26

Microbial Growth Control

Filtration of an aqueous liquid through the tiny pores of a membrane filter traps any microbial cells that were present in the liquid and renders it sterile.

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With this chapter we begin to study the relationships between microorganisms and humans. We start with the agents and methods used for control of microbial growth. The goal is to either reduce or eliminate the microbial load and limit microbial effects.

A few agents eliminate microbial growth entirely by **sterilization**—the killing or removal of all viable organisms from a growth medium or surface. In certain circumstances, however, sterility is not attainable or practical, as in fresh foods. Microorganisms can be effectively controlled by limiting or inhibiting their growth. For example, we wash fresh produce to remove most existing bacteria, limiting their growth. Likewise, we inhibit microbial growth on body surfaces by washing. Neither of these processes, however, kills or removes all microbes.

Methods for inhibiting rapid microbial growth include decontamination and disinfection. **Decontamination** is the treatment of an object or surface to make it safe to handle. For example, simply wiping a table after a meal removes contaminating microorganisms and their potential nutrients. **Disinfection**, in contrast, directly targets pathogens, although it may not eliminate all microorganisms. Specialized chemical or physical agents called *disinfectants* can kill microorganisms or inhibit microbial growth. Bleach (sodium hypochlorite) solution, for example, is a disinfectant used to clean and disinfect food preparation areas.

Under certain circumstances, it may be necessary to destroy all microorganisms. Such measures are necessary, for instance, when making microbiological media or preparing surgical instruments. Sterilization completely eliminates all microorganisms, including endospores, and also eliminates all viruses. Microbial control *in vivo* is much more difficult: Clinically useful bacteriocidal (bacteria killing) agents or bacteriostatic (bacteria inhibiting) agents must selectively prevent or reduce bacterial growth, while causing no harm to the host.

In this chapter we first examine methods of microbial control that are used *in vitro*. We then discuss antimicrobial drugs used in humans and animals.

I Physical Antimicrobial Control

Physical methods are used in industry, medicine, and in the home to achieve microbial decontamination, disinfection, and sterilization. Heat, radiation, and filtration are commonly used to destroy or remove microorganisms. These methods prevent microbial growth or decontaminate areas or materials harboring microorganisms. Here we discuss physical control mechanisms and present some practical examples.

26.1 Heat Sterilization

Perhaps the most widespread method used for controlling microbial growth is the use of heat as a sterilization method. Factors that affect a microorganism's susceptibility to heat include the temperature and duration of the heat treatment and whether the heat is moist or dry.

Measuring Heat Sterilization

All microorganisms have a maximum growth temperature beyond which viability decreases (↻ Section 5.12). Microorganisms lose viability at very high temperatures because most macromolecules lose structure and function, a process called *denaturation*. The effectiveness of heat as a sterilant is measured by the time required for a 10-fold reduction in the viability of a microbial population at a given temperature. This is the *decimal reduction time* or *D*. For example, over the range of temperatures usually used in food preparation (cooking and canning), the relationship between *D* and temperature is exponential; the logarithm of *D* plotted against temperature yields a straight line (**Figure 26.1**). The graph can be used to calculate processing times to achieve sterilization, for instance in a canning operation. The slope of the line indicates the sensitivity of the organism to heat under the conditions employed (↻ Section 36.2). Death from heating is an exponential (first-order) function, proceeding more rapidly as the temperature rises, as shown in **Figure 26.2**.

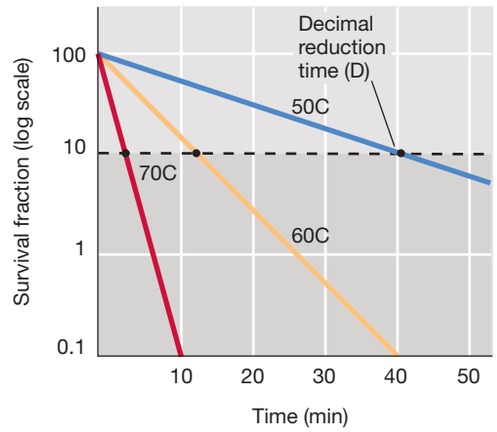


Figure 26.1 The effect of temperature over time on the viability of a mesophilic bacterium. The decimal reduction time, *D*, is the time at which only 10% of the original population of organisms remains viable at a given temperature. For 70°C, $D = 3$ min; for 60°C, $D = 12$ min; for 50°C, $D = 42$ min.

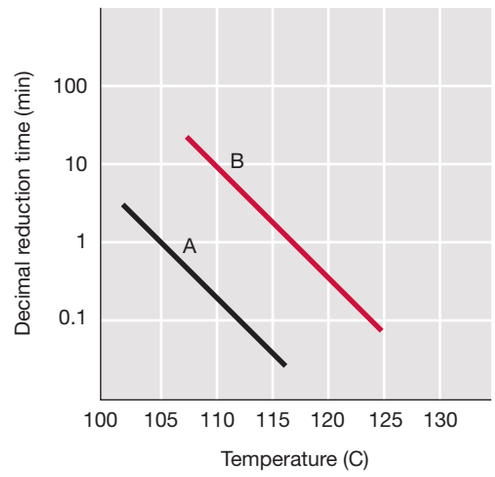


Figure 26.2 The relationship between temperature and the rate of killing in mesophiles and thermophiles. Data were obtained for decimal reduction times, *D*, at several different temperatures, as in Figure 26.1. For organism A, a typical mesophile, exposure to 110°C for less than 20 sec resulted in a decimal reduction, while for organism B, a thermophile, 10 min was required to achieve a decimal reduction.

The time necessary to kill a defined fraction (for example, 90%) of viable cells is independent of the initial cell concentration. As a result, sterilization of a microbial population takes longer at lower temperatures than at higher temperatures. The time and temperature, therefore, must be adjusted to achieve sterilization for each specific set of conditions. The type of heat is also important: Moist heat has better penetrating power than dry heat and, at a given temperature, produces a faster reduction in the number of living organisms.

Determination of a decimal reduction time requires a large number of viable count measurements (↻ Section 5.10). An easier way to characterize the heat sensitivity of an organism is to measure the *thermal death time*, the time it takes to kill all cells at a given temperature. To determine the thermal death time, samples of a cell suspension are heated for different times, mixed with culture medium, and incubated. If all the cells have been killed, no growth is observed in the incubated samples. The thermal death time depends on the size of the population tested; a longer time is required to kill all cells in a large population than in a small one. When the number of cells is standardized, it is possible to compare the heat sensitivities of different organisms by comparing their thermal death times at a given temperature.

Endospores and Heat Sterilization

Some bacteria produce highly resistant cells called *endospores* (↻ Section 3.12). The heat resistance of vegetative cells and endospores from the same organism differs considerably. For instance, in the autoclave (see below) a temperature of 121°C is normally reached. Under these conditions, endospores may require 4–5 minutes for a decimal reduction, whereas vegetative cells may require only 0.1–0.5 min at 65°C. To ensure adequate decontamination of any material, heat sterilization procedures must be designed to destroy endospores.

Endospores can survive heat that would rapidly kill vegetative cells of the same species. A major factor in heat resistance is the amount and state of water within the endospore. During endospore formation, the protoplasm is reduced to a minimum volume as a result of the accumulation of calcium (Ca^{2+})–dipicolinic acid complexes and small acid-soluble spore proteins (SASPs). This mixture forms a cytoplasmic gel, and a thick cortex then forms around the developing endospore. Contraction of the cortex results in a shrunken, dehydrated cell containing only 10–30% of the water of a vegetative cell (↻ Section 3.12).

The water content of the endospore coupled with the concentration of SASPs determines its heat resistance. If endospores have a low concentration of SASPs and high water content, they exhibit low heat resistance. Conversely, if they have a high concentration of SASPs and low water content, they show high heat resistance. Water moves freely in and out of endospores, so it is not the impermeability of the endospore coat that excludes water, but the gel-like material in the endospore protoplast.

The medium in which heating takes place also influences the killing of both vegetative cells and endospores. Microbial death is more rapid at acidic pH, and acid foods such as tomatoes, fruits, and pickles are much easier to sterilize than neutral pH foods such as corn and beans. High concentrations of sugars, proteins, and fats decrease heat penetration and usually increase the resistance of organisms to heat, whereas high salt concentrations may either increase or decrease heat resistance, depending on the organism. Dry cells and endospores are more heat resistant than moist ones; consequently, heat sterilization of dry objects such as endospores always requires higher temperatures and longer heat application times than sterilization of wet objects such as liquid bacterial cultures.

The Autoclave

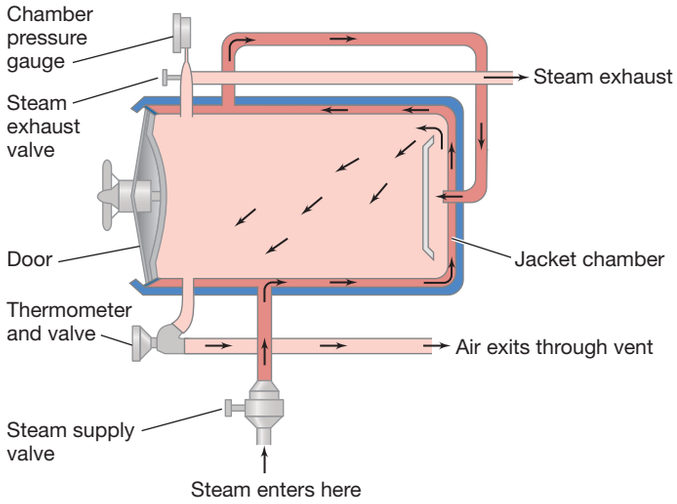
The **autoclave** is a sealed heating device that uses steam under pressure to kill microorganisms (**Figure 26.3*a***). Killing of heat-resistant endospores requires heating at temperatures above 100°C, the boiling point of water at normal atmospheric pressure. The autoclave uses steam under 1.1 kilograms/square centimeter (kg/cm^2) [15 pounds/square inch (lb/in^2)] pressure, which yields a temperature of 121°C. At 121°C, the time to achieve sterilization of endospore-containing material is generally 10–15 minutes (Figure 26.3*b*).

If an object being sterilized is bulky, heat transfer to the interior is retarded, and the total heating time must be extended to ensure that the entire object is at 121°C for 10–15 minutes. Extended times are also required when large volumes of liquids are being autoclaved because large volumes take longer to reach sterilization temperatures. Note that it is not the *pressure* inside the autoclave that kills the microorganisms but the high *temperature* that can be achieved when steam is applied under pressure.

Pasteurization

Pasteurization uses precisely controlled heat to reduce the number of microorganisms found in milk and other heat-sensitive liquids. The process, named for Louis Pasteur (↻ Section 1.7), was first used for controlling the spoilage of wine. Pasteurization does not kill all organisms and is therefore not a method of sterilization. Pasteurization does, however, reduce the *microbial load*, the number of viable microorganisms in a sample. At temperatures and times used for pasteurization of food products such as milk, pathogenic bacteria, especially the organisms causing tuberculosis, brucellosis, Q fever, and typhoid fever, are killed. These pathogens are no longer common in raw foods in developed countries, but pasteurization also controls commonly encountered pathogens such as *Listeria monocytogenes, Campylobacter* species, *Salmonella*, and *Escherichia coli* O157:H7; these pathogenic bacteria can be found in foods such as dairy products and juices (↻ Sections 36.8–36.12). In addition, by decreasing the overall microbial load, pasteurization retards the growth of spoilage organisms, increasing the shelf life of perishable liquids (↻ Sections 36.1 and 36.2).

Pasteurization of milk is usually achieved by passing the milk through a heat exchanger. The milk is pumped through tubing that is in contact with a heat source. Careful control of the milk flow rate and the size and temperature of the heat source raises the temperature of the milk to 71°C for 15 seconds. The milk is then rapidly cooled. This process is aptly called flash pasteurization.



(a)

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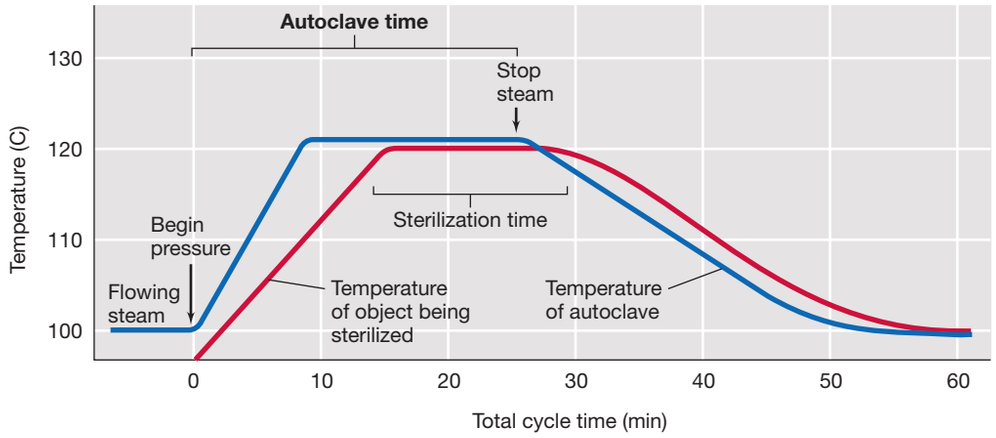


(b)

Figure 26.3 **The autoclave and moist heat sterilization.** *(a)* The flow of steam through an autoclave. *(b)* A typical autoclave cycle. The temporal heating profile of a fairly bulky object is shown. 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. *(c)* A modern research autoclave. Note 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.

Milk can also be pasteurized in large quantities by heating in large vats to 63–66°C for 30 minutes. However, this bulk pasteurization method is less satisfactory because the milk heats and cools slowly and must be held at high temperatures for longer times. This slower heating and cooling of the milk alters the taste of the final product, rendering it generally less palatable for the consumer. Flash pasteurization, sometimes done at even higher temperatures and shorter times, alters the flavor less, kills heat-resistant organisms more effectively, and can be done on a continuous-flow basis, making it more adaptable to large dairy operations.

MiniQuiz

- Why is heat an effective sterilizing agent?
- Why is moist heat more effective than dry heat for sterilization?
- What steps are necessary to ensure the sterility of material contaminated with bacterial endospores?
- Distinguish between the sterilization of microbiological media and the pasteurization of dairy products.

26.2 Radiation Sterilization

Heat is just one form of energy that can sterilize or reduce microbial load. Microwaves, ultraviolet (UV) radiation, X-rays, gamma rays (γ-rays), and electrons can also effectively reduce microbial growth if applied in the proper dose and time. However, each type of energy has a different mode of action. For example, the antimicrobial effects of microwaves are due, at least in part, to thermal effects. Other forms of energy cause other modifications that lead to death or inactivation of microorganisms.

Ultraviolet Radiation

Ultraviolet radiation between 220 and 300 nm in wavelength has enough energy to cause modifications or actual breaks in DNA, sometimes leading to disruption of DNA and death of the exposed organism (↻ Section 10.4). This "near-visible" UV light is useful for disinfecting surfaces, air, and materials such as water that do not absorb the UV waves. For example, laboratory laminar flow hoods, designed to maintain clean work areas, are equipped with a "germicidal" UV light to decontaminate the work surface after use (**Figure 26.4**). UV radiation, however, cannot penetrate solid, opaque, or light-absorbing surfaces, limiting its use to disinfection of exposed surfaces.

Ionizing Radiation

Ionizing radiation is electromagnetic radiation of sufficient energy to produce ions and other reactive molecular species from molecules with which the radiation particles collide. Ionizing radiation generates electrons, e^-; hydroxyl radicals, OH· (↻ Section 5.18), and hydride radicals, H·. Each of these highly reactive molecules is capable of altering and disrupting macromolecules such as DNA, lipids, and protein. The ionization and subsequent degradation of these biologically important molecules leads to the death of irradiated cells.

J. Martinko

Figure 26.4 A laminar flow hood. An ultraviolet 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.

Table 26.1 *Radiation sensitivity of microorganisms and biological functions*

Species or function	*Type of microorganism*	*D10[a] (Gy)*
Clostridium botulinum	Gram-positive, anaerobic, sporulating *Bacteria*	3300
Clostridium tetani	Gram-positive, anaerobic, sporulating *Bacteria*	2400
Bacillus subtilis	Gram-positive, aerobic, sporulating *Bacteria*	600
Escherichia coli O157:H7	Gram-negative *Bacteria*	300
Salmonella typhimurium	Gram-negative *Bacteria*	200
Lactobacillus brevis	Gram-positive *Bacteria*	1200
Deinococcus radiodurans	Gram-negative, radiation-resistant *Bacteria*	2200
Aspergillus niger	Mold	500
Saccharomyces cerevisiae	Yeast	500
Foot-and-mouth	Virus	13,000
Coxsackie	Virus	4500
Enzyme inactivation		20,000–50,000
Insect deinfestation		1000–5000

[a]*D*10 is the amount of radiation necessary to reduce the initial population or activity level 10-fold (1 logarithm). Gy = grays. 1 gray = 100 rads. The lethal dose for humans is 10 Gy.

The unit of radiation is the *roentgen*, which is a measure of the energy output from a radiation source. The standard for biological applications such as sterilization is the absorbed radiation dose, measured in *rads* (100 erg/g) or *grays* (1 Gy = 100 rad). Some microorganisms are much more resistant to radiation than others. **Table 26.1** shows the dose of radiation necessary for a 10-fold (one log) reduction in the numbers of selected microorganisms or biological functions. For example, the amount of energy necessary to achieve a 10-fold reduction (*D*) of a radiation-sensitive bacterium such as *Escherichia coli* O157:H7 is 300 Gy. The *D* value is analogous to the decimal reduction time for heat sterilization: The relationship of the survival fraction plotted on a logarithmic scale versus the radiation dose in grays is essentially linear (**Figure 26.5** and compare to Figure 26.1).

