Antimicrobial Drugs: Mechanism of Action

10

The most important concept underlying antimicrobial therapy is **selective toxicity,** ie, selective inhibition of the growth of the microorganism without damage to the host. Selective toxicity is achieved by exploiting the differences between the metabolism and structure of the microorganism and the corresponding features of human cells. For example, penicillins and cephalosporins are effective antibacterial agents because they prevent the synthesis of peptidoglycan, thereby inhibiting the growth of bacterial but not human cells.

There are four major sites in the bacterial cell that are sufficiently different from the human cell that they serve as the basis for the action of clinically effective drugs: cell wall, ribosomes, nucleic acids, and cell membrane (Table 10–1).

There are far more antibacterial drugs than antiviral drugs. This is a consequence of the difficulty of designing a drug that will selectively inhibit viral replication. Because viruses use many of the normal cellular functions of the host in their growth, it is not easy to develop a drug that specifically inhibits viral functions and does not damage the host cell.

Broad-spectrum antibiotics are active against several types of microorganisms, eg, tetracyclines are active against many gram-negative rods, chlamydiae, mycoplasmas, and rickettsiae. **Narrow-spectrum** antibiotics are active against one or very few types, eg, vancomycin is primarily used against certain gram-positive cocci, namely, staphylococci and enterococci.

■ BACTERICIDAL & BACTERIOSTATIC ACTIVITY

In some clinical situations, it is essential to use a bactericidal drug rather than a bacteriostatic one. A **bactericidal drug kills bacteria,** whereas a **bacteriostatic drug inhibits their growth but does not kill them.** The salient features of the behavior of bacteriostatic drugs are that (1) the bacteria can grow again when the drug is withdrawn, and (2) host defense mechanisms, such as phagocytosis, are required to kill the bacteria. Bactericidal drugs are particularly useful in certain infections, eg, those that are immediately life-threatening; those in patients whose polymorphonuclear leukocyte count is below 500/μL; and endocarditis, in which phagocytosis is limited by the fibrinous network of the vegetations and bacteriostatic drugs do not effect a cure.

■ MECHANISMS OF ACTION

INHIBITION OF CELL WALL SYNTHESIS

1. Inhibition of Bacterial Cell Wall Synthesis

Penicillins

Penicillins (and cephalosporins) act by inhibiting **transpeptidases,** the enzymes that catalyze the final cross-linking step in the synthesis of peptidoglycan (see Figure 2–5). For example, in *Staphylococcus aureus,* transpeptidation occurs between the amino group on the end of the pentaglycine cross-link and the terminal carboxyl group of the D-alanine on the tetrapeptide side chain. Because the stereochemistry of penicillin is similar to that of a dipeptide, D-alanyl-D-alanine, penicillin can bind to the active site of the transpeptidase and inhibit its activity.

Two additional factors are involved in the action of penicillin.

(1) The first is that penicillin binds to a variety of receptors in the bacterial cell membrane and cell wall called **penicillin-binding proteins (PBPs).** Some PBPs are transpeptidases; the function of others is unknown. Changes in PBPs are in part responsible for an organism's becoming resistant to penicillin.

(2) The second factor is that **autolytic enzymes** called murein hydrolases (murein is a synonym for peptidoglycan) are activated in penicillin-treated cells and degrade the peptidoglycan. Some bacteria, eg, strains of

Table 10–1. Mechanism of action of important antibacterial and antifungal drugs.

Mechanism of Action	Drugs
Inhibition of cell wall synthesis	
1. Antibacterial activity	
Inhibition of cross-linking (transpeptidation) of peptidoglycan	Penicillins, cephalosporins, imipenem, aztreonam, vancomycin
Inhibition of other steps in peptidoglycan synthesis	Cycloserine, bacitracin
2. Antifungal activity	
Inhibition of β-glucan synthesis	Caspofungin
Inhibition of protein synthesis	
Action on 50S ribosomal subunit	Chloramphenicol, erythromycin, clindamycin, linezolid
Action on 30S ribosomal subunit	Tetracyclines and aminoglycosides
Inhibition of nucleic acid synthesis	
Inhibition of nucleotide synthesis	Sulfonamides, trimethoprim
Inhibition of DNA synthesis	Quinolones
Inhibition of mRNA synthesis	Rifampin
Alteration of cell membrane function	
Antibacterial activity	Polymyxin
Antifungal activity	Amphotericin B, nystatin, ketoconazole
Other mechanisms of action	
1. Antibacterial activity	Isoniazid, metronidazole, ethambutol, pyrazinamide
2. Antifungal activity	Griseofulvin, pentamidine

S. aureus, are **tolerant** to the action of penicillin, because these autolytic enzymes are not activated. A tolerant organism is one that is inhibited but not killed by a drug that is usually bactericidal, such as penicillin (see page 85).

Penicillin-treated cells die by rupture as a result of the influx of water into the high-osmotic-pressure interior of the bacterial cell. If the osmotic pressure of the medium is raised about 3-fold, by the addition of sufficient KCl, for example, rupture will not occur and the organism can survive as a protoplast. Exposure of the bacterial cell to lysozyme, which is present in human tears, results in degradation of the peptidoglycan and osmotic rupture similar to that caused by penicillin.

Penicillin is bactericidal, but it **kills cells only when they are growing.** When cells are growing, new peptidoglycan is being synthesized and transpeptidation occurs. However, in nongrowing cells, no new cross-linkages are required and penicillin is inactive. Penicillins are therefore **more active during the log phase** of bacterial cell growth than during the stationary phase (see Chapter 3 for the bacterial cell growth cycle).

Penicillins (and cephalosporins) are called β-lactam drugs because of the importance of the β-lactam ring (Figure 10–1). An intact ring structure is essential for antibacterial activity; cleavage of the ring by penicillinases (**β-lactamases**) inactivates the drug. The most important naturally occurring compound is benzylpenicillin (penicillin G), which is composed of the 6-aminopenicillanic acid nucleus that all penicillins have, plus a benzyl side chain (see Figure 10–1). Penicillin G is available in three main forms:

(1) Aqueous penicillin G, which is metabolized most rapidly.

(2) Procaine penicillin G, in which penicillin G is conjugated to procaine. This form is metabolized more slowly and is less painful when injected intramuscularly because the procaine acts as an anesthetic.

(3) Benzathine penicillin G, in which penicillin G is conjugated to benzathine. This form is metabolized very slowly and is often called a "depot" preparation.

Benzylpenicillin is one of the most widely used and effective antibiotics. However, it has four disadvantages,

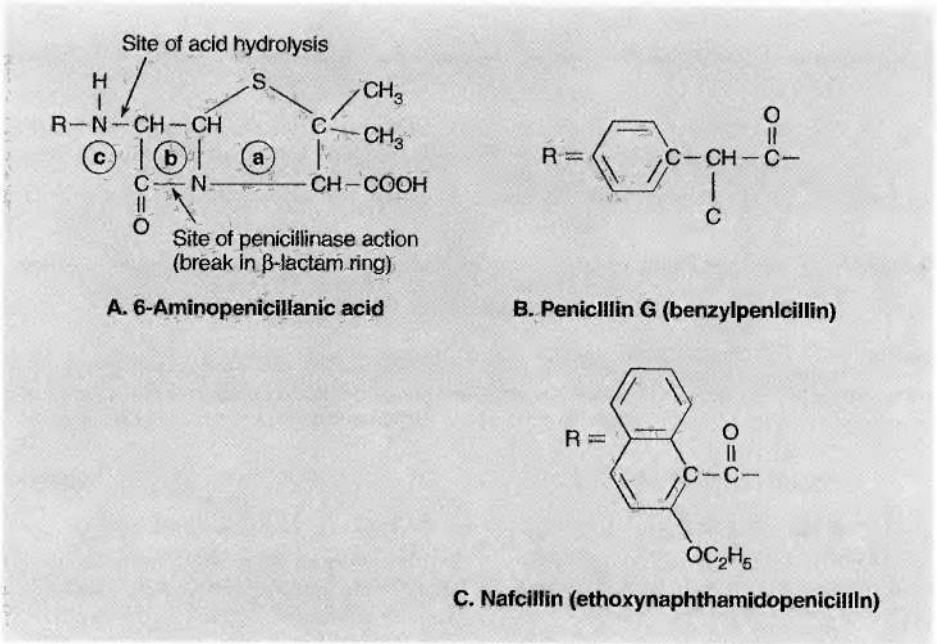

Figure 10–1. Penicillins. **A:** The 6-aminopenicillanic acid nucleus is composed of a thiazolidine ring (a), a β-lactam ring (b), and an amino group (c). The sites of inactivation by stomach acid and by penicillinase are indicated. **B:** The benzyl group, which forms benzylpenicillin (penicillin G) when attached at R. **C:** The large aromatic ring substituent that forms nafcillin, a β-lactamase-resistant penicillin, when attached at R. The large ring blocks the access of β-lactamase to the β-lactam ring.

three of which have been successfully overcome by chemical modification of the side chain. The three disadvantages are (1) limited effectiveness against many gram-negative rods; (2) hydrolysis by gastric acids, so that it cannot be taken orally; and (3) inactivation by β-lactamases. The fourth disadvantage common to all penicillins that has *not* been overcome is hypersensitivity, especially anaphylaxis, in some recipients of the drug.

The effectiveness of penicillins against gram-negative rods has been increased by a series of chemical changes in the side chain (Table 10–2). It can be seen that ampicillin and amoxicillin have activity against several gram-negative rods that the earlier penicillins do not have. However, these drugs are not useful against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Hence, other penicillins were introduced. Generally speaking, as the activity against gram-negative bacteria increases, the activity against gram-positive bacteria decreases.

The second important disadvantage—acid hydrolysis in the stomach—also has been addressed by modification of the side chain. The site of acid hydrolysis is the amide bond between the side chain and penicillanic acid nucleus (see Figure 10–1). Minor modifications of the side chain in that region, such as addition of an oxygen (to produce penicillin V) or an amino group (to produce ampicillin), prevent hydrolysis and allow the drug to be taken orally.

The inactivation of penicillin G by β-lactamases is another important disadvantage, especially in the treatment of *S. aureus* infections. Access of the enzyme to the β-lactam ring is blocked by modification of the side chain with the addition of large aromatic rings containing bulky methyl or ethyl groups (methicillin, oxacillin, nafcillin, etc; Figure 10–1). Another defense against β-lactamases is inhibitors such as clavulanic acid and sulbactam. These are structural analogues of penicillin that have little antibacterial activity but bind strongly to β-lactamases and thus protect the penicillin. Combinations, such as amoxicillin and clavulanic acid (Augmentin), are in clinical use. Some bacteria resistant to these combinations have been isolated from patient specimens.

Penicillins are usually nontoxic at clinically effective levels. The major disadvantage of these compounds is hypersensitivity, which is estimated to occur in 1–10% of patients. The hypersensitivity reactions include ana-

Table 10–2. Activity of selected penicillins.

Drug	Major Organisms[1]
Penicillin G	Gram-positive cocci, gram-positive rods, *Neisseria*, spirochetes such as *Treponema pallidum*, and many anaerobes (except *Bacteroides fragilis*) but none of the gram-negative rods listed below
Ampicillin or amoxicillin	Certain gram-negative rods, such as *Haemophilus influenzae, Escherichia coli, Proteus, Salmonella,* and *Shigella* but not *Pseudomonas aeruginosa*
Carbenicillin or ticarcillin	*P. aeruginosa*, especially when used in synergistic combination with an aminoglycoside
Piperacillin	Similar to carbenicillin but with greater activity against *P. aeruginosa* and *Klebsiella pneumoniae*
Nafcillin or dicloxacillin	Penicillinase-producing *Staphylococcus aureus*

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

phylaxis, skin rashes, hemolytic anemia, nephritis, and drug fever. Anaphylaxis, the most serious complication, occurs in 0.5% of patients. Death as a result of anaphylaxis occurs in 0.002% (1:50,000) of patients.

Cephalosporins

Cephalosporins are β-lactam drugs that act in the same manner as penicillins; ie, they are bactericidal agents that inhibit the cross-linking of peptidoglycan. The structures, however, are different: The cephalosporins have a six-membered ring adjacent to the β-lactam ring and are substituted in two places on the 7-aminocephalosporanic acid nucleus (Figure 10–2), whereas penicillins have a five-membered ring and are substituted in only one place.

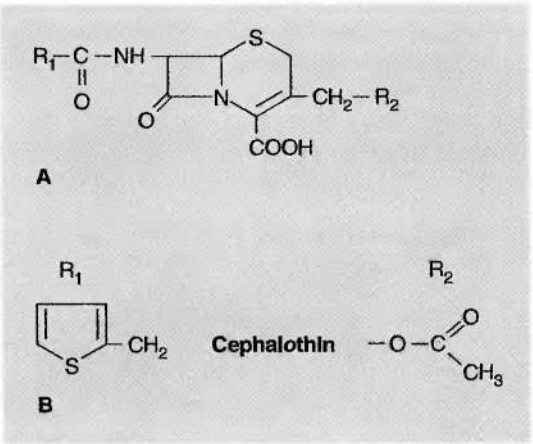

Figure 10–2. Cephalosporins. **A:** The 7-aminocephalosporanic acid nucleus. **B:** The two R groups in the drug cephalothin.

The first-generation cephalosporins are active primarily against gram-positive cocci. Similar to the penicillins, new cephalosporins were synthesized with expansion of activity against gram-negative rods as the goal. These new cephalosporins have been categorized into second, third, and fourth generations, with each generation having expanded coverage against certain gram-negative rods. Cephalosporins are effective against a broad range of organisms, are generally well tolerated, and produce fewer hypersensitivity reactions than do the penicillins. Despite the structural similarity, a patient allergic to penicillin has only about a 10% chance of being hypersensitive to cephalosporins also. Most cephalosporins are the products of molds of the genus *Cephalosporium;* a few, such as cefoxitin, are made by the actinomycete *Streptomyces.*

Carbapenems

Carbapenems are β-lactam drugs that are structurally different from penicillins and cephalosporins. For example, imipenem (*N*-formimidoylthienamycin), the currently used carbapenem, has a methylene group in the ring in place of the sulfur (Figure 10–3). Imipenem has the widest spectrum of activity of the β-lactam drugs. It has excellent bactericidal activity against many gram-positive, gram-negative, and anaerobic bacteria. It is effective against most gram-positive cocci, eg, streptococci and staphylococci; most gram-negative cocci, eg, *Neisseria;* many gram-negative rods, eg, *Pseudomonas, Haemophilus,* and members of the family Enterobacteriaceae such as *E. coli;* and various anaerobes, eg, *Bacteroides* and *Clostridium.* It is prescribed in combination with cilastatin, which is an inhibitor of dehydropeptidase, a kidney enzyme that inactivates imipenem. Imipenem is not inactivated by most β-lactamases.

