34 Antimicrobial Chemotherapy

Many antimicrobial medications are available to combat infections. Nonetheless, they fall into a limited number of classes based on their modes of action.

Preview

- Many infectious diseases are treated with chemotherapeutic agents, such as antibiotics, that inhibit or kill the pathogen while harming the host as little as possible.
- Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes.
- The effectiveness of chemotherapeutic agents depends on many factors: the route of administration and location of the infection, the presence of interfering substances, the concentration of the drug in the body, the nature of the pathogen, the presence of drug allergies, and the resistance of microorganisms to the drug.
- The increasing number and variety of drug-resistant pathogens is a serious public health problem.
- Although antibacterial chemotherapy is more advanced, drugs for the treatment of fungal, protozoan, and viral infections are also becoming increasingly available.

The control of microorganisms is critical for the prevention and treatment of disease. Chapter 7 is concerned principally with the chemical and physical agents used to treat inanimate objects in order to destroy microorganisms or inhibit their growth. Microorganisms also grow on and within other organisms, and microbial colonization can lead to disease, disability, and death. Thus the control or destruction of microorganisms residing within the bodies of humans and other animals is of great importance.

When disinfecting or sterilizing an inanimate object, one naturally must use procedures that do not damage the object itself. The same is true for the treatment of living hosts. The most successful drugs interfere with vital processes that differ between the pathogen and host, thereby seriously damaging the target microorganism while harming its host as little as possible. This chapter introduces the principles of antimicrobial chemotherapy and briefly reviews the characteristics of selected antibacterial, antifungal, and antiprotozoan antiviral drugs.

Modern medicine is dependent on **chemotherapeutic agents,** chemical agents that are used to treat disease. Ideally, chemotherapeutic agents used to treat infectious disease destroy pathogenic microorganisms or inhibit their growth at concentrations low enough to avoid undesirable damage to the host. Most of these agents are **antibiotics** [Greek *anti*, against, and *bios*, life], microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth. Drugs such as the sulfonamides are sometimes called antibiotics although they are synthetic chemotherapeutic agents, not microbially synthesized.

34.1 The Development of Chemotherapy

The modern era of chemotherapy began with the work of the German physician Paul Ehrlich (1854–1915). Ehrlich was fascinated with dyes that specifically bind to and stain microbial cells. He reasoned that one of the dyes could be a chemical that would selectively destroy pathogens without harming human cells—a

It was the knowledge of the great abundance and wide distribution of actinomycetes, which dated back nearly three decades, and the recognition of the marked activity of this group of organisms against other organisms that led me in 1939 to undertake a systematic study of their ability to produce antibiotics.

—Selman A. Waksman

"magic bullet." By 1904 Ehrlich found that the dye trypan red was active against the trypanosome that causes African sleeping sickness (*see figure 25.6*) and could be used therapeutically. Subsequently Ehrlich and a young Japanese scientist named Sahachiro Hata tested a variety of arsenicals on syphilis-infected rabbits and found that arsphenamine was active against the syphilis spirochete. Arsphenamine was made available in 1910 under the trade name Salvarsan, and paved the way to the testing of hundreds of compounds for their selective toxicity and therapeutic potential.

In 1927, the German chemical industry giant, I. G. Farbenindustrie, began a long-term search for chemotherapeutic agents under the direction of Gerhard Domagk. Domagk had screened a vast number of chemicals for other "magic bullets" and discovered that Prontosil Red, a new dye for staining leather, protected mice completely against pathogenic streptococci and staphylococci without apparent toxicity. Jacques and Therese Trefouel later showed that the body metabolized the dye to sulfanilamide. Domagk received the 1939 Nobel Prize in Physiology or Medicine for his discovery of sulfonamides, or sulfa drugs.

In the 1920s, Alexander Fleming, a Scottish physician, found that human tears contained a naturally occurring antibacterial substance that he termed "lysozyme." This substance unfortunately had little therapeutic value because it could not be isolated in large quantities and was not effective against many microorganisms. However, it prepared Fleming for the discovery of penicillin, the first true antibiotic to be used therapeutically.

Penicillin was actually discovered in 1896 by a 21-year-old French medical student named Ernest Duchesne. His work was forgotten until Fleming's accidental rediscovery of the antibiotic in September 1928. After returning from a weekend vacation, Fleming noticed that a petri plate of *Staphylococcus* also had a mold growing on it and, like the lysozyme he had discovered years before, there were no *Staphylococcus* colonies surrounding it (**figure 34.1**). Although the precise events are still unclear, it has been suggested that a *Penicillium notatum* spore had made its way onto the petri dish before it had been inoculated with the staphylococci. The mold apparently grew before the bacteria and produced penicillin. The bacteria nearest the fungus were lysed. Fleming correctly deduced that the mold contaminant produced a diffusible substance, which he called penicillin. In subsequent studies he showed that this substance could diffuse through agar so that even small amounts of it extracted from broth cultures could kill several pathogenic bacteria, including *S. aureus*. Unfortunately, Fleming could not demonstrate that penicillin remained active in vivo long enough to destroy pathogens and thus dropped the research.

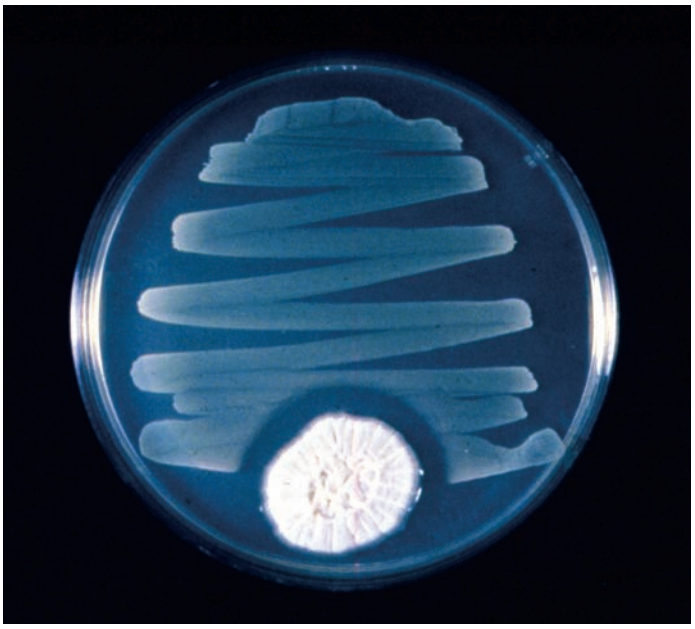


Figure 34.1 Bacteriocidal Action of Penicillin. The *Penicillium* mold colony secretes penicillin that kills *Staphylococcus aureus* that was streaked nearby.

In 1939 Howard Florey, a professor of pathology at Oxford University, was in the midst of testing the bactericidal activity of many substances, including lysozyme and the sulfonamides. After reading Fleming's paper on penicillin, one of Florey's coworkers, Ernst Chain, obtained the *Penicillium* culture from Fleming and set about culturing it and purifying penicillin. Florey and Chain were greatly aided in this by the biochemist Norman Heatley. Heatley devised the original assay, culture, and purification techniques needed to produce crude penicillin for further experimentation. When purified penicillin was injected into mice infected with streptococci or staphylococci, practically all the mice survived. Florey and Chain's success was reported in 1940, and subsequent human trials were equally successful. Fleming, Florey, and Chain received the Nobel Prize in 1945 for the discovery and production of penicillin.

The discovery of penicillin stimulated the search for other antibiotics. Selman Waksman announced in 1944 that he and his associates had found a new antibiotic, streptomycin, produced by the actinomycete *Streptomyces griseus.* This discovery arose from the careful screening of about 10,000 strains of soil bacteria and fungi. The importance of streptomycin cannot be understated, as it was the first drug that could successfully treat tuberculosis. Waksman received the Nobel Prize in 1952, and his success led to a worldwide search for other antibiotic-producing soil microorganisms. Microorganisms producing chloramphenicol, neomycin, terramycin, and tetracycline were isolated by 1953.

The discovery of chemotherapeutic agents and the development of newer, more powerful drugs has transformed modern medicine and greatly alleviated human suffering. Furthermore, antibiotics have proven exceptionally useful in microbiological research (**Techniques & Applications 34.1**).

1. What are chemotherapeutic agents? Antibiotics?
2. What contributions to chemotherapy were made by Ehrlich, Domagk, Fleming, Florey and Chain, and Waksman?

Techniques & Applications

34.1 The Use of Antibiotics in Microbiological Research

Although the use of antibiotics in the treatment of disease is emphasized in this chapter, it should be noted that antibiotics are extremely important research tools. For example, they aid the cultivation of viruses by preventing bacterial contamination. When eggs are inoculated with a virus sample, antibiotics often are included in the inoculum to maintain sterility. Usually a mixture of antibiotics (e.g., penicillin, amphotericin, and streptomycin) also is added to tissue cultures used for virus cultivation and other purposes.

Researchers often use antibiotics as instruments to dissect metabolic processes by inhibiting or blocking specific steps and observing the consequences. Although selective toxicity is critical when antibiotics are employed therapeutically, specific toxicity is more important in this context: the antibiotic must act by a specific and precisely understood mechanism. A clinically useful antimicrobial agent such as ampicillin sometimes may be employed in research, but often an agent with specific toxicity and excellent research potential is too toxic for therapeutic use. The actinomycins, discovered in 1940 by Selman Waksman, are a case in point. They are so toxic to higher organisms that it was suggested they be used as rat poison. Today actinomycin D is a standard research tool specifically used to block RNA synthesis. Other examples of antibiotics useful in research, with the process inhibited, are the following: chloramphenicol (bacterial protein synthesis), cycloserine (peptidoglycan synthesis), nalidixic acid and novobiocin (bacterial DNA synthesis), rifampin (bacterial RNA synthesis), cycloheximide (eucaryotic protein synthesis), daunomycin (fungal RNA synthesis), mitomycin C (eucaryotic DNA synthesis), polyoxin D (fungal cell wall chitin synthesis), and cerulenin (fatty acid synthesis).

In practice, the antibiotic is administered and changes in cell function are monitored. If one desired to study the dependence of bacterial flagella synthesis on RNA transcription, the flagella could be removed by high-speed mixing in a blender, followed by actinomycin D addition to the incubation mixture. The bacterial culture would then be observed for flagella regeneration in the absence of RNA synthesis. The results of such experiments must be interpreted with caution. Flagella synthesis may have been blocked because actinomycin D inhibited some other process, thus affecting flagella regeneration indirectly rather than simply inhibiting transcription of a gene required for flagella synthesis. Furthermore, not all microorganisms respond in the same way to a particular drug.

34.2 General Characteristics of Antimicrobial Drugs

As Ehrlich so clearly saw, to be successful a chemotherapeutic agent must have **selective toxicity:** it must kill or inhibit the microbial pathogen while damaging the host as little as possible. The degree of selective toxicity may be expressed in terms of (1) the therapeutic dose, the drug level required for clinical treatment of a particular infection, and (2) the toxic dose, the drug level at which the agent becomes too toxic for the host. The **therapeutic index** is the ratio of the toxic dose to the therapeutic dose. The larger the therapeutic index, the better the chemotherapeutic agent (all other things being equal).

A drug that disrupts a microbial function not found in eucaryotic animal cells often has a greater selective toxicity and a higher therapeutic index. For example, penicillin inhibits bacterial cell wall peptidoglycan synthesis but has little effect on host cells because they lack cell walls; therefore penicillin's therapeutic index is high. A drug may have a low therapeutic index because it inhibits the same process in host cells or damages the host in other ways. The undesirable effects on the host, or side effects, are of many kinds and may involve almost any organ system (**table 34.1**). Because side effects can be severe, chemotherapeutic agents should be administered with great care.

Some bacteria and fungi are able to naturally produce many of the commonly employed antibiotics (**table 34.2**). In contrast, several important chemotherapeutic agents, such as sulfonamides, trimethoprim, chloramephenicol, ciprofloxacin, isoniazid, and dapsone, are synthetic—that is, manufactured by chemical procedures independent of microbial activity (table 34.1). An increasing number of antibiotics are semisynthetic—they are natural antibiotics that have been structurally modified by the addition of chemical groups to make them less susceptible to inactivation by pathogens (e.g., ampicillin, carbenicillin, and methicillin). In addition, many semisynthetic drugs have a broader spectrum of antibiotic activity than does their parent molecule. This is particularly true of the semisynthetic penicillins (e.g., ampicillin, amoxycillin) versus the naturally produced penicillin G and penicillin V. It is likely that the manufacture of newer semisynthetic antimicrobials will increase in the coming years as the rise in microbes resistant to existing antibiotics continues to grow and newer drugs must be introduced.

