CHAPTER 11

# Antimicrobial Drugs: Resistance

## CHAPTER CONTENTS



## PRINCIPLES OF ANTIBIOTIC RESISTANCE

There are four major mechanisms that mediate bacterial resistance to drugs (Table 11–1). (1) Bacteria produce enzymes that **inactivate the drug** (e.g., 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 a reduced effect (e.g., 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 **reduce permeability** to the drug such that an effective intracellular concentration of the drug is not achieved (e.g., 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 tetracyclines.

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 91.

**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, quinolones |

The term **high-level** resistance refers to resistance that cannot be overcome by increasing the dose of the antibiotic. A different antibiotic, usually from another class of drugs, is used. Resistance mediated by enzymes such as β-lactamases often result in high-level resistance, as all the drug is destroyed. **Low-level** resistance refers to resistance that can be overcome by increasing the dose of the antibiotic. Resistance mediated by mutations in the gene encoding a drug target is often low level, as the altered target can still bind some of the drug but with reduced strength.

To illustrate the use of these terms, strains of *Neisseria gonorrhoeae* that produce penicillinase cannot be treated successfully with penicillin G. They exhibit high-level resistance, and a different drug such as ceftriaxone must be used. However, strains of *N. gonorrhoeae* that synthesize altered penicillin-binding proteins exhibit low-level resistance and can be treated successfully with high-dose penicillin G.

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 that encode the enzymes that mediate resistance.

Table 11–2 describes certain medically important bacteria and the main drugs to which they are resistant.

**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, methicillin/nafcillin |
| *Streptococcus pneumoniae* | Penicillin G |
| *Enterococcus faecalis, E. faecium* | 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** | |
| *Mycobacterium 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.

Note that although these bacteria are resistant to other drugs as well, for simplicity, only the most characteristic drugs are listed. Some strains of the bacteria listed in Table 11–2 are highly resistant to multiple antibiotics, namely methicillin-resistant *S. aureus* (MRSA; see Chapter 15), vancomycin-resistant *Enterococcus faecium* (VRE; see Chapter 15), multidrug-resistant *Streptococcus pneumoniae* (MDR-SP; see Chapter 15), *P. aeruginosa* (see Chapter 18), and multidrug-resistant *Mycobacterium tuberculosis* (MDR-MTB; see Chapter 21).

## 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 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

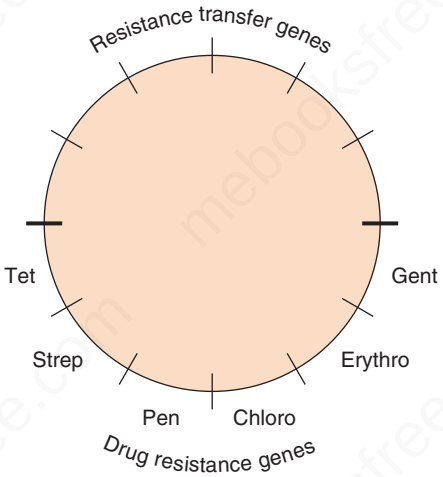


**FIGURE 11–1** Resistance plasmid (R plasmid, R factor). Most resistance plasmids have two sets of genes: (1) resistance transfer genes that encode the sex pilus and other proteins that mediate transfer of the plasmid DNA during conjugation, and (2) drug resistance genes that encode the proteins that 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.

of a plasmid carrying more than one resistance gene is twofold: first and most obvious is that a bacterium containing that plasmid can be resistant to more than one class of antibiotics (e.g., 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 organism resistant not only to penicillin, but also to tetracyclines, aminoglycosides (e.g., 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 (e.g., 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.

**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 |

## 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 10–2). β-Lactamases 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, tazobactam, sulbactam, and avibactam are penicillin analogues that bind strongly to β-lactamases and inactivate them. Combinations of these inhibitors and penicillins (e.g., clavulanic acid and amoxicillin [Augmentin]) can overcome resistance mediated by many but not all β-lactamases.