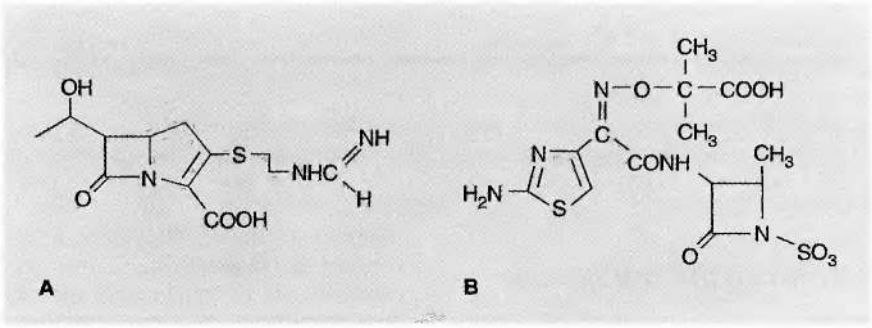

Figure 10–3. **A:** Imipenem. **B:** Aztreonam.

Monobactams

Monobactams are also β-lactam drugs that are structurally different from penicillins and cephalosporins. Monobactams are characterized by a β-lactam ring without an adjacent sulfur-containing ring structure; ie, they are monocyclic (Figure 10–3). Aztreonam, currently the most useful monobactam, has excellent activity against many gram-negative rods, such as Enterobacteriaceae and *Pseudomonas,* but is inactive against gram-positive and anaerobic bacteria. It is resistant to most β-lactamases. It is very useful in patients who are hypersensitive to penicillin, because there is no cross-reactivity.

Vancomycin

Vancomycin is a glycopeptide that **inhibits cell wall synthesis by blocking transpeptidation** but by a mechanism different from that of the β-lactam drugs. Vancomycin binds directly to the D-alanyl-D-alanine portion of the pentapeptide which blocks the transpeptidase from binding, whereas the β-lactam drugs bind to the transpeptidase itself. Vancomycin also inhibits a second enzyme, the bacterial transglycosylase, which also functions in synthesizing the peptidoglycan, but this appears to be less important than inhibition of the transpeptidase.

Vancomycin is a bactericidal agent **effective against certain gram-positive bacteria.** Its most important use is in the treatment of infections by *S. aureus* strains that are resistant to the penicillinase-resistant penicillins such as nafcillin. Note that vancomycin is not a β-lactam drug and, therefore, is not degraded by β-lactamase. Vancomycin is also used in the treatment of infections caused by *Staphylococcus epidermidis* and enterococci. Strains of *S. aureus, S. epidermidis,* and enterococci with partial or complete resistance to vancomycin have been recovered from patients.

Cycloserine & Bacitracin

Cycloserine is a structural analogue of D-alanine that inhibits the synthesis of the cell wall dipeptide D-alanyl-D-alanine. It is used as a second-line drug in the treatment of tuberculosis. Bacitracin is a cyclic polypeptide antibiotic that prevents the dephosphorylation of the phospholipid that carries the peptidoglycan subunit across the cell membrane. This blocks the regeneration of the lipid carrier and inhibits cell wall synthesis. Bacitracin is a bactericidal drug useful in the treatment of superficial skin infections but too toxic for systemic use.

2. Inhibition of Fungal Cell Wall Synthesis

Caspofungin (Cancidas) is a lipopeptide that blocks fungal cell wall synthesis by inhibiting the enzyme that synthesizes β-glucan. β-Glucan is a polysaccharide composed of long chains of D-glucose that is an essential component of certain medically important fungal pathogens. Caspofungin inhibits the growth of *Aspergillus* and *Candida* but not *Cryptococcus* or *Mucor.* Caspofungin is used for the treatment of disseminated candidiasis and for the treatment of invasive aspergillosis that does not respond to amphotericin B.

INHIBITION OF PROTEIN SYNTHESIS

Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells. This selectivity is due to the differences between bacterial and human ribosomal proteins, RNAs, and associated enzymes. Bacteria have 70S[1] ri-

[1] S stands for Svedberg units, a measure of sedimentation rate in a density gradient. The rate of sedimentation is proportionate to the mass of the particle.

bosomes with 50S and 30S subunits, whereas human cells have 80S ribosomes with 60S and 40S subunits.

Chloramphenicol, erythromycin, clindamycin, and linezolid act on the 50S subunit, whereas tetracyclines and aminoglycosides act on the 30S subunit. A summary of the modes of action of these drugs is presented in Table 10–3, and a summary of their clinically useful activity is presented in Table 10–4.

1. Drugs That Act on the 30S Subunit

Aminoglycosides

Aminoglycosides are bactericidal drugs especially useful against many gram-negative rods. Certain aminoglycosides are used against other organisms; eg, streptomycin is used in the multidrug therapy of tuberculosis, and gentamicin is used in combination with penicillin G against enterococci. Aminoglycosides are named for the amino sugar component of the molecule, which is connected by a glycosidic linkage to other sugar derivatives (Figure 10–4).

The two important modes of action of aminoglycosides have been documented best for streptomycin; other aminoglycosides probably act similarly. Both **inhibition of the initiation complex** and **misreading of messenger** RNA (mRNA) occur; the former is probably more important for the bactericidal activity of the drug. An initiation complex composed of a streptomycin-treated 30S subunit, a 50S subunit, and mRNA will not function—ie, no peptide bonds are formed, no polysomes are made, and a frozen "streptomycin monosome" results. Misreading of the triplet codon of mRNA so that the wrong amino acid is inserted into the protein also occurs in streptomycin-treated bacteria. The site of action on the 30S subunit includes both a ribosomal protein and the ribosomal RNA (rRNA). As a result of inhibition of initiation and misreading, membrane damage occurs and the bacterium dies. (In 1993, another possible mode of action was described, namely, that aminoglycosides inhibit ribozyme-mediated self-splicing of rRNA.)

Aminoglycosides have certain limitations in their use: (1) They have a toxic effect both on the kidneys and on the auditory and vestibular portions of the eighth cranial nerve. To avoid toxicity, serum levels of the drug, blood urea nitrogen, and creatinine should be measured. (2) They are poorly absorbed from the gastrointestinal tract and cannot be given orally. (3) They penetrate the spinal fluid poorly and must be given intrathecally in the treatment of meningitis. (4) They are ineffective against anaerobes, because their transport into the bacterial cell requires oxygen.

Tetracyclines

Tetracyclines are a family of antibiotics with bacteriostatic activity against a variety of gram-positive and gram-negative bacteria, mycoplasmas, chlamydiae, and rickettsiae. They inhibit protein synthesis by binding to the 30S ribosomal subunit and by **blocking the aminoacyl transfer RNA (tRNA) from entering the acceptor site** on the ribosome. However, the selective action of tetracycline on bacteria is not at the level of the ribosome, because tetracycline in vitro will inhibit protein synthesis equally well in purified ribosomes from both bacterial and human cells. Its selectivity is based on its greatly increased uptake into susceptible bacterial cells compared with human cells.

Tetracyclines, as the name indicates, have four cyclic rings with different substituents at the three R groups (Figure 10–5). The various tetracyclines (eg, doxycycline, minocycline, oxytetracycline) have similar antimicrobial activity but different pharmacologic proper-

Table 10–3. Mode of action of antibiotics that inhibit protein synthesis.

Antibiotic	Ribosomal Subunit	Mode of Action	Bactericidal or Bacteriostatic
Aminoglycosides	30S	Blocks functioning of initiation complex and causes misreading of mRNA	Bactericidal
Tetracyclines	30S	Blocks tRNA binding to ribosome	Bacteriostatic
Chloramphenicol	50S	Blocks peptidyltransferase	Both[1]
Erythromycin	50S	Blocks translocation	Primarily bacteriostatic
Clindamycin	50S	Blocks peptide bond formation	Primarily bacteriostatic
Linezolid	50S	Blocks early step in ribosome formation	Both[1]

[1] Can be either bactericidal or bacteriostatic, depending on the organism.

Table 10–4. Spectrum of activity of antibiotics that inhibit protein synthesis.

Antibiotic	Clinically Useful Activity	Comments
Aminoglycosides		
Streptomycin	Tuberculosis, tularemia, plague, brucellosis	Ototoxic and nephrotoxic.
Gentamicin and tobramycin	Many gram-negative rod infections including *Pseudomonas aeruginosa*	Most widely used aminoglycosides.
Amikacin	Same as gentamicin and tobramycin	Effective against some organisms resistant to gentamicin and tobramycin.
Neomycin	Preoperative bowel preparation	Too toxic to be used systemically; use orally since not absorbed.
Tetracyclines	Rickettsial and chlamydial infections, *Mycoplasma pneumoniae*	Not given during pregnancy or to young children.
Chloramphenicol	*Haemophilus influenzae* meningitis, typhoid fever, anaerobic infections (especially *Bacteroides fragilis*)	Bone marrow toxicity limits use to severe infections.
Erythromycin	Pneumonia caused by *Mycoplasma* and *Legionella*, infections by gram-positive cocci in penicillin-allergic patients	Generally well tolerated but some diarrhea.
Clindamycin	Anaerobes such as *Clostridium perfringens* and *Bacteroides fragilis*	Pseudomembranous colitis is a major side effect.
Linezolid	Vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, and penicillin-resistant pneumococci	Generally well tolerated.

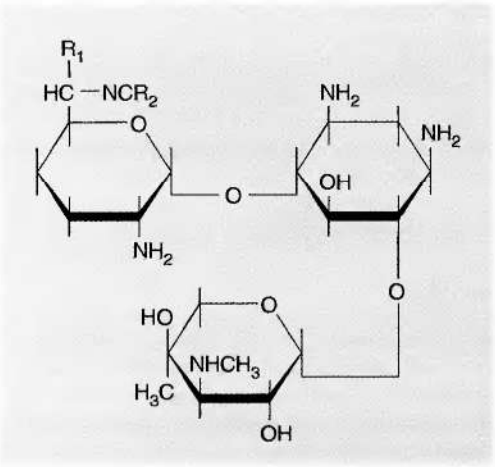

Figure 10–4. Aminoglycosides. Aminoglycosides consist of amino sugars joined by a glycosidic linkage. The structure of gentamicin is shown.

ties. In general, tetracyclines have low toxicity but are associated with two important side effects. One is suppression of the normal flora of the intestinal tract, which can lead to diarrhea and overgrowth by drug-resistant bacteria and fungi. The other is brown staining of the teeth of fetuses and young children as a result of deposition of the drug in developing teeth; tetracyclines are avid calcium chelators. For this reason, tetracycline is contraindicated for use in pregnant women and in children younger than 8 years of age.

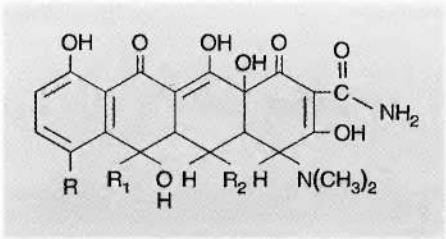

Figure 10–5. Tetracycline structure. The four-ring structure is depicted with its three R sites. Chlortetracycline, for example, has R = Cl, $R_1 = CH_3$, and $R_2 = H$.

2. Drugs That Act on the 50S Subunit

Chloramphenicol

Chloramphenicol is active against a broad range of organisms, including gram-positive and gram-negative bacteria (including anaerobes). It is bacteriostatic against certain organisms, such as *Salmonella typhi,* but has bactericidal activity against the three important encapsulated organisms that cause meningitis: *Haemophilus influenzae, Streptococcus pneumoniae,* and *Neisseria meningitidis.*

Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and **blocking the action of peptidyltransferase;** this prevents the synthesis of new peptide bonds. It inhibits bacterial protein synthesis selectively, because it binds to the catalytic site of the transferase in the 50S bacterial ribosomal subunit but not to the transferase in the 60S human ribosomal subunit. Chloramphenicol inhibits protein synthesis in the mitochondria of human cells to some extent, since mitochondria have a 50S subunit (mitochondria are thought to have evolved from bacteria). This inhibition may be the cause of the dose-dependent toxicity of chloramphenicol to bone marrow (discussed below).

Chloramphenicol is a comparatively simple molecule with a nitrobenzene nucleus (Figure 10–6). Nitrobenzene itself is a bone marrow depressant; therefore, the nitrobenzene portion of the molecule may be involved in the hematologic problems reported with this drug. The most important side effect of chloramphenicol is bone marrow toxicity, of which there are two types. One is a dose-dependent suppression, which is more likely to occur in patients receiving high doses for long periods and which is reversible when administration of the drug is stopped. The other is aplastic anemia, which is caused by an idiosyncratic reaction to the drug. This reaction is not dose-dependent, can occur weeks after administration of the drug has been stopped, and is not reversible. Fortunately, this reaction is rare, occurring in about 1:30,000 patients.

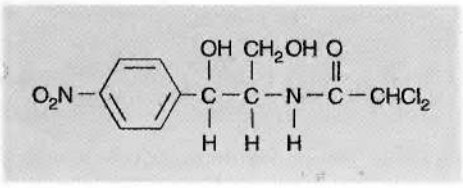

Figure 10–6. Chloramphenicol.