Drugs vary considerably in their range of effectiveness. Many are **narrow-spectrum drugs**—that is, they are effective only against a limited variety of pathogens (table 34.1). Others are **broad-spectrum drugs** that attack many different kinds of pathogens. Drugs may also be classified based on the general microbial group they act against: antibacterial, antifungal, antiprotozoan, and antiviral. Some agents can be used against more than one group; for example, sulfonamides are active against bacteria and some protozoa. Chemotherapeutic agents, like disinfectants, can be either **cidal** or **static.** Static agents reversibly inhibit growth; if the agent is removed, the microorganisms will recover and grow again. The pattern of microbial death (section 7.2)

Although a cidal agent kills the target pathogen, its activity is concentration dependent and the agent may be only static at low

Table 34.1 Properties of Some Common Antibacterial Drugs

Antibiotic Group	Primary Effect	Mechanism of Action	Members	Spectrum	Common Side Effects
Cell Wall Synthesis Inhibition					
Penicillins	Cidal	Inhibit transpeptidation enzymes involved in cross-linking the polysaccharide chains of the bacterial cell wall peptidoglycan. Activate cell wall lytic enzymes.	Penicillin G, penicillin V, methicillin	Narrow (gram-positive)	Allergic responses (diarrhea, anemia, hives, nausea, renal toxicity)
			Ampicillin, carbenicillin	Broad (gram-positive, some gram-negative)	
Cephalosporins	Cidal	Same as above	Cephalothin, cefoxitin, cefaperazone, ceftriaxone	Broad (gram-positive, some gram-negative)	Allergic responses, thrombophlebitis, renal injury
Vancomycin	Cidal	Prevents transpeptidation of peptidoglycan subunits by binding to D-Ala-D-Ala amino acids at the end of peptide cross-bridges. Thus it has a different binding site than that of the penicillins.	Vancomycin	Narrow (gram-positive)	Ototoxic (tinnitus and deafness), nephrotoxic, allergic reactions
Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly inhibiting synthesis and causing misreading of mRNA	Neomycin, kanamycin, gentamicin	Broad (gram-negative, mycobacteria)	Deafness, renal damage, loss of balance, nausea, allergic responses
			Streptomycin	Narrow (aerobic gram-negative)	Same as above
Tetracyclines	Static	Same as above	Oxytetracycline, chlortetracycline	Broad (gram-positive and -negative, rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration, renal, hepatic injury
Macrolides	Static	Bind to 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin, clindamycin	Broad (aerobic and anaerobic gram-positive, some gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic responses
Chloramphenicol	Static	Same as above	Chloramphenicol	Broad (gram-positive and -negative, rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions
Nucleic Acid Synthesis Inhibition					
Quinolones and Fluoroquinolones	Cidal	Inhibit DNA gyrase and topoisomerase IV, thereby blocking DNA replication and transcription	Norfloxacin, ciprofloxacin,	Narrow (gram-negatives better than gram-positives)	Tendonitis, headache, lightheadedness, convulsions, allergic reactions
			Levofloxacin	Broad spectrum	

Rifampin	Cidal	Inhibits bacterial DNA-dependent RNA polymerase	R-Cin, rifacilin, rifamycin, rimactane, rimpin, siticox	*Mycobacterium* infections and some gram-negative such as *Neisseria meningitidis* and *Haemophilus influenzae* b	Nausea, vomiting, diarrhea, fatigue, anemia, drowsiness, headache, mouth ulceration, liver damage
Cell Membrane Disruption					
Polymyxin B	Cidal	Binds to plasma membrane and disrupts its structure and permeability properties	Polymyxin B, polymyxin topical ointment	Narrow—gram-negatives only	Can cause severe kidney damage, drowsiness, dizziness
Antimetabolites					
Sulfonamides	Static	Inhibits folic acid synthesis by competing with ρ-aminobenzoic acid (PABA)	Silver sulfadiazine, sodium sulfacetamide, sulfamethoxazole, sulfanilamide, sulfasalazine, sulfisoxazole	Broad spectrum	Nausea, vomiting, and diarrhea; hypersensitivity reactions such as rashes, photosensitivity
Trimethoprim	Static	Blocks folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase	Trimethoprim (in combination with a sulfamethoxazole [1:5])	Broad spectrum	Same as sulfonamides, but less frequent
Dapsone	Static	Thought to interfere with folic acid synthesis	Dapsone	Narrow—mycobacterial infections, principally leprosy	Back, leg, or stomach pains; discolored fingernails, lips, or skin; breathing difficulties fever, loss of appetite, skin rash, fatigue
Isoniazid	Cidal if bacteria are actively growing, static if bacteria are dormant	Exact mechanism is unclear, but it is thought to inhibit lipid synthesis (especially mycolic acid); putative enoyl-reductase inhabitor	Isoniazid	Narrow—mycobacterial infections, principally tuberculosis	Nausea, vomiting, liver damage, seizures, “pins and needles” in extremities (peripheral neuropathy)

Table 34.2 Microbial Sources of Some Antibiotics

Microorganism	Antibiotic
Bacteria	
Streptomyces spp.	Amphotericin B
	Chloramphenicol (also synthetic)
	Kanamycin
	Neomycin
	Nystatin
	Rifampin
	Streptomycin
	Tetracyclines
	Vancomycin
Micromonospora spp.	Gentamicin
Bacillus spp.	Bacitracin
	Polymyxins
Fungi	
Penicillium spp.	Griseofulvin
	Penicillin
Cephalosporium spp.	Cephalosporins

levels. The effect of an agent also varies with the target species: an agent may be cidal for one species and static for another. Because static agents do not directly destroy the pathogen, elimination of the infection depends on the host's own resistance mechanisms. A static agent may not be effective if the host's resistance is too low.

Some idea of the effectiveness of a chemotherapeutic agent against a pathogen can be obtained from the **minimal inhibitory concentration (MIC).** The MIC is the lowest concentration of a drug that prevents growth of a particular pathogen. On the other hand, the **minimal lethal concentration (MLC)** is the lowest drug concentration that kills the pathogen. A cidal drug generally kills pathogens at levels only two to four times the MIC, whereas a static agent kills at much higher concentrations (if at all).

1. Define the following terms: selective toxicity, therapeutic index, side effect, narrow-spectrum drug, broad-spectrum drug, synthetic and semisynthetic antibiotics, cidal and static agents, minimal inhibitory concentration (MIC), and minimal lethal concentration (MLC).
2. Why is it necessary to make synthetic and semisynthetic antibiotics?
3. Use the MIC and MLC concepts to distinguish between cidal and static agents.

34.3 Determining the Level of Antimicrobial Activity

Determination of antimicrobial effectiveness against specific pathogens is essential to proper therapy. Testing can show which agents are most effective against a pathogen and give an estimate of the proper therapeutic dose.

Dilution Susceptibility Tests

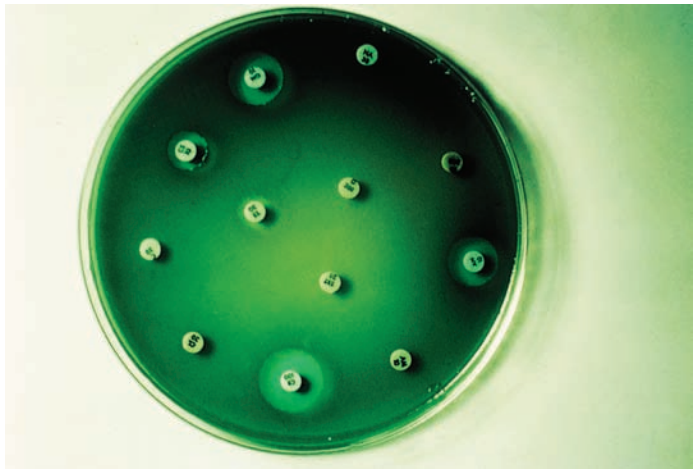
Dilution susceptibility tests can be used to determine MIC and MLC values. Antibiotic dilution tests can be done in both agar and broth. In the broth dilution test, a series of broth tubes (usually Mueller-Hinton broth) containing antibiotic concentrations in the range of 0.1 to 128 μg/ml (2-fold dilutions) is prepared and inoculated with a standard density of the test organism. The lowest concentration of the antibiotic resulting in no growth after 16 to 20 hours of incubation is the MIC. The MLC can be ascertained if the tubes showing no growth are subcultured into fresh medium lacking antibiotic. The lowest antibiotic concentration from which the microorganisms do not grow when transferred to fresh medium is the MLC. The agar dilution test is very similar to the broth dilution test. Plates containing Mueller-Hinton agar and various amounts of antibiotic are inoculated and examined for growth. Several automated systems for susceptibility testing and MIC determination with broth or agar cultures have been developed.

Disk Diffusion Tests

If a rapidly growing aerobic or facultative pathogen like *Staphylococcus* or *Pseudomonas* is being tested, a disk diffusion technique may be used to save time and media. The principle behind the assay technique is fairly simple. When an antibiotic-impregnated disk is placed on agar previously inoculated with the test bacterium, the antibiotic diffuses radially outward through the agar, producing an antibiotic concentration gradient. The antibiotic is present at high concentrations near the disk and affects even minimally susceptible microorganisms (resistant organisms will grow up to the disk). As the distance from the disk increases, the antibiotic concentration decreases and only more susceptible pathogens are harmed. A clear zone or ring is present around an antibiotic disk after incubation if the agent inhibits bacterial growth. The wider the zone surrounding a disk, the more susceptible the pathogen is. Zone width also is a function of the antibiotic's initial concentration, its solubility, and its diffusion rate through agar. Thus zone width cannot be used to compare directly the effectiveness of two different antibiotics.

Currently the disk diffusion test most often used is the **Kirby-Bauer method,** which was developed in the early 1960s at the University of Washington Medical School by William Kirby, A.W. Bauer, and their colleagues. An inoculating loop or needle is touched to four or five isolated colonies of the pathogen growing on agar and then used to inoculate a tube of culture broth. The culture is incubated for a few hours at 35°C until it becomes slightly turbid and is diluted to match a turbidity standard. A sterile cotton swab is dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a Mueller-Hinton agar plate. After the agar surface has dried for about 5 minutes, the appropriate antibiotic test disks are placed on it, either with sterilized forceps or with a multiple applicator device (**figure 34.2**). The plate is immediately placed in a 35°C incubator. After 16 to 18 hours of incubation, the diameters of the zones of inhibition are measured to the nearest mm.

(a)



(b)

Figure 34.2 The Kirby-Bauer Method. **(a)** A multiple antibiotic disk dispenser and **(b)** disk diffusion test results.

Kirby-Bauer test results are interpreted using a table that relates zone diameter to the degree of microbial resistance (**table 34.3**). The values in table 34.3 were derived by finding the MIC values and zone diameters for many different microbial strains. A plot of MIC (on a logarithmic scale) versus zone inhibition diameter (arithmetic scale) is prepared for each antibiotic (**figure 34.3**). These plots are then used to find the zone diameters corresponding to the drug concentrations actually reached in the body. If the zone diameter for the lowest level reached in the body is smaller than that seen with the test pathogen, the pathogen should have an MIC value low enough to be destroyed by the drug. A pathogen with too high a MIC value (too small a zone diameter) is resistant to the agent at normal body concentrations.

The Etest

The Etest from AB Biodisk may be used in sensitivity testing under some conditions. It is particularly convenient for use with anaerobic pathogens. A petri dish of the proper agar is streaked in three different directions with the test organism and special plastic Etest® strips are placed on the surface so that they extend out radially from the center (**figure 34.4**). Each strip contains a gradient of an antibiotic and is labeled with a scale of minimal inhibitory concentration values. The lowest concentration in the strip lies at the center of the plate. After 24 to 48 hours of incubation, an elliptical zone of inhibition appears. As shown in the figure, MICs are determined from the point of intersection between the inhibition zone and the strip's scale of MIC values.

Measurement of Drug Concentrations in the Blood

A drug must reach a concentration at the site of infection above the pathogen's MIC to be effective. In cases of severe, life-threatening disease, it often is necessary to monitor the concentration of drugs in the blood and other body fluids. This may be achieved by microbiological, chemical, immunologic, enzymatic, or chromatographic assays. Extra care is needed to also evaluate antibiotic binding to serum proteins and are thus unavailable for measurement by common antibiotic assays.

1. How can dilution susceptibility tests and disk diffusion tests be used to determine microbial drug sensitivity?
2. Briefly describe the Kirby-Bauer test and its purpose.
3. How is the Etest carried out?

34.4 Antibacterial Drugs

Since Fleming's discovery of penicillin, natural antibiotics (table 34.2) have been found that can damage pathogens in several ways. A few antibacterial drugs are described here and summarized in table 34.1, with emphasis on their mechanisms of action.

Inhibitors of Cell Wall Synthesis

The most selective antibiotics are those that interfere with bacterial cell wall synthesis. Drugs like penicillins, cephalosporins, vancomycin, and bacitracin have a high therapeutic index because they target structures not found in eukaryotic cells. The bacterial cell wall (section 3.6)

Penicillins

Most **penicillins** (e.g., penicillin G or benzylpenicillin) are derivatives of 6-aminopenicillanic acid and differ from one another with

Table 34.3 Inhibition Zone Diameter of Selected Chemotherapeutic Drugs

Chemotherapeutic Drug	Disk Content	Zone Diameter (Nearest mm)		
		Resistant	Intermediate	Susceptible
Carbenicillin (with *Proteus* spp. and *E. coli*)	100 μg	≤17	18–22	≥23
Carbenicillin (with *Pseudomonas aeruginosa*)	100 μg	≤13	14–16	≥17
Ceftriaxone	30 μg	≤13	14–20	≥21
Chloramphenicol	30 μg	≤12	13–17	≥18
Erythromycin	15 μg	≤13	14–17	≥18
Penicillin G (with staphylococci)	10 U[a]	≤20	21–28	≥29
Penicillin G (with other microorganisms)	10 U	≤11	12–21	≥22
Streptomycin	10 μg	≤11	12–14	≥15
Sulfonamides	250 or 300 μg	≤12	13–16	≥17
Tetracycline	30 μg	≤14	15–18	≥19

[a]One milligram of penicillin G sodium = 1,600 units (U).

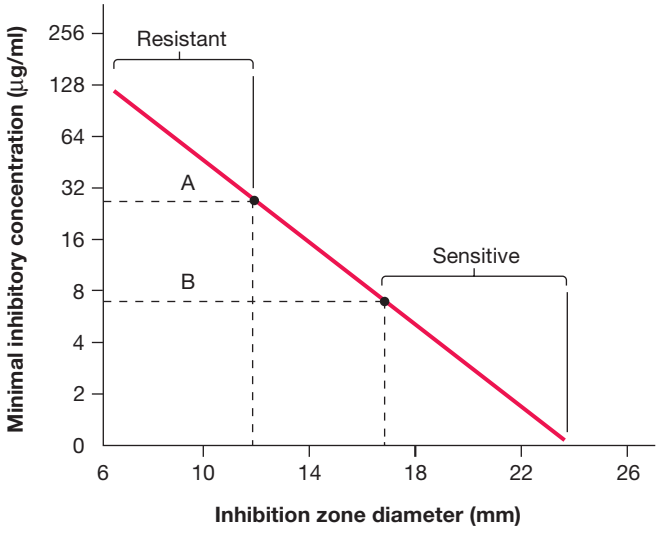


Figure 34.3 Interpretation of Kirby-Bauer Test Results. The relationship between the minimal inhibitory concentrations of a hypothetical drug and the size of the zone around a disk in which microbial growth is inhibited. As the sensitivity of microorganisms to the drug increases, the MIC value decreases and the inhibition zone grows larger. Suppose that this drug varies from 7–28 μg/ml in the body during treatment. Dashed line A shows that any pathogen with a zone of inhibition less than 12 mm in diameter will have an MIC value greater than 28 μg/ml and will be resistant to drug treatment. A pathogen with a zone diameter greater than 17 mm will have an MIC less than 7 μg/ml and will be sensitive to the drug (see line B). Zone diameters between 12 and 17 mm indicate intermediate sensitivity and usually signify resistance.

