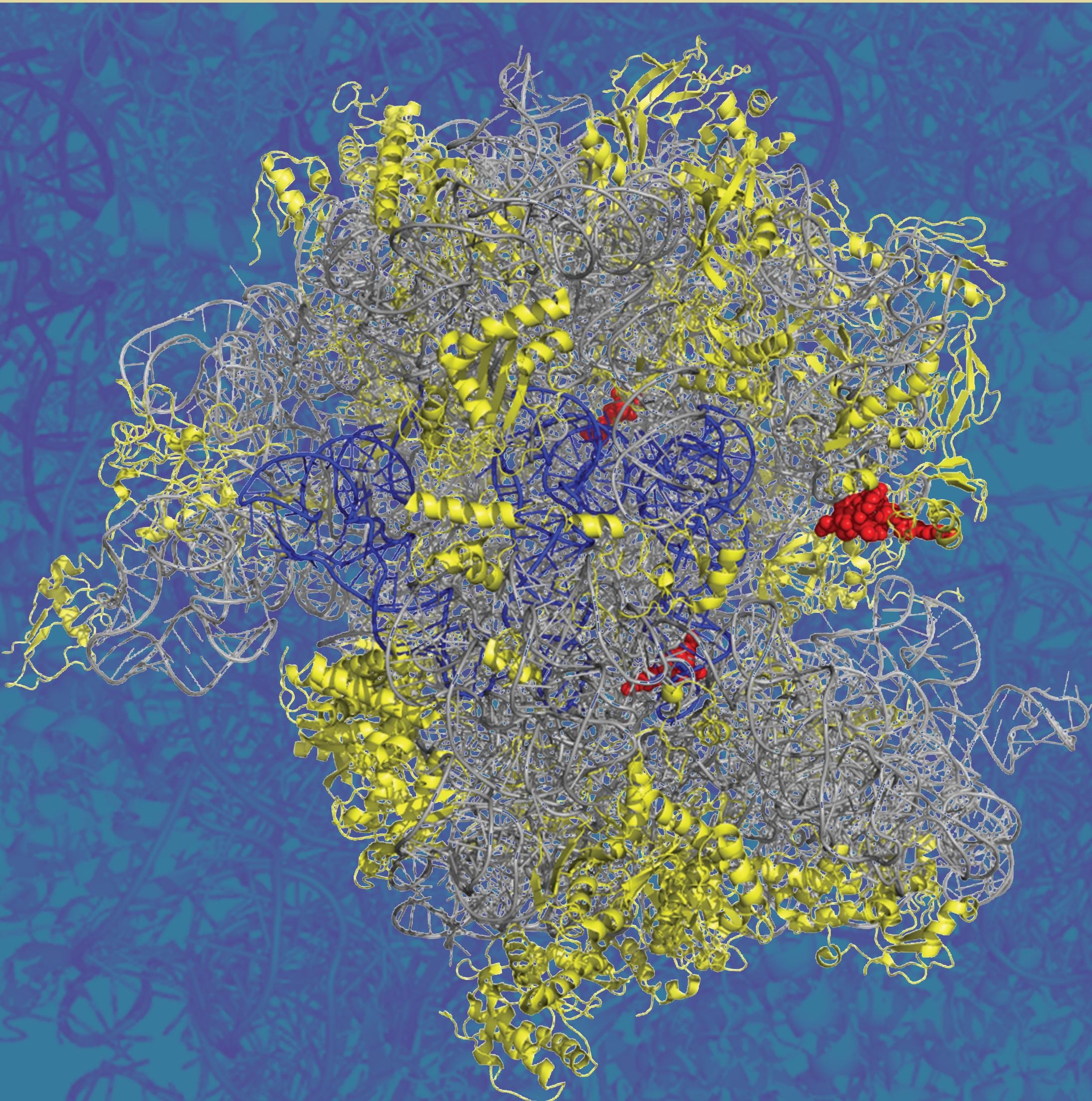
ANTIBIOTICS

CHALLENGES
MECHANISMS
OPPORTUNITIES



CHRISTOPHER WALSH | TIMOTHY WENCEWICZ

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MECHANISMS
OPPORTUNITIES

by

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and

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Washington University in St. Louis

ASM
PRESS
Washington, DC

Cover illustration: Snapshot of the 70S *E. coli* ribosome (PDB for 50S is 2WDL; PDB for 30S and tRNAs is 2WDK) bound to three classes of antibiotics. The figure shows RNA (gray) and protein (yellow) components of the ribosome with associated tRNAs shown as blue cartoons and bound antibiotics shown as red space filling spheres. The terpenoid tiamulin Q23 binds to the 23S rRNA at the peptidyltransferase center (PDB 1XBP). The nonribosomal peptide viomycin binds at the interface of the 50S and 30S subunits at the tRNA A site (PDB 4V7L). The thiazolyl peptide thiostrepton binds at the periphery, interacting with a specific pair of 23S rRNA helices and a proline-rich region of the L11 protein subunit (PDB 3CF5). (Image created using PyMOL.)