Erythromycin

Erythromycin is a bacteriostatic drug with a wide spectrum of activity. It is the treatment of choice for pneumonia caused by *Legionella* (a gram-negative rod) and *Mycoplasma* (a wall-less bacterium) and is also an effective alternative against a variety of infections caused by gram-positive cocci in penicillin-allergic patients. Erythromycin, azithromycin, and clarithromycin are members of a group of drugs called **macrolides,** named for their large ring structure (Figure 10–7).

Erythromycin binds to the 50S subunit and blocks the translocation step by preventing the release of the uncharged tRNA from the donor site after the peptide bond is formed. It has a macrolide structure composed of a large 13-carbon ring to which two sugars are attached by glycosidic linkages (Figure 10–7). Erythromycin is one of the least toxic drugs, with only some gastrointestinal distress associated with oral use.

Two derivatives of erythromycin, azithromycin and clarithromycin, have the same mechanism of action as erythromycin but are effective against a broader range of organisms and have a longer half-life, which means they can be taken only once or twice a day.

Clindamycin

The most useful clinical activity of this bacteriostatic drug is against anaerobes, both gram-positive bacteria such as *Clostridium perfringens* and gram-negative bacteria such as *Bacteroides fragilis.*

Clindamycin binds to the 50S subunit and blocks peptide bond formation by an undetermined mechanism. Its specificity for bacteria arises from its inability to bind to the 60S subunit of human ribosomes.

The most important side effect of clindamycin is pseudomembranous colitis, which, in fact, can occur with virtually any antibiotic, whether taken orally or parenterally. The pathogenesis of this potentially severe complication is suppression of the normal flora of the bowel by the drug and overgrowth of a drug-resistant strain of *Clostridium difficile.* The organism secretes an exotoxin that produces the pseudomembrane in the colon and severe, often bloody diarrhea.

Linezolid

Linezolid is useful for the treatment of vancomycin-resistant enterococci, methicillin-resistant *S. aureus* and *S. epidermidis,* and penicillin-resistant pneumococci. It is bacteriostatic against enterococci and staphylococci but bactericidal against pneumococci.

Linezolid binds to the 23S ribosomal RNA in the 50S subunit and inhibits protein synthesis, but the precise mechanism is unknown. It appears to block some early step (initiation) in ribosome formation.

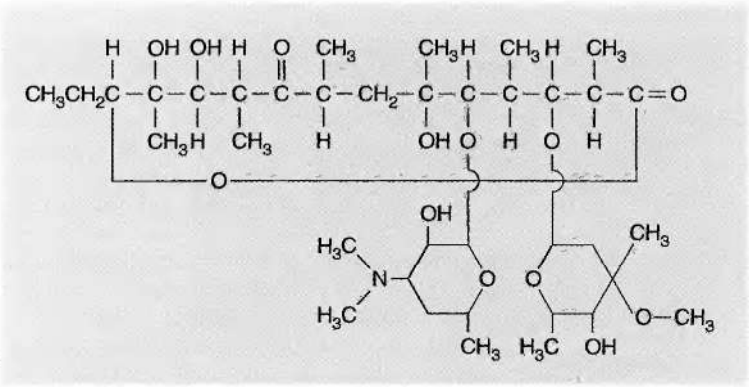

Figure 10–7. Erythromycin.

INHIBITION OF NUCLEIC ACID SYNTHESIS

1. Inhibition of Precursor Synthesis

Sulfonamides

Either alone or in combination with trimethoprim, sulfonamides are useful in a variety of bacterial diseases such as urinary tract infections caused by *Escherichia coli,* otitis media caused by *S. pneumoniae* or *H. influenzae* in children, shigellosis, nocardiosis, and chancroid. In combination, they are also the drugs of choice for two protozoan diseases, toxoplasmosis and *Pneumocystis* pneumonia. The sulfonamides are a large family of bacteriostatic drugs that are produced by chemical synthesis. In 1935, the parent compound, sulfanilamide, became the first clinically effective antimicrobial agent.

The mode of action of sulfonamides is to block the synthesis of tetrahydrofolic acid, which is required as a methyl donor in the synthesis of the nucleic acid precursors adenine, guanine, and thymine. Sulfonamides are **structural analogues of *p*-aminobenzoic acid** (PABA). PABA condenses with a pteridine compound to form dihydropteroic acid, a precursor of tetrahydrofolic acid (Figure 10–8). Sulfonamides compete with PABA for the active site of the enzyme dihydropteroate synthetase. This competitive inhibition can be overcome by an excess of PABA.

The basis of the selective action of sulfonamides on bacteria is that many bacteria synthesize their folic acid from PABA-containing precursors, whereas human cells require preformed folic acid as an exogenous nutrient because they lack the enzymes to synthesize it. Human cells therefore bypass the step at which sulfonamides act. Bacteria that can use preformed folic acid are similarly resistant to sulfonamides.

The *p*-amino group on the sulfonamide is essential for its activity. Modifications are therefore made on the sulfonic acid side chain.

Sulfonamides are inexpensive and rarely cause side effects. However, drug-related fever, rashes, and bone marrow suppression can occur.

Trimethoprim

Trimethoprim also inhibits the production of tetrahydrofolic acid but by a mechanism different from that of the sulfonamides; ie, it inhibits the enzyme **dihydrofolate reductase** (Figure 10–8). Its specificity for bacteria is based on its much greater affinity for bacterial reductase than for the human enzyme.

Trimethoprim is used most frequently together with sulfamethoxazole. Note that both drugs act on the same pathway—but at different sites—to inhibit the synthesis of tetrahydrofolate. The advantages of the combination are that (1) bacterial mutants resistant to one drug will be inhibited by the other and that (2) the two drugs can act **synergistically,** ie, when used together, they cause significantly greater inhibition than the sum of the inhibition caused by each drug separately.

Trimethoprim-sulfamethoxazole is clinically useful in the treatment of urinary tract infections, *Pneumocystis* pneumonia, and shigellosis. It also is used for prophylaxis in granulopenic patients to prevent opportunistic infections.

2. Inhibition of DNA Synthesis

Quinolones

Quinolones are bactericidal drugs that block bacterial DNA synthesis by inhibiting DNA gyrase (**topoisomerase**). Fluoroquinolones, such as ciprofloxacin (Figure 10–9), norfloxacin, ofloxacin, and others, are active against a broad range of organisms that cause infections of the lower respiratory tract, intestinal tract, urinary

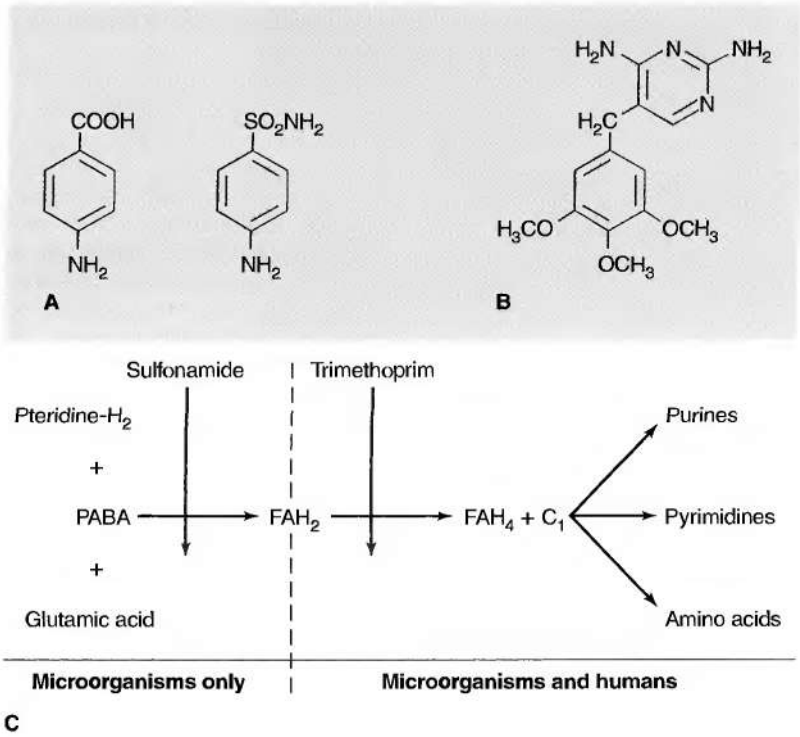

Figure 10–8. Sulfonamide and trimethoprim. **A:** Comparison of PABA (**left**) and sulfonamide (**right**). **B:** Trimethoprim. **C:** Inhibition of the folic acid pathway by sulfonamide and trimethoprim (FAH_2 = dihydrofolate; FAH_4 = tetrahydrofolate). (Modified and reproduced, with permission, from Corcoran JW, Hahn FE [editors]: *Mechanism of Action of Antimicrobial and Antitumor Agents.* Vol. 3 of: *Antibiotics.* Springer-Verlag, 1975.)

tract, and skeletal and soft tissues. Fluoroquinolones should not be given to pregnant women and young children because they damage growing bone. Nalidixic acid, which is not a fluoroquinolone, is much less active and is used only for the treatment of urinary tract infections. Quinolones are not recommended for children and pregnant women because they damage growing cartilage.

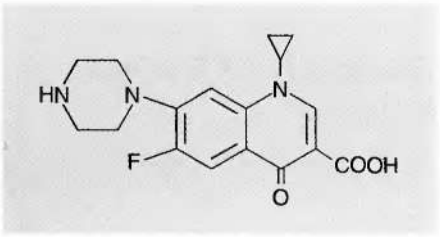

Figure 10–9. Ciprofloxacin. The triangle indicates a cyclopropyl group.

Flucytosine

Flucytosine (fluorocytosine, 5-FC) is an antifungal drug that inhibits DNA synthesis. It is a nucleoside analogue that is metabolized to fluorouracil, which inhibits thymidylate synthetase, thereby limiting the supply of thymidine. It is used in combination with amphotericin B in the treatment of disseminated cryptococcal or candidal infections, especially cryptococcal meningitis. It is not used alone because resistant mutants emerge very rapidly.

3. Inhibition of mRNA Synthesis

Rifampin is used primarily for the treatment of tuberculosis in combination with other drugs and for prophylaxis in close contacts of patients with meningitis caused by either *N. meningitidis* or *H. influenzae.* It is also used in combination with other drugs in the treatment of prosthetic-valve endocarditis caused by *S. epidermidis.* With the exception of the short-term prophylaxis of meningitis, rifampin is given in combination

with other drugs because resistant mutants appear at a high rate when it is used alone.

The selective mode of action of rifampin is based on **blocking mRNA synthesis** by bacterial RNA polymerase without affecting the RNA polymerase of human cells. Rifampin is red, and the urine, saliva, and sweat of patients taking rifampin often turn orange; this is disturbing but harmless. Rifampin is excreted in high concentration in saliva, which accounts for its success in the prophylaxis of bacterial meningitis since the organisms are carried in the throat.

Rifabutin, a rifampin derivative with the same mode of action as rifampin, is useful in the prevention of disease caused by *Mycobacterium avium-intracellulare* in patients with severely reduced numbers of helper T cells, eg, AIDS patients.

ALTERATION OF CELL MEMBRANE FUNCTION

1. Alteration of Bacterial Cell Membranes

There are few antimicrobial compounds that act on the cell membrane, because the structural and chemical similarities of bacterial and human cell membranes make it difficult to provide sufficient selective toxicity.

Polymyxins are a family of polypeptide antibiotics of which the clinically most useful compound is polymyxin E (colistin). It is active against gram-negative rods, especially *P. aeruginosa.* Polymyxins are cyclic peptides composed of 10 amino acids, 6 of which are diaminobutyric acid. The positively charged free amino groups act like a cationic detergent to disrupt the phospholipid structure of the cell membrane.

2. Alteration of Fungal Cell Membranes

Amphotericin B, the most important antifungal drug, is used in the treatment of a variety of disseminated fungal diseases. It is classified as a polyene compound because it has a series of seven unsaturated double bonds in its macrolide ring structure (*poly* means many, and *-ene* is a suffix indicating the presence of double bonds; Figure 10–10). It disrupts the cell membrane of fungi because of its affinity for **ergosterol,** a component of fungal membranes but not of bacterial or human cell membranes. Fungi resistant to amphotericin B have rarely been recovered from patient specimens. Amphotericin B has significant renal toxicity; measurement of serum creatinine levels is used to monitor the dose. Nephrotoxicity is significantly reduced when the drug is administered in lipid vehicles such as liposomes, but these formulations are expensive. Fever, chills, nausea, and vomiting are common side effects.

Nystatin is another polyene antifungal agent, which, because of its toxicity, is used topically for infections caused by the yeast *Candida.*

Azoles are antifungal drugs that act by **inhibiting ergosterol synthesis.** They block cytochrome P-450-dependent demethylation of lanosterol, the precursor of ergosterol. Fluconazole, ketoconazole, voriconazole, and itraconazole are used to treat systemic fungal diseases; clotrimazole and miconazole are used only topically because they are too toxic to be given systemically. The two nitrogen-containing azole rings of fluconazole can be seen in Figure 10–11.

Ketoconazole is useful in the treatment of blastomycosis, chronic mucocutaneous candidiasis, coccidioidomycosis, and skin infections caused by dermatophytes. Fluconazole is useful in the treatment of

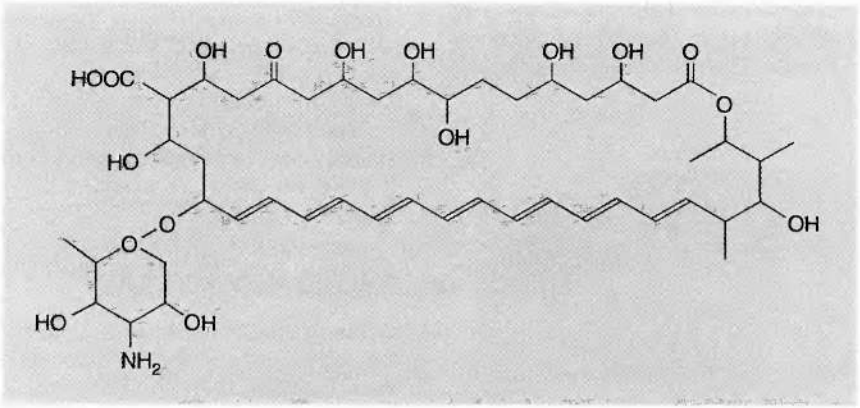

Figure 10–10. Amphotericin B.

