimicrobial Efficacy (cont'd)
  - Only sterilants are effective against bacterial endospores.
  - Endospores are much more resistant to other agents than are vegetative cells because of their low water availability and reduced metabolism.
  - *Mycobacterium tuberculosis*, the causal agent of tuberculosis, are resistant to disinfectants because of the waxy nature of their cell wall.

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26.5 Chemical Antimicrobial Agents for External Use

Table 26.4 *Industrial uses of antimicrobial chemicals*

Industry	Chemicals	Use
Paper	Organic mercurials, phenols,[a] methylisothiazolinone	To prevent microbial growth during manufacture
Leather	Heavy metals, phenols[a]	Antimicrobial agents present in the final product inhibit growth
Plastic	Cationic detergents	To prevent growth of bacteria on aqueous dispersions of plastics
Textile	Heavy metals, phenols[a]	To prevent microbial deterioration of fabrics, such as awnings and tents, that are exposed in the environment
Wood	Metal salts, phenols[a]	To prevent deterioration of wooden structures
Metal working	Cationic detergents	To prevent growth of bacteria in aqueous cutting emulsions
Petroleum	Mercurics, phenols,[a] cationic detergents, methylisothiazolinone	To prevent growth of bacteria during recovery and storage of petroleum and petroleum products
Air conditioning	Chlorine, phenols,[a] methylisothiazolinone	To prevent growth of bacteria (for example, *Legionella*) in cooling towers
Electrical power	Chlorine	To prevent growth of bacteria in condensers and cooling towers
Nuclear	Chlorine	To prevent growth of radiation-resistant bacteria in nuclear reactors

[a]Metallic (mercury, arsenic, and copper) compounds and phenolic compounds may produce environmentally hazardous waste products and create health hazards.

26.5 Chemical Antimicrobial Agents for External Use

Table 26.5 *Antiseptics, sterilants, disinfectants, and sanitizers*

Agent	Use	Mode of action
Sterilants, disinfectants, and sanitizers[c]		
Alcohol (60–85% ethanol or isopropanol in water)[a]	Disinfectant for medical instruments and laboratory surfaces	Lipid solvent and protein denaturant
Cationic detergents (quaternary ammonium compounds, Lysol® and many related disinfectants)	Disinfectant and sanitizer for medical instruments, food and dairy equipment	Interact with phospholipids
Chlorine gas	Disinfectant for purification of water supplies	Oxidizing agent
Chlorine compounds (chloramines, sodium hypochlorite, sodium chlorite, chlorine dioxide)	Disinfectant and sanitizer for dairy and food industry equipment, and water supplies	Oxidizing agent
Copper sulfate	Algicide disinfectant in swimming pools and water supplies	Protein precipitant
Ethylene oxide (gas)	Sterilant for temperature-sensitive materials such as plastics and lensed instruments	Alkylating agent
Formaldehyde	3–8% solution used as surface disinfectant, 37% (formalin) or vapor used as sterilant	Alkylating agent
Glutaraldehyde	2% solution used as high-level disinfectant or sterilant, commonly used fixative in electron microscopy	Alkylating agent
Hydrogen peroxide[a]	Vapor used as sterilant	Oxidizing agent
Iodine-containing iodophor compounds in solution[a] (Wescodyne®)	Disinfectant for medical instruments and laboratory surfaces	Iodinates tyrosine residues
Mercuric dichloride[b]	Disinfectant for laboratory surfaces	Combines with –SH groups
OPA (ortho-phthalaldehyde)	High-level disinfectant for medical instruments	Alkylating agent
Ozone	Disinfectant for drinking water	Strong oxidizing agent
Peroxyacetic acid	Solution used as high-level disinfectant or sterilant	Strong oxidizing agent
Phenolic compounds[b]	Disinfectant for laboratory surfaces	Protein denaturant
Pine oils (Pine-Sol®) (contains phenolics and other detergents)	General disinfectant for household surfaces	Protein denaturant