In practice, this means that at a radiation dose of 300 Gy, 90% of *E. coli* O157:H7 in a given sample would be killed. A dose of 2 *D*, or 600 Gy, would kill 99% of the organism, and so on. A standard killing dose for radiation sterilization is 12 *D*. A killing dose of radiation-resistant endospores of a bacterium such as *Clostridium botulinum* for example, would be 3300 Gy × 12, or 39,600 Gy (Table 26.1). By contrast, the killing dose for *E. coli* O157:H7 is

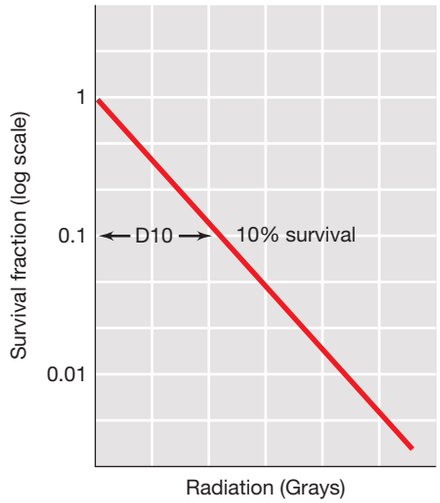


Figure 26.5 Relationship between the survival fraction and the radiation dose of a microorganism. The *D*10, or decimal reduction dose, can be interpolated from the data as shown.

only 3600 Gy. In general, microorganisms are much more resistant to ionizing radiation than are multicellular organisms. For example, the lethal radiation dose for humans can be as low as 10 Gy if delivered over a short time (several minutes)!

Radiation Practices

Several radiation sources are useful for sterilization. Common sources of ionizing radiation include cathode ray tubes that generate electron beams, X-ray machines, and radioactive nuclides ^{60}Co and ^{137}Cs, which are relatively inexpensive by-products of nuclear fission. These sources produce electrons (e^-), X-rays, or γ-rays, respectively, all of which have sufficient energy to efficiently kill microorganisms. In addition, X-rays and γ-rays penetrate solids and liquids, making them ideal for treatment of bulk items such as ground beef or cereal grains.

Radiation is currently used for sterilization and decontamination in the medical supplies and food industries. In the United States, the Food and Drug Administration has approved the use of radiation for sterilization of such diverse items as surgical supplies, disposable labware, drugs, and even tissue grafts (**Table 26.2**). However, because of the required specialized equipment, costs, and hazards associated with radiation techniques, this type of sterilization is limited to large industrial applications or specialized facilities.

Table 26.2 *Medical and laboratory products sterilized by radiation*

Tissue grafts	*Drugs*	*Medical and laboratory supplies*
Cartilage	Chloramphenicol	Disposable labware
Tendon	Ampicillin	Culture media
Skin	Tetracycline	Syringes
Heart valve	Atropine	Surgical equipment
	Vaccines	Sutures
	Ointments	

Table 26.3 *Recommended radiation dose for decontamination of selected foods*

Food type	*kiloGrays*
Fruit	1
Poultry	3
Spices, seasonings	30

Certain foods and food products are also routinely irradiated to ensure sterilization, pasteurization, or insect deinfestation. Radiation is approved by the World Health Organization and can be used in the United States for decontamination of foods particularly susceptible to microbial contamination such as fresh produce, meat products, chicken, and spices (**Table 26.3** and Section 36.2). The use of radiation for these purposes is an established and accepted technology in many countries. However, the practice has not been readily accepted in some countries such as the United States because of fears of possible radioactive contamination, alteration in nutritional value, production of toxic or carcinogenic products, and perceived "off" tastes in irradiated food.

MiniQuiz

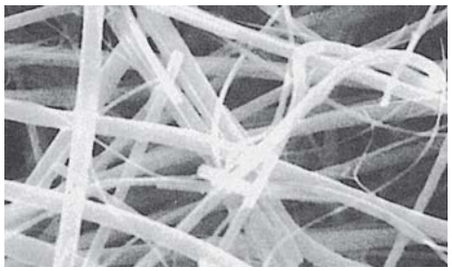
- Define the decimal reduction dose and the killing dose for radiation treatment of microorganisms.
- Why is ionizing radiation more effective than UV radiation for sterilization of food products?

26.3 Filter Sterilization

Heat is an effective way to decontaminate most liquids and can even be used to treat gases. Heat-sensitive liquids and gases, however, must be treated by other methods. Filtration accomplishes decontamination and even sterilization without exposure to denaturing heat. The liquid or gas is passed through a filter, a device with pores too small for the passage of microorganisms, but large enough to allow the passage of the liquid or gas. The selection of filters for sterilization must account for the size range of the contaminants to be excluded. Some microbial cells are greater than 10 μm in diameter, while the smallest bacteria are less than 0.3 μm in diameter. Historically, selective filtration methods were used to define and isolate viruses, most of which range from 25 nm to 200 nm (0.2 μm) in diameter. **Figure 26.6** illustrates major types of filters.

Depth Filters

A depth filter is a fibrous sheet or mat made from a random array of overlapping paper or borosilicate (glass) fibers (Figure 26.6*a*). The depth filter traps particles in the network of fibers in the



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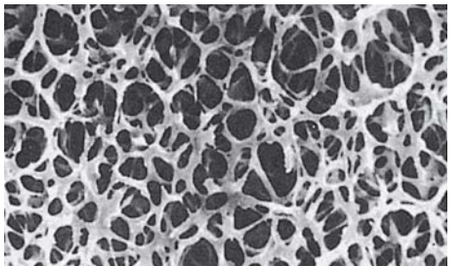

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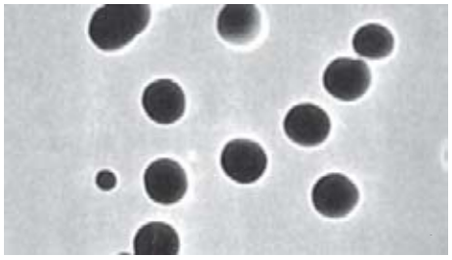


(c)

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.

structure. Because the filtration material is arranged randomly in a thick layer, depth filters resist clogging and are often used as prefilters to remove larger particles from liquid suspensions so that the final filter in the sterilization process is not clogged. Depth filters are also used for the filter sterilization of air in industrial processes. In the home, the filter used in forced air heating and cooling systems is a simple depth filter designed to trap particulate matter such as dust, spores, and allergens.

Depth filters are important for biosafety applications. For example, manipulations of cell cultures, microbial cultures, and growth media require that contamination of both the operator and the experimental materials are minimized. These operations can be efficiently performed in a biological safety cabinet with airflow, both in and out of the cabinet, directed through a depth filter called a **HEPA filter**, or *h*igh-*e*fficiency *p*articulate *a*ir filter (Figure 26.4). A typical HEPA filter is a single sheet of borosilicate glass fibers that has been treated with a water-repellent binder. The filter, pleated to increase the overall surface area, is mounted inside a rigid, supportive frame. HEPA filters come in various shapes and sizes, from several square centimeters for vacuum cleaners, to several square meters for biological containment hoods and room air systems. Control of airborne particulate materials with HEPA filters allows the construction of "clean rooms" and isolation rooms for quarantine, as well as specialized biological safety laboratories (Section 31.4). HEPA filters typically remove 0.3-μm test particles with an efficiency of at least 99.97%; they remove both small and large particles, including most microorganisms, from the airstream.

Membrane Filters

Membrane filters are the most common type of filters used for liquid sterilization in the microbiology laboratory (Figure 26.6*b*). Membrane filters are composed of high tensile strength polymers such as cellulose acetate, cellulose nitrate, or polysulfone, manufactured to contain a large number of tiny holes, or pores. By adjusting the polymerization conditions during manufacture, the size of the holes in the membrane (and thus the size of the molecules that can pass through) can be precisely controlled. The membrane filter differs from the depth filter, functioning more like a sieve and trapping particles on the filter surface. About 80–85% of the membrane surface area consists of open pores. The porosity provides for a relatively high fluid flow rate.

Membrane filters for the sterilization of a liquid are illustrated in **Figure 26.7**. Presterilized membrane filter assemblies for sterilization of small to medium volumes of liquids such as growth media are routinely used in research and clinical laboratories. Filtration is accomplished by using a syringe, pump, or vacuum to force the liquid through the filtration apparatus into a sterile collection vessel.

Another type of membrane filter in common use is the nucleation track (nucleopore) filter. To make these filters, very thin polycarbonate film (10 μm) is treated with nuclear radiation and then etched with a chemical. The radiation causes local damage to the film, and the etching chemical enlarges these damaged locations into holes. The size of the holes can be controlled by varying the strength of the etching solution and the etching time. A typical nucleation track filter therefore has very uniform holes (Figure 26.6*c*). Nucleopore filters are commonly used to isolate specimens for scanning electron microscopy. Microorganisms are removed from liquid and concentrated in a single plane on the filter, where they can be observed with the microscope (**Figure 26.8**). Commonly used filter pore sizes for filter sterilization of small volumes, such as laboratory solutions, are 0.45 μm and 0.2 μm.

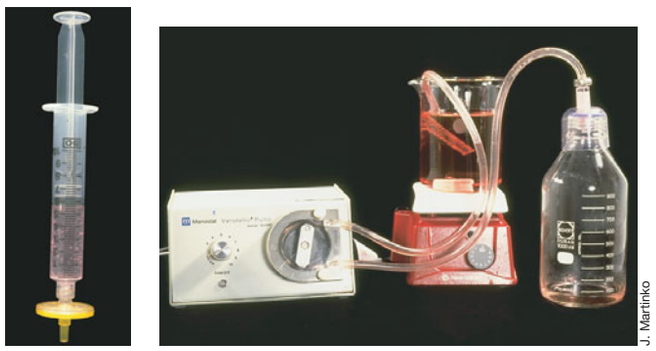
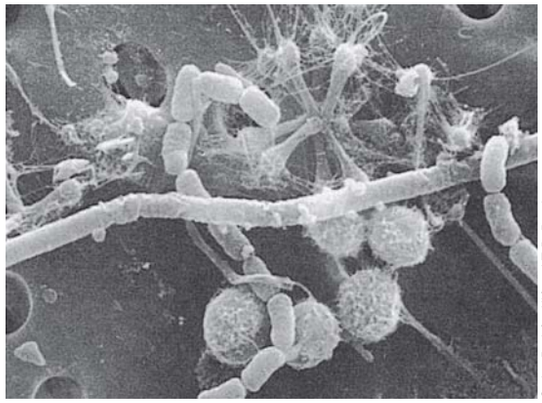
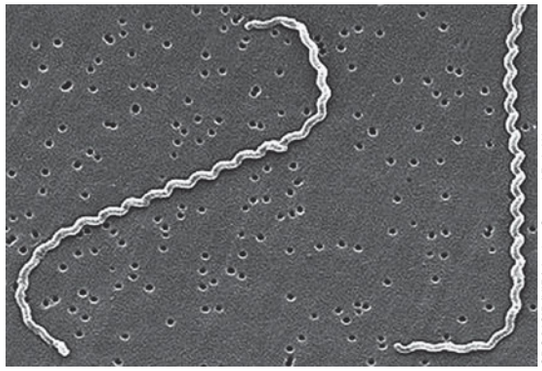


Figure 26.7 **Membrane filters.** Disposable, presterilized, and assembled membrane filter units. Left: a filter system designed for small volumes. Right: a filter system designed for larger volumes.



(a)



(b)

Figure 26.8 **Scanning electron micrographs of bacteria trapped on nucleopore membrane filters.** *(a)* Aquatic bacteria and algae. The pore size is 5 μm. *(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.

MiniQuiz

- Why are filters used for sterilization of heat-sensitive liquids?
- Describe the use of depth filters for maintaining clean air in hospitals, laboratories, and the home.

II Chemical Antimicrobial Control

In the home, workplace, and laboratory, chemicals are routinely used to control microbial growth. An **antimicrobial agent** is a natural or synthetic chemical that kills or inhibits the growth of microorganisms. Agents that kill organisms are called *-cidal* agents, with a prefix indicating the type of microorganism killed. Thus, they are called **bacteriocidal**, **fungicidal**, and **viricidal** agents because they kill bacteria, fungi, and viruses, respectively. Agents that do not kill but only inhibit growth are called *-static* agents. These include **bacteriostatic**, **fungistatic**, and **viristatic** compounds.

26.4 Chemical Growth Control

Antimicrobial agents can differ in their selective toxicity. Nonselective agents have similar effects on all cells. Selective agents are more toxic for microorganisms than for animal tissues. Antimicrobial agents with selective toxicity are especially useful for treating infectious diseases because they kill selected microorganisms *in vivo* without harming the host. They are described later in this chapter. Here we discuss chemical agents that have relatively broad toxicity and are widely used for limiting microbial growth *in vitro*.

Effect of Antimicrobial Agents on Growth

Antibacterial agents can be classified as bacteriostatic, bacteriocidal, and bacteriolytic by observing their effects on bacterial cultures (**Figure 26.9**). Viable cells are measured by plate counts. The number of viable cells for a given organism is proportional to culture turbidity during the log phase of growth. 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 (Figure 26.9*a*). Many antibiotics work by this mechanism, and they will be discussed in Sections 26.6–26.9. Bacteriocidal

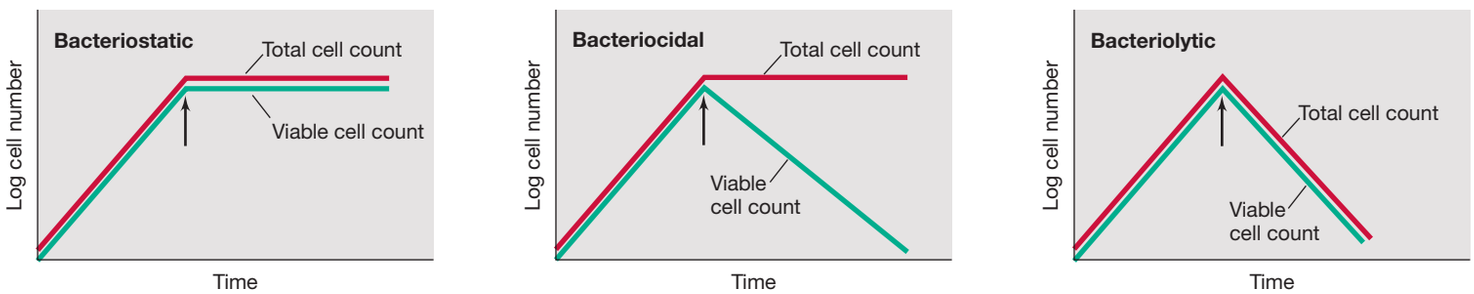


Figure 26.9 **Bacteriostatic, bacteriocidal, and bacteriolytic antimicrobial agents.** At the time indicated by the arrow, a growth-inhibitory concentration of each antimicrobial agent was added to an exponentially growing culture. The turbidity of each culture, coupled with viable plate counts, establishes the relationship between viable and total cell counts.

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 26.9*b*). 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 (Figure 26.9*c*). Bacteriolytic agents include antibiotics that inhibit cell wall synthesis, such as penicillin, and chemicals such as detergents that rupture the cytoplasmic membrane.

Measuring Antimicrobial Activity

Antimicrobial activity is measured by determining the smallest amount of agent needed to inhibit the growth of a test organism, a value called the **minimum inhibitory concentration (MIC)**. To determine the MIC for a given agent against a given organism, a series of culture tubes is prepared and inoculated with the same number of microorganisms. Each tube contains medium with an increasing concentration of the agent. After incubation, the tubes are checked for visible growth (turbidity). The MIC is the lowest concentration of agent that completely inhibits the growth of the test organism (**Figure 26.10**). This is called the *tube dilution technique*.

The MIC is not a constant for a given agent; it varies with the test organism, the inoculum size, the composition of the culture medium, the incubation time, and the conditions of incubation, such as temperature, pH, and aeration. When culture conditions are standardized, however, different antimicrobial agents can be compared to determine which is most effective against a given organism.

Another common assay for antimicrobial activity is the *disc diffusion technique* (**Figure 26.11**). A Petri plate containing an agar medium is inoculated with a culture of the test organism. Known amounts of an antimicrobial agent are added to filter-paper discs, which are then placed on the surface of the agar. During incubation, the agent diffuses from the disc into the agar, establishing a gradient; the farther the chemical diffuses away from the filter paper, the lower is the concentration of the agent. At some distance from the disc, the effective MIC is reached. Beyond this point the microorganism grows, but closer to the disc, growth is absent. A *zone of inhibition* is created with a diameter proportional to the amount of antimicrobial agent added to the disc, the solubility of the agent, the diffusion coefficient, and the overall effectiveness of the agent. The disc diffusion technique and other growth-dependent methods are routinely used to test pathogens for antibiotic susceptibility (Section 31.3).

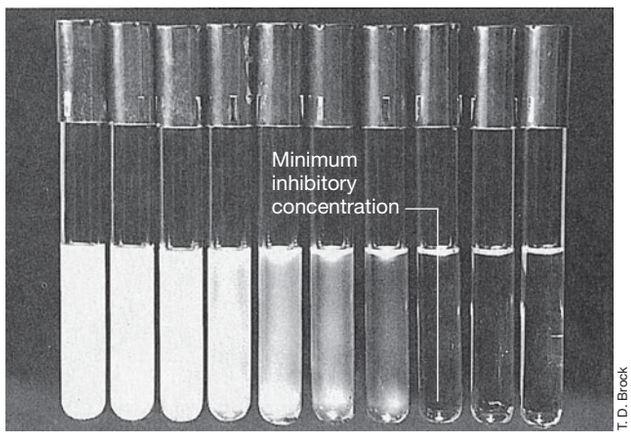


Figure 26.10 Antimicrobial agent susceptibility assay using dilution methods. The assay defines the minimum inhibitory concentration (MIC). A series of increasing concentrations of antimicrobial agent is prepared in the culture medium. Each tube is inoculated with a specific concentration of a test organism, followed by a defined incubation period. Growth, measured as turbidity, occurs in those tubes with antimicrobial agent concentrations below the MIC.

