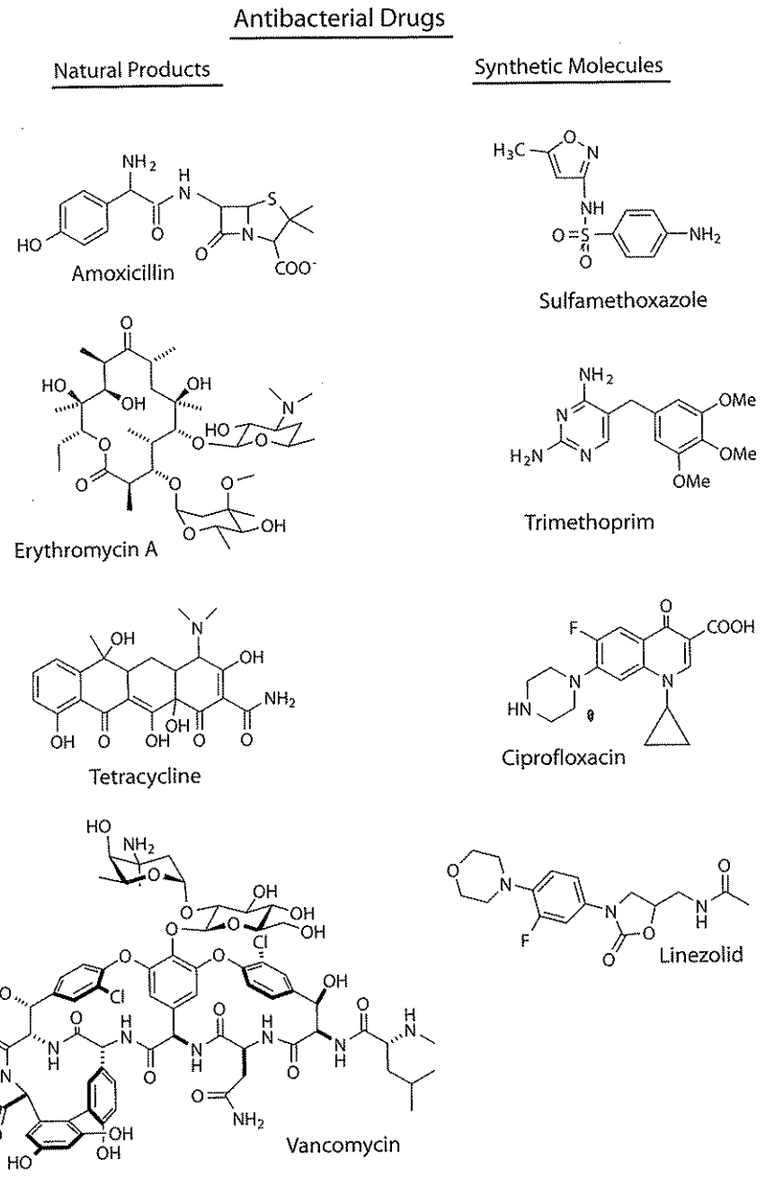


Structures of naturally and synthetically derived antibacterials.

chapter 2

Introduction to Major Antibiotic Classes and Modes of Action

Major classes of antibiotics in human clinical use

While hundreds to thousands of natural product structures have been isolated in screens for new antibiotics, only a small number of structural types have proven efficacious and safe enough to be taken through clinical development and approved for human clinical use to treat bacterial diseases. The major antibacterial drugs in current human use can be categorized in multiple ways.

