1

Antimicrobial Drug Action and Interaction: An Introduction

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Antimicrobial drugs exploit differences in structure or biochemical function between host and parasite. Modern chemotherapy is traced to Paul Ehrlich, a pupil of Robert Koch, who devoted his career to discovering agents that possessed selective toxicity so that they might act as so-called "magic bullets" in the fight against infectious diseases. The remarkable efficacy of modern antimicrobial drugs still retains the sense of the miraculous. Sulfonamides, the first clinically successful broad-spectrum antibacterial agents, were produced in Germany in 1935.

However, it was the discovery of the antimicrobial penicillin, a fungal metabolite, by Fleming in 1929 and its subsequent development by Chain and Florey during World War II that led to the "antibiotic revolution." Within a few years of the introduction of penicillin, many other antimicrobials were described. This was followed by the development of semisynthetic and synthetic antimicrobial agents which has resulted in an increasingly powerful and effective array of compounds used to treat infectious diseases.

The term *antibiotic* has been defined as a low molecular weight substance produced by a microorganism that at low concentrations inhibits or kills other microorganisms. In contrast, the word *antimicrobial* has a broader definition than antibiotic and includes any substance of natural, semisynthetic, or synthetic origin that kills or inhibits the growth of a microorganism but causes little or no damage to the host. Antimicrobial agent and antibiotic are commonly used synonymously. The term *antimicrobial* is preferentially used in this book as the more encompassing term.

The marked structural and biochemical differences between prokaryotic and eukaryotic cells give antimicrobial agents greater opportunities for selective toxicity against bacteria than against other microorganisms such as fungi, which are nucleated like mammalian cells, or viruses, which require their host's genetic material for replication. Nevertheless, in recent years increasingly effective antifungal and antiviral drugs have been introduced into clinical practice.

Important milestones in the development of antibacterial drugs are shown in Figure 1.1. Because of the enormous costs of development, the therapeutic use of these agents in veterinary medicine has usually followed their use in human medicine. However, some antimicrobials have been developed specifically for animal health and production (e.g., tylosin, tiamulin, tilmicosin, ceftiofur, tulathromycin, gamithromycin, tildipirosin), although all these are related to drug classes used in human medicine. A few classes not used because of toxicity for humans, such as the orthosomycins, have been relegated to oral use

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Figure 1.1 Milestones in human infectious disease and their relationship to development of antimicrobial drugs, 1930–2010, illustrating the relationship between the introduction of an antibacterial drug and the emergence of resistance. *Source:* Modified and reproduced with permission from Kammer (1982).

in animals for treatment of enteric infections. Figure 1.1 highlights the relationship between antimicrobial use and the development of resistance in many target microorganisms.

Spectrum of Activity of Antimicrobial Drugs

Antimicrobial drugs may be classified in a variety of ways, based on four basic features.

Class of Microorganism

Antiviral and antifungal drugs generally are active only against viruses and fungi, respectively. However, some imidazole antifungal agents have activity against staphylococci and nocardioform bacteria. Antibacterial agents can be described as *narrow spectrum* if they inhibit only Gram-positive and Gram-negative bacteria or *broad spectrum* if they also inhibit a wider range of bacteria such as chlamydia, mycoplasma, and rickettsia (Table 1.1).

Antibacterial Activity

Within the class description of antibacterial drug activity, antimicrobial drugs can further also be described as narrow spectrum if they inhibit only either Gram-positive or Gram-negative bacteria and as broad-spectrum drugs if they inhibit both Gram-positive and Gram-negative bacteria. This distinction is often not absolute since, although some agents may be primarily active against Gram-positive bacteria, they may also inhibit some Gram negatives (Table 1.2). It seems likely that some antimicrobial drugs developed in the future may be narrow spectrum and targeted to particular pathogens, avoiding the considerable "bystander" effect of broad-spectrum antimicrobials on the nonpathogenic microflora.

Bacteriostatic or Bactericidal Activity

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent required to prevent the growth of the pathogen. In contrast, the minimum

Table 1.1 Spectrum of activity of common antibacterial drugs.