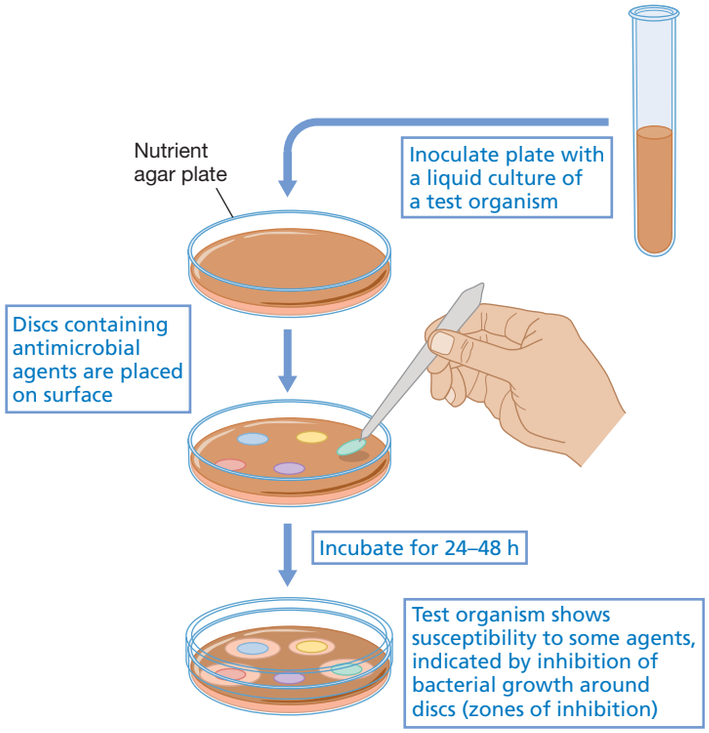


Figure 26.11 Antimicrobial agent susceptibility assay using diffusion methods. The antimicrobial agent diffuses from paper disks into the surrounding agar, inhibiting growth of susceptible microorganisms.

MiniQuiz

- For antimicrobial agents, distinguish between the effects of -static, -cidal, and - lytic agents.
- Describe how the minimum inhibitory concentration of an antibacterial agent is determined.

26.5 Chemical Antimicrobial Agents for External Use

Chemical antimicrobial agents are divided into two categories. The first category contains antimicrobial products used to control microorganisms in industrial and commercial environments. These include chemicals used in foods, air-conditioning cooling

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.

towers, textile and paper products, fuel tanks, and so on; some of these chemicals are so toxic that exposure can affect human health. Table 26.4 provides examples of industrial applications for chemicals used to control microbial growth.

The second category of chemical antimicrobial agents contains products designed to prevent growth of human pathogens in inanimate environments and on external body surfaces. This category is subdivided into sterilants, disinfectants, sanitizers, and antiseptics.

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 (Section 26.1) or radiation (Section 26.2) for decontamination or sterilization. Hospitals and laboratories, for example, must be able to decontaminate and sterilize heat-sensitive materials, such as thermometers, lensed instruments, polyethylene tubing, catheters, and reusable medical equipment such as respirometers. Some form of cold sterilization is usually used for these purposes. Cold sterilization is performed in enclosed devices that resemble autoclaves, but which employ a gaseous chemical agent such as ethylene oxide, formaldehyde, peroxyacetic acid, or hydrogen peroxide. Liquid sterilants such as a sodium hypochlorite (bleach) solution or amylphenol are used for instruments that cannot withstand high temperatures or gas (Table 26.5).

Disinfectants and Sanitizers

Disinfectants are chemicals that kill microorganisms, but not necessarily endospores, and are used on inanimate objects. For example, disinfectants such as ethanol and cationic detergents are used to disinfect floors, tables, bench tops, walls, and so on. These agents are important for infection control in, for example, hospitals and other medical settings. General disinfectants are used in households, swimming pools, and water purification systems (Table 26.5).

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 (Table 26.5).

Antiseptics and Germicides

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 (Microbial Sidebar, "Preventing Antimicrobial Drug Resistance") or for treating surface wounds (Table 26.5). Under some conditions, certain antiseptics are also effective disinfectants; they are effective antimicrobial agents when applied to inanimate surfaces. Ethanol, for example, 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. The Food and Drug Administration in the United States regulates the formulation, manufacture, and use of antiseptics and germicides because these agents involve direct human exposure and contact.

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. Furthermore,

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

Agent	*Use*	*Mode of action*
Antiseptics		
Alcohol (60–85% ethanol or isopropanol in water)[a]	Topical antiseptic	Lipid solvent and protein denaturant
Phenol-containing compounds (hexachlorophene, triclosan, chloroxylenol, chlorhexidine)[b]	Soaps, lotions, cosmetics, body deodorants, topical disinfectants	Disrupts cytoplasmic membrane
Cationic detergents, especially quaternary ammonium compounds (benzalkonium chloride)	Soaps, lotion, topical disinfectants	Interact with phospholipids of cytoplasmic membrane
Hydrogen peroxide[a] (3% solution)	Topical antiseptic	Oxidizing agent
Iodine-containing iodophor compounds in solution[a] (Betadine®)	Topical antiseptic	Iodinates tyrosine residues of proteins; oxidizing agent
Octenidine	Topical antiseptic	Disrupts cytoplasmic membrane
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

[a]Alcohols, hydrogen peroxide, and iodine-containing iodophor compounds can act as antiseptics, disinfectants, sanitizers, or sterilants depending on concentration, length of exposure, and form of delivery.
[b]Use of heavy metal (mercury) compounds and phenolic compounds may produce environmentally hazardous waste products and may create health hazards.
[c]Many water-soluble antimicrobial compounds, with the exception of those containing heavy metals, can be used as sanitizers for food and dairy equipment and preparation areas, provided their use is followed by adequate draining before food contact.

Preventing Antimicrobial Drug Resistance

According to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, antimicrobial resistance is widespread. For example, nearly 2 million patients in the United States develop hospital-acquired (nosocomial) infections each year. Hospital-acquired infections are difficult to treat because up to 70% of the infecting microorganisms are resistant to antimicrobial drugs. For *Staphylococcus aureus*, which causes over 10% of these infections in intensive care units, Figure 1 shows a trend toward methicillin-oxacillin-resistant *Staphylococcus aureus* (MRSA) blood infections over an 11-year period. Since these *S. aureus* isolates are often resistant to other drugs as well, the appearance of MRSA strains further limits the choice of effective therapeutic drugs. Antimicrobial drug resistance in strains of *Mycobacterium tuberculosis*, *Enterococcus faecium*, and *Candida albicans* are also of major concern in clinical settings.

The CDC has promoted a 12-step program to prevent resistance to antimicrobial agents, and the program is summarized below (see also http://www.cdc.gov/drugresistance/healthcare/ha/12steps_HA.htm). The program stresses the importance of preventing infection, rapidly and positively diagnosing and treating infections, using antimicrobial agents wisely, and preventing pathogen transmission.

1. **Immunize to prevent common diseases.** Keep immunizations up to date, especially for likely disease exposures. In addition to required vaccinations (↻ Section 28.7), this should include yearly influenza vaccination for nearly everyone, meningitis vaccines, and pneumococcal immunizations for healthcare providers or those exposed to large numbers of people, as in schools, colleges, and the military.

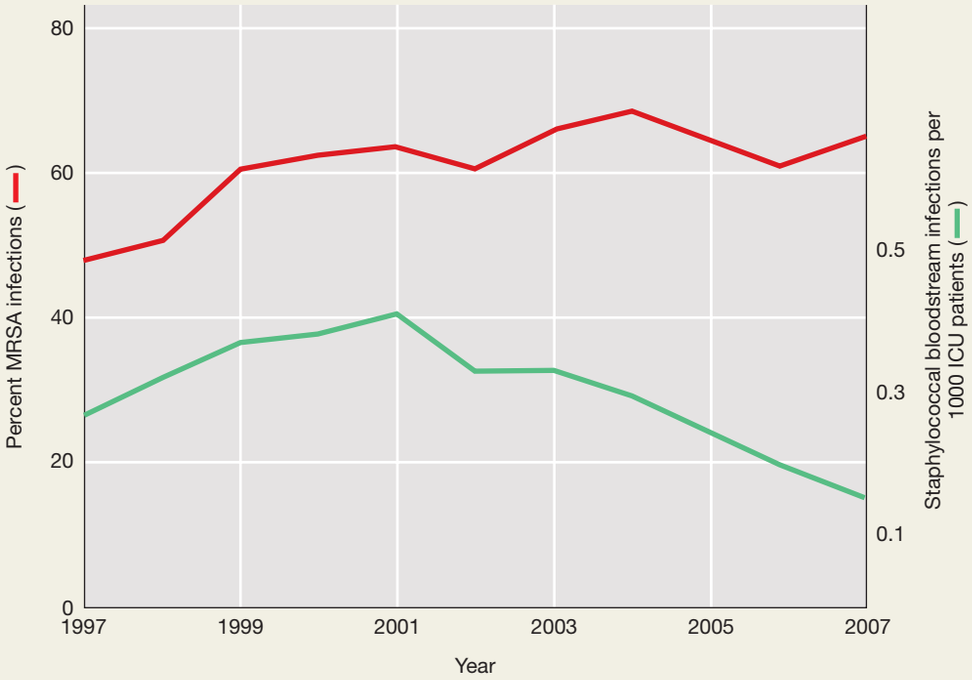


Figure 1 Methicillin-resistant *Staphylococcus aureus* blood infections in intensive care units in the United States. The percentage of methicillin-oxacillin-resistant *S. aureus* (MRSA) infections as compared to total staphylococcal blood infections is shown in red. Although the overall number of staphylococcal blood infections decreased during this time (green), the percentage of staphylococcal bloodstream infections caused by MRSA strains continued to increase. Data are adapted from the CDC.

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 (↻ Chapter 5, Microbial Sidebar, "Microbial Growth in the Real World: Biofilms"). Biofilms may slow or even completely prevent penetration of antimicrobial agents, reducing or negating their effectiveness.

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 (Section 26.1). Some bacteria, such as *Mycobacterium tuberculosis*, the causal agent of tuberculosis, are resistant to the action of common disinfectants because of the waxy nature of their cell wall (↻ Sections 18.5 and 33.4). Thus, the efficacy of antiseptics, disinfectants, sterilants, and other antimicrobial compounds used *in vitro* and *in vivo* for antimicrobial treatment must be empirically determined under the actual conditions of use.

MiniQuiz

- Distinguish between a sterilant, a disinfectant, a sanitizer, and an antiseptic.
- What disinfectants are routinely used for sterilization of water? Why are these disinfectants not harmful to humans?

2. **Avoid unnecessary introduction of parenteral devices such as catheters.** All present a risk of introducing infectious agents into the body. If such devices are necessary, remove them as soon as possible.
3. **Target the pathogen.** Attempt to culture the infectious agent while targeting antimicrobial drug treatment for the most likely pathogens. After positive culture results, adjust the therapy to target the known pathogen and its antibiotic susceptibility.
4. **Access the experts.** For serious infections, follow up with an infectious disease expert. Get a second opinion if conditions do not rapidly improve after treatment has begun.
5. **Practice antimicrobial control.** Be aware and current in knowledge of appropriate antimicrobial drugs and their use. Be sure the treatment offered is current and recommended for the pathogen.
6. **Use local data.** Obtain and understand the antibiotic susceptibility profile for the infectious agent from local healthcare sources.
7. **Treat infection, not contamination.** Antiseptic techniques must be followed to obtain appropriate samples from infected tissues. Contaminating organisms may be present on skin, catheters, or IV lines. Obtain cultures only from the site of infection.
8. **Treat infection, not colonization.** Treat the pathogen and not other colonizing microorganisms that are not causing disease. For example, cultures from normal skin and throat are often colonized with potential pathogens such as *Staphylococcus* species. These may have nothing to do with the current infection.
9. **Treat with the least exotic antimicrobial agent that will eliminate the pathogen.** Treatment with the latest broad-spectrum antibiotic, while efficacious, may not be warranted if other drugs are still effective. The more a drug is used, the greater the chance that resistant organisms will develop. For example, some *Enterococcus* isolates are already resistant to vancomycin, a relatively new broad-spectrum antibiotic, largely because the drug was over-prescribed to treat MRSA infections when it was first introduced.
10. **Monitor antimicrobial use.** Antimicrobial use should be discontinued as soon as the prescribed course of treatment is completed. If an infection cannot be diagnosed, treatment should be discontinued. For example, in the case of pharyngitis (sore throat), antibiotic treatment for *Streptococcus pyogenes* (strep throat) is often started before throat culture results are confirmed. If throat cultures are negative for *S. pyogenes*, treatment with antibiotics should be stopped. Antibiotics are ineffective for treatment of the viruses that are the most probable causes of pharyngitis.
11. **Isolate the pathogen.** Keep areas around infected persons free from contamination. Clean up and contain body fluids appropriately. Decontaminate linens, clothes, and other potential sources of contamination. In a hospital setting, infection control experts should be consulted.
12. **Break the chain of contagion.** Avoid exposing others by staying home from work or school when you are sick. Maintain cleanliness, especially by handwashing (Figure 2), if you are sick or are caring for sick persons.

CDC/Kimberly Smith

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.*

III Antimicrobial Agents Used *In Vivo*

Up to this point, we have considered the effects of physical and chemical agents used to inhibit microbial growth outside the human body. Most of the physical methods are too harsh and most chemicals mentioned are too toxic to be used inside the body; even relatively mild antiseptics can be used only on the skin. For control of infectious disease, chemical compounds that can be used internally are required. Discovery and development of antimicrobial drugs has played a major role in clinical and veterinary medicine, as well as in agriculture.

Antimicrobial drugs are classified based on their molecular structure, mechanism of action (**Figure 26.12**), and spectrum of antimicrobial activity (**Figure 26.13**). Worldwide, probably more than 10,000 metric tons of various antimicrobial drugs are manufactured and used annually (**Figure 26.14**). Antimicrobial agents fall into two broad categories, *synthetic agents* and *antibiotics*. We first concentrate on synthetic antimicrobial compounds. We then discuss naturally produced antibiotics.

26.6 Synthetic Antimicrobial Drugs

Systematic work on antimicrobial drugs was first initiated by the German scientist Paul Ehrlich. In the early 1900s, Ehrlich developed the concept of **selective toxicity**, the ability of a chemical

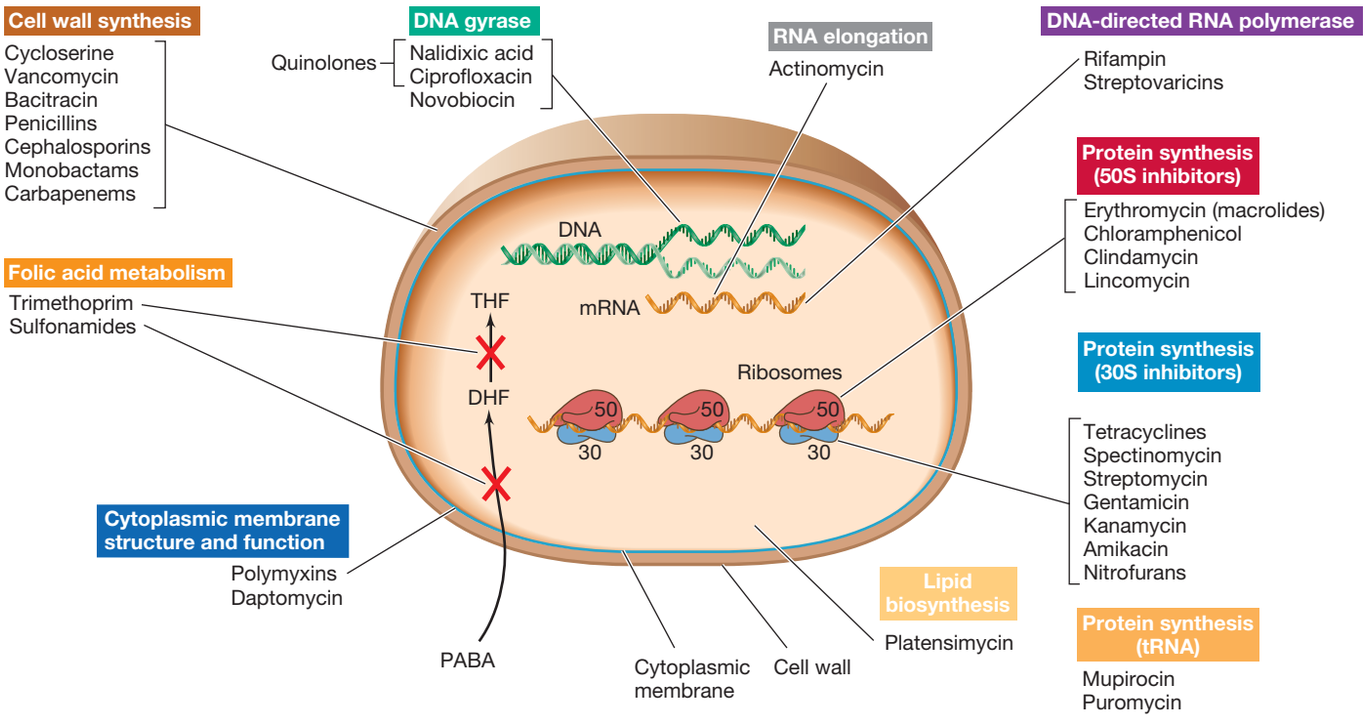


Figure 26.12 **Mode of action of some major antimicrobial agents.** Agents are classified according to their target structures in the bacterial cell. THF, tetrahydrofolate; DHF, dihydrofolate; mRNA, messenger RNA.

agent to inhibit or kill pathogenic microorganisms without adversely affecting the host. In his search for a "magic bullet" that would kill only pathogens, Ehrlich tested large numbers of chemical dyes for selective toxicity and discovered the first effective antimicrobial drugs, of which Salvarsan, an arsenic-containing compound used for the treatment of syphilis, was the most successful (**Figure 26.15**).