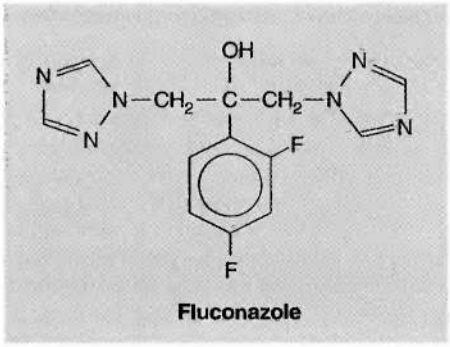

Figure 10–11. Fluconazole.

candidal and cryptococcal infections. Itraconazole is used to treat histoplasmosis and blastomycosis. Miconazole and clotrimazole, two other imidazoles, are useful for topical therapy of *Candida* infections and dermatophytoses. Fungi resistant to the azole drugs have rarely been recovered from patient specimens.

ADDITIONAL DRUG MECHANISMS

1. Antibacterial Activity

Isoniazid, or isonicotinic acid hydrazide (INH), is a bactericidal drug highly specific for *Mycobacterium tuberculosis* and other mycobacteria. It is used in combination with other drugs to treat tuberculosis and by itself to prevent tuberculosis in exposed persons. Because it penetrates human cells well, it is effective against the organisms residing within macrophages. The structure of isoniazid is shown in Figure 10–12.

INH **inhibits mycolic acid synthesis,** which explains why it is specific for mycobacteria and relatively nontoxic for humans. The drug inhibits a reductase required for the synthesis of the long-chain fatty acids called mycolic acids that are an essential constituent of mycobacterial cell walls. The active drug is probably a metabolite of INH formed by the action of catalase-peroxidase, because deletion of the gene for these enzymes results in resistance to the drug. Its main side effect is liver toxicity. It is given with pyridoxine to prevent neurologic complications.

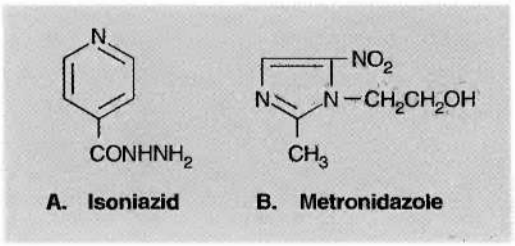

Figure 10–12. **A:** Isoniazid. **B:** Metronidazole.

Metronidazole (Flagyl) is bactericidal against anaerobic bacteria. (It is also effective against certain protozoa such as *Giardia* and *Trichomonas.*) This drug has two possible mechanisms of action, and it is unclear which is the more important. The first, which explains its specificity for anaerobes, is its ability to act as an **electron sink.** By accepting electrons, the drug deprives the organism of required reducing power. In addition, when electrons are acquired, the drug ring is cleaved and a toxic intermediate is formed that damages DNA. The precise nature of the intermediate and its action is unknown. The structure of metronidazole is shown in Figure 10–12.

The second mode of action of metronidazole relates to its ability to inhibit DNA synthesis. The drug binds to DNA and causes strand breakage, which prevents its proper functioning as a template for DNA polymerase.

Ethambutol is a bacteriostatic drug active against *M. tuberculosis* and many of the atypical mycobacteria. It is thought to act by inhibiting the synthesis of arabinogalactan, which functions as a link between the mycolic acids and the peptidoglycan of the organism.

Pyrazinamide (PZA) is a bactericidal drug used in the treatment of tuberculosis but not in the treatment of most atypical mycobacterial infections. PZA is particularly effective against semidormant organisms in the lesion, which are not affected by INH or rifampin. PZA acts by inhibiting a fatty acid synthetase that prevents the synthesis of mycolic acid. It is converted to the active intermediate, pyrazinoic acid by an amidase in the mycobacteria.

2. Antifungal Activity

Griseofulvin is an antifungal drug that is useful in the treatment of hair and nail infections caused by dermatophytes. It binds to tubulin in microtubules and may act by preventing formation of the mitotic spindle.

Pentamidine is active against fungi and protozoa. It is widely used to prevent or treat pneumonia caused by *Pneumocystis carinii.* It inhibits DNA synthesis by an unknown mechanism.

CHEMOPROPHYLAXIS

In most instances, the antimicrobial agents described in this chapter are used for the *treatment* of infectious diseases. However, there are times when they are used to *prevent* diseases from occurring, a process called **chemoprophylaxis.**

Chemoprophylaxis is used in three circumstances: prior to surgery, in immunocompromised patients, and in people with normal immunity who have been exposed to certain pathogens. Table 10–5 describes the drugs and the situations in which they are used. For more information, see the chapters on the individual organisms.

PROBIOTICS

In contrast to the chemical antibiotics previously described in this chapter, probiotics are live, nonpathogenic bacteria that may be effective in the treatment or prevention of certain human diseases. The suggested basis for the possible beneficial effect lies either in providing colonization resistance by which the nonpathogen excludes the pathogen from binding sites on the mucosa, in enhancing the immune response against the pathogen, or in reducing the inflammatory response against the pathogen. For example, the oral administration of live *Lactobacillus rhamnosus* strain GG significantly reduces the number of cases of nosocomial diarrhea in young children.

Table 10–5. Chemoprophylactic use of drugs described in this chapter.

Drug	Use	Number of Chapter for Additional Information
Penicillin	1. Prevent recurrent pharyngitis in those who have had rheumatic fever	15
	2. Prevent syphilis in those exposed to *Treponema pallidum*	24
	3. Prevent pneumococcal sepsis in splenectomized young children	15
Ampicillin	1. Prevent neonatal sepsis and meningitis in children born of mothers carrying group B streptococci	15
	2. Prevent enterococcal endocarditis in those with damaged heart valves who are undergoing GI or GU tract surgery (used in combination with gentamicin)	15
Cefazolin	Prevent staphylococcal surgical wound infections	15
Ceftriaxone	Prevent gonorrhea in those exposed to *Neisseria gonorrhoeae*	16
Ciprofloxacin	1. Prevent meningitis in those exposed to *Neisseria meningitidis*	16
	2. Prevent anthrax in those exposed to *Bacillus anthracis*	17
	3. Prevent infection in neutropenic patients	68
Rifampin	Prevent meningitis in those exposed to *N. meningitidis* and *Haemophilus influenzae*	16, 19
Isoniazid	Prevent progression of *Mycobacterium tuberculosis* in those recently infected who are asymptomatic[1]	21
Erythromycin	1. Prevent pertussis in those exposed to *Bordetella pertussis*	19
	2. Prevent gonococcal and chlamydial conjunctivitis in newborns	16, 25
Tetracycline	Prevent plague in those exposed to *Yersinia pestis*	20
Fluconazole	Prevent cryptococcal meningitis in AIDS patients	50
Clotrimazole	Prevent thrush in AIDS patients and in others with reduced cell-mediated immunity	50
Trimethoprim-sulfamethoxazole	1. Prevent *Pneumocystis* pneumonia in AIDS patients	52
	2. Prevent recurrent urinary tract infections	18
Pentamidine	Prevent *Pneumocystis* pneumonia in AIDS patients	52

GI = gastrointestinal, GU = genitourinary.

[1] Chemoprophylaxis with isoniazid is also viewed as treatment of asymptomatic individuals (see Chapter 21).

PEARLS

- *For an antibiotic to be clinically useful, it must exhibit* ***selective toxicity;*** *that is, it must inhibit bacterial processes significantly more than it inhibits human cell processes.*
- *There are four main targets of antibacterial drugs:* ***cell wall, ribosomes, cell membrane,*** *and* ***nucleic acids.*** *Humans are not affected by these drugs because we do not have a cell wall, and we have different ribosomes, nucleic acid enzymes, and sterols in our membranes.*
- ***Bactericidal*** *drugs kill bacteria, whereas* ***bacteriostatic*** *drugs inhibit the growth of the bacteria but do not kill. Bacteriostatic drugs depend on the phagocytes of the patient to kill the organism. If a patient has too few neutrophils, then bactericidal drugs should be used.*

Inhibition of Cell Wall Synthesis

- ***Penicillins*** *and* ***cephalosporins*** *act by inhibiting* ***transpeptidases,*** *the enzymes that cross-link peptidoglycan. Transpeptidases are also referred to as* ***penicillin-binding proteins.*** *Several medically important bacteria, eg,* Streptococcus pneumoniae, *manifest resistance to penicillins based on mutations in the genes encoding penicillin-binding proteins.*
- *Exposure to penicillins activates* ***autolytic enzymes*** *that degrade the bacteria. If these autolytic enzymes are not activated, eg, in certain strains of* Staphylococcus aureus, *the bacteria are not killed and the strain is said to be* ***tolerant.***
- *Penicillins kill bacteria when they are growing, ie, when they are synthesizing new peptidoglycan. Penicillins are therefore* ***more active during the log phase*** *of bacterial growth than during the lag phase or the stationary phase.*
- *Penicillins and cephalosporins are* ***β-lactam drugs,*** *ie, an* ***intact β-lactam ring is required*** *for activity.* ***β-lactamases,*** *eg, penicillinases and cephalosporinases, cleave the β-lactam ring and inactivate the drug.*
- ***Modification of the side chain*** *adjacent to the β-lactam ring endows these drugs with* ***new properties,*** *such as expanded activity against gram-negative rods, ability to be taken orally, and protection against degradation by β-lactamases. For example, the original penicillin (benzyl penicillin, penicillin G) cannot be taken orally because stomach acid hydrolyzes the bond between the β-lactam ring and the side chain. But ampicillin and amoxicillin can be taken orally because they have a different side chain.*
- ***Hypersensitivity*** *to penicillins, especially* ***IgE-mediated anaphylaxis,*** *remains a significant problem.*
- ***Cephalosporins*** *are structurally* similar *to penicillins: both have a β-lactam ring. The first-generation cephalosporins are active primarily against gram-positive cocci and the second, third, and fourth generations have expanded coverage against gram-negative rods.*
- *Carbapenems, such as imipenem, and monobactams, such as aztreonam, are also β-lactam drugs but are structurally different from penicillins and cephalosporins.*
- ***Vancomycin*** *is a* ***glycopeptide,*** *ie, it is* not *a β-lactam drug, but its mode of action is very similar to that of penicillins and cephalosporins, ie, it* ***inhibits transpeptidases.***
- ***Caspofungin*** *is a lipopeptide that inhibits fungal cell wall synthesis by blocking the synthesis of β-glucan, a polysaccharide component of the cell wall.*

Inhibition of Protein Synthesis

- ***Aminoglycosides*** *and* ***tetracyclines*** *act at the level of the 30S ribosomal subunit, whereas* ***chloramphenicol, erythromycins,*** *and* ***clindamycin*** *act at the level of the 50S ribosomal subunit.*
- ***Aminoglycosides*** *inhibit bacterial protein synthesis by binding to the 30S subunit, which* ***blocks the initiation complex.*** *No peptide bonds are formed and no polysomes are made. Aminoglycosides are a family of drugs that includes gentamicin, tobramycin, and streptomycin.*
- ***Tetracyclines*** *inhibit bacterial protein synthesis by* ***blocking the binding of aminoacyl t-RNA*** *to the 30S ribosomal subunit. The tetracyclines are a family of drugs; doxycycline is used most often.*
- ***Chloramphenicol*** *inhibits bacterial protein synthesis by* ***blocking peptidyl transferase,*** *the enzyme*

that adds the new amino acid to the growing polypeptide. Chloramphenicol can cause bone marrow suppression.

- ***Erythromycin*** *inhibits bacterial protein synthesis by* ***blocking the release of the t-RNA*** *after it has delivered its amino acid to the growing polypeptide. Erythromycin is a member of the macrolide family of drugs that includes azithromycin and clarithromycin.*
- ***Clindamycin*** *binds to the same site on the ribosome as does erythromycin and is thought to act in the same manner. It is effective against many anaerobic bacteria. Clindamycin is one of the antibiotics that predisposes to pseudomembranous colitis caused by* Clostridium difficile *and is infrequently used.*

Inhibition of Nucleic Acid Synthesis

- ***Sulfonamides*** *and* ***trimethoprim*** *inhibit* ***nucleotide*** *synthesis,* ***quinolones*** *inhibit* ***DNA*** *synthesis, and* ***rifampin inhibits RNA*** *synthesis.*
- ***Sulfonamides*** *and* ***trimethoprim*** *inhibit the* ***synthesis of tetrahydrofolic acid,*** *the main donor of the methyl groups that are required to synthesize adenine, guanine, and thymine.* ***Sulfonamides*** *are structural analogues of para-aminobenzoic acid, which is a component of folic acid.* ***Trimethoprim*** *inhibits* ***dihydrofolate reductase,*** *the enzyme that reduces dihydrofolic acid to tetrahydrofolic acid. A combination of sulfamethoxazole and trimethoprim is often used because bacteria resistant to one drug will be inhibited by the other.*
- ***Quinolones*** *inhibit DNA synthesis in bacteria by* ***blocking DNA gyrase*** *(topoisomerase), the enzyme that unwinds DNA strands so that they can be replicated. Quinolones are a family of drugs that includes ciprofloxacin, ofloxacin, and levofloxacin.*
- ***Rifampin*** *inhibits RNA synthesis in bacteria by* ***blocking the RNA polymerase*** *that synthesizes mRNA. Rifampin is typically used in combination with other drugs because there is a* ***high rate of mutation of the RNA polymerase gene,*** *which results in rapid resistance to the drug.*

Alteration of Cell Membrane Function

- ***Antifungal*** *drugs predominate in this category. These drugs have selective toxicity because* ***fungal cell membranes contain ergosterol,*** *whereas human cell membranes have cholesterol.* ***Bacteria, with the exception of Mycoplasma,*** *do not have sterols in their membranes and therefore are resistant to these drugs.*
- ***Amphotericin B*** *disrupts fungal cell membranes by* ***binding at the site of ergosterol*** *in the membrane. It is used to treat the most serious systemic fungal diseases but has significant side effects, especially on the kidney.*
- ***Azoles*** *are antifungal drugs that* ***inhibit ergosterol synthesis.*** *The azole family includes drugs such as ketoconazole, fluconazole, itraconazole, and clotrimazole. They are useful in the treatment of systemic as well as skin and mucous membrane infections.*

Additional Drug Mechanisms

- ***Isoniazid inhibits the synthesis of mycolic acid,*** *a long-chain fatty acid found in the cell wall of mycobacteria. Isoniazid is a* ***prodrug*** *that requires a bacterial* ***peroxidase (catalase) to activate isoniazid*** *to the metabolite that inhibits mycolic acid synthesis. Isoniazid is the most important drug used in the treatment of tuberculosis and other mycobacterial diseases.*
- ***Metronidazole*** *is* ***effective against anaerobic bacteria and certain protozoa*** *because it* ***acts as an electron sink,*** *taking away the electrons that the organisms need to survive. It also forms toxic intermediates that damage DNA.*

Chemoprophylaxis

- *Antimicrobial drugs are used to prevent infectious diseases as well as to treat them. Chemoprophylactic drugs are given primarily in three circumstances: to prevent surgical wound infections, to prevent opportunistic infections in immunocompromised patients, and to prevent infections in those known to be exposed to pathogens that cause serious infectious diseases.*

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part X: USMLE (National Board) Practice Questions starting on page 517. Also see Part XI: USMLE (National Board) Practice Examination starting on page 568.