One is by economic impact as shown in **Table 2.1** for the year 1997. The cephalosporin class of β-lactam antibiotics had the largest sales, while classical penicillin forms of the β-lactams were represented largely by amoxicillin (Amoxil). The combination of β-lactamase inhibitors with β-lactams in amoxicillin-potassium clavulanate (Augmentin), imipenem-cilastatin (Primaxin), and ampicillin-sulbactum (Unasyn) rounds out the β-lactam class at about $6 billion in sales for that year. The two expanded-spectrum macrolide antibiotics of the erythromycin class, clarithromycin (Biaxin) and azithromycin (Zithromax), amounted to almost $2 billion in sales. The third major class, the quinolones, was represented by the $1 billion drug ciprofloxacin. These three categories of drugs block cell wall biosynthesis, protein biosynthesis, and the DNA replication enzyme DNA gyrase, respectively. A somewhat broader cut of the antibiotic market, this time for the year 1995 (see **Table 2.2**), indicates three other classes of antibacterial drugs with over $400 million in sales in that year: tetracyclines, aminoglycosides, and glycopeptides. The antitubercular drug rifampin and the carbapenem version of the β-lactam imipenem are also listed. Representative brand names are indicated, as well as infections for which these drugs have been utilized and where clinically significant resistance had been detected. These classes of antibiotics are taken up in detail in this and later sections of the book, with discussions of mechanisms of action, modes of resistance development, and prospects for development of new versions to overcome resistance. Global sales of these antibiotics approached $24 billion by 2000.

Table 2.1 Antibiotic sales in 1997

Drug	$ millions
Cephalosporins	
Rocephin (Roche)	933
Ceftin (GlaxoWellcome)	640
Ceclor (Lilly)	542
Fortaz (GlaxoWellcome)	449
Claforan (Hofmann LaRoche)	335
Macrolides	
Biaxin (Abbott)	1,150
Zithromax (Pfizer)	619
β-Lactamase inhibitors	
Augmentin (GlaxoSmithKline)	1,354
Primaxin (Merck)	555
Unasyn (Pfizer)	619
Penicillins	
Amoxil (GlaxoSmithKline)	406
Quinolones	
Ciprofloxacin (Bayer)	1,290

A second method of categorizing antibiotics is by the bacterial diseases they are prescribed to treat (Anonymous, 1999; Levy, 1998). **Table 2.3** lists some common infections, broken into two columns based on whether the causative agents are gram-positive versus gram-negative bacteria. The Gram-staining status reflects differences in cell wall complexity (chapter 3) and is a broad index for antibiotic susceptibility. The gram-negative organisms have an intact outer membrane permeability barrier, while gram-positive organisms do not, and in general, such antibiotics as vancomycin can block gram-positive but not gram-

Table 2.2 Antibiotic market in 1995

Class	Worldwide sales ($ millions)	Representative drugs	Infections that have developed resistance
Cephalosporins	8,446	Cefaclor, cefuroxime	Bronchitis, pneumonia, meningitis
Penicillins	4,413	Amoxicillin, ampicillin	Pneumonia, septicemia, bronchitis
Fluoroquinolones	3,309	Ciprofloxacin, ofloxacin	Toxic shock syndrome, meningitis
Macrolides	2,927	Clarithromycin, erythromycin	Toxic shock syndrome, meningitis
Tetracyclines	744	Minocycline	Urinary tract infections, pelvic inflammatory disease
Aminoglycosides	729	Gentamicin	Intestinal infections, septicemia
Glycopeptides	462	Vancomycin	Intestinal infections
All other systemic antibiotics	1,873	Imipenem, rifampin	Bronchitis, tuberculosis