Growth Factor Analogs

We previously defined growth factors as specific chemical substances required in the medium because the organisms cannot synthesize them (↻ Section 4.1). A **growth factor analog** is a synthetic compound that is structurally similar to a growth factor, but subtle structural differences between the analog and

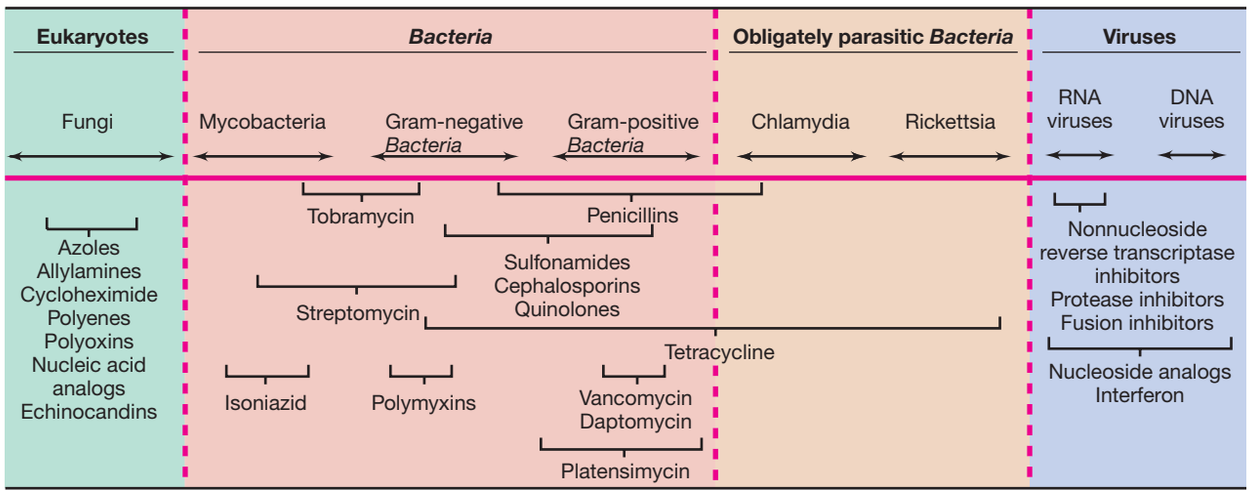


Figure 26.13 **Antimicrobial spectrum of activity.** Each antimicrobial agent affects a limited and well-defined group of microorganisms. A few agents are very specific and affect the growth of only a single genus. For example, isoniazid affects only organisms in the genus *Mycobacterium*.

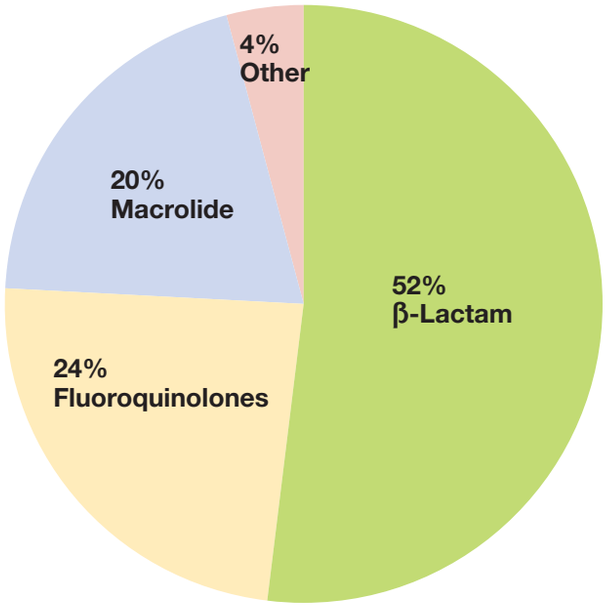


Figure 26.14 **Annual worldwide production and use of antibiotics.** Each year an estimated 10,000 metric tons of antimicrobial agents are manufactured worldwide. The β-lactam antibiotics include cephalosporins (30%), penicillins (7%), and other β-lactams (15%). "Others" includes tetracyclines, aminoglycosides, and all other antimicrobial drugs.

the authentic growth factor prevent the analog from functioning in the cell, disrupting cell metabolism. Analogs are known for many important biomolecules, including vitamins, amino acids, purines and pyrimidines, and other compounds. We begin by considering antibacterial growth factor analogs. Growth factor analogs effective for the treatment of viral and fungal infections will be discussed in Sections 26.10 and 26.11.

Sulfa Drugs

Sulfa drugs, discovered by Gerhard Domagk in the 1930s, were the first widely used growth factor analogs that specifically inhibited the growth of bacteria. The discovery of the first sulfa drug

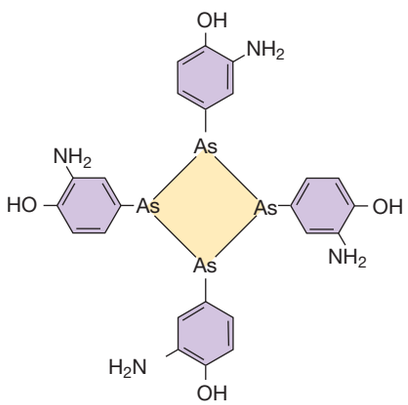


Figure 26.15 **Salvarsan.** This arsenic-containing compound was one of the first useful antimicrobial agents. It was used to treat syphilis.

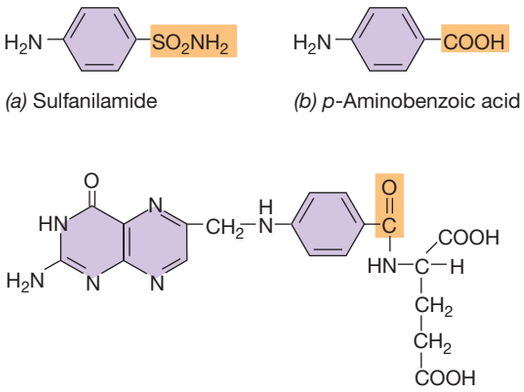


Figure 26.16 **Sulfa drugs.** *(a)* The simplest sulfa drug, sulfanilamide. *(b)* Sulfanilamide is an analog of *p*-aminobenzoic acid, a precursor of *(c)* folic acid, a growth factor.

resulted from the large-scale screening of chemicals for activity against streptococcal infections in experimental animals.

Sulfanilamide, the simplest sulfa drug, is an analog of *p*-aminobenzoic acid, which is itself a part of the vitamin folic acid, a nucleic acid precursor (**Figure 26.16**). Sulfanilamide blocks the synthesis of folic acid, thereby inhibiting nucleic acid synthesis. Sulfanilamide is selectively toxic in bacteria because bacteria synthesize their own folic acid, whereas most animals obtain folic acid from their diet. Initially, sulfa drugs were widely used for treatment of streptococcal infections (↻ Section 33.2). However, resistance to sulfonamides has been increasing because many formerly susceptible pathogens have developed an ability to take up folic acid from their environment. Antimicrobial therapy with sulfamethoxazole (a sulfa drug) plus trimethoprim, a related folic acid synthesis competitor, is still effective in many instances because the drug combination produces sequential blocking of the folic acid synthesis pathway. Resistance to this drug combination requires that two mutations in genes of the same pathway occur, a relatively rare event.

Isoniazid

Isoniazid (↻ Figure 33.11) is an important growth factor analog with a very narrow spectrum of activity (Figure 26.13). Effective only against *Mycobacterium*, isoniazid interferes with the synthesis of mycolic acid, a mycobacterial cell wall component. A nicotinamide (vitamin) analog, isoniazid is the most effective single drug used for control and treatment of tuberculosis (↻ Section 33.4).

Nucleic Acid Base Analogs

Analogs of nucleic acid bases formed by addition of a fluorine or bromine atom are shown in **Figure 26.17**. Fluorine is a relatively small atom and does not alter the overall shape of the nucleic acid base, but changes the chemical properties such that the compound does not function in cell metabolism, thereby blocking nucleic acid synthesis. Examples include fluorouracil, an analog of uracil, and bromouracil, an analog of thymine. Growth

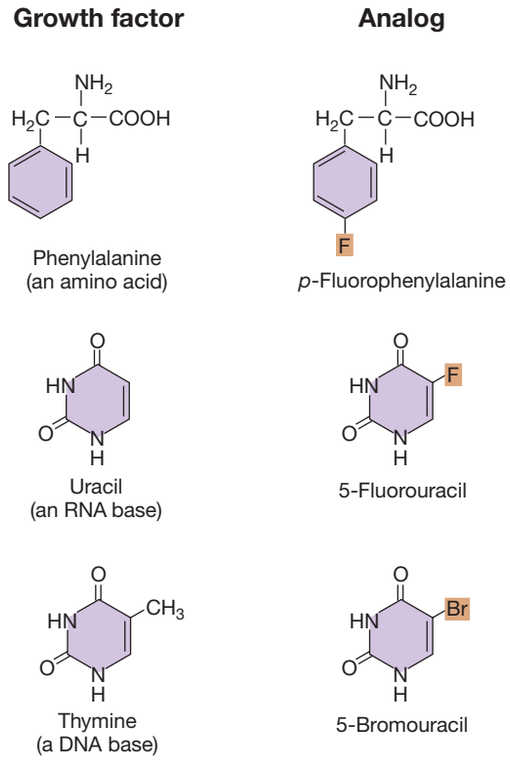


Figure 26.17 Growth factors and antimicrobial analogs. Structurally similar growth factors and their biologically active analogs are shown for comparison. The nutritional aspects of growth factors are discussed in Section 4.1(↻ Table 4.2).

factor analogs of nucleic acids are used in the treatment of viral and fungal infections and are also used as mutagens (Sections 26.10 and 26.11).

Quinolones

The **quinolones** are antibacterial compounds that interfere with bacterial DNA gyrase, preventing the supercoiling of DNA, a required step for packaging DNA in the bacterial cell (Figure 26.12; ↻ Section 6.3). Because DNA gyrase is found in all *Bacteria*, the fluoroquinolones are effective for treating both gram-positive and gram-negative bacterial infections (Figure 26.13). Fluoroquinolones such as ciprofloxacin (**Figure 26.18*a***) are routinely used to treat urinary tract infections in humans. Ciprofloxacin is also the drug of choice for treating anthrax because some strains of *Bacillus anthracis*, the causative agent of anthrax (↻ Section 32.12), are resistant to penicillin. Moxifloxacin, a new fluoroquinolone, has been approved for treatment of tuberculosis, one of the few new drugs proven effective against *Mycobacterium tuberculosis* infections (**Figure 26.18*b***). This drug, in combination with other anti-tuberculosis drugs (↻ Section 33.4), may significantly shorten the time necessary for treatment. Fluoroquinolones have also been widely used in the beef and poultry industries for prevention and treatment of respiratory diseases in animals.

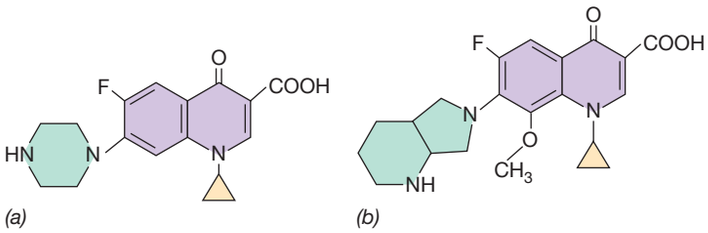


Figure 26.18 Quinolones. *(a)* Ciprofloxacin, a fluorinated derivative of nalidixic acid with broad-spectrum activity, is more soluble than the parent compound, allowing it to reach therapeutic levels in blood and tissues. *(b)* Moxifloxacin, a new fluoroquinolone approved for treatment of *Mycobacterium* infections.

MiniQuiz

- Explain selective toxicity in terms of antibiotic therapy.
- Distinguish the use of synthetic antimicrobial agents from antiseptics and disinfectants.
- Describe the action of any one of the synthetic antimicrobial drugs.

26.7 Naturally Occurring Antimicrobial Drugs: Antibiotics

Antibiotics are antimicrobial agents produced by microorganisms. Antibiotics are produced by a variety of bacteria and fungi and apparently have the sole function of inhibiting or killing other microorganisms. Although thousands of antibiotics are known, less than 1% are clinically useful, often because of host toxicity or lack of uptake by host cells. However, the clinically useful antibiotics have had a dramatic impact on the treatment of infectious diseases. *Natural* antibiotics can often be artificially modified to enhance their efficacy. These are said to be *semisynthetic* antibiotics. The isolation, characterization, and industrial production of antibiotics were discussed in Sections 15.3 and 15.4.

Antibiotics and Selective Antimicrobial Toxicity

The susceptibility of individual microorganisms to individual antimicrobial agents varies significantly (Figure 26.13). For example, gram-positive *Bacteria* and gram-negative *Bacteria* differ in their susceptibility to an individual antibiotic such as penicillin; gram-positive *Bacteria* are generally affected, whereas most gram-negative *Bacteria* are naturally resistant. Certain **broad-spectrum antibiotics** such as tetracycline, however, are effective against both groups. As a result, a broad-spectrum antibiotic finds wider medical use than a narrow-spectrum antibiotic. An antibiotic with a limited spectrum of activity may, however, be quite valuable for the control of pathogens that fail to respond to other antibiotics. A good example is vancomycin, a narrow-spectrum glycopeptide antibiotic that is a highly effective bacteriocidal agent for gram-positive, penicillin-resistant *Bacteria* from the genera *Staphylococcus, Bacillus*, and *Clostridium* (Figures 26.12 and 26.13).

Important targets of antibiotics in *Bacteria* are ribosomes, the cell wall, the cytoplasmic membrane, lipid biosynthesis enzymes, and DNA replication and transcription elements (Figure 26.12).

Antibiotics Affecting Protein Synthesis

Many antibiotics inhibit protein synthesis by interacting with the ribosome and disrupting translation (Figure 26.12). These interactions are quite specific and many involve binding to ribosomal RNA (rRNA). Several of these antibiotics are medically useful, and several are also effective research tools because they block defined steps in protein synthesis (↻ Section 6.19). For instance, streptomycin inhibits protein chain initiation, whereas puromycin, chloramphenicol, cycloheximide, and tetracycline inhibit protein chain elongation.

Even when two antibiotics inhibit the same step in protein synthesis, the mechanisms of inhibition can be quite different. For example, puromycin binds to the A site on the ribosome, and the growing polypeptide chain is transferred to puromycin instead of the aminoacyl–transfer RNA (aminoacyl-tRNA) complex. The puromycin–peptide complex is then released from the ribosome, prematurely halting elongation. By contrast, chloramphenicol inhibits elongation by blocking formation of the peptide bond (↻ Section 6.19).

Many antibiotics specifically inhibit ribosomes of organisms from only one phylogenetic domain. For example, chloramphenicol and streptomycin specifically target the ribosomes of *Bacteria*, whereas cycloheximide only affects the cytoplasmic ribosomes of *Eukarya*. Since the major organelles (mitochondria and chloroplasts) in *Eukarya* also have ribosomes that are similar to those of *Bacteria* (that is, 70S ribosomes), antibiotics that inhibit protein synthesis in *Bacteria* also inhibit protein synthesis in these organelles. For example, tetracycline antibiotics inhibit 70S ribosomes, but are still medically useful because eukaryotic mitochondria are affected only at higher concentrations than are used for antimicrobial therapy.

Antibiotics Affecting Transcription

A number of antibiotics specifically inhibit transcription by inhibiting RNA synthesis (Figure 26.12). For example, rifampin and the streptovaricins inhibit RNA synthesis by binding to the β-subunit of RNA polymerase. These antibiotics have specificity for *Bacteria*, chloroplasts, and mitochondria. Actinomycin inhibits RNA synthesis by combining with DNA and blocking RNA elongation. This agent binds most strongly to DNA at guanine–cytosine base pairs, fitting into the major groove in the double strand where RNA is synthesized.

Some of the most useful antibiotics are directed against unique structural features of *Bacteria*, such as their cell walls. We discuss these antibiotics and their targets in the next section.

MiniQuiz

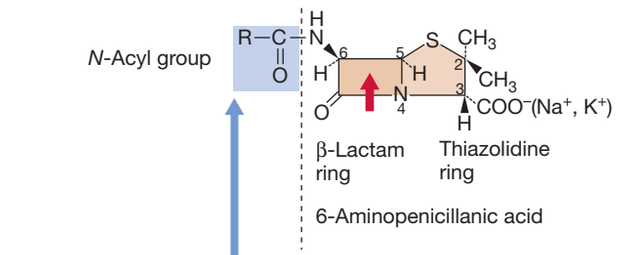
- Distinguish antibiotics from growth factor analogs.
- What is a broad-spectrum antibiotic?
- Identify the potential target sites for the antibiotics that inhibit protein synthesis and transcription.

26.8 β-Lactam Antibiotics: Penicillins and Cephalosporins

One of the most important groups of antibiotics, both historically and medically, is the β-lactam group. **β-lactam antibiotics** include the medically important penicillins, cephalosporins, and cephamycins. These antibiotics share a characteristic structural component, the *β-lactam ring* (**Figure 26.19**). Together, the β-lactam antibiotics account for over one-half of all of the antibiotics produced and used worldwide (Figure 26.14).

Penicillins

In 1929, the British scientist Alexander Fleming characterized the first antibiotic, an antibacterial compound called *penicillin* because it was isolated from the fungus *Penicillium chrysogenum*



N-Acyl group	Designation
$-CH_2-CO-$	**NATURAL PENICILLIN** Benzylpenicillin (penicillin G) Gram-positive activity β-lactamase-sensitive
	SEMISYNTHETIC PENICILLINS
OCH_3, $-CO-$, OCH_3	Methicillin acid-stable, β-lactamase-resistant
$-CO-$ (N, O, CH_3)	Oxacillin acid-stable, β-lactamase-resistant
$-CH(NH_2)-CO-$	Ampicillin broadened spectrum of activity (especially against gram-negative *Bacteria*), acid-stable, β-lactamase-sensitive
$-CH(COOH)-CO-$	Carbenicillin broadened spectrum of activity (especially against *Pseudomonas aeruginosa*), acid-stable but ineffective orally, β-lactamase-sensitive

Figure 26.19 Penicillins. The red arrow (top panel) is the site of activity of most β-lactamase enzymes.