	Class of Microorganism					
Drug	**Bacteria**	**Fungi**	**Mycoplasma**	**Rickettsia**	**Chlamydia**	**Protozoa**
Aminoglycosides	+	–	+	–	–	–
Beta-lactams	+	–	–	–	–	–
Chloramphenicol	+	–	+	+	+	–
Fluoroquinolones	+	–	+	+	+	–
Glycylcyclines	+		+	+	+	+/–
Lincosamides	+	–	+	–	–	+/–
Macrolides	+	–	+	–	+	+/–
Oxazolidinones	+	–	+	–	–	–
Pleuromutilins	+	–	+	–	+	–
Tetracyclines	+	–	+	+	+	+/–
Streptogramins	+	–	+	–	+	+/–
Sulfonamides	+	–	+	–	+	+
Trimethoprim	+	–	–	–	–	+

+/–, activity against some protozoa.

Table 1.2 Antibacterial activity of selected antibiotics.

	Aerobic Bacteria		Anaerobic Bacteria		
Spectrum	**Gram +**	**Gram –**	**Gram +**	**Gram –**	**Examples**
Very broad	+	+	+	+	Carbapenems; chloramphenicol; third-generation fluoroquinolones; glycylcyclines
Intermediately broad	+	+	+	(+)	Third- and fourth-generation cephalosporins
	+	(+)	+	(+)	Second-generation cephalosporins
	(+)	(+)	(+)	(+)	Tetracyclines
Narrow	+	+/–	+	(+)	Ampicillin; amoxicillin; first-generation cephalosporins
	+	–	+	(+)	Penicillin; lincosamides; glycopeptides; streptogramins; oxazolidinones
	+	+/–	+	(+)	Macrolides
	+/–	+	–	–	Monobactams; aminoglycosides
	(+)	+	–	–	Second-generation fluoroquinolones
	(+)	(+)	–	–	Trimethoprim-sulfa
	–	–	+	+	Nitroimidazoles
	+	–	(+)	(+)	Rifamycin

+, excellent activity; (+), moderate activity; +/–, limited activity; –, no or negligible activity.

bactericidal concentration (MBC) is the lowest concentration of an antimicrobial agent required to kill the pathogen. Antimicrobials are usually regarded as bactericidal if the MBC is no more than four times the MIC. This distinction is rarely important for treatment of clinical conditions. Some drugs are routinely bactericidal (e.g., beta-lactams, aminoglycosides) whereas others are usually bacteriostatic (e.g., chloramphenicol, tetracyclines), but this distinction depends on both the drug concentration at the site of infection and the microorganism involved. For example, benzyl penicillin is bactericidal at usual therapeutic concentrations but bacteriostatic at lower concentrations.

Time- or Concentration-dependent Activity

Antimicrobial agents are often classified as exerting either time-dependent or concentration-dependent activity, depending on their pharmacodynamic properties. These properties of a drug address the relationship between drug concentration and antimicrobial activity (Chapter 5). Drug pharmacokinetic features, such as serum concentrations over time and area under the serum concentration-time curve (AUC), when integrated with MIC values, can predict the probability of bacterial eradication and clinical success. These pharmacokinetic and pharmacodynamic relationships are also important in preventing the selection and spread of resistant strains. The most significant factor determining the efficacy of beta-lactams, some macrolides, tetracyclines, trimethoprim-sulfonamide combinations, and chloramphenicol is the length of time that serum concentrations exceed the MIC of a given pathogen. Increasing the concentration of the drug several-fold above the MIC does not significantly increase the rate of microbial killing. Rather, it is the length of time that bacteria are exposed to

concentrations of these drugs above the MIC that dictates their rate of killing. Optimal dosing of such antimicrobial agents involves frequent administration.

Other antimicrobial agents such as the aminoglycosides, fluoroquinolones, and metronidazole exert concentration-dependent killing characteristics. Their rate of killing increases as the drug concentration increases above the MIC for the pathogen and it is not necessary or even beneficial to maintain drug levels above the MIC between doses. Thus, optimal dosing of aminoglycosides and fluoroquinolones involves administration of high doses at long dosing intervals.

Some drugs exert characteristics of both time- and concentration-dependent activity. The best predictor of efficacy for these drugs is the 24-hour area under the serum concentration-time curve (AUC)/MIC ratio. Glycopeptides, rifampin, and, in some situations, fluoroquinolones fall within this category (Chapter 5).

Mechanisms of Action of Antimicrobial Drugs

Antibacterial Drugs

Figure 1.2 summarizes the diverse sites of action of commonly used antibacterial drugs. Their mechanisms of action fall into four categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function, and inhibition of protein synthesis.