Table 2.3 Bacteria that are common causes of infections

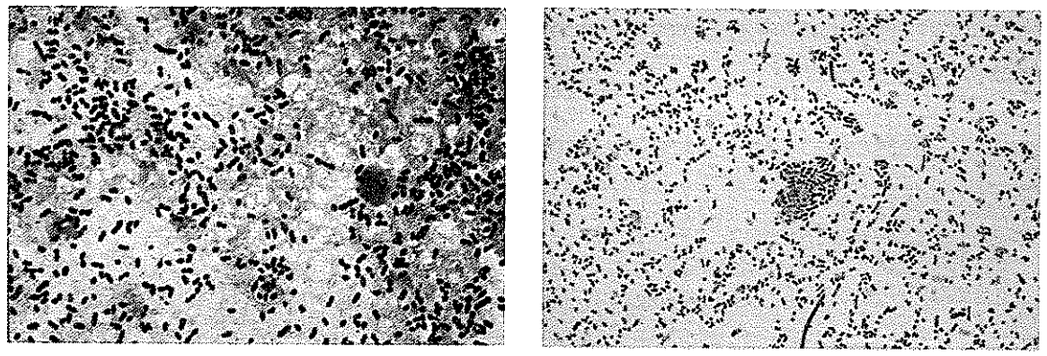
Infections	Gram-negative pathogens	Gram-positive pathogens
Burns	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Skin infections		*S. aureus*
Throat		*Streptococcus pyogenes*
Otitis media	*Haemophilus influenzae*	*Streptococcus pneumoniae*
Pneumonia	*H. influenzae*	*S. pneumoniae*
Endocarditis		*S. aureus, Enterococcus faecalis*
Septicemia	*Escherichia coli*	*S. aureus, S. pyogenes*
Gastrointestinal tract	*Salmonella enterica* serovar Typhimurium *Helicobacter pylori, E. coli, Shigella dysenteriae*	
Urinary tract	*E. coli*	*Enterococcus* sp.

Adapted from Table 1.1 of Scholar and Pratt (2000), with permission.

negative bacterial growth for that reason, as explained in detail in the subsequent chapters of this section. After the staining test, gram-positive bacteria show up as purple/black while gram-negative organisms show red colors. **Color Plate 2.1A** shows a photograph of a gram-positive *Streptococcus pneumoniae* isolated from the cerebrospinal fluid of a meningitis patient. **Color Plate 2.1B** shows a Gram stain of an *Escherichia coli* culture, with the typical red appearance of gram-negative bacteria.

The gram-positive streptococci are important pathogens in pneumonia, meningitis, and middle ear infections, while the gram-positive staphylococci and enterococci are problematic pathogens in postsurgical infections. The gram-positive *Mycobacterium tuberculosis* still causes millions of deaths annually. The historical scourges of plague and cholera are caused by two gram-negative bacteria, *Yersinia pestis* and *Vibrio cholerae*, respectively, while *E. coli, Salmonella,*

Color Plate 2.1 Gram stains of gram-positive *Streptococcus pneumoniae* (A) and gram-negative *E. coli* (B). (From Elliot et al., 1997.)



and *Shigella* strains are common causes of diarrheal diseases. The gram-negative *Pseudomonas aeruginosa* is often described as an opportunistic pathogen, causing disease in settings where the patient may have compromised immunity and/or some underlying disease such as cystic fibrosis. We shall note that *P. aeruginosa* has many facets that contribute to decreased susceptibility to many classes of antibacterial drugs, making it a difficult pathogen to treat.

This volume will not focus on the clinical pharmacology of existing antimicrobial drugs or the regimens used in infectious disease therapies. Two excellent texts for thorough and up-to-date coverage on those key aspects of antibiotics are *The Antimicrobial Drugs* (2nd edition), by Scholar and Pratt (2000), and *Antimicrobial Chemotherapy*, edited by Greenwood (2000). The latter book has a section dedicated to the treatment of bacterial infections in different tissues, e.g., respiratory tract infections, urinary tract infections, skin and soft tissue infections, bacteremias, endocarditis, and tuberculosis.

Some bacterial infections such as pneumonia are most often acquired in community settings, as are plague, cholera, and diarrheal diseases, while others may be acquired during hospital stays, so-called nosocomial infections. The staphylococci and enterococci that cause infections in postsurgical patients fall into the latter category, and since they exist in environments where antibiotics are in constant use, many staphylococcal and enterococcal strains are antibiotic resistant and are especially problematic bacteria (Lowy, 1998). Staphylococci with resistance to penicillins and in particular to methicillin can occur with high incidence (40% infection rate with methicillin-resistant *S. aureus* [MRSA] and 50% with methicillin-resistant *S. epidermidis* [MRSE] in some clinical wards). These pathogens have high mortality rates (25 to 63%) in hospital infections of the blood (bacteremias). In the late 1990s enterococci accounted for up to 12% of hospital infections in some U.S. cities, with >15% incidence of vancomycin resistance. Vancomycin-resistant enterococcal (VRE) infections produced mortality rates of 42 to 81%.