Antimicrobial Drugs: Resistance

11

There are four major mechanisms that mediate bacterial resistance to drugs (Table 11–1). (1) Bacteria produce enzymes that **inactivate the drug;** eg, β-lactamases can inactivate penicillins and cephalosporins by cleaving the β-lactam ring of the drug. (2) Bacteria **synthesize modified targets** against which the drug has no effect; eg, a mutant protein in the 30S ribosomal subunit can result in resistance to streptomycin, and a methylated 23S rRNA can result in resistance to erythromycin. (3) Bacteria **decrease their permeability** such that an effective intracellular concentration of the drug is not achieved; eg, changes in porins can reduce the amount of penicillin entering the bacterium. (4) Bacteria **actively export drugs** using a "multidrug resistance pump" (MDR pump, or "efflux" pump). The MDR pump imports protons and, in an exchange-type reaction, exports a variety of foreign molecules including certain antibiotics, such as quinolones.

Most drug resistance is due to a genetic change in the organism, either a chromosomal **mutation** or the acquisition of a **plasmid** or **transposon.** Nongenetic changes, which are of lesser importance, are discussed on page 86.

Hospital-acquired infections are significantly more likely to be caused by antibiotic-resistant organisms than are community-acquired infections. This is especially true for hospital infections caused by *Staphylococcus aureus* and enteric gram-negative rods such as *Escherichia coli* and *Pseudomonas aeruginosa.* Antibiotic-resistant organisms are common in the hospital setting because widespread antibiotic use in hospitals selects for these organisms. Furthermore, hospital strains are often resistant to multiple antibiotics. This resistance is usually due to the acquisition of plasmids carrying several genes thar encode the enzymes that mediate resistance.

Table 11–2 describes certain medically important bacteria and the main drugs to which they are resistant. Note that although these bacteria are resistant to other drugs as well, for simplicity, only the most characteristic drugs are listed.

GENETIC BASIS OF RESISTANCE

Chromosome-Mediated Resistance

Chromosomal resistance is due to a mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug. The frequency of spontaneous mutations usually ranges from 10^{-7} to 10^{-9}, which is much

Table 11–1. Mechanisms of drug resistance.

Mechanism	Important Example	Drugs Commonly Affected
Inactivate drug	Cleavage by β-lactamase	β-Lactam drugs such as penicillins, cephalosporins
Modify drug target in bacteria	1. Mutation in penicillin-binding proteins	Penicillins
	2. Mutation in protein in 30S ribosomal subunit	Aminoglycosides, such as streptomycin
	3. Replace alanine with lactate in peptidoglycan	Vancomycin
	4. Mutation in DNA gyrase	Quinolones
	5. Mutation in RNA polymerase	Rifampin
	6. Mutation in catalase-peroxidase	Isoniazid
Reduce permeability of drug	Mutation in porin proteins	Penicillins, aminoglycosides, and others
Export of drug from bacteria	Multidrug resistance pump	Tetracyclines, sulfonamides

lower than the frequency of acquisition of resistance plasmids. Therefore, chromosomal resistance is less of a clinical problem than is plasmid-mediated resistance.

The treatment of certain infections with two or more drugs is based on the following principle. If the frequency that a bacterium mutates to become resistant to antibiotic A is 10^{-7} (1 in 10 million) and the frequency that the same bacterium mutates to become resistant to antibiotic B is 10^{-8} (1 in 100 million), then the chance that the bacterium will become resistant to both antibiotics (assuming that the antibiotics act by different mechanisms) is the product of the two probabilities, or 10^{-15}. It is therefore highly unlikely that the bacterium will become resistant to *both* antibiotics. Stated another way, although an organism may be resistant to one antibiotic, it is likely that it will be effectively treated by the other antibiotic.

Plasmid-Mediated Resistance

Plasmid-mediated resistance is very important from a clinical point of view for three reasons:

(1) It occurs in many different species, especially gram-negative rods

(2) Plasmids frequently mediate resistance to multiple drugs

(3) Plasmids have a high rate of transfer from one cell to another, usually by conjugation.

Resistance plasmids (resistance factors, R factors) are extrachromosomal, circular, double-stranded DNA molecules that carry the genes for a variety of enzymes that can degrade antibiotics and modify membrane transport systems (Figure 11–1). Table 11–3 describes the most important mechanisms of resistance for several important drugs.

R factors may carry one antibiotic resistance gene or may carry two or more of these genes. The medical implications of a plasmid carrying more than one resistance gene is 2-fold: first and most obvious is that a bacterium containing that plasmid can be resistant to more than one class of antibiotics (eg, penicillins and aminoglycosides) and second, that the use of an antibiotic that selects for an organism resistant to one antibiotic will select for an organism that is resistant to all the antibiotics whose resistance genes are carried by the plasmid. For example, if an organism has the R plasmid depicted in Figure 11–1, then the use of penicillin will select for an

Table 11–2. Medically important bacteria that exhibit significant drug resistance.

Type of Bacteria	Clinically Significant Drug Resistance
Gram-positive cocci	
Staphylococcus aureus	Penicillin G, nafcillin
Streptococcus pneumoniae	Penicillin G
Enterococcus faecalis	Penicillin G, aminoglycosides, vancomycin
Gram-negative cocci	
Neisseria gonorrhoeae	Penicillin G
Gram-positive rods	
None	
Gram-negative rods	
Haemophilus influenzae	Ampicillin
Pseudomonas aeruginosa	β-Lactams,[1] aminoglycosides
Enterobacteriaceae[2]	β-Lactams,[1] aminoglycosides
Mycobacteria	
M. tuberculosis[3]	Isoniazid, rifampin
M. avium-intracellulare	Isoniazid, rifampin, and many others

[1]β-Lactams are penicillins and cephalosporins.

[2]The family Enterobacteriaceae includes bacteria such as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae,* and *Serratia marcescens.*

[3]Some strains of *M. tuberculosis* are resistant to more than two drugs.

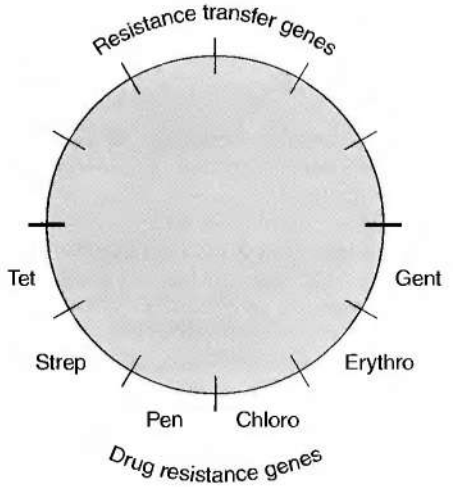

Figure 11–1. Resistance plasmid (R plasmid, R factor). Most resistance plasmids have two sets of genes: (1) resistance transfer genes that encode the sex pilus and other proteins that mediate transfer of the plasmid DNA during conjugation and (2) drug resistance genes that encode the proteins that mediate drug resistance. The bottom half of the figure depicts, from left to right, the genes that encode resistance to tetracycline, streptomycin, penicillin (β-lactamase), chloramphenicol, erythromycin, and gentamicin.

organism resistant not only to penicillin but also to tetracyclines, aminoglycosides (such as streptomycin and gentamicin), chloramphenicol, and erythromycin.

In addition to producing drug resistance, R factors have two very important properties: (1) They can replicate independently of the bacterial chromosome; therefore, a cell can contain many copies; and (2) they can be transferred not only to cells of the same species but also to other species and genera. Note that this conjugal transfer is under the control of the genes of the R plasmid and not of the F (fertility) plasmid, which governs the transfer of the bacterial chromosome (see Chapter 4).

R factors exist in two broad size categories: large plasmids with molecular weights of about 60 million and small ones with molecular weights of about 10 million. The large plasmids are conjugative R factors, which contain the extra DNA to code for the conjugation process. The small R factors are not conjugative and contain only the resistance genes.

In addition to conveying antibiotic resistance, R factors impart two other traits: (1) resistance to metal ions (eg, they code for an enzyme that reduces mercuric ions to elemental mercury); and (2) resistance to certain bacterial viruses by coding for restriction endonucleases that degrade the DNA of the infecting bacteriophages.

Transposon-Mediated Resistance

Transposons are genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmids. A typical drug resistance transposon is composed of three genes flanked on both sides by shorter DNA sequences, usually a series of inverted repeated bases that mediate the interaction of the transposon with the larger DNA (see Figure 2–7). The three genes code for (1) transposase, the enzyme that catalyzes excision and reintegration of the transposon; (2) a repressor that regulates synthesis of the transposase; and (3) the drug resistance gene.

SPECIFIC MECHANISMS OF RESISTANCE

Penicillins & Cephalosporins. There are several mechanisms of resistance to these drugs. Cleavage by **β-lactamases** (penicillinases and cephalosporinases) is by far the most important (see Figure 10–1). β-Lactamases

Table 11–3. R-factor–mediated resistance mechanisms.

Drug	Mechanism of Resistance
Penicillins and cephalosporins	β-Lactamase cleavage of β-lactam ring
Aminoglycosides	Modification by acetylation, adenylylation, or phosphorylation
Chloramphenicol	Modification by acetylation
Erythromycin	Change in receptor by methylation of rRNA
Tetracycline	Reduced uptake or increased export
Sulfonamides	Active export out of the cell and reduced affinity of enzyme

produced by various organisms have different properties. For example, staphylococcal penicillinase is inducible by penicillin and is secreted into the medium. In contrast, some β-lactamases produced by several gram-negative rods are constitutively produced, are located in the periplasmic space near the peptidoglycan, and are not secreted into the medium. The β-lactamases produced by various gram-negative rods have different specificities; some are more active against cephalosporins, others against penicillins. Clavulanic acid and sulbactam are penicillin analogues that bind strongly to β-lactamases and inactivate them. Combinations of these inhibitors and penicillins, eg, clavulanic acid and amoxicillin (Augmentin), can overcome resistance mediated by many but not all β-lactamases.

Resistance to penicillins can also be due to changes in the **penicillin-binding proteins** in the bacterial cell membrane. These changes account for both the low-level and the high-level resistance exhibited by *Streptococcus pneumoniae* to penicillin G and for the resistance of *S. aureus* to nafcillin and other β-lactamase–resistant penicillins. The relative resistance of *Enterococcus faecalis* to penicillins may be due to altered penicillin-binding proteins. Low-level resistance of *Neisseria gonorrhoeae* to penicillin is attributed to **poor permeability** to the drug. High-level resistance is due to the presence of a plasmid coding for penicillinase.

Some isolates of *S. aureus* demonstrate yet another form of resistance, called **tolerance,** in which growth of the organism is inhibited by penicillin but the organism is not killed. This is attributed to a failure of activation of the autolytic enzymes, murein hydrolases, which degrade the peptidoglycan.

Vancomycin. Resistance to vancomycin is caused by a change in the peptide component of peptidoglycan from D-alanyl-D-alanine, which is the normal binding site for vancomycin, to D-alanine-D-lactate, to which the drug does not bind. Of the four gene loci mediating vancomycin resistance, VanA is the most important. It is carried by a transposon on a plasmid and provides high-level resistance to both vancomycin and teichoplanin. (Teichoplanin is used in Europe but is not approved in the United States.) The VanA locus encodes the enzymes that synthesize D-ala-D-lactate as well as several regulatory proteins.

Vancomycin-resistant strains of enterococci have been recovered from clinical specimens. Rare isolates of *S. aureus* that exhibit resistance to vancomycin have also been recovered from patient specimens. Rare isolates of *S. pneumoniae* that exhibit tolerance to vancomycin have been recovered as well.

Aminoglycosides. Resistance to aminoglycosides occurs by three mechanisms: (1) modification of the drugs by plasmid-encoded phosphorylating, adenylylating, and acetylating enzymes (the most important mechanism); (2) chromosomal mutation, eg, a mutation in the gene that codes for the target protein in the 30S subunit of the bacterial ribosome; and (3) decreased permeability of the bacterium to the drug.

Tetracyclines. Resistance to tetracyclines is the result of failure of the drug to reach an inhibitory concentration inside the bacteria. This is due to plasmid-encoded processes that either reduce uptake of the drug or **enhance its transport** out of the cell.

Chloramphenicol. Resistance to chloramphenicol is due to a plasmid-encoded acetyltransferase that acetylates the drug, thus inactivating it.

Erythromycin. Resistance to erythromycin is due primarily to a plasmid-encoded enzyme that methylates the 23S rRNA, thereby blocking binding of the drug. An efflux pump that reduces the concentration of erythromycin within the bacterium causes low-level resistance to the drug.