**Extended-spectrum β-lactamases** (ESBLs) are produced by several enteric bacteria, notably *E. coli, Klebsiella, Enterobacter*, and *Proteus*. ESBLs endow the bacteria with resistance to all penicillins, cephalosporins, and monobactams. However, these bacteria remain sensitive to combinations such as piperacillin/tazobactam. In 2009, a new strain of highly resistant *Klebsiella* was isolated in India carrying a plasmid that encoded **New Delhi metallo-β-lactamase** (NDM-1). This plasmid confers high-level resistance to many antibiotics and has spread from *Klebsiella* to other member of the Enterobacteriaceae. Resistant Enterobacteriaceae carrying NDM-1 have emerged in many countries, including the United States.

Resistance to penicillins can also be due to changes in the **penicillin-binding proteins** (PBPs) in the bacterial cell membrane. These changes account for both the low-level and high-level resistance exhibited by *S. pneumoniae* to penicillin G and for the resistance of *S. aureus* to nafcillin and other β-lactamase–resistant penicillins. The resistance of MRSA to almost all β-lactams is attributed to the presence of PBP2a, which is found particularly in MRSA. The relative resistance of *Enterococcus faecalis* to penicillins may be due to altered penicillin-binding proteins. Low-level resistance of *N. 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.

**Carbapenems**—Resistance to carbapenems, such as imipenem, is caused by carbapenemases that degrade the β-lactam ring. This enzyme endows the organism with resistance to penicillins and cephalosporins as well. Carbapenemases are produced by many enteric gram-negative rods, especially *Klebsiella, Escherichia*, and *Pseudomonas*. Carbapenem-resistant strains of *Klebsiella pneumoniae* are an important cause of hospital-acquired infections and are resistant to almost all known antibiotics.

**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 teicoplanin. (Teicoplanin is used in Europe but is not approved in the United States.) The VanA locus encodes those enzymes that synthesize D-alanine-D-lactate as well as several regulatory proteins.

Vancomycin-resistant strains of enterococci (VRE) 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 (e.g., 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 the 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. An esterase produced primarily by enteric gram-negative rods cleaves the macrolide ring, which inactivates 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.

**Rifampin**—Resistance to rifampin is due to a chromosomal mutation in the gene encoding 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 10–8).

**Isoniazid**—Resistance of *M. 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 (i.e., 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 (e.g., 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 multidrug-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 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

### Antibiogram

An antibiogram is the term used to describe the results of antibiotic susceptibility tests performed on the bacteria isolated from the patient. These results are the most important factor in determining the choice of antibiotic with which to treat the patient. Other factors such as the patient's renal function and hypersensitivity profile must also be considered in choosing the antibiotic.

There are two types of tests used to determine the antibiogram: (1) the tube dilution test that determines the minimal inhibitory concentration and (2) the disk diffusion (Kirby-Bauer) test that determines the diameter of the zone of inhibition (see following discussion and Figures 11–2 and 11–3).