A typical first-line approach for antibiotic treatment, as published in *The Medical Letter* (Anonymous, 2001), is summarized in **Table 2.4**.

Table 2.4 intersects with the data of Tables 2.1 and 2.2 on the use of cephalosporins, macrolides, quinolones, aminoglycosides, and vancomycin. It also notes the trimethoprim-sulfamethoxazole combination, fosfomycin, and the antitubercular drug cocktail, all of which will be evaluated further in the chapters of this section. A more extensive list of antibiotic recommendations for bacterial diseases and likely causative agents can be found in Table 1-3 of Scholar and Pratt (2000), and their Table 1-5 includes antibiotics recommended in surgical prophylaxis based on clean versus contaminated surgical sites.

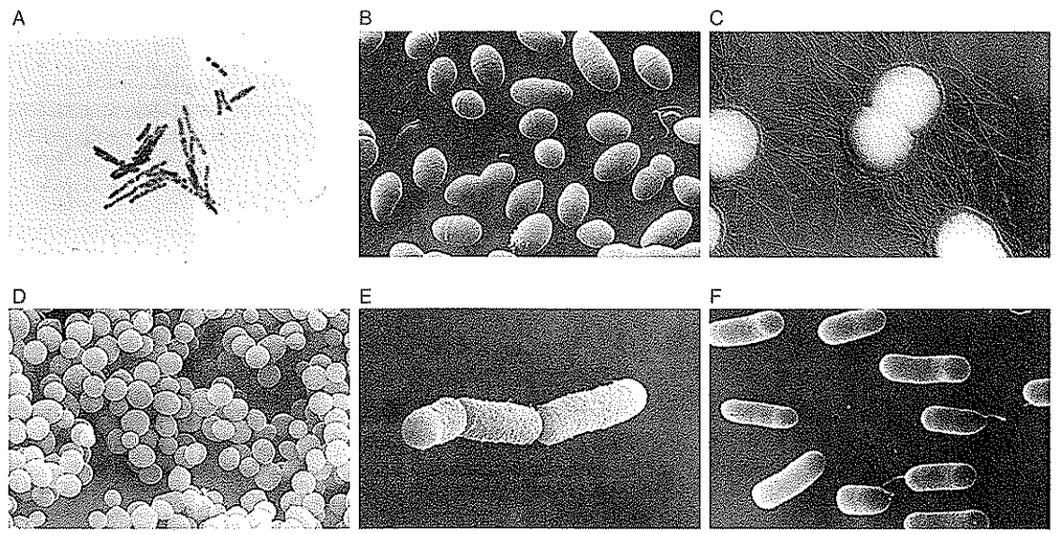
Many other antibiotics are used in specific situations and against particular pathogens, for example, bacitracin used topically against skin infections and tetracyclines for Helicobacter, *V. cholerae*, and brucella-derived infections. Some of the disease-causing pathogens are shown in **Fig. 2.1**, which highlights their distinct morphology.

Each of the antibiotic classes displayed in Table 2.5 has been shown by accrued experience to be more useful against certain bacterial pathogens than others in different clinical situations. The constraints are probably a mixture of both antibiotic levels and penetration efficacy and the intrinsic sensitivity of the

Table 2.4 Summary of typical first-line approach for antibiotic treatment

Infection	Likely pathogen	Reasonable first-choice therapy
Community-acquired pneumonia	*Streptococcus pneumoniae*	For hospitalized patients: broad-spectrum or "fourth-generation" cephalosporin; for ambulatory patients: an orally available macrolide or fluoroquinolone
Hospital-acquired pneumonia	Gram-negative bacterium or *Staphylococcus*	For *Pseudomonas aeruginosa*: broad-spectrum or "fourth-generation" cephalosporin, imipenem, and aminoglycoside; for MRSA: vancomycin
Meningitis	*S. pneumoniae* or *Neisseria meningitidis*	Broad-spectrum cephalosporin + vancomycin + rifampin
Sepsis syndrome	Gram-negative bacilli but also gram-positive cocci such as MRSA	Cephalosporin + aminoglycoside; vancomycin
Urinary tract infections	Gram-negative bacterium such as *E. coli*	Sulfamethoxazole + trimethoprim; fluoroquinolones; fosfomycin
Tuberculosis	*Mycobacterium tuberculosis*	Isoniazid + rifampin + pyrizinamide + ethambutol