Sulfonamides. Resistance to sulfonamides is mediated primarily by two mechanisms: (1) a plasmid-encoded transport system that **actively exports** the drug out of the cell; and (2) a chromosomal mutation in the gene coding for the target enzyme dihydropteroate synthetase, which reduces the binding affinity of the drug.

Trimethoprim. Resistance to trimethoprim is due primarily to mutations in the chromosomal gene that encodes dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate.

Quinolones. Resistance to quinolones is due primarily to chromosomal mutations that modify the bacterial DNA gyrase. Resistance can also be caused by changes in bacterial outer-membrane proteins that result in reduced uptake of drug into the bacteria.

Rifampin. Resistance to rifampin is due to a chromosomal mutation in the gene for the β subunit of the bacterial RNA polymerase, resulting in ineffective binding of the drug. Because resistance occurs at high frequency (10^{-5}), rifampin is not prescribed alone for the *treatment* of infections. It is used alone for the *prevention* of certain infections because it is administered for only a short time (see Table 10–5).

Isoniazid. Resistance of *Mycobacterium tuberculosis* to isoniazid is due to mutations in the organism's catalase-peroxidase gene. Catalase or peroxidase enzyme activity is required to synthesize the metabolite of isoniazid that actually inhibits the growth of *M. tuberculosis.*

Ethambutol. Resistance of *M. tuberculosis* to ethambutol is due to mutations in the gene that encodes arabinosyl transferase, the enzyme that synthesizes the arabinogalactan in the organism's cell wall.

Pyrazinamide. Resistance of *M. tuberculosis* to pyrazinamide (PZA) is due to mutations in the gene that encodes bacterial amidase, the enzyme that converts PZA to the active form of the drug, pyrazinoic acid.

NONGENETIC BASIS OF RESISTANCE

There are several nongenetic reasons for the failure of drugs to inhibit the growth of bacteria.

(1) Bacteria can be walled off within an abscess cavity that the drug cannot penetrate effectively. Surgical drainage is therefore a necessary adjunct to chemotherapy.

(2) Bacteria can be in a resting state, ie, not growing; they are therefore insensitive to cell wall inhibitors such as penicillins and cephalosporins. Similarly, *M. tuberculosis* can remain dormant in tissues for many years, during which time it is insensitive to drugs. If host defenses are lowered and the bacteria begin to multiply, they are again susceptible to the drugs, indicating that a genetic change did not occur.

(3) Under certain circumstances, organisms that would ordinarily be killed by penicillin can lose their cell walls, survive as **protoplasts,** and be insensitive to cell-wall–active drugs. Later, if such organisms resynthesize their cell walls, they are fully susceptible to these drugs.

(4) The presence of foreign bodies makes successful antibiotic treatment more difficult. This applies to foreign bodies such as surgical implants and catheters as well as materials that enter the body at the time of penetrating injuries, such as splinters and shrapnel.

(5) Several artifacts can make it appear that the organisms are resistant, eg, administration of the wrong drug or the wrong dose or failure of the drug to reach the appropriate site in the body. (A good example of the latter is the poor penetration into spinal fluid by several early-generation cephalosporins.) Failure of the patient to take the drug (noncompliance, nonadherence) is another artifact.

SELECTION OF RESISTANT BACTERIA BY OVERUSE & MISUSE OF ANTIBIOTICS

Serious outbreaks of diseases caused by gram-negative rods resistant to multiple antibiotics have occurred in many developing countries. In North America, many hospital-acquired infections are caused by multiple-resistant organisms. Three main points of overuse and misuse of antibiotics increase the likelihood of these problems by enhancing the selection of resistant mutants:

(1) Some physicians use multiple antibiotics when one would be sufficient, prescribe unnecessarily long courses of antibiotic therapy, use antibiotics in self-limited infections for which they are not needed, and overuse antibiotics for prophylaxis before and after surgery.

(2) In many countries, antibiotics are sold over the counter to the general public; this practice encourages the inappropriate and indiscriminate use of the drugs.

(3) Antibiotics are used in animal feed to prevent infections and promote growth. This selects for resistant organisms in the animals and may contribute to the pool of resistant organisms in humans.

ANTIBIOTIC SENSITIVITY TESTING

Minimal Inhibitory Concentration

For many infections, the results of sensitivity testing are important in the choice of antibiotic. These results are commonly reported as the **minimal inhibitory concentration (MIC),** which is defined as the lowest concentration of drug that inhibits the growth of the organism. The MIC is determined by inoculating the organism isolated from the patient into a series of tubes or cups containing 2-fold dilutions of the drug (Figure 11–2). After incubation at 35°C for 18 hours, the lowest concentration of drug that prevents visible growth of the organism is the MIC. This provides the physician with a precise concentration of drug to guide the choice of both the drug and the dose.

A second method of determining antibiotic sensitivity is the disk diffusion method, in which disks impregnated with various antibiotics are placed on the surface of an agar plate that has been inoculated with the organism isolated from the patient (Figure 11–3). After incubation at 35°C for 18 hours, during which time the antibiotic diffuses outward from the disk, the diameter of the zone of inhibition is determined. The size of the zone of inhibition is compared with standards to determine the sensitivity of the organism to the drug.

Minimal Bactericidal Concentration

For certain infections, such as endocarditis, it is important to know the concentration of drug that actually kills the organism rather than the concentration that merely inhibits growth. This concentration, called the **minimal bactericidal concentration (MBC),** is determined by taking a small sample (0.01 or 0.1 mL) from the tubes used for the MIC assay and spreading it over the surface of a drug-free blood agar plate (Figure 11–2). Any organisms that were inhibited but not killed now have a chance to grow, because the drug has been diluted significantly. After incubation at 35°C for 48 hours, the lowest concentration that has reduced the number of colonies by 99.9%, compared with the drug-free control, is the MBC. Bactericidal drugs usually have an MBC equal or very similar to the MIC, whereas bacteriostatic drugs usually have an MBC significantly higher than the MIC.

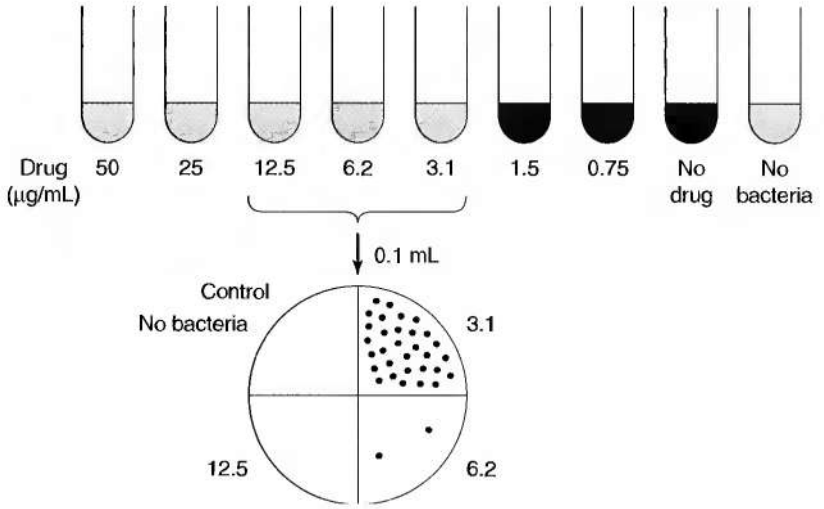

Figure 11–2. Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). In the top part of the figure, the patient's organism is added to tubes containing decreasing amounts of the antibiotic. After incubation at 37°C overnight, growth of the bacteria is observed visually. The lowest concentration of drug that inhibits growth, ie, 3.1 μg/mL, is the MIC. However, at this point, it is not known whether the bacteria have been killed or whether the drug has only inhibited their growth. To determine whether that concentration of drug is bactericidal, ie, to determine its MBC, an aliquot (0.1 mL) from the tubes is plated on an agar plate that does not contain any drug. The concentration of drug that inhibits at least 99.9% of the bacterial colonies, ie, 6.2 μg/mL, is the MBC.

Serum Bactericidal Activity

In the treatment of endocarditis, it can be useful to determine whether the drug is effective by assaying the ability of the drug in the patient's serum to kill the organism. This test, called the **serum bactericidal activity,** is performed in a manner similar to that of the MBC determination, except that it is a serum sample from the patient, rather than a standard drug solution, that is diluted in 2-fold steps. After a standard inoculum of the organism has been added and the mixture has been incubated at 35°C for 18 hours, a small sample is subcultured onto blood agar plates, and the serum dilution that kills 99.9% of the organisms is determined. Clinical experience has shown that a peak[1] serum bactericidal activity of 1:8 or 1:16 is adequate for successful therapy of endocarditis.

β-Lactamase Production

For severe infections caused by certain organisms, such as *S. aureus* and *Haemophilus influenzae,* it is important to know as soon as possible whether the organism isolated from the patient is producing β-lactamase. For this purpose, rapid assays for the enzyme can be used that yield an answer in a few minutes, as opposed to an MIC test or a disk diffusion test, both of which take 18 hours.

A commonly used procedure is the chromogenic β-lactam method, in which a colored β-lactam drug is added to a suspension of the organisms. If β-lactamase is made, hydrolysis of the β-lactam ring causes the drug to turn a different color in 2–10 minutes. Disks impregnated with a chromogenic β-lactam can also be used.

USE OF ANTIBIOTIC COMBINATIONS

In most cases, the single best antimicrobial agent should be selected for use because this minimizes side effects. However, there are several instances in which two or more drugs are commonly given:

(1) To treat serious infections before the identity of the organism is known

(2) To achieve a synergistic inhibitory effect against certain organisms

(3) To prevent the emergence of resistant organisms. (If bacteria become resistant to one drug, the second drug will kill them, thereby preventing the emergence of resistant strains.)

[1] One variable in this test is whether the serum is drawn shortly after the drug has been administered (at the "peak concentration") or shortly before the next dose is due (at the "trough"). Another variable is the inoculum size.

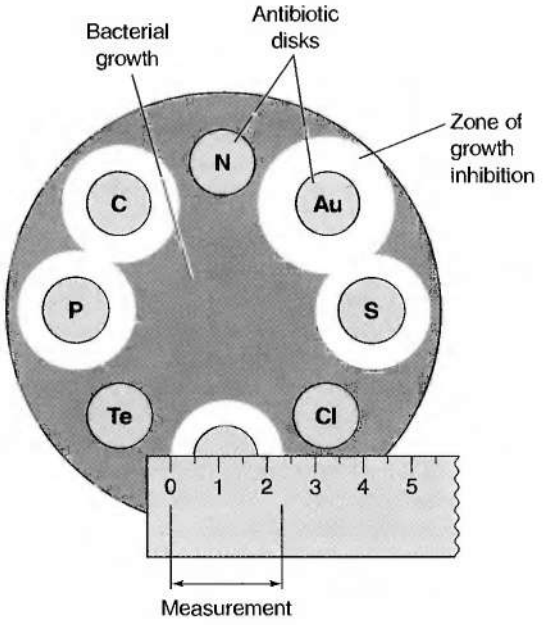

Figure 11–3. Antibiotic sensitivity testing. A zone of inhibition surrounds several antibiotic-containing disks. A zone of certain diameter or greater indicates that the organism is sensitive. Some resistant organisms will grow all the way up to the disk, eg, disk N. (Modified and reproduced, with permission, from Wistreich GA, Lechtman MD: *Laboratory Exercises in Microbiology,* 5th ed. Macmillan, 1984.)

Two drugs can interact in one of several ways (Figure 11–4). They are usually indifferent to each other, ie, additive only. Sometimes there is a **synergistic** interaction, in which the effect of the two drugs together is significantly greater than the sum of the effects of the two drugs acting separately. Rarely, the effect of the two drugs together is **antagonistic,** in which the result is significantly lower activity than the sum of the activities of the two drugs alone.

A synergistic effect can result from a variety of mechanisms. For example, the combination of a penicillin and an aminoglycoside such as gentamicin has a synergistic action against enterococci (*E. faecalis*), because penicillin damages the cell wall sufficiently to enhance the entry of aminoglycoside. When given alone, neither drug is effective. A second example is the combination of a sulfonamide with trimethoprim. In this instance, the two drugs act on the same metabolic pathway, such that if one drug does not inhibit folic acid synthesis sufficiently, the second drug provides effective inhibition by blocking a subsequent step in the pathway.

Although antagonism between two antibiotics is unusual, one example is clinically important. This involves the use of penicillin G combined with the bacteriostatic drug tetracycline in the treatment of meningitis caused by *S. pneumoniae.* Antagonism occurs because the tetracycline inhibits the growth of the organism, thereby preventing the bactericidal effect of penicillin G, which kills growing organisms only.

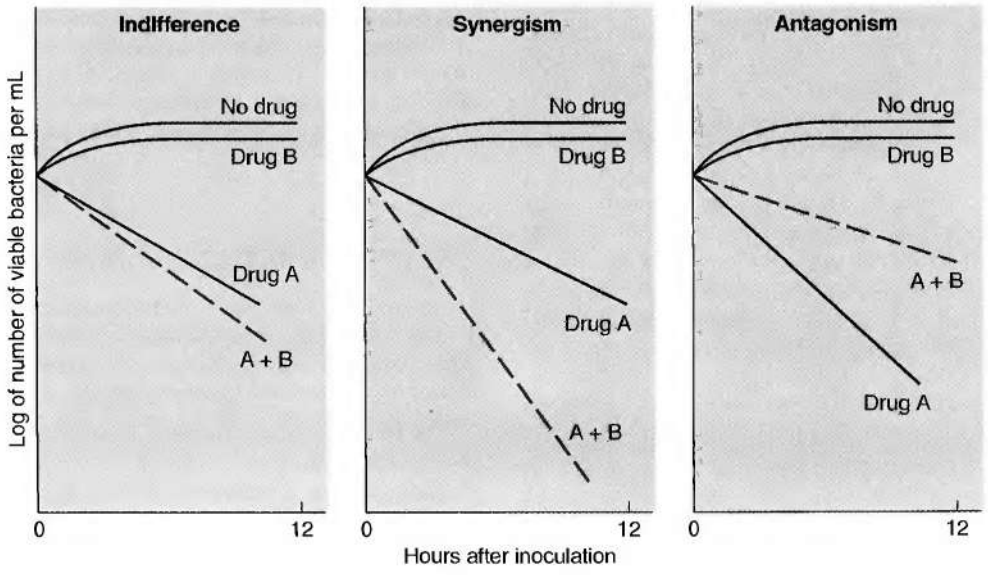

Figure 11–4. Drug interaction. The solid lines represent the response of bacteria to drug A alone, drug B alone, or no drug. The dotted lines represent the response to drug A and drug B together.