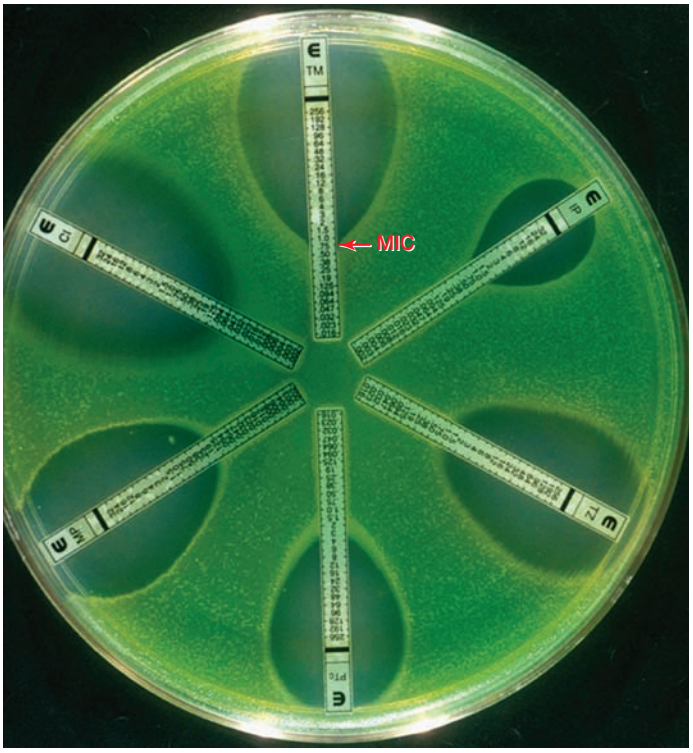


Figure 34.4 The Etest®. An example of a bacterial culture plate with Etest® strips arranged radially on it. The strips are arranged so that the lowest antibiotic concentration in each is at the center. The MIC concentration is read from the scale at the point it intersects the zone of inhibition as shown by the arrow in this example. Etest® is a registered trademark of AB BIODISK and patented in all major markets.

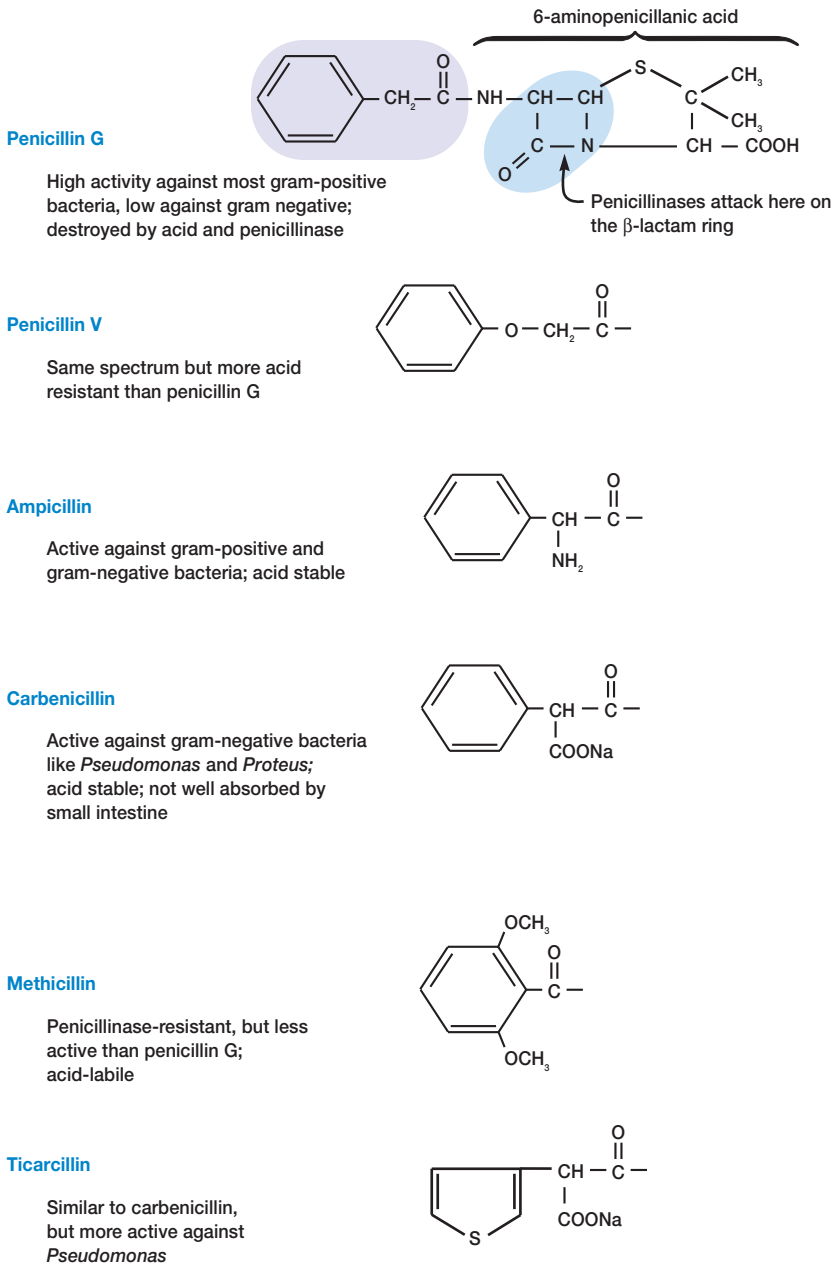


Figure 34.5 Penicillins. The structures and characteristics of representative penicillins. All are derivatives of 6-aminopenicillanic acid; in each case the shaded portion of penicillin G is replaced by the side chain indicated. The β-lactam ring is also shaded (blue), and an arrow points to the bond that is hydrolyzed by penicillinase.

respect to the side chain attached to its amino group (**figure 34.5**). The most crucial feature of the molecule is the **β-lactam ring,** which is essential for bioactivity. Many penicillin-resistant bacteria produce **penicillinase** (also called **β-lactamase**), an enzyme that inactivates the antibiotic by hydrolyzing a bond in the β-lactam ring.

Although the complete mechanism of action of penicillins is still not completely known, their structures resemble the terminal D-alanyl-D-alanine found on the peptide side chain of the peptidoglycan subunit. It has been proposed that this structural similarity blocks the enzyme catalyzing the transpeptidation reaction that forms the peptidoglycan cross-links (*see figure 10.12*). Thus formation of a complete cell wall is blocked, leading to osmotic lysis. This mechanism is consistent with the observation that penicillins act only on growing bacteria that are synthesizing new peptidoglycan.

Evidence has indicated that the mechanism of penicillin action is even more complex than previously imagined. It has been discovered that penicillins bind to several periplasmic proteins (penicillin-binding proteins, or PBPs) and may also destroy bacteria by activating their own autolytic enzymes. However, there is also some evidence that penicillin kills bacteria even in the absence of autolysins or murein hydrolases. Lysis could occur after bacterial viability has already been lost. Penicillin may stimulate special proteins called bacterial holins to form holes or lesions in the plasma membrane, leading directly to membrane leakage and

death. Murein hydrolases also could move through the holes, disrupt the peptidoglycan, and lyse the cell.

Penicillins differ from each other in several ways. The two naturally occurring penicillins, penicillin G and penicillin V, are narrow-spectrum drugs. Penicillin G is effective against gonococci, meningococci, and several gram-positive pathogens such as streptococci and staphylococci. However, it must be administered by injection (parenterally) because it is destroyed by stomach acid. Penicillin V (figure 34.5) is similar to penicillin G in spectrum of activity, but can be given orally because it is more resistant to acid. The semisynthetic penicillins, on the other hand, have a broader spectrum of activity. Ampicillin can be administered orally and is effective against gram-negative bacteria such as *Haemophilus*, *Salmonella*, and *Shigella*. Carbenicillin and ticarcillin are potent against *Pseudomonas* and *Proteus*.

An increasing number of bacteria have become resistant to natural penicillins and many of the semisynthetic analogs. Physicians frequently employ specific semisynthetic penicillins that are not destroyed by β-lactamases to combat antibiotic-resistant pathogens. These include methicillin (figure 34.5), nafcillin, and oxacillin. However, this practice has been confounded by the emergence of meticillin-resistant bacteria.

Although penicillins are the least toxic of the antibiotics, about 1 to 5% of the adults in the United States are allergic to them. Occasionally, a person will die of a violent allergic response; therefore, patients should be questioned about penicillin allergies before treatment is begun. Immune disorders: Hypersensitivities (section 32.11)

Cephalosporins

Cephalosporins are a family of antibiotics originally isolated in 1948 from the fungus *Cephalosporium*. They contain a **β-lactam** structure that is very similar to that of the penicillins (**figure 34.6**). As might be expected from their structural similarities to penicillins, cephalosporins also inhibit the transpeptidation reaction during peptidoglycan synthesis. They are broad-spectrum drugs frequently given to patients with penicillin allergies (although about 10% of patients allergic to penicillin are also allergic to cephalosporins).

Many cephalosporins are in use. Cephalosporins are broadly categorized into four generations (groups of drugs that are sequentially developed) based on their spectrum of activity. First-generation cephalosporins are more effective against gram-positive pathogens than gram-negatives. Second-generation drugs, developed after the first generation, have improved effects on gram-

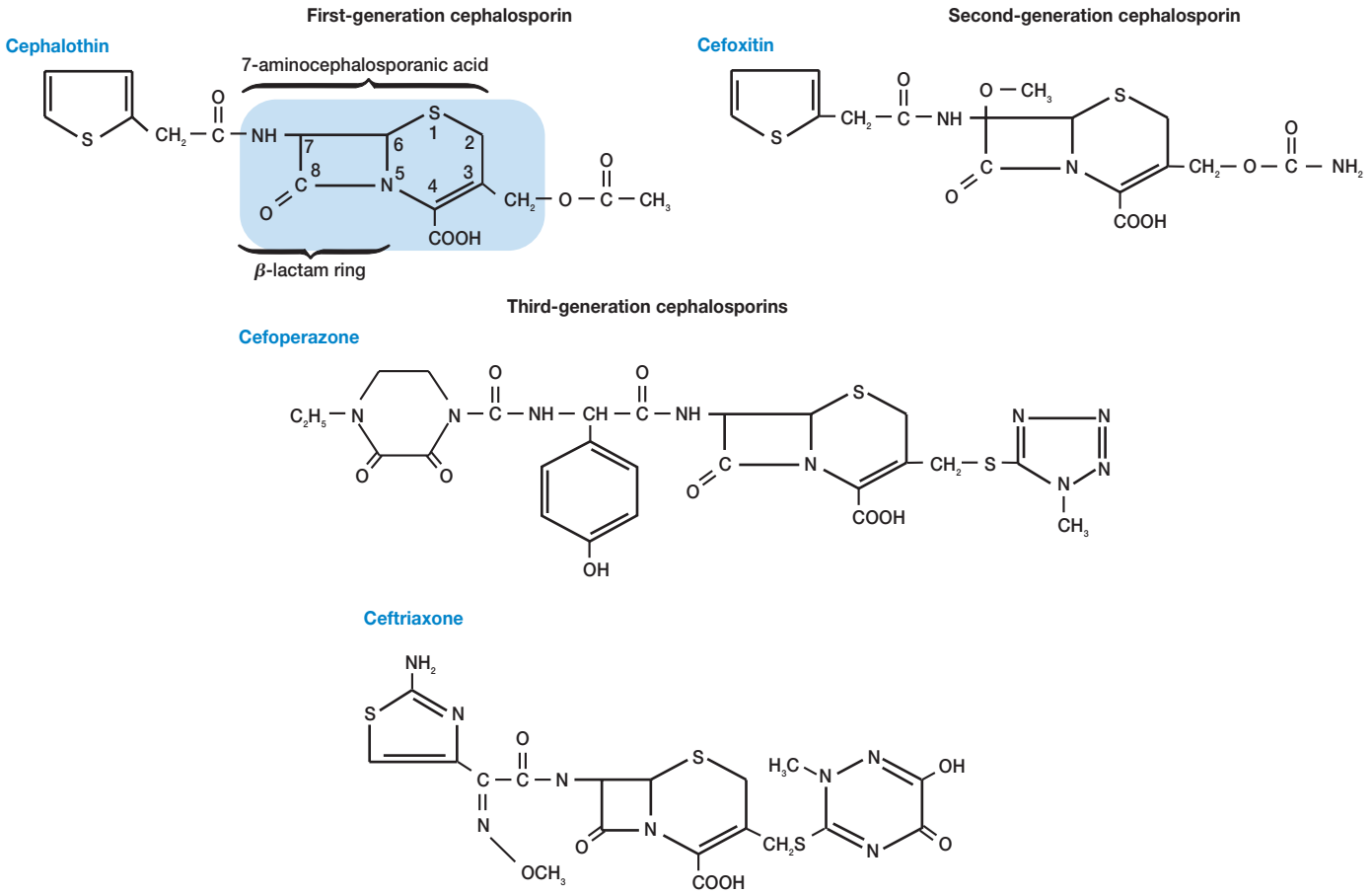


Figure 34.6 Cephalosporin Antibiotics. These drugs are derivatives of 7-aminocephalosporanic acid and contain a β-lactam ring.

negative bacteria with some anaerobe coverage. Third-generation drugs are particularly effective against gram-negative pathogens, and some reach the central nervous system. This is of particular note because many antimicrobial agents do not cross the blood-brain barrier. Finally, fourth-generation cephalosporins are broad spectrum with excellent gram-positive and gram-negative coverage and, like their third-generation predecessors, inhibit the growth of the difficult opportunistic pathogen *Pseudomonas aeruginosa*.

Vancomycin and Teicoplanin

Vancomycin is a glycopeptide antibiotic produced by *Streptomyces oreintalis*. It is a cup-shaped molecule composed of a peptide linked to a disaccharide. Vancomycin's peptide portion blocks the transpeptidation reaction by binding specifically to the D-alanine-D-alanine terminal sequence on the pentapeptide portion of peptidoglycan. The antibiotic is bactericidal for *Staphylococcus* and some members of the genera *Clostridium*, *Bacillus*, *Streptococcus*, and *Enterococcus*. It is given both orally and intravenously and has been particularly important in the treatment of antibiotic-resistant staphylococcal and enterococcal infections. However, vancomycin-resistant strains of *Enterococcus* have become widespread and cases of resistant *Staphylococcus aureus* have appeared. This poses a serious public health threat—vancomycin has been considered the "drug of last resort" in cases of antibiotic-resistant *S. aureus*. Clearly newer drugs must be developed.

Teicoplanin is a glycopeptide antibiotic from the actinomycete *Actinoplanes teichomyceticus* that is similar in structure and mechanism of action to vancomycin, but has fewer side effects. It is active against staphylococci, enterococci, streptococci, clostridia, *Listeria*, and many other gram-positive pathogens.

Protein Synthesis Inhibitors

Many antibiotics inhibit protein synthesis by binding with the procaryotic ribosome. Because these drugs discriminate between procaryotic and eucaryotic ribosomes, their therapeutic index is fairly high, but not as high as that of cell wall inhibitors. Some drugs bind to the 30S (small) ribosomal subunit, while others attach to the 50S (large) subunit. Several different steps in protein synthesis can be affected: aminoacyl-tRNA binding, peptide bond formation, mRNA reading, and translocation. Translation (section 11.8)

Aminoglycosides

Although there is considerable variation in structure among several important **aminoglycoside antibiotics,** all contain a cyclohexane ring and amino sugars (**figure 34.7**). **Streptomycin,** kanamycin, neomycin, and tobramycin are synthesized by different species of the genus *Streptomyces*, whereas gentamicin comes from another actinomycete, *Micromonospora purpurea*. Aminoglycosides bind to the 30S (small) ribosomal subunit and interfere with protein synthesis by directly inhibiting the synthesis process and also by causing misreading of the mRNA.