UNIT 8

(Figure 26.19). The antibiotic, however, was not immediately recognized as a potentially important clinical drug. Even though sulfa drugs were widely available in the 1930s, their efficacy was mostly limited to the treatment of infections by gram-positive organisms such as *Streptococcus*; most other bacterial diseases were uncontrollable. However, in 1939, Howard Florey and his colleagues, motivated by the impending world war, developed a process for the large-scale production of **penicillin**. Penicillin G was the first clinically useful antibiotic. This new β-lactam antibiotic was dramatically effective in controlling staphylococcal and pneumococcal infections among military personnel and was more effective for treating streptococcal infections than sulfa drugs. By the end of World War II in 1945, penicillin became available for general use and pharmaceutical companies began to look for and develop other antibiotics, leading to drugs that revolutionized the treatment of infectious diseases.

Penicillin G is active primarily against gram-positive *Bacteria* because gram-negative *Bacteria* are impermeable to the antibiotic. Chemical modification of the penicillin G structure, however, significantly changes the properties of the resulting antibiotic. Many chemically modified semisynthetic penicillins are quite effective against gram-negative *Bacteria*. Figure 26.19 shows the structures of some of the penicillins. For example, ampicillin and carbenicillin, semisynthetic penicillins, are effective against some gram-negative *Bacteria*. The structural differences in the *N*-acyl groups of these semisynthetic penicillins allow them to be transported inside the gram-negative outer membrane (↻ Section 3.7), where they inhibit cell wall synthesis. Penicillin G is also sensitive to β-lactamase, an enzyme produced by a number of penicillin-resistant *Bacteria* (Section 26.12). Oxacillin and methicillin are widely used β-lactamase-resistant semisynthetic penicillins.

Mechanism of Action

The β-lactam antibiotics are inhibitors of cell wall synthesis. An important feature of bacterial cell wall synthesis is *transpeptidation*, the reaction that results in the cross-linking of two glycan-linked peptide chains (↻ Section 5.4 and Figure 5.7). The transpeptidase enzymes bind to penicillin or other β-lactam antibiotics. Thus, these transpeptidases are called *penicillin-binding proteins* (PBPs). When PBPs bind penicillin, they cannot catalyze the transpeptidase reaction, but cell wall synthesis continues. As a result, the newly synthesized bacterial cell wall is no longer cross-linked and cannot maintain its strength. In addition, the antibiotic–PBP complex stimulates the release of autolysins, enzymes that digest the existing cell wall. The result is a weakened, self-degrading cell wall. Eventually the osmotic pressure differences between the inside and outside of the cell cause lysis. By contrast, vancomycin, also a cell wall synthesis inhibitor, does not bind to PBPs, but binds directly to the terminal D-alanyl-D-alanine peptide on the peptidoglycan precursors (↻ Figure 5.7); this effectively blocks transpeptidation.

Because the cell wall and its synthesis mechanisms are unique to *Bacteria*, the β-lactam antibiotics are highly selective and are not toxic to host cells. However, some individuals develop allergies to β-lactam antibiotics after repeated courses of antibiotic therapy.

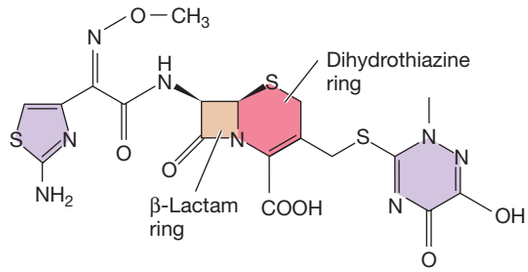


Figure 26.20 Ceftriaxone. Ceftriaxone is a β-lactam antibiotic that is resistant to most β-lactamases due to the adjacent six-member dihydrothiazine ring. Compare this structure to the five-member thiazolidine ring of the β-lactamase-sensitive penicillins (Figure 26.19).

Cephalosporins

The cephalosporins are another group of clinically important β-lactam antibiotics. Cephalosporins, produced by the fungus *Cephalosporium* sp., differ structurally from the penicillins. They retain the β-lactam ring but have a six-member dihydrothiazine ring instead of the five-member thiazolidine ring. The cephalosporins have the same mode of action as the penicillins; they bind irreversibly to PBPs and prevent the cross-linking of peptidoglycan. Clinically important cephalosporins are semisynthetic antibiotics with a broader spectrum of antibiotic activity than the penicillins. In addition, cephalosporins are typically more resistant to the enzymes that destroy β-lactam rings, the β-lactamases. For example, ceftriaxone (**Figure 26.20**) is highly resistant to β-lactamases and has replaced penicillin for treatment of *Neisseria gonorrhoeae* (gonorrhea) infections because many *N. gonorrhoeae* strains are now resistant to penicillin (Section 26.12, ↻ Section 33.12).

MiniQuiz

- Draw the structure of the β-lactam ring and indicate the site of β-lactamase activity.
- How do the β-lactam antibiotics function?
- Of what clinical value are semisynthetic penicillins over natural penicillin?

26.9 Antibiotics from Prokaryotes

Many antibiotics active against *Bacteria* are also produced by *Bacteria*. These include many antibiotics that have major clinical applications, and we discuss their general properties here.

Aminoglycosides

Antibiotics that contain amino sugars bonded by glycosidic linkage are called **aminoglycosides**. Clinically useful aminoglycosides include streptomycin (produced by *Streptomyces griseus*) and its relatives, kanamycin (**Figure 26.21**), neomycin, gentamicin, tobramycin, netilmicin, spectinomycin, and amikacin. The aminoglycosides target the 30S subunit of the ribosome, inhibiting protein synthesis (Figure 26.12), and are clinically useful against gram-negative *Bacteria* (Figure 26.13).

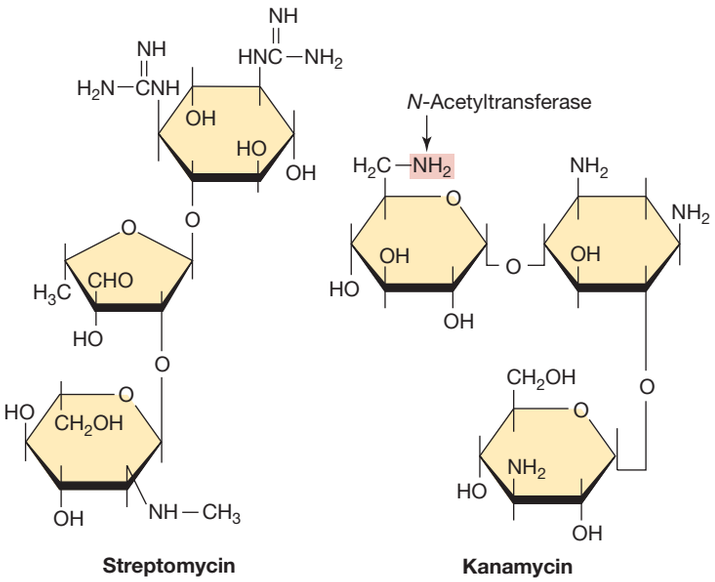


Figure 26.21 **Aminoglycoside antibiotics: streptomycin and kanamycin.** The amino sugars are in yellow. At the position indicated, kanamycin can be modified by a resistance plasmid that encodes *N*-acetyltransferase. Following acetylation, the antibiotic is inactive. Both kanamycin and streptomycin are synthesized by *Streptomyces* species.

Streptomycin was the first effective antibiotic used for the treatment of tuberculosis. The aminoglycoside antibiotics, however, are not widely used today, and together the aminoglycosides account for less than 4% of the total of all antibiotics produced and used. Because of serious side effects such as neurotoxicity and nephrotoxicity (kidney toxicity), streptomycin has been replaced by several synthetic antimicrobials for tuberculosis treatment. Bacterial resistance to aminoglycosides also develops readily. The use of aminoglycosides for treatment of gram-negative infections has decreased since the development of the semisynthetic penicillins (Section 26.8) and the tetracyclines (discussed later in this section). Aminoglycoside antibiotics are now considered reserve antibiotics used primarily when other antibiotics fail.

Macrolides

Macrolide antibiotics contain lactone rings bonded to sugars (**Figure 26.22**). Variations in both the lactone ring and the sugars result in a large number of macrolide antibiotics. The best-known macrolide is erythromycin (produced by *Streptomyces erythreus*). Other clinically useful macrolides include dirithromycin, clarithromycin, and azithromycin. The macrolides account for about 20% of the total world production and use of antibiotics (Figure 26.14). Erythromycin is a broad-spectrum antibiotic that targets the 50S subunit of the bacterial ribosome, inhibiting protein synthesis (Figure 26.12). Often used clinically in place of penicillin in patients allergic to penicillin or other β-lactam antibiotics, erythromycin is particularly useful for treating legionellosis (Section 35.7).

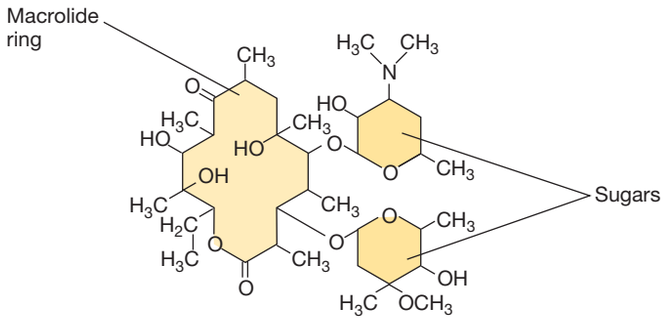
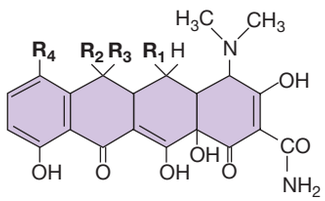


Figure 26.22 **Erythromycin, a macrolide antibiotic.** Erythromycin is a widely used broad-spectrum antibiotic.

Tetracyclines

The **tetracyclines**, produced by several species of *Streptomyces*, are an important group of antibiotics that find widespread medical use in humans (Section 15.4). They were some of the first broad-spectrum antibiotics, inhibiting almost all gram-positive and gram-negative *Bacteria*. The basic structure of the tetracyclines consists of a naphthacene ring system (**Figure 26.23**). Substitutions to the basic naphthacene ring occur naturally and form new tetracycline analogs. Semisynthetic tetracyclines having substitutions in the naphthacene ring system have also been developed. Like erythromycin and the aminoglycoside antibiotics, tetracycline is a protein synthesis inhibitor, interfering with bacterial 30S ribosome subunit function (Figure 26.12).

The tetracyclines and the β-lactam antibiotics comprise the two most important groups of antibiotics in the medical field. The tetracyclines are also widely used in veterinary medicine and in some countries are used as nutritional supplements for poultry and swine. Because extensive nonmedical uses of medically important antibiotics have contributed to widespread antibiotic resistance, this use is now discouraged.



Tetracycline analog	R_1	R_2	R_3	R_4
Tetracycline	H	OH	CH_3	H
7-Chlortetracycline (aureomycin)	H	OH	CH_3	Cl
5-Oxytetracycline (terramycin)	OH	OH	CH_3	H

Figure 26.23 **Tetracycline.** The structure of tetracycline and its semisynthetic analogs.

Daptomycin

Daptomycin is another antibiotic produced by a member of the *Streptomyces* genus. This novel antibiotic is a cyclic lipopeptide (**Figure 26.24**) with a unique mode of action. Used mainly to treat infections by gram-positive *Bacteria* such as the pathogenic staphylococci and streptococci, daptomycin binds specifically to bacterial cytoplasmic membranes, forms a pore, and induces rapid depolarization of the membrane. The depolarized cell quickly loses its ability to synthesize macromolecules such as nucleic acids and proteins, resulting in cell death. Alterations in cytoplasmic membrane structure may account for rare instances of resistance.

Platensimycin

Platensimycin is the first member of a new structural class of antibiotics. Produced by *Streptomyces platensis*, this antibiotic (**Figure 26.25**) selectively inhibits a bacterial enzyme central to fatty acid biosynthesis, thus disrupting lipid biosynthesis. Platensimycin is effective against a broad range of gram-positive *Bacteria*, including nearly untreatable infections caused by methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. Already shown to be effective in eradicating *S. aureus* infections in mice, this antibiotic shows no toxicity. Platensimycin has a unique mode of action, and there is no known potential for development of resistance by pathogens. We discuss the discovery of platensimycin in Section 26.13.

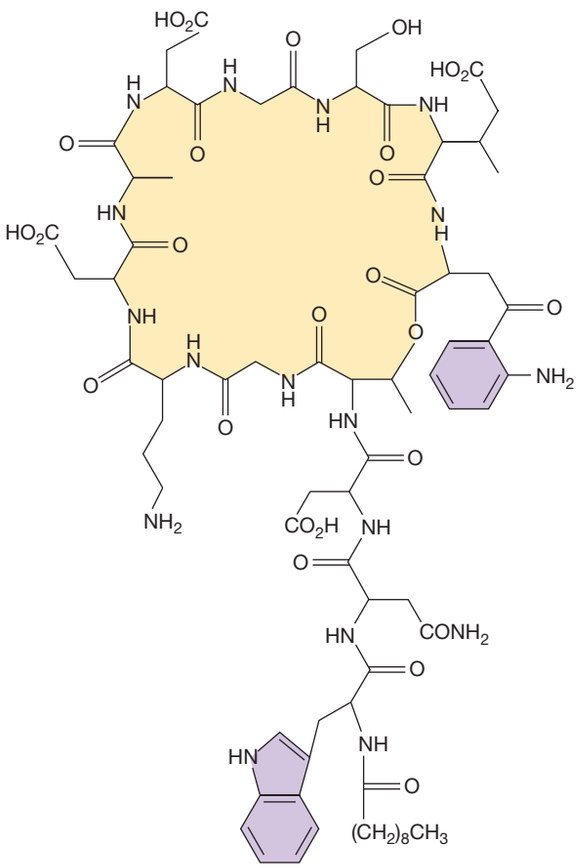


Figure 26.24 **Daptomycin.** Daptomycin is a cyclic lipopeptide that depolarizes cytoplasmic membranes in gram-positive *Bacteria*.

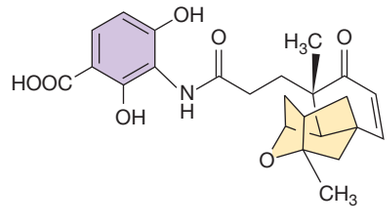


Figure 26.25 **Platensimycin.** Platensimycin selectively inhibits lipid biosynthesis in *Bacteria*.

MiniQuiz

- What are the biological sources of aminoglycosides, tetracyclines, macrolides, daptomycin, and platensimycin?
- How does the activity of each antibiotic class lead to death of the affected cell?

IV Control of Viruses and Eukaryotic Pathogens

Drugs that control growth of viruses and eukaryotic pathogens such as fungi and parasites are available, but they often affect eukaryotic host cells as well. As a result, selective toxicity for eukaryotic pathogens is very difficult to attain; only agents that preferentially affect pathogen-specific metabolic pathways or structural components are useful. There are a limited number of these drugs, and we discuss some important ones that affect viruses and fungi here. Drugs specific for treatment of parasitic diseases are discussed with the diseases themselves because they are extremely specific for individual parasites.

26.10 Antiviral Drugs

Because viruses use their eukaryotic hosts to reproduce and perform metabolic functions, most antiviral drugs also target host structures, resulting in host toxicity. However, several compounds are more toxic for viruses than for the host, and a few agents specifically target viruses. Largely because of efforts to find effective measures to control infections with the human immunodeficiency virus (HIV), the cause of AIDS (Section 33.14), significant achievements have been made in the development and use of antiviral agents.

Antiviral Agents

The most successful and commonly used agents for antiviral chemotherapy are the nucleoside analogs (**Table 26.6**). The first compound to gain universal acceptance in this category was zidovudine, or azidothymidine (AZT) (Figure 33.43). AZT

Table 26.6 *Antiviral compounds*

Category/drug	*Mechanism of action*	*Virus affected*
Fusion inhibitor		
Enfuvirtide	Blocks HIV–T lymphocyte membrane fusion	HIV[a]
Interferons		
α-Interferon	Induces proteins that inhibit viral replication	Broad spectrum (host-specific)
β-Interferon		
γ-Interferon		
Neuraminidase inhibitors		
Oseltamivir (Tamiflu®)	Block active site of influenza neuraminidase	Influenza A and B
Zanamivir (Relenza®)		Influenza A and B
Nonnucleoside reverse transcriptase inhibitor (NNRTI)		
Nevirapine	Reverse transcriptase inhibitor	HIV
Nucleoside analogs		
Acyclovir	Viral polymerase inhibitors	Herpes viruses, *Varicella zoster*
Ganciclovir		Cytomegalovirus
Trifluridine		Herpesvirus
Valacyclovir		Herpesvirus
Vidarabine		Herpesvirus, vaccinia, hepatitis B virus
Abacavir (ABC)	Reverse transcriptase inhibitors	HIV
Didanosine (dideoxyinosine or ddI)		HIV
Emtricitabine (FTC)		HIV
Lamivudine (3TC)		HIV, hepatitis B virus
Stavudine (d4T)		HIV
Zalcitabine (ddC)		HIV
Zidovudine (AZT) (↻ Figure 33.43)		HIV
Ribavirin	Blocks capping of viral RNA	Respiratory syncytial virus, influenza A and B, Lassa fever
Nucleotide analogs		
Cidofovir	Viral polymerase inhibitor	Cytomegalovirus, herpesviruses
Tenofovir (TDF)	Reverse transcriptase inhibitor	HIV
Protease inhibitors		
Amprenavir	Viral protease inhibitor	HIV
Indinavir (Figure 26.31)		HIV
Lopinavir		HIV
Nelfinavir		HIV
Saquinavir (Figure 26.31)		HIV
Pyrophosphate analog		
Phosphonoformic acid (foscarnet)	Viral polymerase inhibitor	Herpesviruses, HIV, hepatitis B virus
RNA polymerase inhibitor		
Rifamycin	RNA polymerase inhibitor	Vaccinia, pox viruses
Synthetic amines		
Amantadine	Viral uncoating blocker	Influenza A
Rimantadine		Influenza A

[a]Human immunodeficiency virus

inhibits retroviruses such as HIV (↻ Sections 33.14 and 9.12). Azidothymidine is chemically related to thymidine but is a dideoxy derivative, lacking the 3′-hydroxyl group. AZT inhibits multiplication of retroviruses by blocking reverse transcription and production of the virally encoded DNA intermediate. This inhibits multiplication of HIV. A number of other nucleoside analogs having analogous mechanisms have been developed for the treatment of HIV and other viruses.