PEARLS

- *The four main mechanisms of antibiotic resistance are: (1)* ***enzymatic degradation*** *of the drug, (2)* ***modification of the drug's target,*** *(3)* ***reduced permeability*** *of the drug, and (4)* ***active export*** *of the drug.*
- *Most drug resistance is the result of a genetic change in the organism, caused either by a chromosomal mutation or the acquisition of a plasmid or transposon.*

Genetic Basis of Resistance

- ***Chromosomal mutations*** *typically either* ***change the target of the drug*** *so that the drug does not bind or* ***change the membrane*** *so that the drug does not penetrate well into the cell. Chromosomal mutations occur at a low frequency (perhaps 1 in 10 million organisms), and often affect only one drug or one family of drugs.*
- ***Plasmids cause drug resistance by encoding enzymes*** *that degrade or modify drugs. Plasmid-mediated resistance occurs at a* ***higher frequency*** *than chromosomal mutations, often affecting* ***multiple drugs*** *or families of drugs.*
- ***Resistance plasmids*** *(R plasmids, R factors) usually carry two sets of genes. One set encodes the enzymes that degrade or modify drugs and the other encodes the proteins that* ***mediate conjugation,*** *the main process by which resistance genes are transferred from one bacterium to another.*
- ***Transposons*** *are small pieces of DNA that* ***move from one site on the bacterial chromosome to another*** *or from the bacterial chromosome to plasmid DNA.* ***Transposons often carry drug resistance genes.*** *Many R plasmids carry one or more transposons.*

Specific Mechanisms of Resistance

- ***Resistance to penicillins and cephalosporins*** *is mediated by three main mechanisms: (1) degradation by β-lactamases, (2) mutations in the genes for penicillin-binding proteins, and (3) reduced permeability.* ***Degradation by β-lactamases is the most important.***
- *Resistance to vancomycin is caused by a change in the* ***D-ala-D-ala part of the peptide in peptidoglycan to D-ala-D-lactate,*** *resulting in an inability of vancomycin to bind.*
- *Resistance to aminoglycosides is mediated by three main mechanisms: modification of the drug by* ***phosphorylating, adenylylating, and acetylating enzymes,*** *mutations in the genes encoding one of the 30S ribosomal proteins, and reduced permeability.*
- *Resistance to* ***tetracyclines*** *is often caused by either reduced permeability or* ***active export*** *of the drug from the bacterium.*
- *Resistance to erythromycins is primarily caused by a plasmid-encoded enzyme that* ***methylates the 23S ribosomal RNA,*** *thereby blocking binding of the drug.*
- *Resistance to sulfonamides is due primarily to plasmid-encoded enzymes that* ***actively export*** *the drug from the bacterium.*
- *Resistance to quinolones is primarily caused by* ***mutations*** *in the gene encoding the bacterial DNA gyrase.*
- *Resistance to rifampin is primarily caused by* ***mutations*** *in the gene encoding the bacterial RNA polymerase.*
- *Resistance to isoniazid is due primarily to the* ***loss of the bacterial peroxidase (catalase)*** *that activates isoniazid to the metabolite that inhibits mycolic acid synthesis.*

Nongenetic Basis of Resistance

- *Nongenetic reasons why bacteria may not be inhibited by antibiotics are that drugs may not reach bacteria located in the center of an abscess and that certain drugs, such as penicillins, will not affect bacteria that are not growing. Also the presence of foreign bodies makes successful antibiotic treatment more difficult.*

Antibiotic Sensitivity Testing

- *The* ***minimal inhibitory concentration (MIC)*** *is the lowest concentration of drug that* ***inhibits the growth*** *of the bacteria isolated from the patient. In this test, it is not known whether the inhibited bacteria have been killed or just have stopped growing.*

- *The **minimal bactericidal concentration (MBC)** is the lowest concentration of drug that **kills** the bacteria isolated from the patient. In certain diseases, such as endocarditis, it is often necessary to use a concentration of drug that is bactericidal.*

Use of Antibiotic Combinations

- *Two or more antibiotics are used under certain circumstances, such as to treat life-threatening infections before the cause has been identified, to prevent the emergence of resistant bacteria during prolonged treatment regimens, and to achieve a synergistic (augmented) effect.*
- *A **synergistic effect** is one in which the effect of two drugs given together is much greater than the sum of the effect of the two drugs given individually. The best example of synergy is the marked killing effect of the combination of a penicillin and an aminoglycoside on enterococci compared to the minor effect of either drug given alone.*

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part X: USMLE (National Board) Practice Questions starting on page 517. Also see Part XI: USMLE (National Board) Practice Examination starting on page 568.

Bacterial Vaccines

12

Bacterial diseases can be prevented by the use of immunizations that induce either active or passive immunity. **Active** immunity is induced by vaccines prepared from bacteria or their products. This chapter will present a summary of the types of vaccines (Table 12–1); detailed information regarding each vaccine is located in the chapters on the specific organisms. **Passive** immunity is provided by the administration of preformed antibody in preparations called immune globulins. The immune globulins useful against bacterial diseases are described below. **Passive-active** immunity involves giving both immune globulins to provide immediate protection and a vaccine to provide long-term protection. This approach is described below in the section on tetanus antitoxin.

Active Immunity

Bacterial vaccines are composed of capsular polysaccharides, inactivated protein exotoxins (toxoids), killed bacteria, or live, attenuated bacteria. The available bacterial vaccines and their indications are as follows.

A. Capsular Polysaccharide Vaccines

(1) *Streptococcus pneumoniae* vaccine contains the capsular polysaccharides of the 23 most prevalent types. It is recommended for persons over 60 years of age and patients of any age with such chronic diseases as diabetes and cirrhosis or with compromised spleen function or splenectomy. A second vaccine containing the capsular polysaccharide of 7 pneumococcal serotypes coupled to a carrier protein (diphtheria toxoid) is available for the protection of young children who do not respond well to the unconjugated vaccine.

A potential problem regarding the use of the pneumococcal vaccine containing 7 serotypes is that of **serotype replacement.** Will the vaccine reduce the incidence of disease caused by the serotypes in the vaccine but not the overall incidence of pneumococcal disease because other serotypes that are not in the vaccine will now cause disease? This important question is being evaluated at this time.

(2) *Neisseria meningitidis* vaccine contains capsular polysaccharide of four important types (A, C, W-135, and Y). It is given when there is a high risk of meningitis, eg, during an outbreak, when military recruits enter boot camp, or for travelers to areas where meningitis is hyperendemic.

(3) *Haemophilus influenzae* vaccine contains the type b polysaccharide conjugated to diphtheria toxoid or other carrier protein. It is given to children between the ages of 2 and 15 months to prevent meningitis. The capsular polysaccharide alone is a poor immunogen in young children, but coupling it to a carrier protein greatly enhances its immunogenicity. A combined vaccine consisting of this vaccine plus the diphtheria, pertussis, and tetanus (DPT) vaccines is available.

(4) One of the vaccines against typhoid fever contains the capsular polysaccharide of *Salmonella typhi.* It is indicated for persons living or traveling in areas where there is a high risk of typhoid fever and for persons in close contact with either infected patients or chronic carriers.

B. Toxoid Vaccines

(1) *Corynebacterium diphtheriae* vaccine contains the toxoid (formaldehyde-treated exotoxin). Immunization against diphtheria is indicated for every child and is given in three doses at 2, 4, and 6 months of age, with boosters given 1 year later and at intervals thereafter.

(2) *Clostridium tetani* vaccine contains tetanus toxoid and is given to everyone both early in life and later as boosters for protection against tetanus.

(3) *Bordetella pertussis* vaccine contains pertussis toxoid but includes other proteins as well. It is, therefore, described in the next section.

C. Purified Protein Vaccines

(1) There are two types of *B. pertussis* vaccines: an acellular vaccine containing purified proteins and a vaccine containing whole killed bacteria. The acellular vaccine is now recommended in the United States. The principal antigen in the acellular vaccine is inactivated pertussis toxin (pertussis toxoid), but other proteins, such as filamentous hemagglutinin and pertactin, are also required for full protection. Pertussis toxin for the vaccine is inactivated *genetically* by introducing two

Table 12–1. Current bacterial vaccines (2004).

Usage	Bacterium	Disease	Antigen
Common usage	*Corynebacterium diphtheriae*	Diphtheria	Toxoid
	Clostridium tetani	Tetanus	Toxoid
	Bordetella pertussis	Whooping cough	Acellular (purified proteins) or killed organisms
	Haemophilus influenzae	Meningitis	Capsular polysaccharide conjugated to carrier protein, eg, diphtheria toxoid
	Streptococcus pneumoniae	Pneumonia	Capsular polysaccharide or capsular polysaccharide conjugated to carrier protein
Special situations	*Neisseria meningitidis*	Meningitis	Capsular polysaccharide
	Salmonella typhi	Typhoid fever	Live organisms or capsular polysaccharide
	Vibrio cholerae	Cholera	Killed organisms
	Yersinia pestis	Plague	Killed organisms
	Bacillus anthracis	Anthrax	Partially purified proteins
	Mycobacterium bovis (BCG)	Tuberculosis	Live organisms
	Francisella tularensis	Tularemia	Live organisms
	Rickettsia prowazekii	Typhus	Killed organisms
	Coxiella burnetii	Q fever	Killed organisms
	Borrelia burgdorferi	Lyme disease[1]	Recombinant outer surface protein A

[1]The Lyme disease vaccine has been withdrawn and is no longer available in the United States.

amino acid changes that eliminate its toxic (ADP-ribosylating) activity but retain its antigenicity. It is the first vaccine to contain a genetically inactivated toxoid. The vaccine is indicated for every child as a protection against whooping cough. It is usually given in combination with diphtheria and tetanus toxoids (DPT or DtaP vaccine).

(2) The vaccine against Lyme disease contains a purified outer surface protein (OspA) of *Borrelia burgdorferi* as the immunogen. OspA is made by recombinant DNA techniques. It is recommended for those who live in areas of endemic disease and whose occupation or recreation makes them likely to be exposed.

(3) *Bacillus anthracis* vaccine contains "protective antigen" purified from the organism. It is given to persons whose occupations place them at risk of exposure to the organism.

D. Live, Attenuated Bacterial Vaccines

(1) The vaccine against tuberculosis contains a live, attenuated strain of *Mycobacterium bovis* called BCG and is recommended for children at high risk for exposure to active tuberculosis in some countries.

(2) One of the vaccines against typhoid fever contains live, attenuated *Salmonella typhi.* It is indicated for persons living or traveling in areas where there is a high risk of typhoid fever and for persons in close contact with either infected patients or chronic carriers.

(3) The vaccine against tularemia contains live, attenuated *Francisella tularensis* organisms and is used primarily in people who are exposed in their occupation, such as laboratory personnel, veterinarians, and hunters.

E. Killed Bacterial Vaccines

(1) *Vibrio cholerae* vaccine contains killed organisms and is given to persons traveling to areas where cholera is endemic.

(2) *Yersinia pestis* vaccine contains killed organisms and is indicated for persons at high risk for contracting plague.

(3) The vaccine against typhus contains killed *Rickettsia rickettsiae* organisms and is used primarily to immunize members of the armed forces.

(4) The vaccine against Q fever contains killed *Coxiella burnetii* organisms and is used to immunize those who are at high risk for being exposed to animals infected with the organism.

Passive Immunity

Antitoxins (immune globulins) can be used for either the treatment or prevention of certain bacterial diseases. The following preparations are available.

(1) **Tetanus** antitoxin is used in the treatment of tetanus and in its prevention (prophylaxis). In treatment, because the goal is to neutralize any unbound toxin to prevent the disease from getting worse, the antitoxin should be given promptly. In prevention, the antitoxin is given to inadequately immunized persons with contaminated ("dirty") wounds. The antitoxin is made in humans to avoid hypersensitivity reactions. In addition to the antitoxin, these people should receive tetanus toxoid. This is an example of **passive-active** immunity. The toxoid and the antitoxin should be given at different sites in the body to prevent the antitoxin from neutralizing the toxoid.

(2) **Botulinum** antitoxin is used in the treatment of botulism. Because the antitoxin can neutralize unbound toxin to prevent the disease from progressing, it should be given promptly. It contains antibodies against botulinum toxins A, B, and E, the most commonly occurring types. The antitoxin is made in horses, so hypersensitivity may be a problem.

(3) **Diphtheria** antitoxin is used in the treatment of diphtheria. The antitoxin can neutralize unbound toxin to prevent the disease from progressing; therefore, the antitoxin should be given promptly. The antitoxin is made in horses, so hypersensitivity may be a problem.