Antibacterial drugs that affect cell wall synthesis (beta-lactam antimicrobials, bacitracin, glycopeptides) or inhibit protein synthesis (aminoglycosides, chloramphenicol, lincosamides, glycylcyclines, macrolides, oxazolidinones, streptogramins, pleuromutilins, tetracyclines) are more numerous than those that affect cell membrane function (polymyxins) or nucleic acid function (fluoroquinolones, nitroimidazoles, nitrofurans, rifampin). Agents that affect intermediate metabolism (sulfonamides, trimethoprim) have greater selective toxicity than those that affect nucleic acid synthesis.

Developing New Antibacterial Drugs

Infection caused by antimicrobial-resistant bacteria has been an increasing and rapidly developing problem and has reached a crisis in medicine. The speed with which some bacteria develop resistance considerably outpaces the slow development of new antimicrobial drugs. Since 1980, the number of antimicrobial agents approved for use in people has fallen steadily. What has been approved are variations of existing drugs; no new classes of antimicrobials have been discovered since the 1980s.

Several factors contribute to driving large pharmaceutical companies out of the antimicrobial drug market. These include expensive regulatory requirements, the challenges of drug discovery and the high cost of drug development coupled with the low rate of return on investment compared with drugs for the treatment of chronic "life-style" conditions. This has left limited treatment options for infections caused by methicillin-resistant staphylococci and vancomycin-resistant enterococci. The picture is even bleaker for infections caused by some Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, extended-spectrum beta-lactamase (ESBL)-resistant *E. coli*, *Klebsiella* spp., and *Enterobacter* spp., which are occasionally resistant to all safe antimicrobial agents. Judicious use of the antimicrobials currently available and better infection control practices, discussed in Chapters 20–24, will prolong the effectiveness of the drugs that are currently available. However, even if we improve these practices, resistant bacteria will continue to emerge and to spread, and new drugs will be needed.

While improvements in some existing classes of antimicrobial drugs continue to be laboriously made, numerous technological advances and improved understanding of bacterial pathogens hold considerable promise for the