These antibiotics are bactericidal and tend to be most effective against gram-negative pathogens. Streptomycin's usefulness has decreased greatly due to widespread drug resistance, but it is still

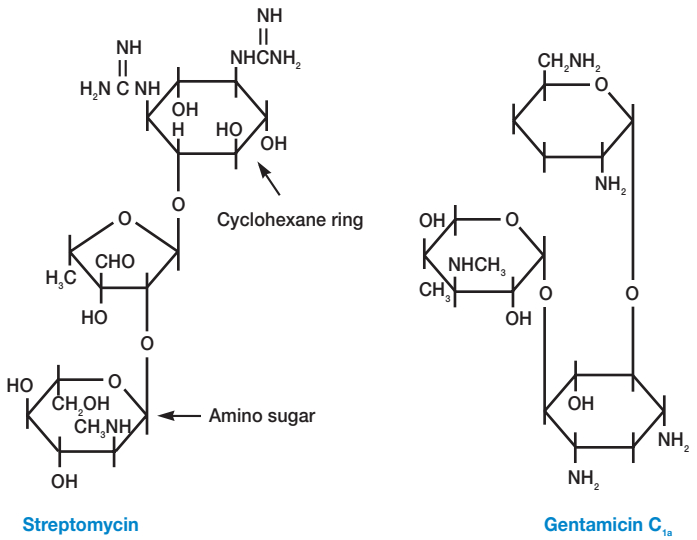


Figure 34.7 Representative Aminoglycoside Antibiotics.

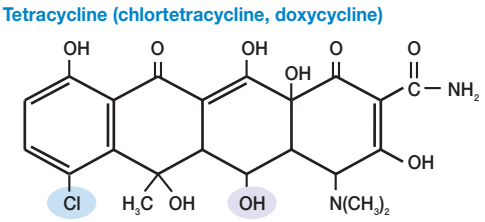


Figure 34.8 Tetracyclines. Three members of the tetracycline family. Tetracycline lacks both of the groups that are shaded. Chlortetracycline (aureomycin) differs from tetracycline in having a chlorine atom (blue): doxycycline consists of tetracycline with an extra hydroxyl (light blue).

effective in treating tuberculosis and plague. Gentamicin is used to treat *Proteus*, *Escherichia*, *Klebsiella*, and *Seratia* infections. Aminoglycosides can be quite toxic, however, and can cause deafness, renal damage, loss of balance, nausea, and allergic responses.

Tetracyclines

The **tetracyclines** are a family of antibiotics with a common four-ring structure to which a variety of side chains are attached (**figure 34.8**). Oxytetracycline and chlortetracycline are produced naturally by *Streptomyces* species while others are semisynthetic drugs. These antibiotics are similar to the aminoglycosides and combine with the 30S (small) subunit of the ribosome. This inhibits the binding of aminoacyl-tRNA molecules to the A site of the ribosome. Because their action is only bacteriostatic, the effectiveness of treatment depends on active host resistance to the pathogen.

Tetracyclines are broad-spectrum antibiotics that are active against gram-negative, as well as gram-positive, bacteria, rickettsias, chlamydiae, and mycoplasmas. High doses may result in

nausea, diarrhea, yellowing of teeth in children, and damage to the liver and kidneys. Although their use has declined in recent years, they are still sometimes used to treat acne.

Macrolides

The **macrolide antibiotics** contain 12- to 22-carbon lactone rings linked to one or more sugars (**figure 34.9**). **Erythromycin** is usually bacteriostatic and binds to the 23S rRNA of the 50S (large) ribosomal subunit to inhibit peptide chain elongation during protein synthesis. Erythromycin is a relatively broad-spectrum antibiotic effective against gram-positive bacteria, mycoplasmas, and a few gram-negative bacteria. It is used with patients who are allergic to penicillins and in the treatment of whooping cough, diphtheria, diarrhea caused by *Campylobacter,* and pneumonia from *Legionella* or *Mycoplasma* infections. Clindamycin is effective against a variety of bacteria including staphylococci, and anaerobes such as *Bacteroides*. Azithromycin, which has surpassed erythromycin in use, is particularly effective against *Chlamydia trachomatis*.

Chloramphenicol

Chloramphenicol (**figure 34.10**) was first produced from cultures of *Streptomyces venezuelae* but it is now synthesized chemically. Like erythromycin, this antibiotic binds to 23S rRNA on the 50S ribosomal subunit to inhibit the peptidyl transferase reaction. It has a very broad spectrum of activity but, unfortunately, is quite toxic. One may see allergic responses or neurotoxic reactions. The most common side effect is depression of bone marrow function, leading to aplastic anemia and a decreased number of white blood cells. Consequently, this antibiotic is used only in life-threatening situations when no other drug is adequate.

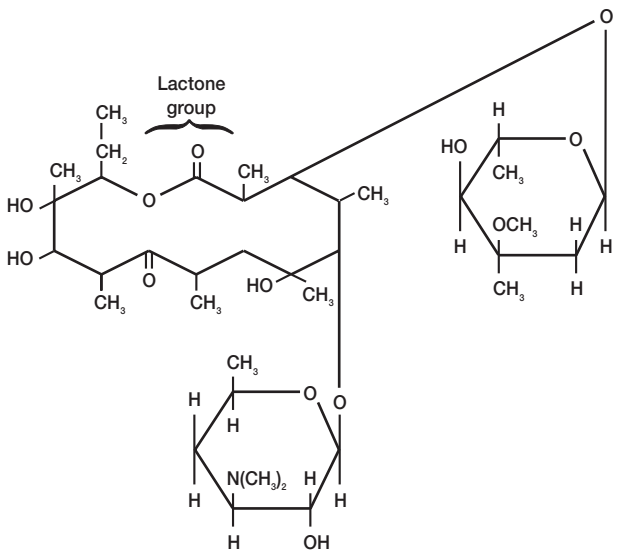


Figure 34.9 Erythromycin, a Macrolide Antibiotic. The 14-member lactone ring is connected to two sugars.

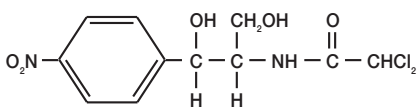


Figure 34.10 Chloramphenicol.

Metabolic Antagonists

Several valuable drugs act as **antimetabolites**—they antagonize, or block, the functioning of metabolic pathways by competitively inhibiting the use of metabolites by key enzymes. These drugs can act as structural analogs, molecules that are structurally similar to naturally occurring metabolic intermediates. These analogs compete with intermediates in metabolic processes because of their similarity, but are just different enough so that they prevent normal cellular metabolism. As such they are bacteriostatic but broad spectrum.

Sulfonamides or Sulfa Drugs

The first antimetabolites to be used successfully as chemotherapeutic agents were the sulfonamides, discovered by G. Domagk. **Sulfonamides,** or sulfa drugs, are structurally related to sulfanilamide, an analog of *p*-aminobenzoic acid, or PABA (**figures 34.11** and **34.12**). PABA is used in the synthesis of the cofactor folic acid (folate). When sulfanilamide or another sulfonamide enters a bacterial cell, it competes with PABA for the active site of an enzyme involved in folic acid synthesis, causing a decline in folate concentration. This decline is detrimental to the bacterium because folic acid is a precursor of purines and pyrimidines, the bases used in the construction of DNA, RNA, and other important cell constituents. The resulting inhibition of purine and pyrimidine synthesis leads to cessation of protein synthesis and DNA replication, thus the pathogen dies. Sulfonamides are selectively toxic for many pathogens because these bacteria manufacture their own fo-

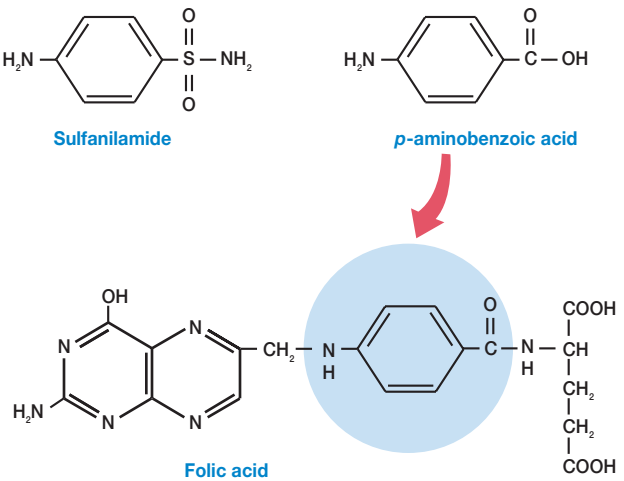


Figure 34.11 Sulfanilamide. Sulfanilamide and its relationship to the structure of folic acid.

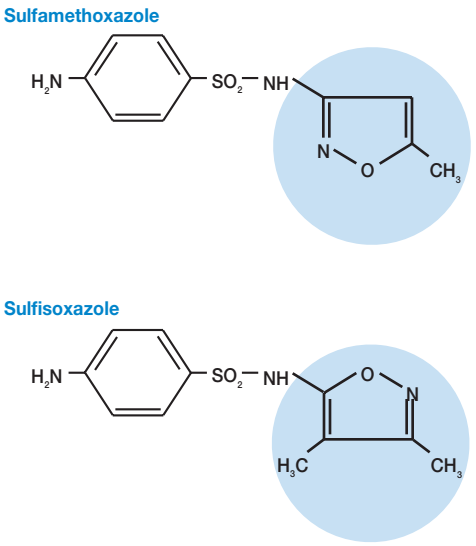


Figure 34.12 Two Sulfonamide Drugs. The blue shaded areas are side chains substituted for a hydrogen in sulfanilamide (figure 35.4).

late and cannot effectively take up this cofactor, whereas humans do not synthesize folate (we must obtain it in our diet). Sulfonamides thus have a high therapeutic index.

The increasing resistance of many bacteria to sulfa drugs limits their effectiveness. Furthermore, as many as 5% of the patients receiving sulfa drugs experience adverse side effects, chiefly allergic responses such as fever, hives, and rashes.

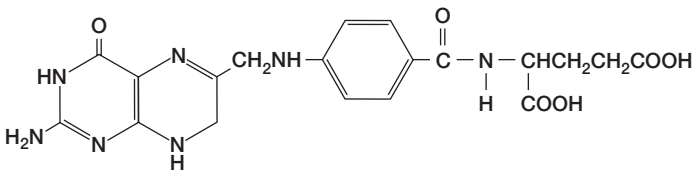
Trimethoprim

Trimethoprim is a synthetic antibiotic that also interferes with the production of folic acid. It does so by binding to dihydrofolate reductase (DHFR), the enzyme responsible for converting dihydrofolic acid to tetrahydrofolic acid, competing against the dihydrofolic acid substrate (**figure 34.13**). It is a broad-spectrum antibiotic often used to treat respiratory and middle ear infections, urinary tract infections, and traveler's diarrhea. It can be combined with sulfa drugs to increase efficacy of treatment by blocking two key steps in the folic acid pathway (**figure 34.14**). The inhibition of two successive steps in a single biochemical pathway means that less of each drug is needed in combination than when used alone. This is termed a synergistic drug interaction.

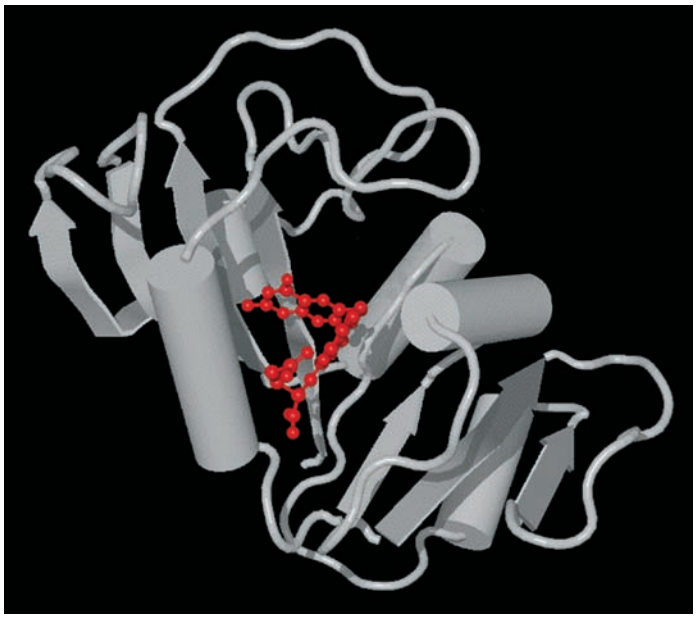
The most common side effects associated with trimethoprim are abdominal pain, abnormal taste, diarrhea, loss of appetite, nausea, swelling of the tongue, and vomiting. Taking trimethoprim with food may reduce some of these side effects. Some patients are allergic to trimethoprim, exhibiting rash and itching. Some patients develop photosensitivity reactions (i.e., rashes due to sun exposure).

Nucleic Acid Synthesis Inhibition

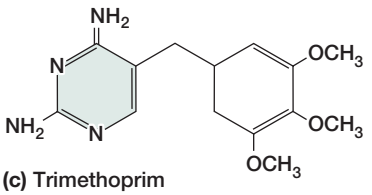
The antibacterial drugs that inhibit nucleic acid synthesis function by inhibiting DNA polymerase and DNA helicase or RNA polymerase, thus blocking processes of replication or transcription, respectively. These drugs are not as selectively toxic as other antibiotics because procaryotes and eucaryotes do not differ greatly with respect to nucleic acid synthesis.



(a) Dihydrofolic acid (DFA)



(b) Dihydrofolate reductase



(c) Trimethoprim

Figure 34.13 Competitive Inhibition of Dihydrofolate Reductase (DHFR) by Trimethoprim. **(a)** Dihydrofolic acid (DFA) is the natural substrate for the DHFR enzyme of the folic acid pathway. **(b)** DHFR structure and its interaction with DFA (red). Note the chemical structure and how it fits into the active site of the enzyme. **(c)** Trimethoprim mimics the structural orientation of the DFA and thus competes for the active site of the enzyme. The consequence of this is delayed or absent folic acid synthesis because the DFA cannot be converted to tetrahydrofolic acid when trimethoprim occupies the DHFR active site.