PEARLS

- *Immunity to certain bacterial diseases can be induced either by immunization with bacterial antigens (**active immunity**) or by administration of preformed antibodies (**passive immunity**).*

Active Immunity

- *Active immunity can be achieved by vaccines consisting of (1) **bacterial capsular polysaccharides, toxoids, whole bacteria** (either killed or live, attenuated) or (2) **purified proteins** isolated from bacteria.*
- ***Vaccines containing capsular polysaccharide** as the immunogen are directed against* Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, *and* Salmonella typhi. *The capsular polysaccharide in the pneumococcal vaccine and in the* H. influenzae *vaccine is coupled to a protein carrier to enhance the antibody response, especially in children younger than 2 years.*
- *Two vaccines contain **toxoids** as the immunogen, the vaccines against **diphtheria** and **tetanus.** A **toxoid is an inactivated toxin** that has lost its ability to cause disease but has retained its immunogenicity. (The pertussis vaccine also contains toxoid but contains other bacterial proteins as well and is described in the next section.)*
- *Two vaccines contain purified bacterial proteins as the immunogen. The most commonly used is the **acellular pertussis vaccine,** which in combination with diphtheria and tetanus toxoids is recommended for all children. The **vaccine against anthrax** also contains purified proteins but is recommended only for individuals who are likely to be exposed to the organism.*
- *The **BCG vaccine** against tuberculosis **contains live, attenuated** **Mycobacterium bovis** and is used in countries where the disease is endemic. One of the vaccines against typhoid fever contains live, attenuated* Salmonella typhi.
- *The vaccines against cholera, plague, typhus, and Q fever contain whole killed bacteria. These vaccines are used only to protect those likely to be exposed.*

Passive Immunity

- *Passive immunity in the form of **antitoxins** is available for the prevention and treatment of **tetanus, botulism,** and **diphtheria.** These three diseases are caused by exotoxins. **Antitoxins** (antibodies against the exotoxins) neutralize the exotoxins and prevent their toxic effects.*

Passive-Active Immunity

- *This involves providing both immediate (but short-term) protection in the form of antibodies and long-term protection in the form of active immunization. An excellent example of the use of passive-active immunity is the prevention of tetanus in an unimmunized person who has sustained a contaminated wound. Both tetanus antitoxin and tetanus toxoid should be given. They should be given at different sites so that the antibodies in the antitoxin do not neutralize the toxoid.*

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

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Sterilization & Disinfection

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Sterilization is the killing or removal of *all* microorganisms, including bacterial spores, which are highly resistant. Sterilization is usually carried out by autoclaving, which consists of exposure to steam at 121°C under a pressure of 15 lb/in^2 for 15 minutes. Surgical instruments that can be damaged by moist heat are usually sterilized by exposure to ethylene oxide gas, and most intravenous solutions are sterilized by filtration.

Disinfection is the killing of many, but not all, microorganisms. For adequate disinfection, pathogens must be killed, but some organisms and bacterial spores may survive. Disinfectants vary in their tissue-damaging properties from the corrosive phenol-containing compounds, which should be used only on inanimate objects, to less toxic materials such as ethanol and iodine, which can be used on skin surfaces. Chemicals used to kill microorganisms on the surface of skin and mucous membranes are called **antiseptics.**

■ RATE OF KILLING OF MICROORGANISMS

Death of microorganisms occurs at a certain rate dependent primarily upon two variables: the concentration of the killing agent and the length of time the agent is applied. The rate of killing is defined by the relationship

$$N \propto 1/CT$$

which shows that the number of survivors, N, is inversely proportionate to the concentration of the agent, C, and to the time of application of the agent, T. Collectively, CT is often referred to as the dose. Stated alternatively, the number of microorganisms killed is directly proportionate to CT. The relationship is usually stated in terms of survivors, because they are easily measured by colony formation. Death is defined as the inability to reproduce. In certain circumstances, the physical remains of dead bacteria can still cause problems (see page 47).

■ CHEMICAL AGENTS

Chemicals vary greatly in their ability to kill microorganisms. A quantitative measure of this variation is expressed as the **phenol coefficient,** which is the ratio of the concentration of phenol to the concentration of the agent required to cause the same amount of killing under the standard conditions of the test.

Chemical agents act primarily by one of three mechanisms: (1) disruption of the lipid-containing cell membrane, (2) modification of proteins, or (3) modification of DNA. Each of the following chemical agents has been classified into one of the three categories, but some of the chemicals act by more than one mechanism.

DISRUPTION OF CELL MEMBRANES

Alcohol

Ethanol is widely used to clean the skin before immunization or venipuncture. It acts mainly by disorganizing the lipid structure in membranes, but it denatures proteins as well. Ethanol requires the presence of water for maximal activity; ie, it is far more effective at 70% than at 100%. Seventy percent ethanol is often used as an antiseptic to clean the skin prior to venipuncture. However, because it is not as effective as iodine-containing compounds, the latter should be used prior to obtaining a blood culture and installing intravenous catheters.

Detergents

Detergents are "surface-active" agents composed of a long-chain, lipid-soluble, hydrophobic portion and a polar hydrophilic group, which can be a cation, an anion, or a nonionic group. These surfactants interact with the lipid in the cell membrane through their hydrophobic chain and with the surrounding water through their polar group and thus disrupt the membrane. Quaternary ammonium compounds, eg, benzalkonium chloride, are cationic detergents widely used for skin antisepsis.

Phenols

Phenol was the first disinfectant used in the operating room (by Lister in the 1860s), but it is rarely used as a disinfectant today because it is too caustic. Hexachlorophene, which is a biphenol with six chlorine atoms, is used in germicidal soaps, but concern over possible neurotoxicity has limited its use. Another phenol derivative is cresol (methylphenol), the active ingredient in Lysol. Phenols not only damage membranes but also denature proteins.

MODIFICATION OF PROTEINS

Chlorine

Chlorine is used as a disinfectant to purify the water supply and to treat swimming pools. It is also the active component of hypochlorite (bleach, Clorox), which is used as a disinfectant in the home and in hospitals. Chlorine is a powerful oxidizing agent that kills by cross-linking essential sulfhydryl groups in enzymes to form the inactive disulfide.

Iodine

Iodine is the most effective skin antiseptic used in medical practice and should be used prior to obtaining a blood culture and installing intravenous catheters because contamination with skin flora such as *Staphylococcus epidermidis* can be a problem. Iodine is supplied in two forms:

(1) Tincture of iodine (2% solution of iodine and potassium iodide in ethanol) is used to prepare the skin prior to blood culture. Because tincture of iodine can be irritating to the skin, it should be removed with alcohol.

(2) Iodophors are complexes of iodine with detergents that are frequently used to prepare the skin prior to surgery because they are less irritating than tincture of iodine. Iodine, like chlorine, is an oxidant that inactivates sulfhydryl-containing enzymes. It also binds specifically to tyrosine residues in proteins.

Heavy Metals

Mercury and silver have the greatest antibacterial activity of the heavy metals and are the most widely used in medicine. They act by binding to sulfhydryl groups, thereby blocking enzymatic activity. Thimerosal (Merthiolate) and merbromin (Mercurochrome), which contain mercury, are used as skin antiseptics. Silver nitrate drops are useful in preventing gonococcal ophthalmia neonatorum. Silver sulfadiazine is used to prevent infection of burn wounds.

Hydrogen Peroxide

Hydrogen peroxide is used as an antiseptic to clean wounds and to disinfect contact lenses. Its effectiveness is limited by the organism's ability to produce catalase, an enzyme that degrades H_2O_2. (The bubbles produced when peroxide is used on wounds are formed by oxygen arising from the breakdown of H_2O_2 by tissue catalase.) Hydrogen peroxide is an oxidizing agent that attacks sulfhydryl groups, thereby inhibiting enzymatic activity.

Formaldehyde & Glutaraldehyde

Formaldehyde, which is available as a 37% solution in water (Formalin), denatures proteins and nucleic acids. Both proteins and nucleic acids contain essential $-NH_2$ and –OH groups, which are the main sites of alkylation by the hydroxymethyl group of formaldehyde. Glutaraldehyde, which has two reactive aldehyde groups, is 10 times more effective than formaldehyde and is less toxic. In hospitals, it is used to sterilize respiratory therapy equipment.

Ethylene Oxide

Ethylene oxide gas is used extensively in hospitals for the sterilization of heat-sensitive materials such as surgical instruments and plastics. It kills by alkylating both proteins and nucleic acids; ie, the hydroxyethyl group attacks the reactive hydrogen atoms on essential amino and hydroxyl groups.

Acids & Alkalis

Strong acids and alkalis kill by denaturing proteins. Although most bacteria are susceptible, it is important to note that *Mycobacterium tuberculosis* and other mycobacteria are relatively resistant to 2% NaOH, which is used in the clinical laboratory to liquefy sputum prior to culturing the organism. Weak acids, such as benzoic, propionic, and citric acids, are frequently used as food preservatives because they are bacteriostatic. The action of these acids is partially a function of the organic moiety, eg, benzoate, as well as the low pH.

MODIFICATION OF NUCLEIC ACIDS

A variety of dyes not only stain microorganisms but also inhibit their growth. One of these is crystal violet (gentian violet), which is used as a skin antiseptic. Its action is based on binding of the positively charged dye molecule to the negatively charged phosphate groups of the nucleic acids. Malachite green, a triphenylamine dye like crystal violet, is a component of Löwenstein-Jensen's medium, which is used to grow *M. tuberculosis.*

The dye inhibits the growth of unwanted organisms in the sputum during the 6-week incubation period.

■ PHYSICAL AGENTS

The physical agents act either by imparting energy in the form of heat or radiation or by removing organisms through filtration.

HEAT

Heat energy can be applied in three ways: in the form of moist heat (either boiling or autoclaving) or dry heat or by pasteurization. In general, heat kills by denaturing proteins, but membrane damage and enzymatic cleavage of DNA may also be involved. Moist heat sterilizes at a lower temperature than dry heat, because water aids in the disruption of noncovalent bonds, eg, hydrogen bonds, which hold protein chains together in their secondary and tertiary structures.

Moist-heat sterilization, usually **autoclaving**, is the most frequently used method of sterilization. Because bacterial **spores are resistant to boiling** (100°C at sea level), they must be exposed to a higher temperature; this cannot be achieved unless the pressure is increased. For this purpose, an autoclave chamber is used in which steam, at a pressure of 15 lb/in^2, reaches a temperature of 121°C and is held for 15–20 minutes. This kills even the highly heat-resistant spores of *Clostridium botulinum*, the cause of botulism, with a margin of safety.

Sterilization by dry heat, on the other hand, requires temperatures in the range of 180°C for 2 hours. This process is used primarily for glassware and is used less frequently than autoclaving.

Pasteurization, which is used primarily for milk, consists of heating the milk to 62°C for 30 minutes followed by rapid cooling. ("Flash" pasteurization at 72°C for 15 seconds is often used.) This is sufficient to kill the vegetative cells of the milk-borne pathogens, eg, *Mycobacterium bovis, Salmonella, Streptococcus, Listeria,* and *Brucella,* but not to sterilize the milk.

RADIATION

The two types of radiation used to kill microorganisms are **ultraviolet** (UV) **light and x-rays.** The greatest antimicrobial activity of UV light occurs at 250–260 nm, which is the wavelength region of maximum absorption by the purine and pyrimidine bases of DNA. The most significant lesion caused by UV irradiation is the formation of thymine dimers, but addition of hydroxyl groups to the bases also occurs. As a result, DNA replication is inhibited and the organism cannot grow. Cells have repair mechanisms against UV-induced damage that involve either cleavage of dimers in the presence of visible light (photoreactivation) or excision of damaged bases, which is not dependent upon visible light (dark repair). Because UV radiation can damage the cornea and skin, the use of UV irradiation in medicine is limited. However, it is used in hospitals to kill airborne organisms, especially in operating rooms when they are not in use. Bacterial spores are quite resistant and require a dose up to 10 times greater than do the vegetative bacteria.

X-rays have higher energy and penetrating power than UV radiation and kill mainly by the production of free radicals, eg, production of hydroxyl radicals by the hydrolysis of water. These highly reactive radicals can break covalent bonds in DNA, thereby killing the organism. Sulfhydryl-containing compounds, such as the amino acid cysteine, can protect DNA from free-radical attack. Another mechanism is a direct hit on a covalent bond in DNA, resulting in chain breakage, but this is probably less important than the mechanism involving free radicals.

X-rays kill vegetative cells readily, but spores are remarkably resistant, probably because of their lower water content. X-rays are used in medicine for sterilization of heat-sensitive items, such as sutures and surgical gloves, and plastic items, such as syringes.

FILTRATION

Filtration is the preferred method of sterilizing certain solutions, eg, those with heat-sensitive components. In the past, solutions for intravenous use were autoclaved, but heat-resistant endotoxin in the cell walls of the dead gram-negative bacteria caused fever in recipients of the solutions. Therefore, solutions are now filtered to make them **pyrogen-free** prior to autoclaving.

The most commonly used filter is composed of nitrocellulose and has a pore size of 0.22 μm. This size will retain all bacteria and spores. Filters work by physically trapping particles larger than the pore size and by retaining somewhat smaller particles via electrostatic attraction of the particles to the filters.

PEARLS

- *Sterilization is the **killing of all** forms of microbial life including bacterial spores. **Spores** are **resistant to boiling,** so sterilization of medical equipment is typically achieved at 121°C for 15 minutes in an autoclave. Sterilization of heat-sensitive materials is achieved by exposure to ethylene oxide, and liquids can be sterilized by filtration.*
- ***Disinfection** is **reducing the number of bacteria** to a level low enough that disease is unlikely to occur. Spores and some bacteria will survive. For example, disinfection of the water supply is achieved by treatment with chlorine. Disinfection of the skin prior to venipuncture is achieved by treatment with 70% ethanol. Disinfectants that are mild enough to use on skin and other tissues, such as 70% ethanol, are called **antiseptics.***
- *The killing of microbes by either chemicals or radiation is proportional to the **dose,** which is defined as the product of the concentration multiplied by the time of exposure.*
- *Chemical agents kill bacteria by one of three actions: disruption of lipid in cell membranes, modification of proteins, or modification of DNA.*
- *Physical agents kill (or remove) bacteria by one of three processes: heat, radiation, or filtration.*
- *Heat is usually applied at temperatures above boiling (121°C) to kill spores, but heat-sensitive materials such as milk are exposed to temperatures below boiling (**pasteurization**) that kills the pathogens in milk but does not sterilize it.*
- *Radiation, such as **ultraviolet light** and x-radiation, is often used to sterilize heat-sensitive items. Ultraviolet light and x-radiation **kill by damaging DNA.***
- *Filtration can sterilize liquids if the pore size of the filter is small enough to retain all bacteria and spores. Heat-sensitive liquids, eg, intravenous fluids, are often sterilized by filtration.*

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

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