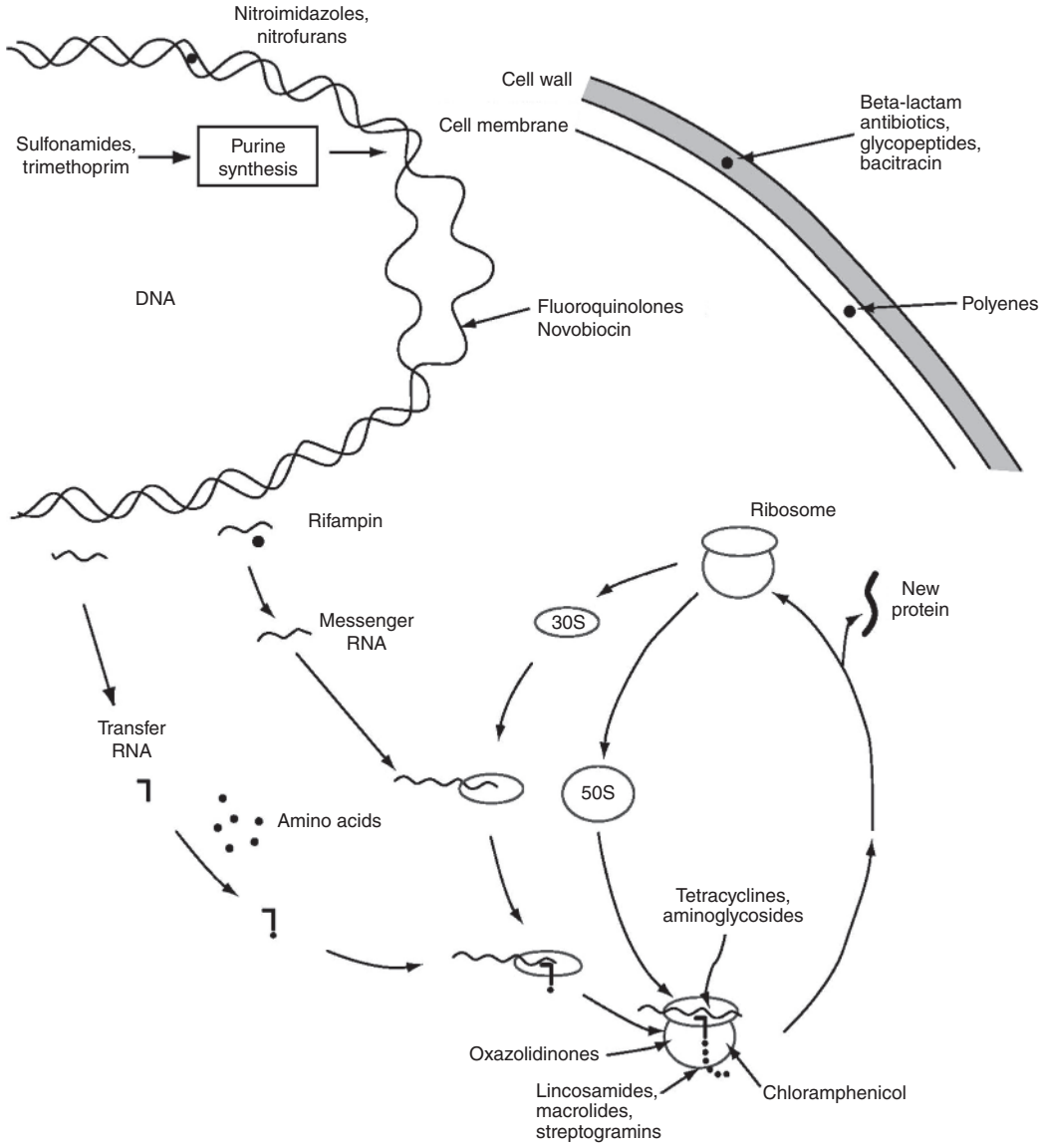

Figure 1.2 Sites of action of commonly used antibacterial drugs that affect virtually all the important processes in a bacterial cell. *Source:* Modified and reproduced with permission after Aharonowitz and Cohen (1981).

development of novel antimicrobial drugs. However, such development is challenging and extremely expensive. Novel targets for antimicrobial drugs that have been identified include those involved in essential amino acid biosynthesis, in cell wall lipid biosynthesis, in metal chelator biosynthesis, in quorum sensing, in efflux pumps, and in regulation of gene expression, among others. The investigation of novel antimicrobial sources has undergone a revival, and many novel antimicrobials have been identified, including numerous peptides. Development of antimicrobial drugs targeting specific pathogens is more straightforward than developing broad-spectrum compounds and, combined with increasing sensitivity of specific

agent diagnosis, is likely to be an important part of the future of antimicrobial therapy in human medicine.

Despite a degree of optimism about the future development of new antimicrobials, the costs are considerable and this and other reasons are likely to preclude veterinary application. Bringing a novel antimicrobial into human clinical use takes an estimated 10–15 years and costs an estimated US$1 billion, with the constant threat of development of resistance among important pathogens, which appear increasingly adept at spreading resistance. Many multinational companies have abandoned the search for new antimicrobials.

If candidate drugs found in preclinical development are identified, they are moved into three phases of human clinical trials, the last of which can consume 80% of the total research and development costs, which include the high costs of regulatory approval, all of which have to be recovered. Historically, only about 60% of drugs entering Phase 3 clinical trials are approved. Such expensive antimicrobials will therefore tend to have restricted use as "last resort" drugs, further limiting the return on investment.

A record of bankruptcy of companies that have brought novel drugs to market does not inspire private investment. Public–private philanthropic initiatives such as CARB-X (Combating Antibiotic-resistant Bacteria Biopharmaceutical Accelerator; www.carb-x.org) have been developed to help support drug discovery. In 2022, there were 62 antibacterial compounds of various types under clinical development for human medicine, with three antibacterials introduced since 2020 (Butler et al., 2023). As a result of funding initiatives, an encouraging increasing number of compounds are entering early Phase 1 evaluation studies. However, the clinical pipelines and recently introduced drugs are insufficient to address the emergence and spread of antimicrobial resistance. Most are direct-acting small molecules including peptides, but others include bacteriophage-related or antivirulence products.

The development of new antimicrobial drugs is inevitably focused on human rather than veterinary medicine, but research continues into new antimicrobial drugs targeted to topic use in specific veterinary infections (Greco et al., 2019; Bellavita et al., 2020).