Figure 2.1 Electron microscopic view of some bacterial pathogens. (A) *Mycobacterium tuberculosis* in sputum; (B) *Enterococcus faecalis*; (C) *Streptococcus pneumoniae*; (D) *Staphylococcus aureus*; (E) *Pseudomonas fluorescens*; (F) *Haemophilus influenzae*. (Courtesy Visuals Unlimited; all © D. M. Phillips except panel A © M. Abbey.)



antibiotic target in the recipient bacteria. The β-lactam antibiotics have progressed through several stages of optimization of the five-ring penicillins and up to four iterations of the six-ring cephalosporins to combat emergence of strains resistant to the prior generations of these classes of antibiotics. Similarly, in the macrolide antibiotics, both the original erythromycins and the successors, clarithromycin and azithromycin, are on the market. Structures of some of the penicillins and cephalosporins currently in wide use are in **Table 2.5**, as is that of the combination of amoxicillin and clavulanate, sold under the trade name Augmentin. The narrow-spectrum (erythromycin) and expanded-spectrum (azithromycin and clarithromycin) members of the macrolide polyketide antibiotics are widely used. Fluoroquinolones and aminoglycosides are also represented, along with the glycopeptide antibiotics vancomycin and teicoplanin. The trimethoprim-sulfamethoxazole combination, among the oldest antibiotics, is still on the market decades after its clinical introduction.

Validated targets in bacteria for antibiotics

The mechanisms of action of most antibacterial drugs were worked out after the discovery that the molecules had effects on bacterial growth, either slowing growth dramatically (bacteriostatic) or killing the bacteria (bactericidal). Molecules of clear therapeutic utility and potential were then examined for the molecular basis of their antibacterial properties, their selectivity, and their associated toxicity. Four major targets in bacterial pathogens have emerged from decades of study on mechanism of antibiotic action (**Fig. 2.2**): cell wall biosynthesis; protein biosynthesis; DNA replication and repair; and folate coenzyme biosynthesis. Each of these targets and the mechanisms of the major classes of antibiotics that interdict one or more steps in these pathways will be discussed in detail in chapters 3 to 7. Figure 2.2 serves as a master control diagram for chapters 3 to 10, and those chapters will focus on and illustrate distinct pathways of antibiotic action and antibiotic resistance.

Table 2.5 Major antibiotics: structural classes, targets, and resistance mechanisms

Antibiotic	Target	Resistance mechanism
Cell wall		
β-Lactams	Transpeptidases/ transglycosylases (PBPs[1])	β-Lactamases, PBP mutants
Vancomycin Teicoplanin	D-Ala-D-Ala termini of peptidoglycan and of lipid II	Reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser
Protein synthesis		
Erythromycins	Peptidyltransferase/ribosome	rRNA methylation/efflux
Tetracyclines	Peptidyltransferase	Drug efflux
Aminoglycosides	Peptidyltransferase	Drug modification
Oxazolidinones	Peptidyltransferase	Unknown
DNA replication/repair		
Fluoroquinolones	DNA gyrase	Gyrase mutations

[1]PBP, penicillin-binding protein.