Nearly all nucleoside analogs, or **nucleoside reverse transcriptase inhibitors (NRTI)**, work by the same mechanism, inhibiting elongation of the viral nucleic acid chain by a nucleic acid polymerase. The nucleotide analog cidofovir works in the same way (Table 26.6). Because the normal cell function of nucleic acid replication is targeted, these drugs usually induce some host toxicity. Many NRTIs also lose their antiviral potency with time due to the emergence of drug-resistant viruses (↻ Section 33.14).

Several other antiviral agents target the key enzyme of retroviruses, reverse transcriptase. Nevirapine, a **nonnucleoside reverse transcriptase inhibitor (NNRTI)**, binds directly to reverse transcriptase and inhibits reverse transcription. Phosphonoformic acid, an analog of inorganic pyrophosphate, inhibits normal internucleotide linkages, preventing synthesis of viral nucleic acids. As with the NRTIs, the NNRTIs generally induce some level of host toxicity because their action also affects normal host cell nucleic acid synthesis.

Protease inhibitors are another class of antiviral drugs that are effective for treatment of HIV (Table 26.6 and see Figure 26.31). These drugs prevent viral replication by binding the active site of HIV protease, inhibiting this enzyme from processing large viral proteins into individual viral components, thus preventing virus maturation (↻ Sections 21.11, 26.13, and 33.14).

A final category of anti-HIV drugs is represented by a single drug, enfuvirtide, a **fusion inhibitor** composed of a 36-amino acid synthetic peptide that binds to the gp41 membrane protein of HIV (Table 26.6 and ↻ Section 33.14). Binding of the gp41 protein by enfuvirtide stops the conformational changes necessary for the fusion of HIV and T lymphocyte membranes, thus preventing infection of cells by HIV.

Influenza Antiviral Agents

Two categories of drugs effectively limit influenza infection. The adamantanes amantadine and rimantadine are synthetic amines that interfere with an influenza A ion transport protein, inhibiting virus uncoating and subsequent replication. The neuraminidase inhibitors oseltamivir (brand name Tamiflu) and zanamivir (Relenza) block the active site of neuraminidase in influenza A and B viruses, inhibiting virus release from infected cells. Zanamivir is used only for treatment of influenza, whereas oseltamivir is used for both treatment and prophylaxis. The adamantanes are less useful than the neuraminidase inhibitors because resistance to adamantanes develops rapidly in strains of influenza virus (↻ Section 33.8).

Interferons

Virus interference is a phenomenon in which infection with one virus interferes with subsequent infection by another virus. Several small proteins are the cause of interference; the proteins are called interferons. **Interferons** are small proteins in the cytokine family (↻ Section 30.10) that prevent viral replication by stimulating the production of antiviral proteins in uninfected cells. Interferons are formed in response to live virus, inactivated virus, and viral nucleic acids. Interferon is produced in large amounts by cells infected with viruses of low virulence, but little is produced against highly virulent viruses. Highly virulent viruses inhibit cell protein synthesis before interferon can be produced. Interferons are also induced by natural and synthetic double-stranded RNA (dsRNA) molecules. In nature, dsRNA exists only in virus-infected cells as the replicative form of RNA viruses such as rhinoviruses (cold viruses) (↻ Section 33.7); the dsRNA from the infecting virus signals the animal cell to produce interferon.

Interferons from virus-infected cells interact with receptors on uninfected cells, promoting the synthesis of antiviral proteins that function to prevent further virus infection. Interferons are produced in three molecular forms: *IFN-*α is produced by leukocytes, *IFN-*β is produced by fibroblasts, and *IFN-*γ is produced by immune lymphocytes.

Interferon activity is *host*-specific rather than *virus*-specific. That is, interferon produced by a member of one species can only activate receptors on cells from the same species. As a result, interferon produced by cells of an animal in response to, for example, a rhinovirus, could also inhibit multiplication of, for example, influenza viruses in cells within the same species, but has no effect on the multiplication of any virus in cells from other animal species.

Interferons produced *in vitro* have potential as possible antiviral and anticancer agents. Several approved recombinant interferons are available. However, the use of interferons as antiviral agents is not widespread because interferon must be delivered locally in high concentrations to stimulate the production of antiviral proteins in uninfected host cells. Thus, the clinical utility of these antiviral agents depends on our ability to deliver interferon to local areas in the host through injections or aerosols. Alternatively, appropriate interferon-stimulating signals such as viral nucleotides, nonvirulent viruses, or even synthetic nucleotides, if given to host cells prior to viral infection, might stimulate natural production of interferon.

MiniQuiz

- Why are there relatively few effective antiviral agents? Such agents are not used to treat common viral illnesses such as colds; why not?
- What steps in the viral maturation process are inhibited by nucleoside analogs? By protease inhibitors? By interferons?

26.11 Antifungal Drugs

Fungi, like viruses, pose special problems for the development of chemotherapy. Because fungi are *Eukarya*, much of their cellular machinery is the same as that of animals and humans; antifungal agents that act on metabolic pathways in fungi often affect corresponding pathways in host cells, making the drugs toxic. As a result, many antifungal drugs can be used only for topical (surface) applications. However, a few drugs are selectively toxic for

fungi because they target unique fungal structures or metabolic processes. Fungus-specific drugs are becoming increasingly important as fungus infections in immunocompromised individuals become more prevalent (↻ Sections 33.14 and 34.8). We examine here the selective action and targets of several effective antifungal agents.

Ergosterol Inhibitors

Ergosterol in fungal cytoplasmic membranes replaces the cholesterol found in animal cytoplasmic membranes. Two types of antifungal compounds work by interacting with ergosterol or inhibiting its synthesis (**Table 26.7**). These include the polyenes, a group of antibiotics produced by species of *Streptomyces*. Polyenes bind to ergosterol, disrupting membrane function, causing membrane permeability and cell death (**Figure 26.26**). A second major type of antifungal compound includes the azoles and allylamines, synthetic agents that selectively inhibit ergosterol biosynthesis and therefore have broad antifungal activity. Treatment with azoles results in abnormal fungus cytoplasmic membranes, leading to membrane damage and alteration of critical membrane transport activities. Allylamines also inhibit ergosterol biosynthesis, but are restricted to topical use because they are not readily taken up by animal tissues.

Echinocandins

Echinocandins act by inhibiting 1,3-β-D-glucan synthase, the enzyme that forms glucan polymers in the fungal cell wall (Figure 26.26 and Table 26.7). Because mammalian cells do not have 1,3-β-D-glucan synthase (or cell walls), the action of these

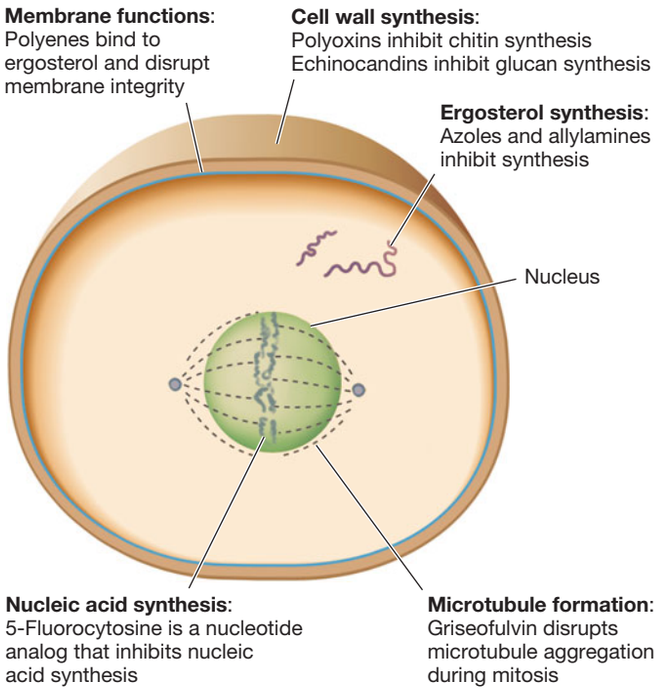


Figure 26.26 Action of some antifungal agents. Traditional antibacterial agents are generally ineffective because fungi are eukaryotic cells. The cytoplasmic membrane and cell wall targets shown here are unique structures not present in vertebrate host cells.

Table 26.7 *Antifungal agents*

Category	*Target*	*Examples*	*Use*
Allylamines	Ergosterol synthesis	Terbinafine	Oral, topical
Aromatic antibiotic	Mitosis inhibitor	Griseofulvin	Oral
Azoles	Ergosterol synthesis	Clotrimazole	Topical
		Fluconazole	Oral
		Itraconazole	Oral
		Ketoconazole	Oral
		Miconazole	Topical
		Posaconazole	Experimental
		Ravuconazole	Experimental
		Voriconazole	Oral
Chitin synthesis inhibitor	Chitin synthesis	Nikkomycin Z	Experimental
Echinocandins	Cell wall synthesis	Caspofungin	Intravenous
Nucleic acid analogs	DNA synthesis	5-Fluorocytosine	Oral
Polyenes	Ergosterol synthesis	Amphotericin B	Oral, intravenous
		Nystatin	Oral, topical
Polyoxins	Chitin synthesis	Polyoxin A	Agricultural
		Polyoxin B	Agricultural

agents is specific, resulting in selective fungal cell death. These agents are used to treat infections with fungi such as *Candida* and some fungi that are resistant to other agents (Sections 33.14 and 34.8).

Other Antifungal Agents

Other antifungal drugs interfere with fungus-specific structures and functions (Table 26.7). For example, fungal cell walls contain chitin, a polymer of *N*-acetylglucosamine found only in fungi and insects. Several polyoxins inhibit cell wall synthesis by interfering with chitin biosynthesis. Polyoxins are widely used as agricultural fungicides, but are not used clinically. Other antifungal drugs inhibit folate biosynthesis, interfere with DNA topology during replication, or, in the case of drugs such as griseofulvin, disrupt microtubule aggregation during mitosis. Moreover, the nucleic acid analog 5-fluorocytosine (flucytosine) is an effective nucleic acid synthesis inhibitor in fungi. Some very effective antifungal drugs also have other applications. For example, vincristine and vinblastine are effective antifungal agents and also have anticancer properties.

Predictably, the use of antifungal drugs has resulted in the emergence of populations of resistant fungi and the emergence of opportunistic fungal pathogens. For example, *Candida* species, which are normally not pathogenic, are known to produce disease in immunocompromised individuals. In addition, drug-resistant pathogenic *Candida* strains have developed in individuals who have been treated with antifungal drugs, and some are now resistant to all of the currently used antifungal agents (see Figure 26.29).

MiniQuiz

- Why are there very few clinically effective antifungal agents?
- What factors are contributing to an increased incidence of fungal infections?

V Antimicrobial Drug Resistance and Drug Discovery

Antimicrobial drug resistance is a major problem when dealing with many pathogenic microorganisms, especially in healthcare settings. Here we explore some of the mechanisms for drug resistance in microorganisms and present strategies for developing new antimicrobial agents. Practical methods for preserving and enhancing the efficacy of currently used antimicrobial drugs are discussed in the Microbial Sidebar, "Preventing Antimicrobial Drug Resistance."

26.12 Antimicrobial Drug Resistance

Antimicrobial drug resistance is the acquired ability of a microorganism to resist the effects of an antimicrobial agent to which it is normally susceptible. No single antimicrobial agent inhibits all microorganisms, and some form of antimicrobial drug resistance is an inherent property of virtually all microorganisms. As we have discussed, antibiotic producers are microorganisms. In order to survive, the antibiotic-producing microorganism itself must be able to neutralize or destroy its own antibiotic. Thus, genes encoding antibiotic resistance must be present in virtually every organism that makes an antibiotic. Widespread antimicrobial drug resistance can then occur by horizontal transfer of resistance genes between and among microorganisms.

Resistance Mechanisms

For any of at least six different reasons, some microorganisms are naturally resistant to certain antibiotics.

1. The organism may lack the structure an antibiotic inhibits. For instance, some bacteria, such as the mycoplasmas, lack a bacterial cell wall and are therefore naturally resistant to penicillins.
2. The organism may be impermeable to the antibiotic. For example, most gram-negative *Bacteria* are impermeable to penicillin G and platensimycin.
3. The organism may be able to alter the antibiotic to an inactive form. Many staphylococci contain β-lactamases, an enzyme that cleaves the β-lactam ring of most penicillins (**Figure 26.27**).
4. The organism may modify the target of the antibiotic. In the laboratory, for example, antibiotic-resistant cells can be isolated from cultures that were grown from strains uniformly susceptible to the selecting antibiotic. The resistance of these isolates is usually due to mutations in chromosomal genes. In most cases, antibiotic resistance mediated by chromosomal genes arises because of a modification of the *target* of antibiotic activity (for example, a ribosome).

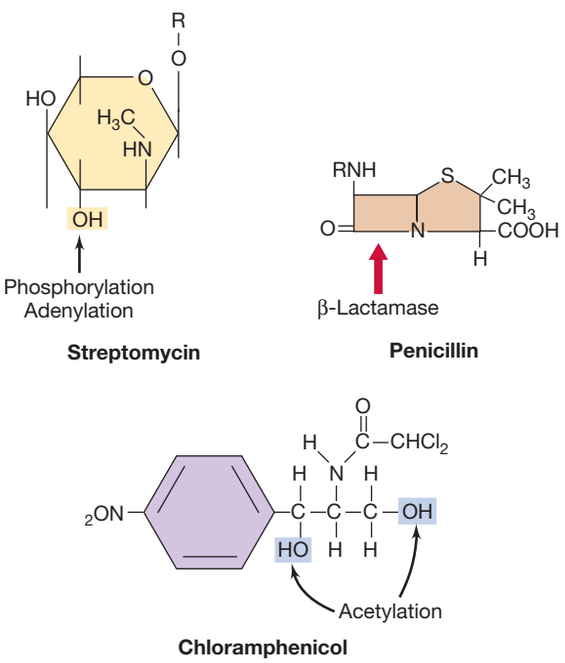


Figure 26.27 Sites at which antibiotics are attacked by enzymes encoded by R plasmid genes. Antibiotics may be selectively inactivated by chemical modification or cleavage. For the complete structure of streptomycin, see Figure 26.21 and for penicillin, Figure 26.19.

5. The organism may develop a resistant biochemical pathway. For example, many pathogens develop resistance to sulfa drugs that inhibit the production of folic acid in *Bacteria* (Section 26.6 and Figure 26.16). Resistant bacteria modify their metabolism to take up preformed folic acid from the environment, avoiding the need for the pathway blocked by the sulfa drugs.
6. The organism may be able to pump out an antibiotic entering the cell, a process called *efflux*.

Some specific examples of bacterial resistance to antibiotics are shown in **Table 26.8**.

Antibiotic resistance can be genetically encoded by the microorganism on either the bacterial chromosome or on a plasmid called an *R* (for *resistance*) *plasmid* (↻ Sections 6.6 and 6.7) (Table 26.8). Because of widespread existing antibiotic resistance and continual emergence of new resistance, bacteria isolated from clinical specimens must be tested for antibiotic susceptibility using the MIC method or an agar diffusion method (Section 26.4). Details of the antibiotic susceptibility testing of clinical isolates are described in Section 31.3.

Mechanism of Resistance Mediated by R Plasmids

Most drug-resistant bacteria isolated from patients contain drug-resistance genes located on R plasmids rather than on the chromosome. Resistance is typically due to genes on the R plasmid that encode enzymes that modify and inactivate the drug (Figure 26.27) or genes that encode enzymes that prevent uptake of the drug or actively pump it out. For instance, the aminoglycoside antibiotics streptomycin, neomycin, kanamycin, and spectinomycin have similar chemical structures. Strains carrying R plasmids that encode resistance to these drugs make enzymes that phosphorylate, acetylate, or adenylate the drug. The modified drug then lacks antibiotic activity.

For the penicillins, R plasmids encode the enzyme *penicillinase* (a β-lactamase that splits the β-lactam ring, inactivating the antibiotic). Chloramphenicol resistance is due to an R plasmid–encoded enzyme that acetylates the antibiotic. Many R plasmids contain several different resistance genes and can confer multiple antibiotic resistance on a cell previously sensitive to each individual antibiotic.

Origin of Resistance Plasmids

R plasmids predated the widespread artificial use of antibiotics. A strain of *Escherichia coli* that was freeze-dried in 1946 contained a plasmid with genes conferring resistance to both tetracycline and streptomycin, even though neither of these antibiotics was used clinically until several years later. Similarly, R plasmid genes for resistance to semisynthetic penicillins existed before the semisynthetic penicillins had been synthesized.

Of perhaps even more ecological significance, R plasmids with antibiotic resistance genes are found in some nonpathogenic gram-negative soil bacteria. In the soil, R plasmids may confer selective advantages because major antibiotic-producing organisms (*Streptomyces* and *Penicillium*) are also soil organisms. R plasmids probably arose long before antibiotics were discovered, but, as we shall see, these naturally occurring plasmids have been propagated and spread as antibiotics were increasingly used in medicine and agriculture.