Antifungal Drugs

Most currently used systemic antifungal drugs damage cell membrane function by binding ergosterols that are unique to the fungal cell membrane (polyenes, azoles) (Chapter 19). The increase in the number of HIV-infected individuals and of people undergoing organ or bone marrow transplants has resulted in increased numbers of immunosuppressed individuals in many societies. The susceptibility of these people to fungal infections has renewed interest in the discovery and development of new antifungal agents. The focus of antifungal drug development has shifted to cell wall structures unique to fungi (1,3-beta-D-glucan synthase inhibitors, chitin synthase inhibitors, mannoprotein binders).

Antimicrobial Drug Combinations: Synergism, Antagonism, and Indifference

In general, the use of combinations should be avoided because the toxicity of the antimicrobials will be at least additive and may be synergistic, because the ready availability of broad-spectrum bactericidal drugs has made use of combinations largely unnecessary, and because they may be more likely to lead to bacterial superinfection. There are, however, well-established circumstances, discussed in Chapter 6, in which combinations of drugs are more effective and often less toxic than drugs administered alone.

Knowledge of the different mechanisms of action of antimicrobials provides some ability to predict their interaction when they are used in combination. It was clear from the early days of their use that combinations of

antimicrobials might give antagonistic rather than additive or synergistic effects. Concerns regarding combinations include the difficulty in defining synergism and antagonism, particularly their method of determination *in vitro*; the difficulty of predicting the effect of a combination against a particular organism; and the uncertainty of the clinical relevance of *in vitro* findings. The clinical use of antimicrobial drug combinations is described in Chapter 6. Antimicrobial combinations are used most frequently to provide broad-spectrum empiric coverage in the treatment of patients who are critically ill. With the availability of broad-spectrum antimicrobial drugs, combinations of different drugs are less commonly used than in the early days of antimicrobial therapy, except for specific purposes.

An antimicrobial combination is *additive* or *indifferent* if the combined effects of the drugs equal the sum of their independent activities measured separately; *synergistic* if the combined effects are significantly greater than the independent effects; and *antagonistic* if the combined effects are significantly less than their independent effects. Synergism and antagonism are not absolute characteristics. Such interactions are often hard to predict, vary with bacterial species and strains, and may occur only over a narrow range of concentrations or ratios of drug components. Because antimicrobial drugs may interact with each other in many ways, it is apparent that no single *in vitro* method will detect all such interactions. Although the techniques to quantify and detect interactions are relatively crude, the observed interactions occur clinically.

The two methods commonly used, the *checkerboard* and the *killing curve*, measure two different effects (growth inhibition and killing, respectively) and have sometimes shown poor clinical and laboratory correlation. In the absence of simple methods for detecting synergism or antagonism, the following general guidelines may be used.

Synergism of Antimicrobial Combinations

Antimicrobial combinations are frequently synergistic if they involve: (1) sequential inhibition of successive steps in metabolism (e.g., trimethoprim-sulfonamide); (2) sequential inhibition of cell wall synthesis (e.g., mecillinam-ampicillin); (3) facilitation of drug entry of one antibiotic by another (e.g., beta-lactam-aminoglycoside); (4) inhibition of inactivating enzymes (e.g., amoxicillin-clavulanic acid); and (5) prevention of emergence of resistant populations (e.g., combination therapy in the treatment of tuberculosis).

Antagonism of Antimicrobial Combinations

To some extent, the definition of antagonism as it relates to antimicrobial combinations reflects a laboratory artifact. However, there have been only a few well-documented clinical situations where antagonism is clinically important. Antagonism may occur if antimicrobial combinations involve: (1) inhibition of bactericidal activity such as combination therapy with tetracycline and penicillin in which the bacteriostatic tetracycline prevents the critically required bactericidal activity of the penicillin; (2) competition for drug-binding sites such as macrolide-chloramphenicol combinations (of uncertain clinical significance); (3) inhibition of cell permeability mechanisms such as chloramphenicol-aminoglycoside combinations (of uncertain clinical significance); and (4) induction of beta-lactamases by beta-lactam drugs such as imipenem and cefoxitin combined with older beta-lactam drugs which are beta-lactamase unstable.

The impressive complexity of the interactions of antimicrobials, the fact that such effects may vary depending on the bacterial species, and the uncertainty of the applicability of *in vitro* findings to clinical settings make predicting the effects of some combinations hazardous. For example, the same

combination may cause both antagonism and synergism in different strains of the same bacterial species. Laboratory determinations are required but may give conflicting results depending on the test used. Knowledge of the mechanism of action is probably the best approach to predicting the outcome of the interaction in the absence of other guidelines.

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