Quinolones

The **quinolones** are synthetic drugs that contain the 4-quinolone ring. The quinolones are important antimicrobial agents that inhibit nucleic acid synthesis. They are increasingly used to treat a wide variety of infections. The first quinolone, nalidixic acid

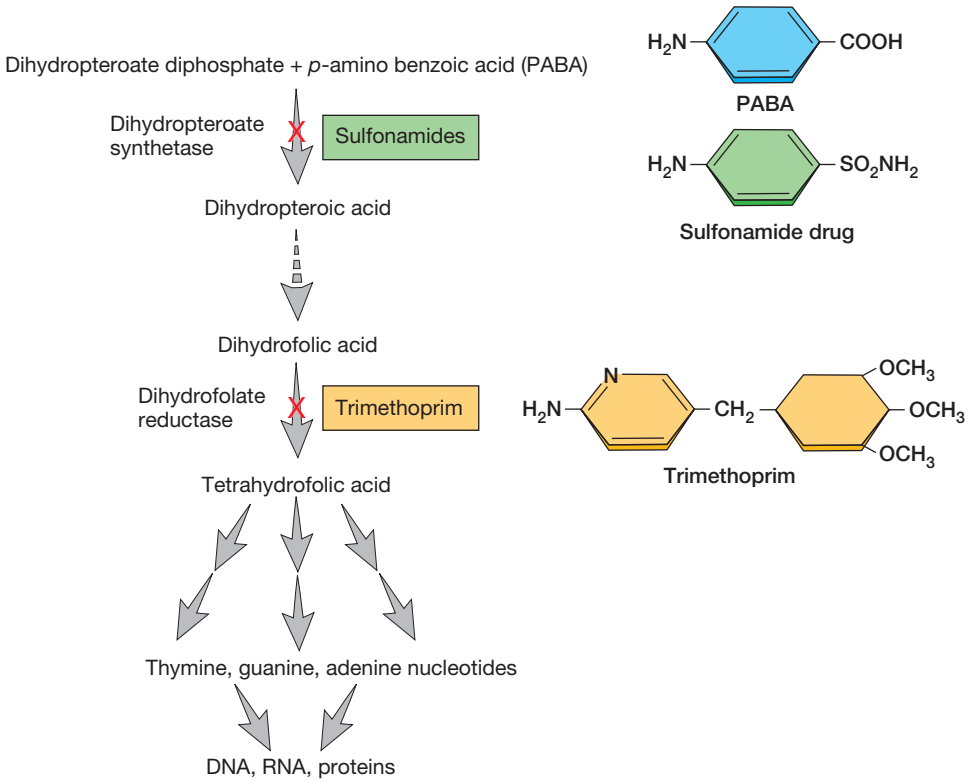


Figure 34.14 Synergistic Drug Interaction Between the Sulfonamides and Trimethoprim. Two successive steps in the biochemical pathway for folic acid synthesis are blocked by these drugs. Thus the efficacy of the drug combination is greater than that of either drug used alone.

(**figure 34.15**), was synthesized in 1962. Since that time, generations of fluoroquinolones have been produced. Three of these—ciprofloxacin, norfloxacin, and ofloxacin—are currently used in the United States, and more fluoroquinolones are being synthesized and tested. Ciprofloxacin (Cipro) gained notoriety during the 2001 bioterror attacks in the United States as one treatment for anthrax. Bioterrorism preparedness (section 36.9)

Quinolones act by inhibiting the bacterial DNA gyrase and topoisomerase II. DNA gyrase introduces negative twist in DNA and helps separate its strands (**figure 34.16**). Inhibition of DNA gyrase disrupts DNA replication and repair, bacterial chromosome separation during division, and other cell processes involving DNA. Fluoroquinolones also inhibit topoisomerase II, another enzyme that untangles DNA during replication. It is not surprising that quinolones are bactericidal. DNA replication (section 11.4)

The quinolones are broad-spectrum antibiotics. They are highly effective against enteric bacteria such as *E. coli* and *Klebsiella pneumoniae*. They can be used with *Haemophilus*, *Neiserria*, *P. aeruginosa*, and other gram-negative pathogens. The quinolones also are active against gram-positive bacteria such as *S. aureus*, *Streptococcus pyogenes*, and *Mycobacterium tuberculosis*. Currently, they are used in treating urinary tract infections, sexually transmitted diseases caused by *Neisseria* and *Chlamydia*, gastrointestinal infections, respiratory infections, skin infections, and osteomyelitis (bone infection). Quinolones are effective when administered orally but can sometimes cause diverse side effects, particularly gastrointestinal upset.

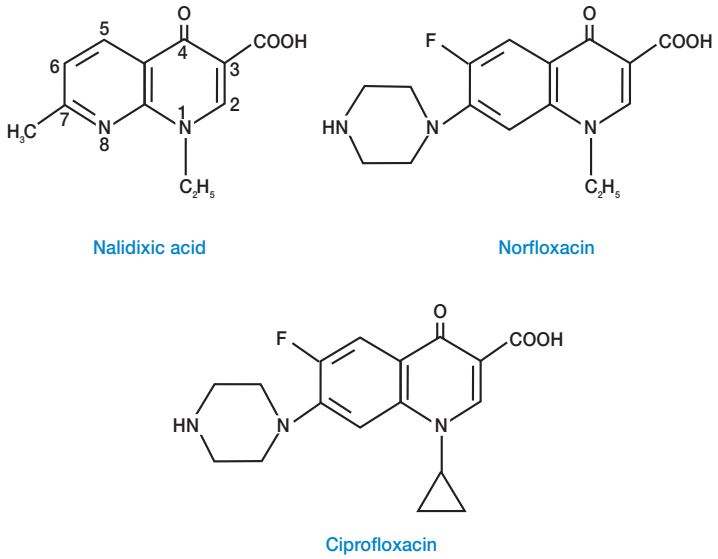


Figure 34.15 Quinolone Antimicrobial Agents. Ciprofloxacin and norfloxacin are newer generation fluoroquinolones. The 4-quinolone ring in nalidixic acid has been numbered.

1. Explain five ways in which chemotherapeutic agents kill or damage bacterial pathogens.
2. Why do penicillins and cephalosporins have a higher therapeutic index than most other antibiotics?

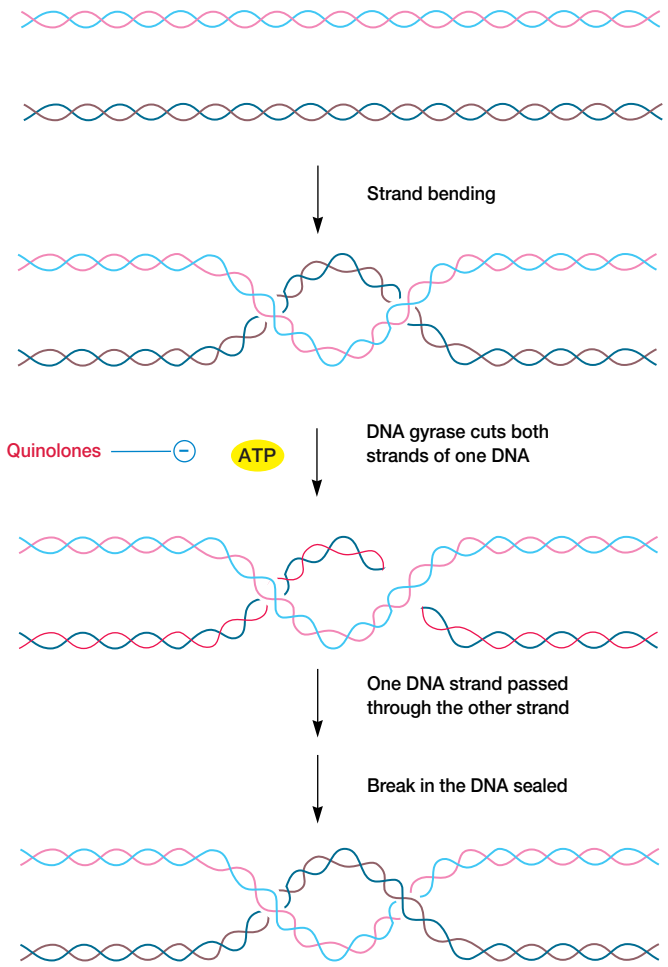


Figure 34.16 DNA Gyrase Action and Quinolone Inhibition.

3. Would there be any advantage to administering a bacteriostatic agent along with penicillins? Any disadvantage?
4. What are antimetabolites?
5. Why are some antibiotics toxic?

34.5 Factors Influencing Antimicrobial Drug Effectiveness

It is crucial to recognize that drug therapy is not a simple matter. Drugs may be administered in several different ways, and they do not always spread rapidly throughout the body or immediately kill all invading pathogens. A complex array of factors influence the effectiveness of drugs.

First, the drug must actually be able to reach the site of infection. Understanding the factors that control drug activity, stability, and metabolism in vivo are essential in drug formulation. For example, the mode of administration plays an important role. A drug such as penicillin G is not suitable for oral administration because it is relatively unstable in stomach acid. Some antibiotics—for example, gentamicin and other aminoglycosides—are not well absorbed from the intestinal tract and must be injected intramuscularly or given intravenously. Other antibiotics (neomycin, bacitracin) are so toxic that they can only be applied topically to skin lesions. Non-oral routes of administration often are called **parenteral routes.** Even when an agent is administered properly, it may be excluded from the site of infection. For example, blood clots or necrotic tissue can protect bacteria from a drug, either because body fluids containing the agent may not easily reach the pathogens or because the agent is absorbed by materials surrounding it.

Second, the pathogen must be susceptible to the drug. Bacteria in biofilms or abscesses may be replicating very slowly and are therefore resistant to chemotherapy, because many agents affect pathogens only if they are actively growing and dividing. A pathogen, even though growing, may simply not be susceptible to a particular agent. For example, penicillins and cephalosporins, which inhibit cell wall synthesis (table 34.1), do not harm mycoplasmas, which lack cell walls. To control resistance, drug cocktails can be used to treat some infections. A notable example of this is the use of clavulonic acid (to inactivate penicillinase) combined with ampicillin (Augmentin) to treat penicillin-resistant bacteria.

Third, the chemotherapeutic agent must exceed the pathogen's MIC value if it is going to be effective. The concentration reached will depend on the amount of drug administered, the route of administration and speed of uptake, and the rate at which the drug is cleared or eliminated from the body. It makes sense that a drug will remain at high concentrations longer if it is absorbed over an extended period and excreted slowly.

Finally, chemotherapy has been rendered less effective and much more complex by the spread of drug-resistance genes.

1. Briefly discuss the factors that influence the effectiveness of antimicrobial drugs.
2. What is parenteral administration of a drug?

34.6 Drug Resistance

The spread of drug-resistant pathogens is one of the most serious threats to public health in the 21st century (**Disease 34.2**). This section describes the ways in which bacteria acquire drug resistance and how resistance spreads within a bacterial population.

Mechanisms of Drug Resistance

The long-awaited "superbug" arrived in the summer of 2002. *S. aureus,* a common but sometimes deadly bacterium, had acquired a new antibiotic-resistance gene. The new strain was isolated from foot ulcers on a diabetic patient in Detroit, Michigan. Meticillin-resistant (formerly methicillin-resistant) *S. aureus* (MRSA) had been well known as the bane of hospitals. This newer strain had developed resistance to vancomycin, one of the few antibiotics that was still able to control *S. aureus*. This new vancomycin-resistant

34.2 Antibiotic Misuse and Drug Resistance

The sale of antimicrobial drugs is big business. In the United States millions of pounds of antibiotics valued at billions of dollars are produced annually. As much as 70% of these antibiotics are added to livestock feed.

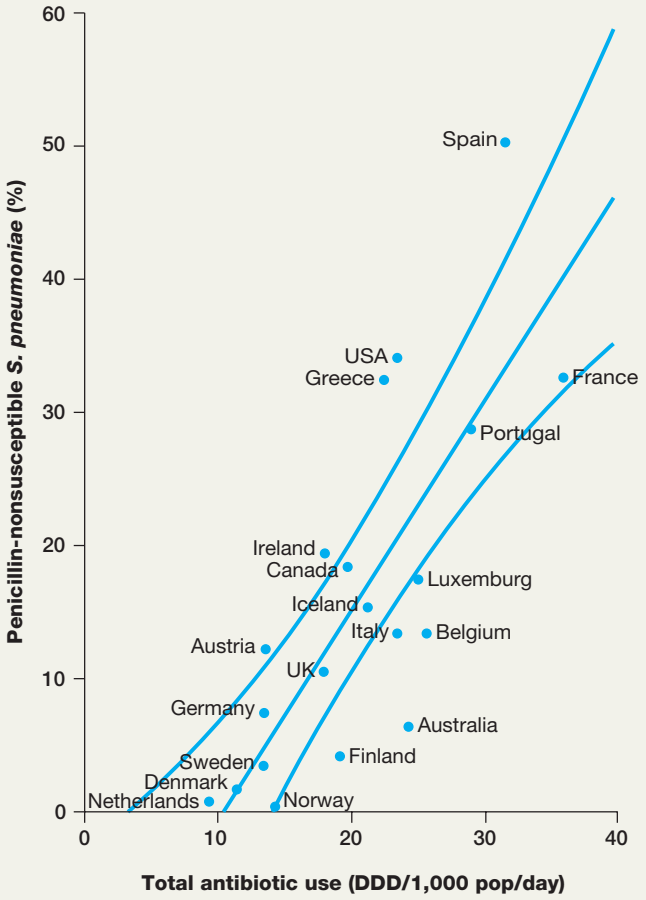
Because of the massive quantities of antibiotics being prepared and used, an increasing number of diseases are resisting treatment due to the spread of drug resistance. A good example is *Neisseria gonorrhoeae*, the causative agent of gonorrhea. Gonorrhea was first treated successfully with sulfonamides in 1936, but by 1942 most strains were resistant and physicians turned to penicillin. Within 16 years a penicillin-resistant strain emerged in Asia. A penicillinase-producing gonococcus reached the United States in 1976 and is still spreading in this country. Thus penicillin is no longer used to treat gonorrhea.

In late 1968 an epidemic of dysentery caused by *Shigella* broke out in Guatemala and affected at least 112,000 persons; 12,500 deaths resulted. The strains responsible for this devastation carried an R plasmid conferring resistance to chloramphenicol, tetracycline, streptomycin, and sulfonamide. In 1972 a typhoid epidemic swept through Mexico producing 100,000 infections and 14,000 deaths. It was due to a *Salmonella* strain with the same multiple-drug-resistance pattern seen in the previous *Shigella* outbreak.

Haemophilus influenzae type b is responsible for many cases of childhood pneumonia and middle ear infections, as well as respiratory infections and meningitis. It is now becoming increasingly resistant to tetracyclines, ampicillin, and chloramphenicol. Similarly, the worldwide rate of penicillin-nonsusceptible (i.e., resistant) *Streptococcus pneumoniae* (PNSP) continues to increase. There is a direct correlation between the daily use of antibiotics (expressed as defined daily dose [DDD] per day) and the percent of PNSP isolates cultured (**Box figure**). This dramatic correlation is alarming. More alarming is the continued indiscriminant use of antibiotics in light of these data.