### 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 twofold 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.

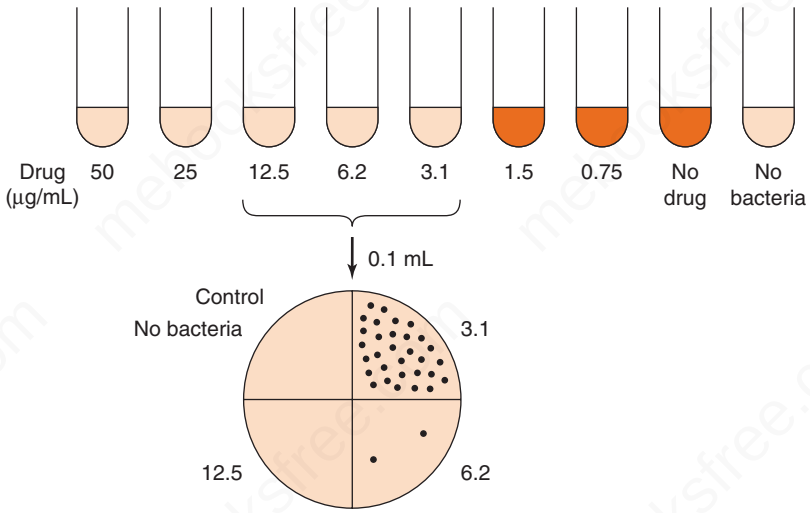


**FIGURE 11–2** Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **Top:** The patient's organism is added to tubes containing decreasing amounts of the antibiotic. After incubation at 37°C overnight, growth of the bacteria is observed visually. The lowest concentration of drug that inhibits growth (i.e., 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. **Bottom:** To determine whether that concentration of drug is bactericidal (i.e., 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 (i.e., 6.2 µg/mL) is the MBC.

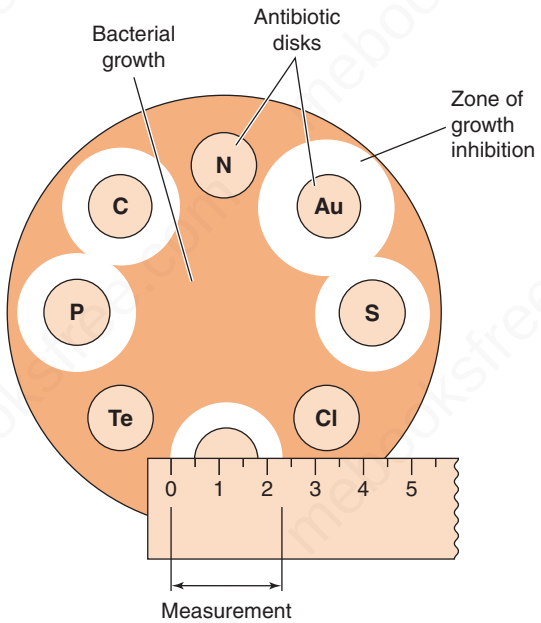


**FIGURE 11–3** Antibiotic sensitivity testing. A zone of inhibition surrounds several antibiotic-containing disks. A zone of certain diameter or greater indicates that the organism is sensitive. Some resistant organisms will grow all the way up to the disk (e.g., disk N). (Wistreich GA. Laboratory Exercises in Microbiology. 5th edition © 1984. Reprinted by permission of Pearson Education Inc, New York, New York.)

## 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.

## 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 used. 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

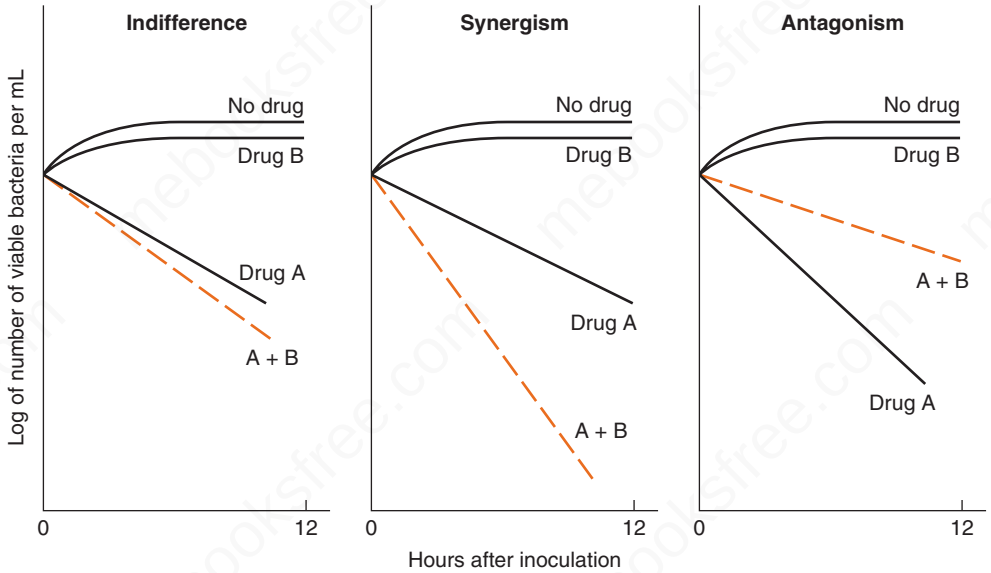


**FIGURE 11–4** Drug interaction. The solid lines represent the response of bacteria to drug A alone, drug B alone, or no drug. The dotted lines represent the response to drug A and drug B together.