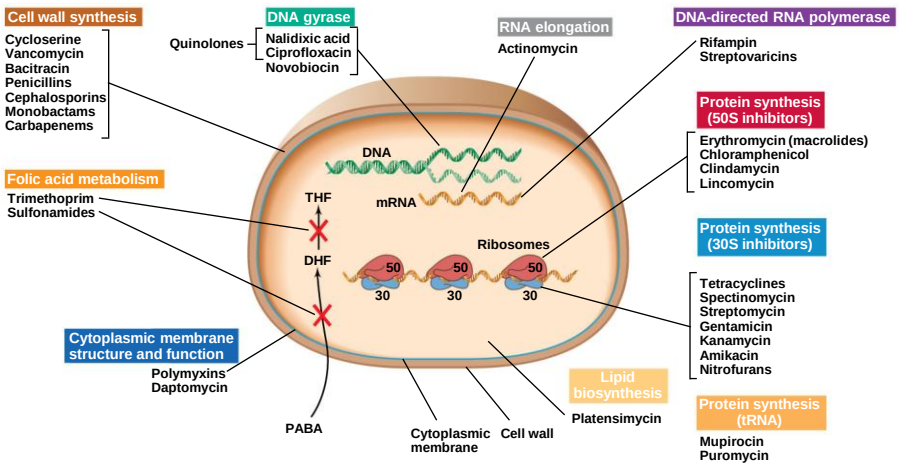
III. Antimicrobial Agents Used *In Vivo*

- 26.6 Synthetic Antimicrobial Drugs
- 26.7 Naturally Occurring Antimicrobial Drugs: Antibiotics
- 26.8 β-Lactam Antibiotics: Penicillins and Cephalosporins
- 26.9 Antibiotics from Prokaryotes

III. Antimicrobial Agents Used *In Vivo*

- Antimicrobial drugs are classified on the basis of
  - Molecular structure
  - Mechanism of action (Figure 26.12)
  - Spectrum of antimicrobial activity (Figure 26.13)

Figure 26.12 Mode of action of some major antimicrobial agents

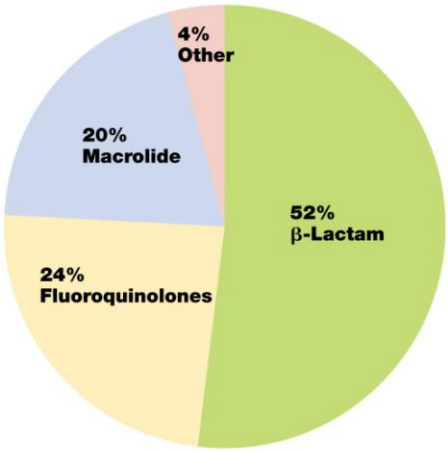


Agents are classified according to their target structures in the bacterial cell.

26.6 Synthetic Antimicrobial Drugs

- Paul Ehrlich studied *selective toxicity* in the early 1900s
  - Selective toxicity is ability to inhibit or kill a pathogen without affecting the host
  - *Salvarsan* – one of the first antimicrobial drugs
- *Growth factor analogs* are structurally similar to growth factors but do not function in the cell
  - Analogs similar to vitamins, amino acids, and other compounds

Figure 26.14 Annual worldwide production and use of antibiotics



Each year an estimated 10,000 metric tons of antimicrobial agents are manufactured worldwide. The β-lactam antibiotics include cephahalosporins (30%), penicillins (7%), and other β-lactams (15%). "Others" includes tetracyclines, aminoglycosides, and all other antimicrobial drugs

26.7 Naturally Occurring Antimicrobial Drugs: Antibiotics

- *Antibiotics* are *naturally produced* antimicrobial agents
  - Less than 1% of known antibiotics are clinically useful
    - Can be modified to enhance efficacy (*semisynthetic*)
- The susceptibility of microbes to different antibiotics varies greatly
  - Gram-positive and gram-negative bacteria vary in their sensitivity to antibiotics
  - Broad-spectrum antibiotics are effective against both groups of bacteria

26.8 β-Lactam Antibiotics: Penicillins and Cephalosporins

- β-Lactam antibiotics are one of the most important groups of antibiotics of all time
  - Include penicillins, cephalosporins, and cephamycins
  - Over half of all antibiotics used worldwide
- *Penicillins*
  - Discovered by Alexander Fleming
  - Primarily effective against gram-positive bacteria
  - Some synthetic forms are effective against some gram-negative bacteria
  - Target cell wall synthesis

26.9 Antibiotics from Prokaryotes

- Many antibiotics effective against *Bacteria* are also produced by *Bacteria*
- These antibiotics include:
  - *Aminoglycosides*
  - *Macrolides*
  - *Tetracyclines*
  - *Daptomycin*
  - *Platensimycin*
- They have major clinical applications