In 1946 almost all strains of *Staphylococcus* were penicillin sensitive. Today most hospital strains are resistant to penicillin G, and some are now also resistant to methicillin and/or gentamicin and only can be treated with vancomycin. Some strains of *Enterococcus* have become resistant to most antibiotics, including vancomycin. Recently a few cases of vancomycin-resistant *S. aureus* have been reported in the United States and Japan. At present these strains are only intermediately resistant to vancomycin. If full vancomycin resistance spreads, *S. aureus* may become untreatable.

It is clear from these and other examples (e.g., multiresistant *Mycobacterium tuberculosis*) that drug resistance is an extremely serious public health problem. Much of the difficulty arises from drug misuse. Drugs frequently have been overused in the past. It has been estimated that over 50% of the antibiotic prescriptions in hospitals are given without clear evidence of infection or adequate medical indication. Many physicians have administered antibacterial drugs to patients with colds, influenza, viral pneumonia, and other viral diseases. A recent study showed that over 50% of the patients diagnosed with colds and upper respiratory infections and 66% of those with chest colds (bronchitis) are given antibiotics, even though over 90% of these cases are caused by viruses. Frequently antibiotics are prescribed without culturing and identifying the pathogen or without determining bacterial sensitivity to the drug. Toxic, broad-spectrum antibiotics are sometimes given in place of narrow-spectrum drugs as a substitute for culture and sensitivity testing, with the consequent risk of dangerous side effects, opportunistic infections, and the selection of drug-resistant mutants. The situation is made worse by patients not completing their course of medication. When antibiotic treatment is ended too early, drug-resistant mutants may survive. Drugs are available without prescription to the public in many countries; people may practice self-administration of antibiotics and further increase the prevalence of drug-resistant strains.



The use of antibiotics in animal feeds is undoubtedly another contributing factor to increasing drug resistance. The addition of low levels of antibiotics to livestock feeds raises the efficiency and rate of weight gain in cattle, pigs, and chickens (partially because of infection control in overcrowded animal populations). However, this also increases the number of drug-resistant bacteria in animal intestinal tracts. There is evidence for the spread of bacteria such as *Salmonella* from animals to human populations. In 1983, 18 people in four midwestern states were infected with a multiple-drug-resistant strain of *Salmonella newport.* Eleven were hospitalized for salmonellosis and one died. All 18 patients had recently been infected by eating hamburger from beef cattle fed subtherapeutic doses of chlortetracycline for growth promotion. Resistance to some antibiotics has been traced to the use of specific farmyard antibiotics. Avoparcin resembles vancomycin in structure, and virginiamycin resembles Synercid. There is good circumstantial evidence that extensive use of these two antibiotics in animal feed has led to an increase in vancomycin and Synercid resistance among enterococci. The use of the quinolone antibiotic enrofloxacin in swine herds appears to have promoted ciprofloxacin resistance in pathogenic strains of *Salmonella*. In 2005, the use of fluoroquinolones in U.S. poultry farming was banned in recognition of this public health threat.

The spread of antibiotic resistance can be due to quite subtle factors. For example, products such as soap and deodorants often now contain triclosan and other germicides. There is increasing evidence that the widespread use of triclosan actually favors an increase in antibiotic resistance (*see section 7.5*).

S. aureus (VRSA) strain also resisted most other antibiotics including ciprofloxacin, methicillin, and penicillin. Isolated from the same patient was another dread of hospitals—vancomycin-resistant enterococci (VRE). Genetic analyses revealed that the patient's own vancomycin-sensitive *S. aureus* had acquired the vancomycin-resistance gene, *vanA*, from VRE through conjugation. So was born a new threat to the health of the human race. Bacterial conjugation (section 13.7); Bacterial plasmids (sections 3.5 and 13.6)

Bacteria often become resistant in several different ways (**figure 34.17**). Unfortunately, a particular type of resistance mechanism is not confined to a single class of drugs (**figure 34.18**). Two bacteria may use different resistance mechanisms to withstand the same chemotherapeutic agent. Furthermore, resistant mutants arise spontaneously and are then selected for in the presence of the drug.

Pathogens often become resistant simply by preventing entrance of the drug. Many gram-negative bacteria are unaffected by penicillin G because it cannot penetrate the envelope's outer membrane. Genetic mutations that lead to changes in penicillin binding proteins also render a cell resistant. A decrease in permeability can lead to sulfonamide resistance. Mycobacteria resist many drugs because of the high content of mycolic acids (*see figure 24.11*) in a complex lipid layer outside their peptidoglycan. This layer is impermeable to most water soluble drugs. Suborder *Corynebacterineae:* Genus *Mycobacterium* (section 24.4)

A second resistance strategy is to pump the drug out of the cell after it has entered. Some pathogens have plasma membrane translocases, often called efflux pumps, that expel drugs. Because they are relatively nonspecific and can pump many different drugs, these transport proteins often are called multidrug-resistance pumps. Many are drug/proton antiporters—that is, protons enter the cell as the drug leaves. Such systems are present in *E. coli*, *P. aeruginosa*, and *S. aureus* to name a few.

Many bacterial pathogens resist attack by inactivating drugs through chemical modification. The best-known example is the hydrolysis of the β-lactam ring of penicillins by the enzyme penicillinase. Drugs also are inactivated by the addition of chemical groups. For example, chloramphenicol contains two hydroxyl groups (figure 34.10) that can be acetylated in a reaction catalyzed by the enzyme chloramphenicol acyltransferase with acetyl CoA as the donor. Aminoglycosides (figure 34.7) can be modified and inactivated in several ways. Acetyltransferases catalyze the acetylation of amino groups. Some aminoglycoside-modifying enzymes catalyze the addition to hydroxyl groups of either phosphates (phosphotransferases) or adenyl groups (adenyltransferases).

Because each chemotherapeutic agent acts on a specific target, resistance arises when the target enzyme or cellular structure is modified so that it is no longer susceptible to the drug.

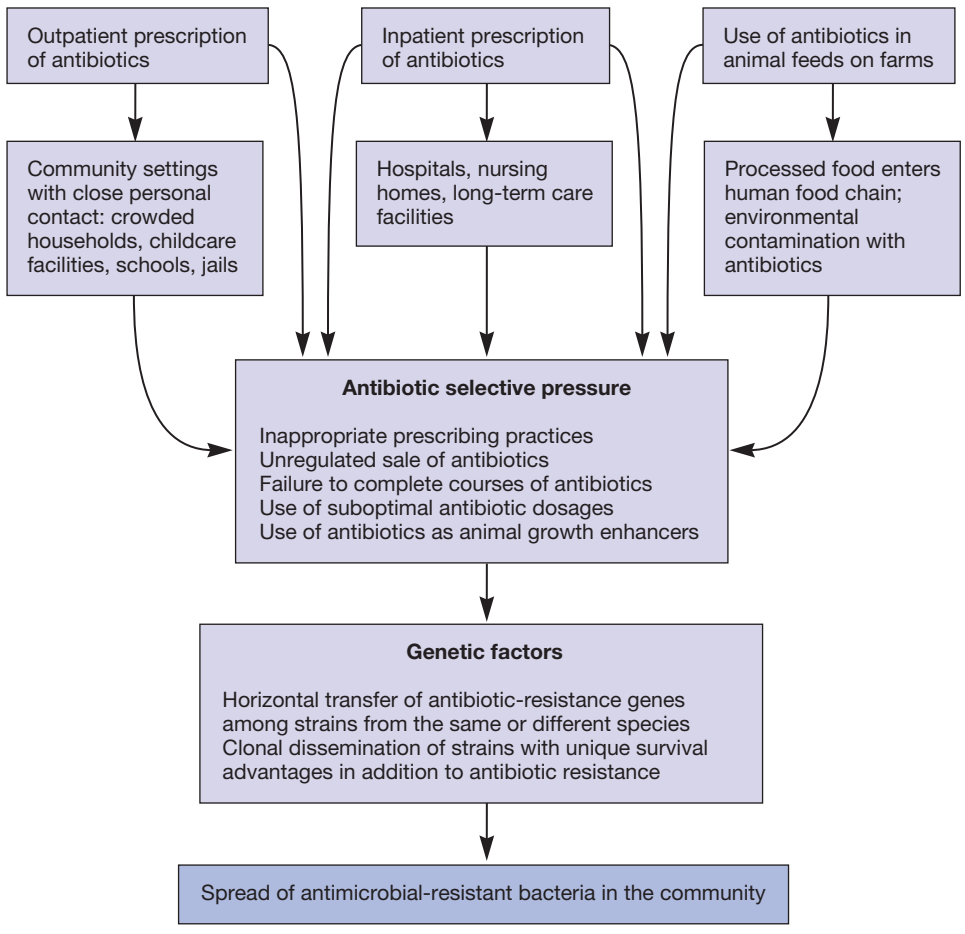


Figure 34.17 Antibiotic Resistance Has Many Sources. Incomplete and indiscriminant use of antibiotics in people and animals leads to increased selective pressure on bacteria. Bacteria capable of resisting antibiotics survive and spread these traits by horizontal gene transfer.

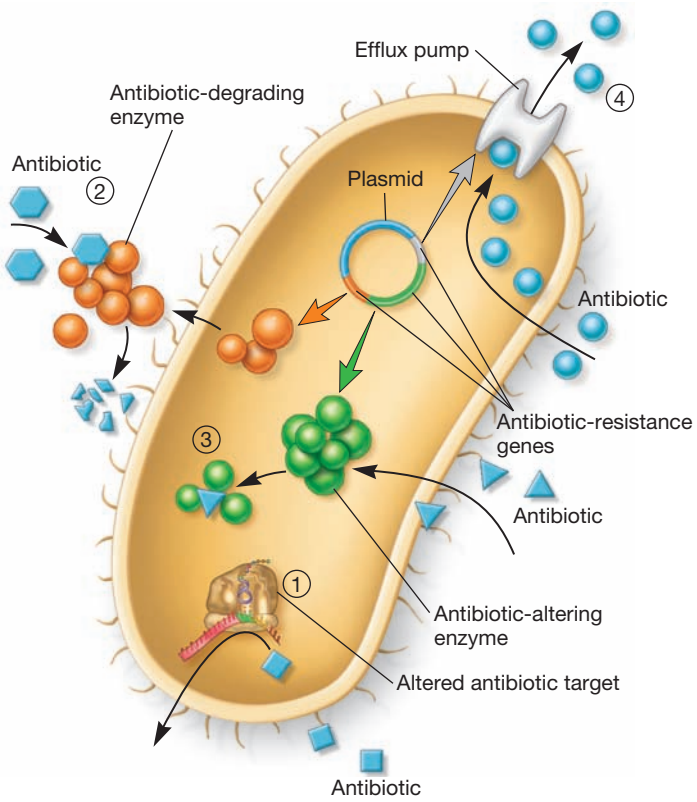


Figure 34.18 Antibiotic Resistance Mechanisms. Bacteria can resist the action of antibiotics by (1) preventing access to (or altering) the target of the antibiotic, (2) degrading the antibiotic, (3) altering the antibiotic, and/or (4) rapid extrusion of the antibiotic.

For example, the affinity of ribosomes for erythromycin and chloramphenicol can be decreased by a change in the 23S rRNA to which they bind. Enterococci become resistant to vancomycin by changing the terminal D-alanine-D-alanine in their peptidoglycan to a D-alanine-D-lactate. This drastically reduces antibiotic binding. Antimetabolite action may be resisted through alteration of susceptible enzymes. In sulfonamide-resistant bacteria the enzyme that uses *p*-aminobenzoic acid during folic acid synthesis (the dihydropteroic acid synthetase; figure 34.14) often has a much lower affinity for sulfonamides.

Finally, resistant bacteria may either use an alternate pathway to bypass the sequence inhibited by the agent or increase the production of the target metabolite. For example, some bacteria are resistant to sulfonamides simply because they use preformed folic acid from their surroundings rather than synthesize it themselves. Other strains increase their rate of folic acid production and thus counteract sulfonamide inhibition.

The Origin and Transmission of Drug Resistance

Genes for drug resistance may be present on bacterial chromosomes, plasmids, transposons, and integrons. Because they are often found on mobile genetic elements, they can freely exchange between bacteria. Spontaneous mutations in the bacterial chromosome, although they do not occur very often, can make bacteria drug resistant. Usually such mutations result in a change in the drug target; therefore the antibiotic cannot bind and inhibit growth (e.g., the protein target to which streptomycin binds on bacterial ribosomes). Many mutants are probably destroyed by natural host resistance mechanisms. However, when a patient is being treated extensively with antibiotics, some resistant mutants may survive and flourish because of their competitive advantage over nonresistant strains.