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 to 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.)

Two drugs can interact in one of several ways (Figure 11–4). They are usually indifferent to each other (i.e., 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 only growing organisms.

[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.

## 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-alanyl-D-alanine part of the peptide in peptidoglycan to D-alanine-D lactate, resulting in an inability of vancomycin to bind.
- Resistance to aminoglycosides is mediated by three main mechanisms: (1) modification of the drug by **phosphorylating, adenylylating, and acetylating enzymes;** (2) mutations in the genes encoding one of the 30S ribosomal proteins; and (3) 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 with the minor effect of either drug given alone.

## SELF-ASSESSMENT QUESTIONS

**1.** The spread of antibiotic resistance from one bacterium to another is a well-recognized and clinically important phenomenon. Which one of the following mechanisms is most likely to be involved with the spread of resistance?

**(A)** Acetylation
**(B)** Conjugation
**(C)** Programmed rearrangement
**(D)** Protoplast mobility
**(E)** Translation

**2.** Regarding the specific mechanisms by which bacteria become resistant to antimicrobial drugs, which one of the following is the most accurate?

**(A)** Some bacteria contain an enzyme that cleaves the ring of aminoglycosides.
**(B)** Some bacteria contain clavulanic acid, which binds to penicillin G and inactivates it.
**(C)** Some bacteria contain a mutated gene encoding an altered transpeptidase, which makes it resistant to doxycycline.
**(D)** Some bacteria contain a mutated gene that encodes an altered RNA polymerase, which makes it resistant to rifampin.
**(E)** Some bacteria contain an altered ribosomal protein, which makes it resistant to isoniazid.

**3.** The susceptibility of bacteria to an antibiotic is often determined by using the minimal inhibitory concentration (MIC) assay. Regarding the MIC assay, which one of the following is the most accurate?

**(A)** MIC is the lowest concentration of the bacteria isolated from the patient that inhibits the activity of a standard dose of antibiotic.
**(B)** MIC is the lowest concentration of antibiotic that inhibits the growth of the bacteria isolated from the patient.
**(C)** MIC is the lowest concentration of antibiotic that kills the bacteria isolated from the patient.
**(D)** MIC is the lowest concentration of antibiotic in the patient's serum that inhibits the activity of a standard dose of antibiotic.

**4.** The minimal inhibitory concentration (MIC) of the patient's organism to penicillin is 1 μg/mL and the MIC to gentamicin is 8 μg/mL. However, the MIC to a combination of penicillin and gentamicin is 0.01 μg/mL. Which one of the following terms is the most accurate to describe this effect?

**(A)** Activation
**(B)** Antagonism
**(C)** Reassortment
**(D)** Recombination
**(E)** Synergism

**5.** Regarding the mechanisms of resistance to specific drugs, which one of the following is most accurate?

**(A)** Certain strains of *Enterococcus faecalis* produce D-lactate rather than D-alanine, which causes them to be resistant to vancomycin.
**(B)** Certain strains of *Escherichia coli* produce ergosterol, which causes them to be resistant to gentamicin.
**(C)** Certain strains of *Neisseria gonorrhoeae* produce a mutant peptidyl transferase, which causes them to be resistant to tetracycline.
**(D)** Certain strains of *Streptococcus pyogenes* produce a β-lactamase, which causes them to be resistant to erythromycin.

## ANSWERS

1. **(B)**
2. **(D)**
3. **(B)**
4. **(E)**
5. **(A)**

## PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.