Table 26.8 *Bacterial resistance to antibiotics*

Resistance mechanism	*Antibiotic example*	*Genetic basis of resistance*	*Mechanism present in:*
Reduced permeability	Penicillins	Chromosomal	*Pseudomonas aeruginosa* Enteric *Bacteria*
Inactivation of antibiotic (for example, penicillinase; modifying enzymes such as methylases, acetylases, phosphorylases, and others)	Penicillins	Plasmid and chromosomal	*Staphylococcus aureus* Enteric *Bacteria*
	Chloramphenicol	Plasmid and chromosomal	*Neisseria gonorrhoeae* *Staphylococcus aureus*
	Aminoglycosides	Plasmid	Enteric *Bacteria* *Staphylococcus aureus*
Alteration of target (for example, RNA polymerase, rifamycin; ribosome, erythromycin and streptomycin; DNA gyrase, quinolones)	Erythromycin Rifamycin Streptomycin Norfloxacin	Chromosomal	*Staphylococcus aureus* Enteric *Bacteria* Enteric *Bacteria* Enteric *Bacteria* *Staphylococcus aureus*
Development of resistant biochemical pathway	Sulfonamides	Chromosomal	Enteric *Bacteria* *Staphylococcus aureus*
Efflux (pumping out of cell)	Tetracyclines Chloramphenicol	Plasmid Chromosomal	Enteric *Bacteria* *Staphylococcus aureus* *Bacillus subtilis*
	Erythromycin	Chromosomal	*Staphylococcus* spp.

Spread of Antimicrobial Drug Resistance

The widespread use of antibiotics in medicine, veterinary medicine, and agriculture provides highly selective conditions for the spread of R plasmids. The resistance genes on R plasmids confer an immediate selective advantage and thus antibiotic resistance due to R plasmids is a predictable outcome of natural selection. The R plasmids and other sources of resistance genes pose significant limits on the long-term use of any single antibiotic as an effective antimicrobial agent.

Inappropriate use of antimicrobial drugs is the leading cause of rapid development of drug-specific resistance in disease-causing microorganisms. The discovery and clinical use of the many known antibiotics have been paralleled by the emergence of bacteria that resist them. **Figure 26.28** shows a correlation between the amounts of antibiotics used and the numbers of bacteria resistant to each antibiotic.

Overuse of antibiotics results in development of resistance. Increasingly, the antimicrobial agent prescribed for treatment of a particular infection must be changed because of increased resistance of the microorganism causing the disease. A classic example is the development of resistance to penicillin and other antimicrobial drugs in *Neisseria gonorrhoeae*, the bacterium that causes the sexually transmitted disease gonorrhea (Figure 26.28*b*). Prior to 1980, penicillin had been in continuous use for treatment for gonorrhea since it became available in the 1940s. However, penicillin is no longer a first-line treatment of gonorrhea because a significant percentage of clinical *N. gonorrhoeae* isolates now produce β-lactamase, conferring penicillin resistance. Virtually all of these resistant isolates have developed since 1980; by 1990, penicillin-resistant strains were so common that fluoroquinolones such as ciprofloxacin replaced penicillin as the drug of choice for treatment. Soon after, however, the growing prevalence of fluoroquinolone-resistant *N. gonorrhoeae* strains isolated from Asia, Hawaii, and California in men who have sex with men again prompted a change in first-line drug recommendations for treatment of gonorrhea from the fluoroquinolone ciprofloxacin to ceftriaxone, a penicillinase-resistant β-lactam antibiotic (Figure 26.28*c*). Treatment guidelines are updated nearly every year to control continually emerging drug resistance in this organism (↻ Section 33.12).

Antibiotics are still used in clinical practice far more often than necessary. Antibiotic treatment is warranted in about 20% of individuals who are seen for infectious disease, but antibiotics are prescribed up to 80% of the time. Furthermore, in up to 50% of cases, prescribed doses or duration of treatments are not correct. This is compounded by patient noncompliance: Many patients stop taking medications, particularly antibiotics, as soon as they feel better. For example, the emergence of isoniazid-resistant tuberculosis correlates with a patient's failure to take the oral medication daily for the full course of 6–9 months (↻ Section 33.4). Exposure of virulent pathogens to sublethal doses of antibiotics for inadequate periods of time may select for drug-resistant strains.

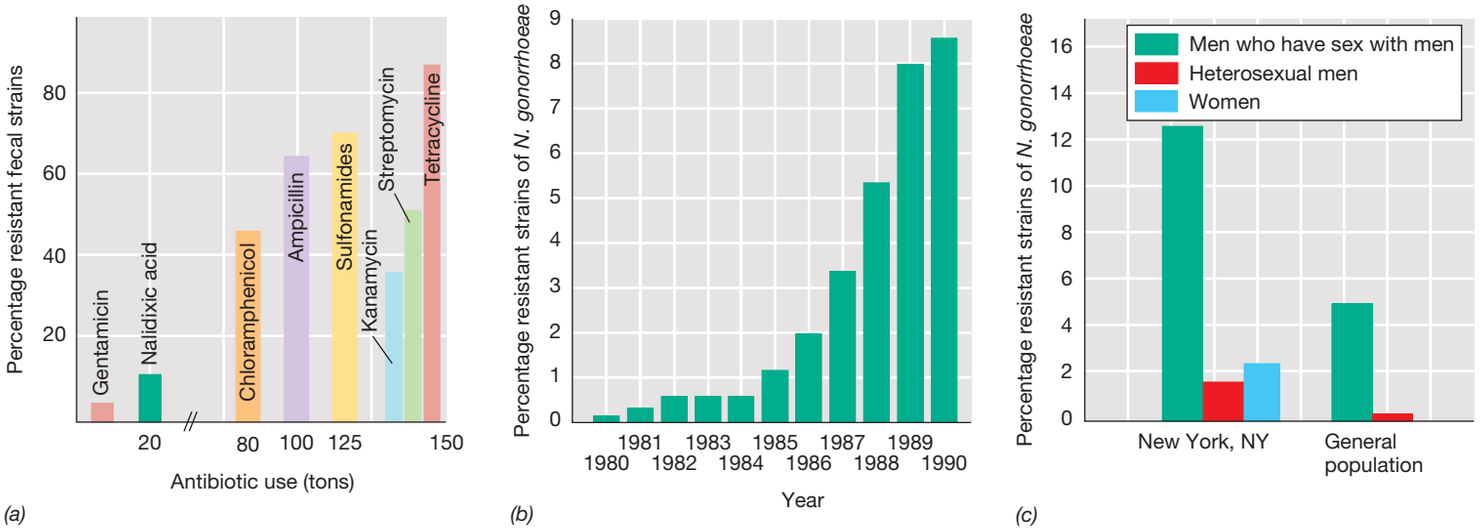


Figure 26.28 Patterns of drug resistance in pathogens. *(a)* The relationship between antibiotic use and the percentage of antibiotic-resistant bacteria isolated from diarrheal patients. Those agents that have been used in the largest amounts, as indicated by the amount produced commercially, are those for which drug-resistant strains are most frequent. *(b)* Percentage of reported cases of gonorrhea caused by drug-resistant strains. The actual number of reported drug-resistant cases in 1985 was 9,000. This number rose to 59,000 in 1990. Greater than 95% of reported drug-resistant cases were due to penicillinase-producing strains of *Neisseria gonorrhoeae*. Since 1990, penicillin has not been recommended for treatment of gonorrhea because of the emerging drug resistance. *(c)* The prevalence of fluoroquinolone-resistant *N. gonorrhoeae* in certain populations in the United States in 2003. Ciprofloxacin, a fluoroquinolone, is no longer recommended as a primary choice for treatment of *N. gonorrhoeae* infections.
Source: Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Other recent studies, however, indicate that this trend is changing in the United States. Physicians prescribe about one-third fewer antibiotics for treatment of childhood infections than they did 10 years ago. This reduction has been done largely through efforts aimed at educating physicians, healthcare providers, and patients concerning the proper use of antibiotic therapy.

Indiscriminant, nonmedical use of antibiotics has also contributed to the emergence of resistant strains. In addition to their traditional use as a treatment for infections, antibiotics are used in agriculture as supplements to animal feeds both as growth-promoting substances and as prophylactic additives to prevent the occurrence of disease. Worldwide, about 50% of all antibiotics made are used in animal agriculture applications. Antibiotics are also extensively used in aquaculture (fish farming) and even in fruit production! Antibiotics used in the food supply far too frequently, over extended periods of time, and in high doses are a proven source of food infection outbreaks due to the selection of antibiotic-resistant pathogens. For example, fluoroquinolones, a group of broad-spectrum antibiotics that include clinically important therapeutic drugs such as ciprofloxacin, have been extensively used for over 20 years as growth-promoting and prophylactic agents in agriculture. As a result, fluoroquinolone-resistant *Campylobacter jejuni* has already emerged as a foodborne pathogen (↻ Section 36.10), presumably because of the routine treatment of poultry flocks with fluoroquinolones to prevent respiratory diseases. Voluntary guidelines used by both poultry and drug producers are in place to monitor and reduce the use of fluoroquinolones. These measures may prevent development of resistance to new fluoroquinolone antibiotics.

Antibiotic-Resistant Pathogens

Largely as a result of failures to properly use antibiotics and monitor resistance, almost all pathogenic microorganisms have developed resistance to some antimicrobial agents since widespread use of antimicrobial drugs began in the 1950s (**Figure 26.29**). Penicillin and sulfa drugs, the first widely used antimicrobial agents, are not used as extensively today because many pathogens have acquired some resistance. Even the organisms that are still uniformly sensitive to penicillin, such as *Streptococcus pyogenes* (the bacterium that causes strep throat, scarlet fever, and rheumatic fever), now require larger doses of penicillin for successful treatment as compared to a decade ago.

A few pathogens have developed resistance to all known antimicrobial agents (Figure 26.29). Among these are several isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) (methicillin is a semisynthetic penicillin; Section 26.8). Although MRSA is usually associated with healthcare settings, it also causes a significant number of community-associated infections. An increasing number of independently derived MRSA strains have developed reduced susceptibility to even vancomycin and are termed "vancomycin intermediate *Staphylococcus aureus*" (VISA) strains (↻ Section 33.9). Vancomycin-resistant *Enterococcus faecium* (VRE) and some isolates of *Mycobacterium tuberculosis* and *Candida albicans* have also developed resistance to all known antimicrobial drugs. Antibiotic resistance can

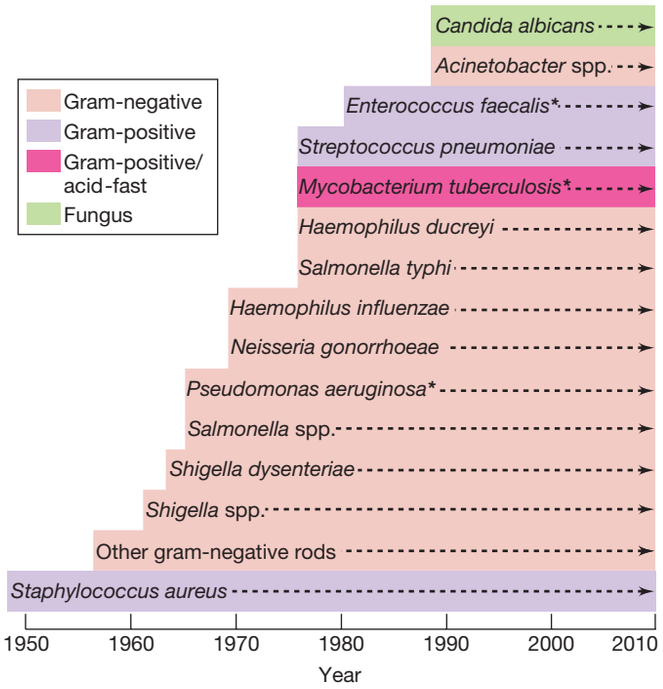


Figure 26.29 The appearance of antimicrobial drug resistance in some human pathogens. The asterisks indicate that some strains of these pathogens are now untreatable with known antimicrobial drugs.

be minimized if drugs are used only for treatment of susceptible diseases and are given in sufficiently high doses and for sufficient lengths of time to reduce the microbial population before resistant mutants can develop. Combining two unrelated antimicrobial agents may also reduce resistance; it is less likely that a mutant strain resistant to one antibiotic will also be resistant to the second antibiotic. However, certain common R plasmids confer multiple drug resistance and make multiple antibiotic therapy less useful as a clinical treatment strategy.

We now know that if the use of a particular antibiotic is stopped, the resistance to that antibiotic can be reversed over the course of several years. On the other hand, antibiotic-resistant organisms may persist in the gut for some time. This information implies that the efficacy of some antibiotics may be reestablished by withdrawing the antibiotic from use, but only by following a carefully monitored plan of prudent use upon reintroduction. Finally, as we discuss below, new antimicrobial agents are actively being developed using various strategies for drug design and discovery.

MiniQuiz

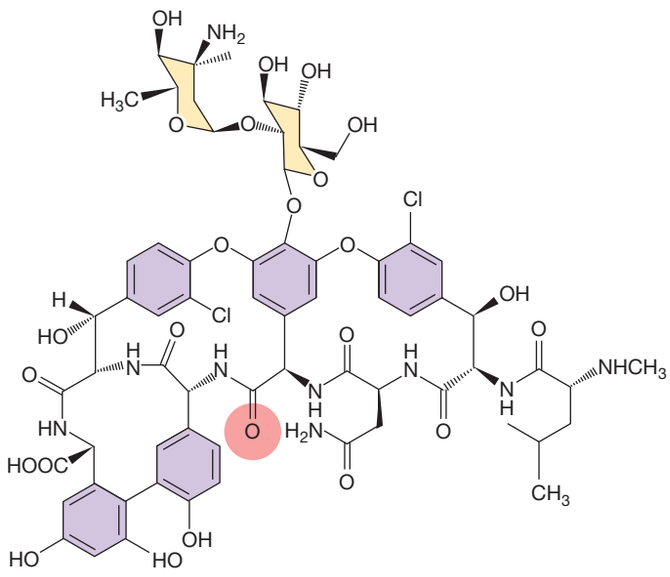
- Identify the six basic mechanisms of antibiotic resistance among bacteria.
- Identify the primary sources of antibiotic resistance genes.
- What practices encourage the development of antibiotic-resistant pathogens?

26.13 The Search for New Antimicrobial Drugs

Resistance will develop to all known antimicrobial drugs, given sufficient drug exposure and time. Conservative, appropriate use of antibiotics is necessary to prolong the useful clinical life of these drugs. The long-term solution to antimicrobial drug resistance, however, resides in our ability to develop new antimicrobial drugs. Several strategies are being used to identify and produce useful analogs of existing agents or to design or discover novel antimicrobial compounds.

New Analogs of Existing Antimicrobial Compounds

New analogs of existing antimicrobial compounds are often effective, largely because new compounds that are structural mimics of older ones have a proven mechanism of action. In many cases, parameters such as solubility and affinity can be optimized by introducing minor modifications to the chemical structure of a drug without altering structures critical to drug action. The new compound may actually be more effective than the parent compound and, because resistance is based on structural recognition, the new compound may not be recognized by resistance factors. For example, Figure 26.23 shows the structure of tetracycline and two bioactive derivatives. Using authentic tetracycline as the lead compound, systematic chemical substitutions at the four R group sites can generate an almost endless series of tetracycline analogs. Using this basic strategy, new tetracycline-related compounds (Section 26.9), new β-lactam antibiotics (Section 26.8), and new analogs of vancomycin (**Figure 26.30**) have been synthesized.



Vancomycin

Figure 26.30 Vancomycin. Intermediate drug resistance to the parent structure of vancomycin has developed in recent years. However, modification at the position shown in red by substitution of a methylene ($=CH_2$) group for the carbonyl oxygen restores much of the lost activity.

Some of these derivatives are as much as 100 times more potent than the parent compound.

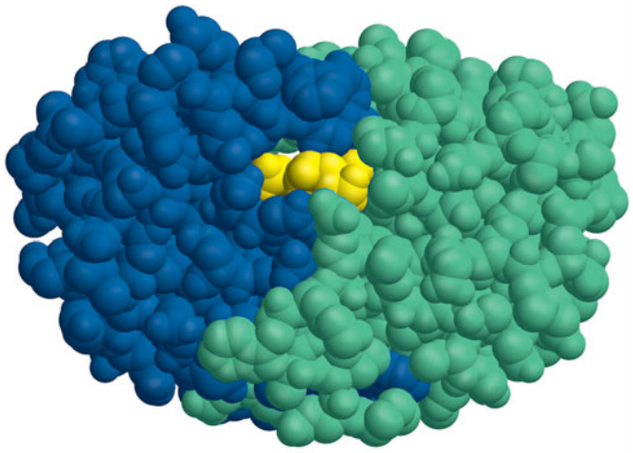
The application of automated chemistry methods to drug discovery has dramatically increased our ability to rapidly generate new antimicrobial compounds. These automated methods, called *combinatorial chemistry*, initiate systematic modifications of a known antimicrobial product to yield large numbers of new analogs. For instance, using automated combinatorial chemistry and starting with pure tetracycline (Figure 26.23), five different reagents might be used to introduce substitutions at the four different tetracycline R groups. The substituted sites would yield $5 \times 5 \times 5 \times 5$ (five derivatives at each of four sites), or 625 different tetracycline derivatives from only five different reagents, all in a few hours' time! These compounds would then be assayed for *in vitro* biological activity on different test organisms using automated testing methods for antibiotic susceptibility. The automated synthesis and screening processes dramatically shorten drug discovery time and increase the number of new candidate drugs by a factor of 10 or more each year.

According to pharmaceutical industry estimates, about 7 million candidate compounds must be screened to yield a single useful clinical drug. Drugs effective in the laboratory must then be tested for efficacy and toxicity in animals and finally in clinical trials in humans. Animal testing requires multiple trials over several years to ensure that the candidate drug is both effective and safe. Clinical trials in humans to check efficacy and safety take additional years for each drug. Each year, the pharmaceutical industry spends up to $4 billion on new antimicrobial drug development. Discovery and development for each drug typically takes 10–25 years before it is approved for clinical use. The cost of discovery and development, from the laboratory through clinical trials, is estimated at over $500 million for each new drug approved for human use. This is a major reason why pharmaceutical drugs are so expensive.