Frequently a bacterial pathogen is drug resistant because it has a plasmid bearing one or more resistance genes; such plasmids are called **R plasmids** (*r*esistance plasmids). Plasmid resistance genes often code for enzymes that destroy or modify drugs; for example, the hydrolysis of penicillin or the acetylation of chloramphenicol and aminoglycoside drugs. Plasmid-associated genes have been implicated in resistance to the aminoglycosides, choramphenicol, penicillins and cephalosporins, erythromycin, tetracyclines, sulfonamides, and others. Once a bacterial cell possesses an R plasmid, the plasmid (or its genes) may be transferred to other cells quite rapidly through normal gene exchange processes such as conjugation, transduction, and transformation (**figure 34.19**). Because a single plasmid may carry genes for resistance to several drugs, a pathogen population can become resistant to several antibiotics simultaneously, even though the infected patient is being treated with only one drug. Bacterial conjugation (section 13.7); Transduction (section 13.9); DNA transformation (section 13.8); Bacterial plasmids (sections 3.5 and 13.6)

Antibiotic resistance genes can be located on genetic elements other than plasmids. Many composite transposons contain genes for antibiotic resistance, and some bear more than one resistance gene. They are found in both gram-negative and gram-positive bacteria. Some examples and their resistance markers are Tn*5* (kanamycin, bleomycin, streptomycin), Tn*9* (chloramphenicol), Tn*10* (tetracycline), Tn*21* (streptomycin, spectinomycin, sulfonamide), Tn*551* (erythromycin), and Tn*4001* (gentamicin, tobramycin, kanamycin). Resistance genes on composite transposons can move rapidly between plasmids and through a bacterial population. Often several resistance genes are carried together as gene cassettes in association with a genetic element known as an integron. An **integron** is composed of an integrase gene and sequences for site-specific recombination. Thus integrons can capture genes and gene cassettes. **Gene cassettes** are genetic elements that may exist as circular nonreplicating DNA when moving from one site to another, but which normally are a linear part of a transposon, plasmid, or bacterial chromosome. Cassettes usually carry one or two genes and a recombination site. Several cassettes can be integrated sequentially in an integron. Thus integrons also are important in spreading resistance genes. Finally, conjugative transposons, like composite transposons, can carry resistance genes. Because they are capable of moving between bacteria by conjugation, they are also effective in spreading resistance. Transposable elements (section 13.5)

Extensive drug treatment favors the development and spread of antibiotic-resistant strains because the antibiotic destroys

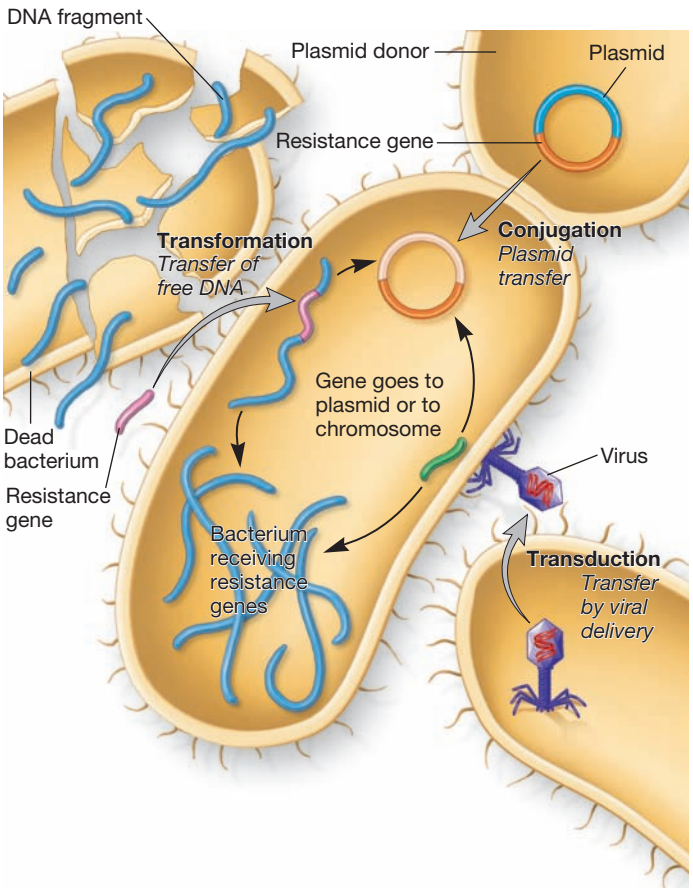


Figure 34.19 Horizontal Gene Exchange. Bacteria exchange genetic information, like antibiotic resistance genes, through conjugation, transformation, and/or transduction.

susceptible bacteria that would usually compete with drug-resistant strains. The result may be the emergence of drug-resistant pathogens. Several strategies can be employed to discourage the emergence of drug resistance. The drug can be given in a high enough concentration to destroy susceptible bacteria and most spontaneous mutants that might arise during treatment. Sometimes two or even three different drugs can be administered simultaneously with the hope that each drug will prevent the emergence of resistance to the other. This approach is used in treating tuberculosis and malaria. Finally, chemotherapeutic drugs, particularly broad-spectrum drugs, should be used only when definitely necessary. If possible, the pathogen should be identified, drug sensitivity tests run, and the proper narrow-spectrum drug employed.

Despite efforts to control the emergence and spread of drug resistance, the situation continues to worsen. Of course, antibiotics should be used in ways that reduce the development of resistance. Another approach is to search for new antibiotics that microorganisms have never encountered. Pharmaceutical companies collect and analyze samples from around the world in a search for completely new antimicrobial agents. Structure-based or rational drug design is a third option. If the three-dimensional structure of a susceptible target molecule such as an enzyme essential to microbial function is known, computer programs can be used to design drugs that precisely fit the target molecule. These drugs might be able to bind to the target and disrupt its function sufficiently to destroy the pathogen. Pharmaceutical companies are using this approach to attempt to develop drugs for the treatment of AIDS, cancer, septicemia caused by lipopolysaccharide (LPS), and the common cold. At least one company is developing "enhancers." These are cationic peptides that disrupt bacterial membranes by displacing their magnesium ions. Antibiotics then penetrate and rapidly exert their effects. Other pharmaceutical companies are developing efflux-pump inhibitors to administer with antibiotics and prevent their expulsion by the resistant pathogen.

There has been some progress in developing new antibiotics that are effective against drug-resistant pathogens. Two new drugs are fairly effective against vancomycin-resistant enterococci. Synercid is a mixture of the streptogramin antibiotics quinupristin and dalfopristin that inhibits protein synthesis. A second drug, linezolid (Zyvox), is the first drug in a new family of antibiotics, the oxazolidinones. It inhibits protein synthesis and is active against both vancomycin-resistant enterococci and meticillin-resistant *Staphylococcus aureus*.

Information that is coming from the sequencing and analysis of pathogen genomes is also useful in identifying new targets for antimicrobial drugs. For example, genomics studies are providing data for research on inhibitors of both aminoacyl-tRNA synthetases and the enzyme that removes the formyl group from the N-terminal methionine during bacterial protein synthesis. Bacteria must synthesize the fatty acids they require for growth rather than acquiring the acids from their environment. The drug susceptibility of enzymes in the fatty acid synthesis system is being analyzed by screening pathogens for potential targets. Genomics (chapter 15)

A most interesting response to the current crisis is the renewed interest in an idea first proposed early in the twentieth century by Felix d'Herelle, one of the discoverers of bacterial viruses or bacteriophages. d'Herelle proposed that bacteriophages could be used to treat bacterial diseases. Although most microbiologists did not pursue his proposal actively due to technical difficulties and the advent of antibiotics, Russian scientists developed the medical use of bacteriophages. Currently Russian physicians use bacteriophages to treat many bacterial infections. Bandages are saturated with phage solutions, phage mixtures are administered orally, and phage preparations are given intravenously to treat *Staphylococcus* infections. Three American companies are actively conducting research on phage therapy and preparing to carry out clinical trials. Viruses of *Bacteria* and *Archaea* (chapter 17)

1. Briefly describe the five major ways in which bacteria become resistant to drugs and give an example of each.
2. Define plasmid, R plasmid, integron, and gene cassette. How are R plasmids involved in the spread of drug resistance?
3. List several ways in which the development of antibiotic-resistant pathogens can be slowed or prevented.

34.7 Antifungal Drugs

Treatment of fungal infections generally has been less successful than that of bacterial infections largely because eucaryotic fungal cells are much more similar to human cells than are bacteria. Many drugs that inhibit or kill fungi are therefore quite toxic for humans. In addition, most fungi have a detoxification system that modifies many antifungal agents. As a result the added antibiotics are fungistatic only as long as repeated application maintains high levels of unmodified antibiotic. Despite their relatively low therapeutic index, a few drugs are useful in treating many major fungal diseases. Effective antifungal agents frequently either extract membrane sterols or prevent their synthesis. Similarly, because animal cells do not have cell walls, the enzyme chitin synthase is the target for fungal-active antibiotics such as polyoxin D and nikkomycin.

Fungal infections are often subdivided into infections of superficial tissues or superficial mycoses and systemic mycoses. Treatment for these two types of disease is very different. Several drugs are used to treat superficial mycoses. Three drugs containing imidazole—miconazole, ketoconazole (**figure 34.20**), and clotrimazole—are broad-spectrum agents available as creams and solutions for the treatment of dermatophyte infections such as athlete's foot, and oral and vaginal candidiasis. They are thought to disrupt fungal membrane permeability and inhibit sterol synthesis. Tolnaftate is used topically for the treatment of cutaneous infections, but is not as effective against infections of the skin and hair. **Nystatin** (figure 34.20), a polyene antibiotic from *Streptomyces*, is used to control *Candida* infections of the skin, vagina, or alimentary tract. It binds to sterols and damages the membrane, leading to fungal membrane leakage. **Griseofulvin** (figure 34.20), an antibiotic formed by *Penicillium*, is given orally to treat chronic dermatophyte infections. It is thought to disrupt the mitotic spindle and inhibit cell division; it also may inhibit protein and nucleic acid synthesis. Side effects of griseofulvin include headaches, gastrointestinal upset, and allergic reactions. Human diseases caused by fungi and protists (chapter 39)

Systemic infections are very difficult to control and can be fatal. Three drugs commonly used against systemic mycoses are **amphotericin B,** 5-flucytosine, and fluconazole (figure 34.20). Amphotericin B from *Streptomyces* spp. binds to the sterols in fungal membranes, disrupting membrane permeability and caus-

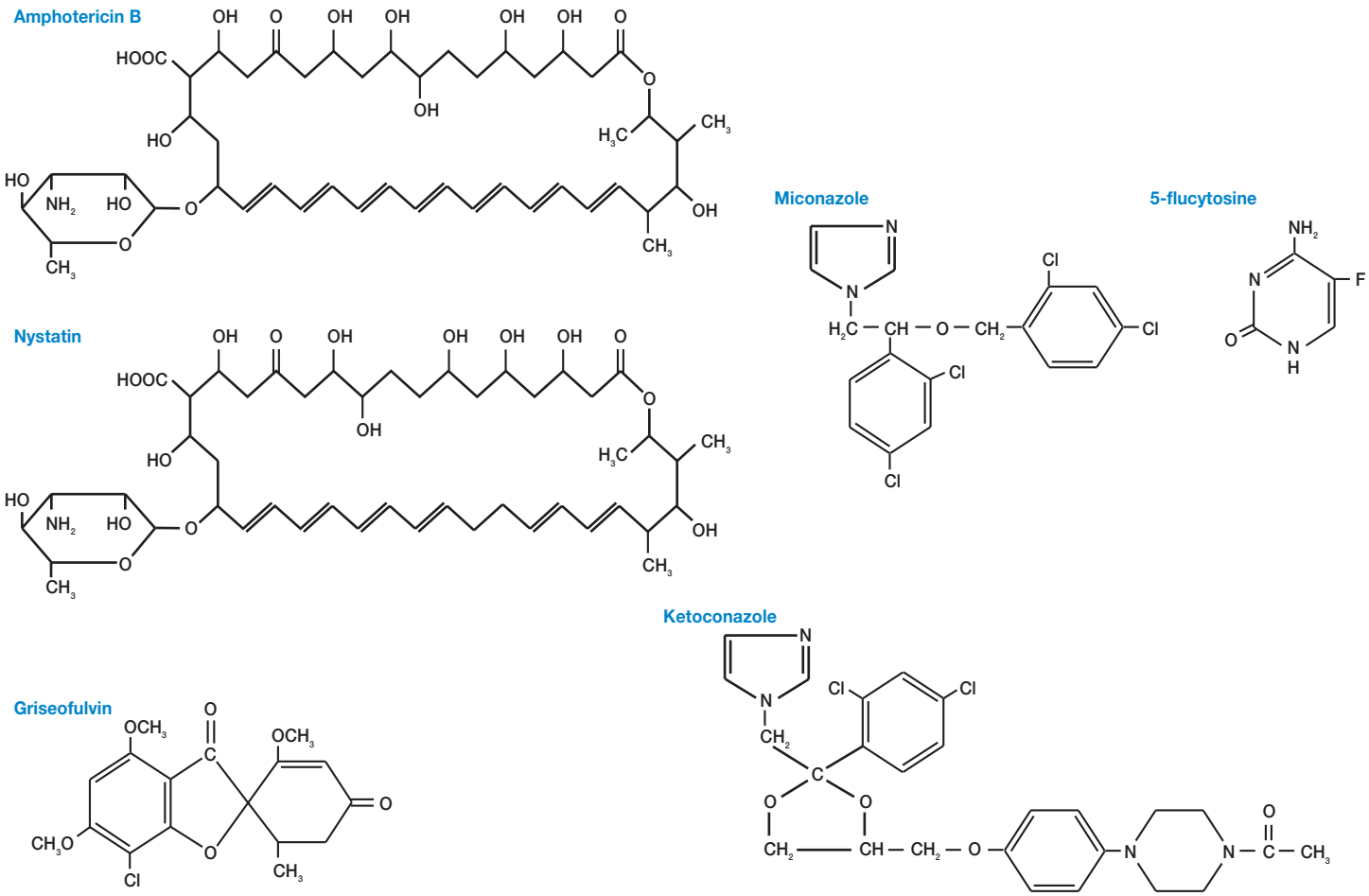


Figure 34.20 Antifungal Drugs. Six commonly used drugs are shown here.

ing leakage of cell constituents. It is quite toxic and used only for serious, life-threatening infections. The synthetic oral antimycotic agent 5-flucytosine (5-fluorocytosine) is effective against most systemic fungi, although drug resistance often develops rapidly. The drug is converted to 5-fluorouracil by the fungi, incorporated into RNA in place of uracil, and disrupts RNA function. Its side effects include skin rashes, diarrhea, nausea, aplastic anemia, and liver damage. Fluconazole is used in the treatment of candidiasis, cryptococcal meningitis, and coccidioidal meningitis. Because adverse effects to fluconazole are relatively uncommon, it is used prophylactically to prevent life-threatening fungal infections in AIDS patients and other individuals who are severely immunosuppressed.

1. Summarize the mechanism of action and the therapeutic use of the following antifungal drugs: miconazole, nystatin, griseofulvin, amphotericin B, and 5-flucytosine.