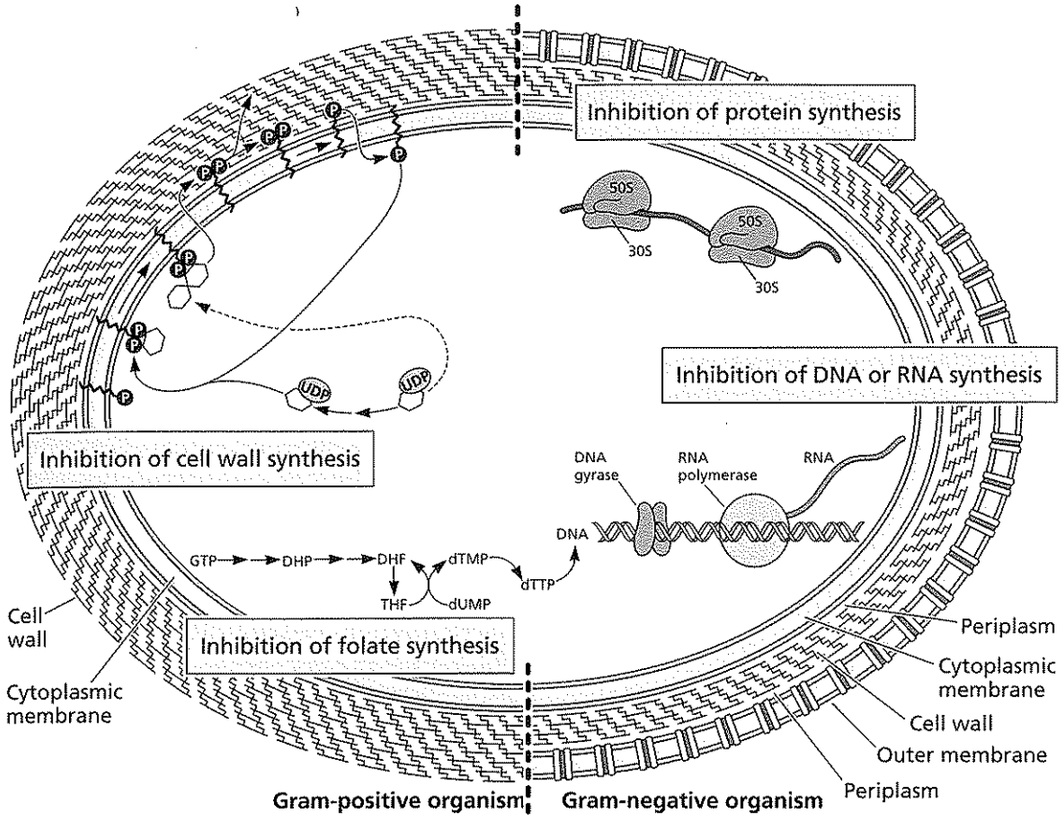


Figure 2.2 Major targets for antibacterial action. (Adapted from a poster on Mechanisms of Antibiotic Action and Resistance, C. Walsh, J. Trauger, P. Courvalin, and J. Davies [2001], *Trends in Microbiology, The Lancet Infectious Disease, Current Opinion in Microbiology, Trends in Molecular Medicine.*)

One guiding precept for selectively killing bacteria while sparing the human host taking the antibiotic would be for the antibiotic to act against a target present in bacteria but not found in animals and humans. This precept holds for two of the established targets, the enzymes of bacterial cell wall biosynthesis and the folic acid biosynthetic pathway enzymes, which do not have counterparts in humans. The other two major targets for antibacterial drugs, protein biosynthesis and DNA replication and repair machinery, clearly have human counterparts, but there are enough differences structurally between the prokaryotic and eukaryotic DNA and protein synthesis machinery that selective inhibition is achievable.

The cell wall biosynthetic processes and protein biosynthesis on the ribosome historically have been the site of action of the largest number of antibiotics, perhaps because of the many enzymatic steps, which offer multiple opportunities for disrupting these key attributes of a healthy bacterial cell. Genomic sequencing of the major bacterial pathogens is essentially complete, and efforts to delineate

essential genes or virulence-enhancing genes are well under way, as noted in chapter 15, offering a new molecular and genetic approach to validated novel targets that have not been targeted by existing natural product antibiotics. Those will be prime candidates for synthetic library-based screens to develop effective new antibiotics.