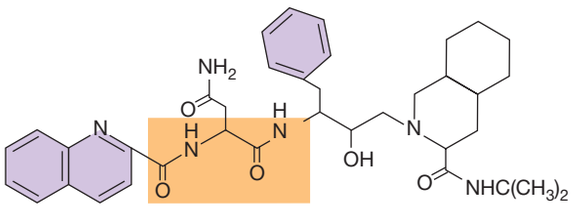
Computer Drug Design

Novel antimicrobial compounds are much more difficult to identify than analogs of existing drugs because new antimicrobial compounds must work at unique sites in metabolism and biosynthesis or be structurally dissimilar to existing compounds to avoid inducing known resistance mechanisms. Computer technology and structural biology methods make it possible to design a drug to interact with specific microbial structures. Thus, drug discovery can now begin at the computer, where new drugs can be rapidly synthesized and tested for binding and efficacy in the computer environment at relatively low cost.

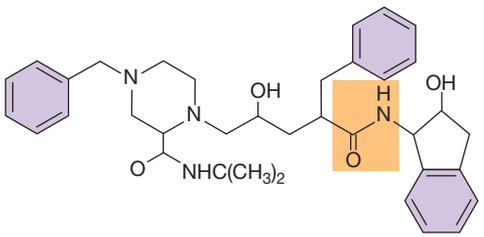
One of the most dramatic successes in computer-directed drug design is the development of *saquinavir*, a protease inhibitor that is used to slow the growth of the human immunodeficiency virus (HIV) in infected individuals (**Figure 26.31**). Designed by computer, saquinavir binds the active site of the HIV protease enzyme. The structure of saquinavir was based on the known three-dimensional structure of the protease–substrate complex. The HIV protease normally cleaves a virus-encoded precursor protein to produce the mature viral core and activate



(a) **HIV protease**



Saquinavir



Indinavir

(b)

Figure 26.31 Computer-generated anti-HIV drugs. *(a)* The HIV protease homodimer. Individual polypeptide chains are shown in green and blue. A peptide (yellow) is bound in the active site. HIV protease cleaves an HIV precursor protein, a necessary step in virus maturation (↻ Section 21.11). Blocking of the protease site by the bound peptide inhibits precursor processing and HIV maturation. This structure is derived from information in the Protein Data Bank. *(b)* These anti-HIV drugs are peptide analogs called protease inhibitors that were designed by computer to block the active site of HIV protease. The areas highlighted in orange show the regions analogous to peptide bonds in proteins.

the reverse transcriptase enzyme necessary for replication (↻ Section 21.11). Saquinavir is a high-affinity peptide analog of the HIV precursor protein that displaces the authentic protein substrate, inhibiting virus maturation and growth in the human host. A number of other computer-designed protease inhibitors are in use as antiviral drugs for the treatment of AIDS (Table 26.6, Figure 26.31, and ↻ Figure 33.43). Computer design and testing based on structural and biochemical modeling is a practical, rapid, and cost-effective method for designing antimicrobial drugs.

Natural Products as Antibiotics

As the first antibiotics were discovered and brought into clinical use in the 1930s and 1940s, researchers developed standard methods to isolate more new antibiotics. Candidate drugs were routinely isolated from natural sources such as *Streptomyces* or *Penicillium* cultures and systematically screened for antimicrobial activity using standard MIC or agar diffusion methods to find new antimicrobial compounds. As time passed, the yield from these traditional methods decreased, supplanted by higher yields from the combinatorial chemistry and computer design methods discussed above. Most of the effective natural antibiotics produced at reasonable levels by antibiotic-producing microorganisms had already been isolated. Remaining effective antibiotics, presumably present in concentrations so low that they were ineffective against test organisms, could not be identified.

Platensimycin (Figure 26.25), however, is an exception to this rule. This antibiotic was discovered using a modification of direct methods for screening natural products. Platensimycin represents a new class of antimicrobials that selectively inhibits bacterial lipid biosynthesis and is especially active against gram-positive pathogens, including MRSA, VISA, and VRE (Section 26.12). A key feature in the discovery of platensimycin was its selection using a novel method that may have broad applications for targeted drug discovery. To select an agent for a defined target, in this case an enzyme in the lipid synthesis pathway of gram-positive bacteria, scientists introduced a defect in the β-ketoacyl-(acyl-carrier protein) synthase I/II (FabF/B) gene in *Staphylococcus aureus* by using a strain expressing antisense FabF RNA (↻ Section 8.14). The gene-specific antisense RNA decreased expression of FabF, reducing fatty acid synthesis and increasing the sensitivity of the crippled *S. aureus* strain to antibiotics that inhibit fatty acid synthesis. By screening 250,000 natural product extracts from 83,000 strains of potential antibiotic producers, the scientists were able to identify and isolate platensimycin from a soil microorganism, *Streptomyces platensis*. Although the screening of large numbers of strains is a huge task, the method identifies target-specific antibiotics present in low concentrations. This strategy is applicable to virtually any target for which the gene sequence (and, hence, the corresponding antisense RNA sequence) is known.

Drug Combinations

The efficacy of some antibiotics can be retained if they are given with compounds that inhibit antibiotic resistance. Several β-lactam antibiotics can be combined with β-lactamase inhibitors to preserve antibiotic activity in β-lactamase-resistant microorganisms. For example, the broad-spectrum β-lactam antibiotic ampicillin (Figure 26.19) can be mixed with sulbactam, a β-lactamase inhibitor. The inhibitor binds β-lactamase irreversibly, preventing degradation of the ampicillin and permitting it to disrupt cell wall formation in the affected cell. This combination preserves the effectiveness of the β-lactamase-sensitive ampicillin against β-lactamase producers such as staphylococci and certain gram-negative pathogens. Likewise, we have already mentioned the use of sulfamethoxazole–trimethoprim, a mixture of two folic acid synthesis inhibitors, to prevent the loss of efficacy through mutation and selection for resistance (Section 26.6).

Drug combination therapy approaches have revolutionized treatment of HIV infections. Currently, a combination therapy consisting of nucleoside analogs and a protease inhibitor is recommended. This drug treatment protocol is termed HAART, for highly active anti-retroviral therapy. As with antimicrobial combination regimens, HAART is designed to target two independent viral functions; the nucleoside analogs target virus replication and the protease inhibitors target virus maturation. Because the probability of a single virus developing resistance to multiple drugs is less than the probability of developing resistance to a single drug, HAART-resistant strains are relatively uncommon (↻ Section 33.14).

Bacteriophages

Bacteriophages are viruses that infect bacteria (↻ Sections 9.8–9.10). *Bacteriophage therapy* has been used on a limited basis for over 80 years to treat infections in animals and, in a few instances, in humans. Phages interact with individual bacterial cell surface components and show specificity for particular bacterial species. The attached phage enters the cell, replicates, and kills the bacterial host in the process. The efficacy and efficiency of these agents for human treatment is largely untested and somewhat controversial, although clinical trials of several products are ongoing. However, because bacteria can acquire resistance to a phage infection through mutations that alter receptors or reduce the susceptibility of the cell wall to phage enzymes, bacteriophage therapy will likely be susceptible to resistance, just as for most chemical antimicrobial agents.

MiniQuiz

- Explain the advantages and disadvantages of developing new drugs based on existing drug analogs.
- How can computer drug design aid in the search for new drugs?
- Explain the use of antisense RNA for drug discovery.

Big Ideas

26.1
Sterilization is the killing of all organisms and viruses, and heat is the most widely used method of sterilization. The temperature employed must eliminate the most heat-resistant organisms, usually bacterial endospores. An autoclave permits applications of steam heat under pressure, achieving temperatures above the boiling point of water, which kills endospores. Pasteurization does not sterilize liquids, but reduces microbial load, kills most pathogens, and inhibits the growth of spoilage microorganisms.

26.2
Controlled doses of electromagnetic radiation effectively inhibit microbial growth. Ultraviolet radiation is used for decontaminating surfaces and materials that do not absorb light, such as air and water. Ionizing radiation that can penetrate solid or light-absorbing materials is used for sterilization and decontamination in the medical and food industries.

26.3
Filters remove microorganisms from air or liquids. Depth filters, including HEPA filters, are used to remove microorganisms and other contaminants from liquids or air. Membrane filters are used for sterilization of heat-sensitive liquids, and nucleation track filters are used to isolate specimens for electron microscopy.

26.4
Chemicals are often used to control microbial growth. Chemicals that kill organisms are called -cidal agents; those that inhibit growth are called -static agents; those that lyse organisms are called -lytic agents. Antimicrobial agents are tested for efficacy by determining their ability to inhibit growth in vitro.

26.5
Sterilants, disinfectants, and sanitizers are used to decontaminate nonliving material. Antiseptics and germicides are used to reduce microbial growth on living tissues. Antimicrobial compounds have commercial, healthcare, and industrial applications.

26.6
Synthetic antimicrobial agents are selectively toxic for *Bacteria*, viruses, and fungi. Synthetic growth factor analogs such as sulfa drugs, isoniazid, and nucleic acid analogs are metabolic inhibitors. Quinolones inhibit the action of DNA gyrase in *Bacteria*.

26.7
Antibiotics are a chemically diverse group of antimicrobial compounds that are produced by microorganisms. Although many antibiotics are known, only a few are clinically effective. Each antibiotic works by inhibiting a specific cellular process or function in the target microorganisms.

26.8
The β-lactam compounds, including the penicillins and the cephalosporins, are the most important single class of clinical antibiotics. These antibiotics and their semisynthetic derivatives target cell wall synthesis in *Bacteria*. They have low host toxicity and collectively have a broad spectrum of activity.

26.9
The aminoglycosides, macrolides, and tetracycline antibiotics are structurally complex molecules produced by *Bacteria* and are active against other *Bacteria*. These antibiotics selectively interfere with protein synthesis in *Bacteria*. Daptomycin and

platensimycin are structurally novel antibiotics that target cytoplasmic membrane functions and lipid biosynthesis, respectively.

26.10
Effective antiviral agents selectively target virus-specific enzymes and processes. Clinically useful antiviral agents include nucleoside analogs and other drugs that inhibit nucleic acid polymerases and viral genome replication. Agents such as protease inhibitors interfere with viral maturation steps. Host cells also produce the antiviral interferon proteins that stop viral replication.

26.11
Antifungal agents fall into many chemical categories. Because fungi are *Eukarya*, selective toxicity is hard to achieve, but some effective antifungal agents are available. Treatment of fungal infections is an emerging human health issue.

26.12
The use of antimicrobial drugs inevitably leads to resistance in the targeted microorganisms. The development of resistance can be accelerated by the indiscriminate use of antimicrobial drugs. A few pathogens have developed resistance to all known antimicrobial drugs.

26.13
New antimicrobial compounds are constantly being discovered and developed to deal with drug-resistant pathogens and to enhance our ability to treat infectious diseases. Analogs of existing drugs are often synthesized and used as next-generation antimicrobial compounds. Computer drug design is an important tool for drug discovery.

Review of Key Terms

Aminoglycoside an antibiotic such as streptomycin, containing amino sugars linked by glycosidic bonds
Antibiotic a chemical substance produced by a microorganism that kills or inhibits the growth of another microorganism
Antimicrobial drug resistance the acquired ability of a microorganism to grow in the presence of an antimicrobial drug to which the microorganism is usually susceptible
Antimicrobial agent a chemical compound that kills or inhibits the growth of microorganisms
Antiseptic (germicide) a chemical agent that kills or inhibits growth of microorganisms and is sufficiently nontoxic to be applied to living tissues
Autoclave a sealed heating device that destroys microorganisms with temperature and steam under pressure
Bacteriocidal agent an agent that kills bacteria
Bacteriostatic agent an agent that inhibits bacterial growth
Beta (β)-lactam antibiotic an antibiotic, including penicillin, that contains the four-membered heterocyclic β-lactam ring
Broad-spectrum antibiotic an antibiotic that acts on both gram-positive and gram-negative *Bacteria*
Decontamination a treatment that renders an object or inanimate surface safe to handle
Disinfectant an antimicrobial agent used only on inanimate objects
Disinfection the elimination of pathogens from inanimate objects or surfaces
Fungicidal agent an agent that kills fungi
Fungistatic agent an agent that inhibits fungal growth
Fusion inhibitor a peptide that blocks the fusion of viral and target cytoplasmic membranes
Germicide (antiseptic) a chemical agent that kills or inhibits growth of microorganisms and is sufficiently nontoxic to be applied to living tissues
Growth factor analog a chemical agent that is related to and blocks the uptake of a growth factor
HEPA filter a *h*igh-*e*fficiency *p*articulate *a*ir filter that removes particles, including microorganisms, from intake or exhaust air flow
Interferon a cytokine protein produced by virus-infected cells that induces signal transduction in nearby cells, resulting in transcription of antiviral genes and expression of antiviral proteins
Minimum inhibitory concentration (MIC) the minimum concentration of a substance necessary to prevent microbial growth
Nonnucleoside reverse transcriptase inhibitor (NNRTI) a nonnucleoside analog used to inhibit viral reverse transcriptase
Nucleoside reverse transcriptase inhibitor (NRTI) a nucleoside analog used to inhibit viral reverse transcriptase
Pasteurization the use of controlled heat to reduce the microbial load, including disease-producing microorganisms and spoilage microorganisms, in heat-sensitive liquids
Penicillin a class of antibiotics that inhibit bacterial cell wall synthesis, characterized by a β-lactam ring
Protease inhibitor an inhibitor of a viral protease
Quinolone a synthetic antibacterial compound that interacts with DNA gyrase and prevents supercoiling of bacterial DNA
Sanitizer an agent that reduces microorganisms to a safe level, but may not eliminate them
Selective toxicity the ability of a compound to inhibit or kill pathogenic microorganisms without adversely affecting the host
Sterilant (sterilizer, sporicide) a chemical agent that destroys all forms of microbial life
Sterilization the killing or removal of all living organisms and viruses from a growth medium
Tetracycline an antibiotic characterized by the four-ring naphthacene structure
Viricidal agent an agent that stops viral replication and activity
Viristatic agent an agent that inhibits viral replication

Review Questions

1. Why is the decimal reduction time (*D*) important in heat sterilization? How would the presence of bacterial endospores affect *D* (Section 26.1)?
2. Describe the effects of a lethal dose of ionizing radiation at the molecular level (Section 26.2).
3. What are the principal advantages of membrane filters? Of depth filters? Of nucleopore filters (Section 26.3)?
4. Describe the procedure for obtaining the minimum inhibitory concentration (MIC) for a chemical that is bacteriocidal for *Escherichia coli* (Section 26.4).
5. Contrast the action of disinfectants and antiseptics. Disinfectants normally cannot be used on living tissue; why not (Section 26.5)?
6. Growth factor analogs are generally distinguished from antibiotics by a single important criterion. Explain (Section 26.6).
7. Identify common sources for naturally occurring antimicrobial drugs (Section 26.7).
8. Describe the mode of action that characterizes a β-lactam antibiotic. Why are these antibiotics generally more effective against gram-positive bacteria than against gram-negative bacteria (Section 26.8)?
9. Distinguish between the modes of action of at least three of the protein synthesis–inhibiting antibiotics (Section 26.9).
10. Why do antiviral drugs generally exhibit host toxicity (Section 26.10)?
11. Identify the targets that allow selective toxicity of antifungal agents (Section 26.11).
12. Identify six mechanisms responsible for antibiotic resistance (Section 26.12).
13. Explain how application of antisense RNA methods can extend traditional natural product selection methods for antibiotic discovery (Section 26.13).

Application Questions

1. What are some potential drawbacks to the use of radiation in food preservation? Do you think these drawbacks could be manifested as health hazards? Why or why not? How would you distinguish between radiation-damaged and radiation-contaminated food?
2. Filtration is an acceptable means of pasteurization for some liquids. Design a filtration system for pasteurization of a heat-sensitive liquid. For a liquid of your choice, identify the advantages and disadvantages of a filtration system over a heat pasteurization system. Explain in terms of product quality, shelf life, and price.
3. Although growth factor analogs may inhibit microbial metabolism, only a few of these agents are useful in practice. Many growth factor analogs, including some in wide use, such as azidothymidine, exhibit significant host cell toxicity. Describe a growth factor analog that is effective and has low toxicity for host cells. Why is the toxicity low for the agent you chose? Also describe a growth factor analog that is effective against an infectious disease, but exhibits toxicity for host cells. Why might a toxic agent such as AZT still be used in certain situations to treat infectious diseases? What precautions would you take to limit the toxic effects of such a drug while maximizing the therapeutic activity? Explain your answer.
4. Although many antibiotics demonstrate clear selective toxicity for *Bacteria*, many groups of *Bacteria* are innately resistant to their effects. Indicate why gram-negative *Bacteria* are resistant to the effects of many, but not all, antibiotics. Further explain why some antibiotics are effective against these organisms.
5. List the features of an ideal antiviral drug, especially with regard to selective toxicity. Do such drugs exist? What factors might limit the use of such a drug?
6. Like viruses, fungi present special problems for drug therapy. Explain the problems inherent in drug treatment of both groups and explain whether or not you agree with the preceding statement. Give specific examples and suggest at least one group of agents that might target both types of infectious agents.
7. Explain the genetic basis of acquired resistance to β-lactam antibiotics in *Staphylococcus aureus*. Design experiments to reverse resistance to the β-lactam antibiotics. Do you think this can be done in the laboratory? Can your experiment be applied "in the field" to promote deselection of antibiotic-resistant organisms?
8. Design experiments to examine microorganisms for production of novel antibiotics. Which group or groups of microorganisms would you choose to screen for antibiotic production? Where could you find and isolate these organisms in a natural environment? What advantage, if any, would the production of an antibiotic provide for these organisms in nature? What *in vitro* methods would you use to test the efficacy of your potential new antibiotics? How might you increase the sensitivity of assays for natural products? Why are members of the genus *Streptomyces* still productive sources of novel antibiotics?

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