34.8 Antiviral Drugs

For many years the possibility of treating viral infections with drugs appeared remote because viruses enter host cells and make use of host cell enzymes and constituents. A drug that would block virus reproduction also was thought to be toxic for the host. Inhibitors of virus-specific enzymes and life cycle processes have now been discovered, and several drugs are used therapeutically. Some important examples are shown in **figure 34.21.** Reproduction of vertebrate viruses (section 18.2)

Most antiviral drugs disrupt either critical stages in the virus life cycle or the synthesis of virus-specific nucleic acids. **Amantadine** and rimantadine can be used to prevent influenza A infections. When given early in the infection (in the first 48 hours), they reduce the incidence of influenza by 50 to 70% in an exposed population. Amantadine blocks the penetration and uncoating of influenza virus particles. **Adenine arabinoside** or **vidarabine**

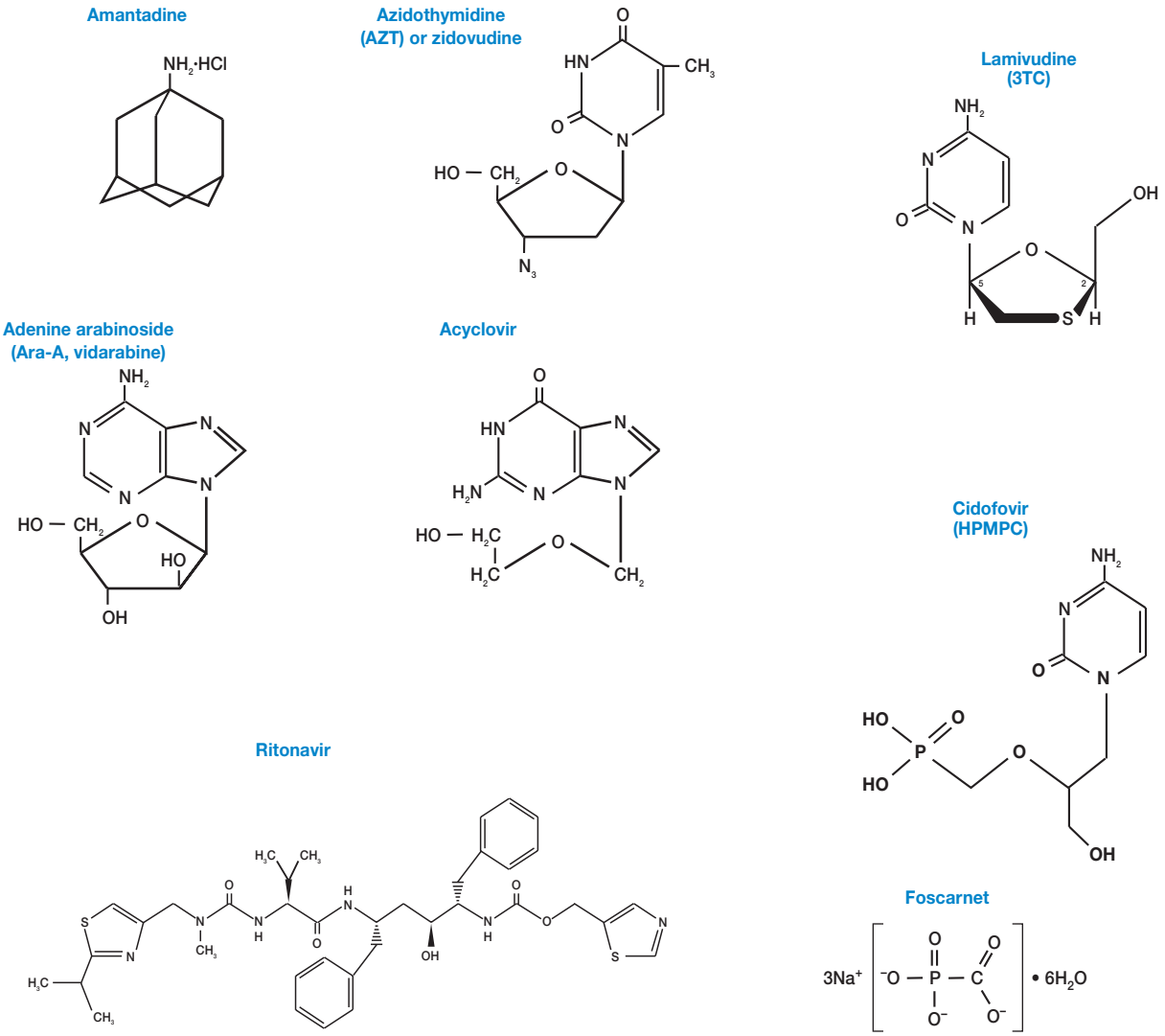


Figure 34.21 Representative Antiviral Drugs.

disrupts the activity of DNA polymerase and several other enzymes involved in DNA and RNA synthesis and function. It is given intravenously or applied as an ointment to treat herpes infections. A third drug, **acyclovir,** is also used in the treatment of herpes infections. Upon phosphorylation, acyclovir resembles deoxy-GTP and inhibits the virus DNA polymerase. Unfortunately acyclovir-resistant strains of herpes are already developing. Effective acyclovir derivatives and relatives are now available. Valacyclovir is an orally administered prodrug form of acyclovir. Prodrugs are inactive until metabolized. Ganciclovir, penciclovir, and its oral form famciclovir are effective in treatment of herpesviruses. Another kind of drug, foscarnet, inhibits the virus DNA polymerase in a different way. Foscarnet is an organic analog of pyrophosphate (figure 34.21) that binds to the polymerase active site and blocks the cleavage of pyrophosphate from nucleoside triphosphate substrates. It is used in treating herpes and cytomegalovirus infections. Airborne diseases: Influenza (section 37.1)

Several broad-spectrum anti-DNA virus drugs have been developed. A good example is the drug HPMPC or cidofovir (figure 34.21). It is effective against papovaviruses, adenoviruses, herpesviruses, iridoviruses, and poxviruses. The drug acts on the viral DNA polymerase as a competitive inhibitor and alternative substrate of dCTP. It has been used primarily against cytomegalovirus but also against herpes simplex and human papillomavirus infections.

Research on anti-HIV drugs has been particularly active. Many of the first drugs to be developed were reverse transcriptase inhibitors such as **azidothymidine (AZT)** or **zidovudine,** lamivudine (3TC), didanosine (ddI), zalcitabine (ddC), and stavudine (d4T) (figure 34.21). These interfere with reverse transcriptase activity and therefore block HIV reproduction. More recently **HIV protease inhibitors** have also been developed. Three of the most used are saquinvir, indinavir, and ritonavir (figure 34.21). Protease inhibitors are effective because HIV, like many viruses, translates multiple proteins as a single polypeptide. This polypeptide must then be cleaved into individual proteins required for virus replication. Protease inhibitors mimic the peptide bond that is normally attacked by the protease. The most successful treatment regimen involves a cocktail of agents given at high dosages to prevent the development of drug resistance. For example, the combination of AZT, 3TC, and ritonavir is very effective in reducing HIV plasma concentrations almost to zero. However, the treatment does not eliminate latent proviral HIV DNA that still resides in memory T cells, and possibly elsewhere. Reproduction of vertebrate viruses: Genome replication, transcription, and protein synthesis in RNA viruses (section 18.2); Direct contact diseases: Acquired immune deficiency syndrome (AIDS) (section 37.3)

Probably the most publicized antiviral agent has been Tamiflu (generically, oseltamivir phosphate). Tamiflu is a neuraminidase inhibitor that has received much attention in light of 21st-century predictions of an influenza pandemic, including avian influenza ("bird flu"). While Tamiflu is not a cure for neurominidase-expressing viruses, two clinical trials showed that patients who took Tamiflu were relieved of flu symptoms 1.3 days faster than patients who did not take Tamiflu. Prophylactic use has resulted in viral resistance to Tamiflu. Tamiflu is not a substitute for yearly flu vaccination and frequent hand-washing.

34.9 Antiprotozoan Drugs

The mechanism of drug action for most antiprotozoan drugs is not completely elucidated. Drugs such as chloroquine, atovaquone, mefloquine, iodoquinol, metronidazole, nitazoxanide, and pentamidine, for example, have potent antiprotozoan action but a clear mechanism of action for each class of protozoa is unknown. It may be that each drug has more than one activity and that the relative role of each mechanism to the overall antiprotozoan activity may be different for the various species of protozoa. However, most antiprotozoan drugs appear to act on protozoan nucleic acid or some metabolic event.

Chloroquine is used to treat malaria. Several mechanisms of action have been reported. It can raise the internal pH, clump the plasmodial pigment, and intercalate into plasmodial DNA. Chloroquine also inhibits heme polymerase, an enzyme that converts toxic heme into nontoxic hemazoin. Inhibition of this enzyme leads to a buildup of toxic heme. Mefloquine is also used to treat malaria and has been found to swell the *Plasmodium falciparum* food vacuoles, where it may act by forming toxic complexes that damage membranes and other plasmodial components.

Metronidazole is used to treat *Entamoeba* infections. Anaerobic organisms readily reduce it to the active metabolite within the cytoplasm. Aerobic organisms appear to reduce it using ferrodoxin (a protein of the electron transport system). Reduced metronidazole interacts with DNA altering its helical structure and causing DNA fragmentation; it prevents normal nucleic acid synthesis, resulting in cell death.

A number of antibiotics that inhibit bacterial protein synthesis are also used to treat protozoan infection. These include the aminoglycosides clindamycin, and paromomycin. Aminoglycosides can be considered polycationic molecules that have a high affinity for nucleic acids. Specifically, aminoglycosides possess high affinities for RNAs. Different aminoglycoside antibiotics bind to different sites on RNAs. RNA binding interferes with the normal expression and function of the RNA, resulting in cell death.

Interference of eucaryotic electron transport is one common activity of some antiprotozoan drugs. Atovaquone is used to treat *Pneumocystis jiroveci* (formerly called *P. carinii*) and *Toxoplasma gondii*. It is an analog of ubiquinone, an integral component of the eucaryotic electron transport system. As an analog of ubiquinone, atovaquone can act as a competitive inhibitor and thus suppress electron transport. The ultimate metabolic effects of electron transport blockade include inhibited or delayed synthesis of nucleic acids and ATP. Another drug that interferes with electron transport is nitazoxanide, which used to treat cryptosporidiosis. It appears to exert its effect through interference with the pyruvate:ferredoxin oxidoreductase. It has also been reported to form toxic free radicals once the nitro group is reduced intracellularly.

Pentamidine is used to treat *Pneumocystis* infection. Some reports indicate that it interferes with protozoan metabolism, although the drug only moderately inhibits glucose metabolism, protein synthesis, RNA synthesis, and intracellular amino acid transport in vitro. Pyrimethamine, used to treat *Toxoplasma* infection, and dapsone, used for *Pneumocystis* infection, appear to

act in the same way as trimethoprim—interfering with folic acid synthesis by inhibition of dihydrofolate reductase.

As with other antimicrobial therapies, traditional drug development starts by identifying a unique target to which a drug can bind and thus prevent some vital function. A second consideration is often related to drug spectrum—how many different species have that target so that the proposed drug can be used broadly as a chemotherapeutic agent. This is also true for use of agents needed to remove protozoan parasites from their hosts. However, because protozoa are eucaryotes, the potential for drug action on host cells and tissues is greater than it is when targeting procaryotes. Most of the drugs used to treat protozoan infection have significant side effects; nonetheless, the side effects are usually acceptable when weighed against the parasitic alternative.

1. Why do you think drugs that inhibit bacterial protein synthesis are also effective against some protists?
2. Why do you think malaria, like tuberculosis, is now treated with several drugs simultaneously?
3. What special considerations must be taken into account when treating infections caused by protozoan parasites?

Summary

Chemotherapeutic agents are compounds that destroy pathogenic microorganisms or inhibit their growth and are used in the treatment of disease. Most are antibiotics: microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth.

34.1 The Development of Chemotherapy

a. The modern era of chemotherapy began with Paul Ehrlich's work on drugs against African sleeping sickness and syphilis. Other early pioneers were Gerhard Domagk, Alexander Fleming, Howard Florey, Ernst Chain, Norman Heatley, and Selman Waksman.

34.2 General Characteristics of Antimicrobial Drugs

a. An effective chemotherapeutic agent must have selective toxicity. A drug with great selective toxicity has a high therapeutic index and usually disrupts a structure or process unique to the pathogen. It has fewer side effects.

b. Antibiotics can be classified in terms of the range of target microorganisms (narrow spectrum versus broad spectrum); their source (natural, semisynthetic, or synthetic); and their general effect (static versus cidal) (**table 34.1**).

34.3 Determining the Level of Antimicrobial Activity

a. Antibiotic effectiveness can be estimated through the determination of the minimal inhibitory concentration and the minimal lethal concentration with dilution susceptibility tests. Tests like the Kirby-Bauer test (a disk diffusion test) and the Etest are often used to estimate a pathogen's susceptibility to drugs quickly (**figures 34.2, 34.3,** and **34.4**).

34.4 Antibacterial Drugs

a. Members of the penicillin family contain a β-lactam ring and disrupt bacterial cell wall synthesis, resulting in cell lysis (**figure 34.5**). Some, like penicillin G, are usually administered by injection and are most effective against gram-positive bacteria. Others can be given orally (penicillin V), are broad spectrum (ampicillin, carbenicillin), or are penicillinase resistant (methicillin).

b. Cephalosporins are similar to penicillins, and are given to patients with penicillin allergies (**figure 34.6**).

c. Vancomycin is a glycopeptide antibiotic that inhibits the transpeptidation reaction during peptidoglycan synthesis. It is used against drug-resistant staphylococci, enterococci, and clostridia.

d. Aminoglycoside antibiotics like streptomycin and gentamicin bind to the small ribosomal subunit, inhibit protein synthesis, and are bactericidal (**figure 34.7**).

e. Tetracyclines are broad-spectrum antibiotics having a four-ring nucleus with attached groups (**figure 34.8**). They bind to the small ribosomal subunit and inhibit protein synthesis.

f. Erythromycin is a bacteriostatic macrolide antibiotic that binds to the large ribosomal subunit and inhibits protein synthesis (**figure 34.9**).

g. Chloramphenicol is a broad-spectrum, bacteriostatic antibiotic that inhibits protein synthesis (**figure 34.10**). It is quite toxic and used only for very serious infections.

h. Sulfonamides or sulfa drugs resemble *p*-aminobenzoic acid and competitively inhibit folic acid synthesis (**figure 34.12**).

i. Trimethoprim is a synthetic antibiotic that inhibits the dihydrofolate reductase, which is required by organisms in the manufacture of folic acid (**figure 34.13**).

j. Quinolones are a family of bactericidal synthetic drugs that inhibit DNA gyrase and thus inhibit such processes as DNA replication (**figure 34.15**).

34.5 Factors Influencing Antimicrobial Drug Effectiveness

a. A variety of factors can greatly influence the effectiveness of antimicrobial drugs during actual use.

34.6 Drug Resistance

a. Bacteria can become resistant to a drug by excluding it from the cell, pumping the drug out of the cell, enzymatically altering it, modifying the target enzyme or organelle to make it less drug sensitive, as examples. The genes for drug resistance may be found on the bacterial chromosome, a plasmid called an R plasmid, or other genetic elements such as transposons (**figures 34.18** and **34.19**).

b. Chemotherapeutic agent misuse fosters the increase and spread of drug resistance, and may lead to superinfections.

34.7 Antifungal Drugs

a. Because fungi are more similar to human cells than bacteria, antifungal drugs generally have lower therapeutic indexes than antibacterial agents and produce more side effects.

b. Superficial mycoses can be treated with miconazole, ketoconazole, clotrimazole, tolnaftate, nystatin, and griseofulvin (**figure 34.20**). Amphotericin B, 5-flucytosine, and fluconazole are used for systemic mycoses.

34.8 Antiviral Drugs

a. Antiviral drugs interfere with critical stages in the virus life cycle (amantadine, rimantadine, and ritonavir) or inhibit the synthesis of virus-specific nucleic acids (zidovudine, adenine arabinoside, acyclovir) (**figure 34.21**).

34.9 Antiprotozoan Drugs

a. The mechanisms by which most drugs used to treat protozoan infection are unknown.

b. Antiprotozoan drugs interfere with critical steps in nucleic acid synthesis, protein synthesis, and electron transport of folic acid synthesis.