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Library of Congress Cataloging-in-Publication Data

Names: Walsh, Christopher, author. | Wencewicz, Timothy A., author.
Title: Antibiotics : challenges, mechanisms, opportunities / by Christopher T. Walsh and Timothy A. Wencewicz.
Description: Washington, DC : ASM Press, [2016] | ?2016 | Includes bibliographical references and index.
Identifiers: LCCN 2015045498 (print) | LCCN 2015047879 (ebook) | ISBN 9781555819309 (hardcover) | ISBN 9781555819316 ()
Subjects: LCSH: Antibiotics. | Drug resistance in microorganisms.
Classification: LCC RM267 .W358 2016 (print) | LCC RM267 (ebook) | DDC 615.3/29–dc23
LC record available at http://lccn.loc.gov/2015045498

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10 9 8 7 6 5 4 3 2 1

Address editorial correspondence to ASM Press, 1752 N St., N.W.,
Washington, DC 20036-2904, USA

Send orders to ASM Press, P.O. Box 605, Herndon, VA 20172, USA
Phone: 800-546-2416; 703-661-1593
Fax: 703-661-1501
E-mail: books@asmusa.org
Online: http://www.asmscience.org

Dedicated by CTW to Diana, Allison, Thomas, and Sean.

Dedicated by TAW to Rachel, Grace, and Lucy.

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Preface

Over the past decade, many lines of evidence have come together to emphasize the critical need for new antibiotics to treat bacterial pathogens. These realizations have galvanized the scientific community as well as the global citizenry more generally. Infectious disease groups, government-appointed expert panels, high political officials, and the popular press have noted the prospect of life-threatening bacterial infections triggering global societal crises.

There has been a parallel realization and set of efforts seeking new antiviral treatments as potentially global epidemics arise. Historically, antiviral drugs and antibacterial drugs have been discovered and developed essentially independently with almost no crossover targets or benefits. Viruses tend to be targeted by small molecules that block nucleic acid replication and inhibit proteases needed to cut viral polyproteins into functional subunits; these approaches have not readily been reframed for useful antibacterials. This book then deals only with antibacterial agents, antibiotics, and not antivirals; albeit the need for a contemporary integrated summary is pressing.

The need for next-generation versions of antibiotics is not new. This was the motivation in writing a precursor to this book in 2003: *Antibiotics: Actions, Origins, Resistance*. It has been true for every class of antibiotic introduced into wide human clinical use since the antibiotic era began in the 1930s that resistant organisms are selected for and eventually become abundant in human infections.

Others have noted, appropriately, the complexity of the cycles of development of antibiotics, development of resistance, and need for new antibiotics. The need for conservation and prolongation of useful lifetimes of the existing antibiotics portfolio has both health and economic consequences. Market incentives over the past 2 decades have been viewed as insufficient for large pharma companies to invest in antibiotics research. Alternatives to traditional small-molecule antibiotics range from vaccines with a preventative role to biologics, whether antibodies or lytic phage treatments, but the use of these therapies has not yet become widespread.

Amid this set of complexities, one constant is that new antibiotics are always needed to deal with waves of resistant bacteria that become inured to any antibiotic class that is widely disseminated for treatment. Given our belief that new classes of antibiotics are acutely needed to fill the front end of the antibiotic cycles, the approach in this monograph, *Antibiotics: Challenges, Mechanisms, Opportunities*, is chemocentric.

The **challenges** are many and layered, as noted in passing above, but chief among them is how to find new molecules, whether through the discovery of novel variants from nature or the creativity of synthetic and medicinal chemists. To understand how one might find, discover, and engineer new antibiotic classes, we focus on **mechanisms**. Three aspects of mechanisms seem relevant. One is to examine the major classes of antibiotics in human clinical use and understand as fully as possible what targets in bacterial cells are hit and what are the bases of selectivity compared to human hosts. A second aspect of mechanism is to understand the molecular bases of resistance as a prelude to next-generation antibiotic design. A third aspect of mechanism is delving into the chemical logic and enzymatic machinery by which producer microbes make the major classes of natural antibiotics. This gives insight into what chemistry is available to producing bacteria and fungi and how it might be modified by chemical and biological engineering approaches to generate optimized antibiotic scaffolds.

The book ends with a section on **opportunities**, again in the molecular sense, realizing all the many layers of complexities that await even if new molecular treasures are discovered. The approach here is to reexamine bacterial targets that may have been underutilized in past approaches, in part because genomics, proteomics, and metabolomics have given a much more sharply focused picture of bacterial physiology and pathology over the past decade. This section also asks the question: where will new antibiotics come from? Will the dual process of antibiotics from nature and synthetic antimicrobial bullets from human medicinal chemistry continue into the future?

As with any area of therapeutic research and inquiry today, interdisciplinary inputs are required to convert basic scientific observations into clinical candidates with reasonable prospects for safety and efficacy in humans. In the infectious disease arenas—antibacterial, antiviral, and antiprotozoal—the confluence of understanding the structure of active molecules and how they interact with affinity and specificity for their microbial targets are a key substratum for success. This volume provides such a starting molecular framework for investigators focused on antibiotics and members of the broader microbiology community.

Conflict of interest statement: CTW is a director of Achaogen Inc., South San Francisco, CA. TAW reports no conflicts.

Christopher Walsh
Timothy Wencewicz

SECTION I

Challenges for Antibiotics

This first section of the book consists of two introductory chapters that provide initial concepts about what antibiotics are, how they work, why they stop working, and what can be done about it. In a real sense, antibiotics are communitarian drugs. When they stop working because of the rise of resistant bacterial pathogens, they put the health of the commons, not just the individual patient, at risk.

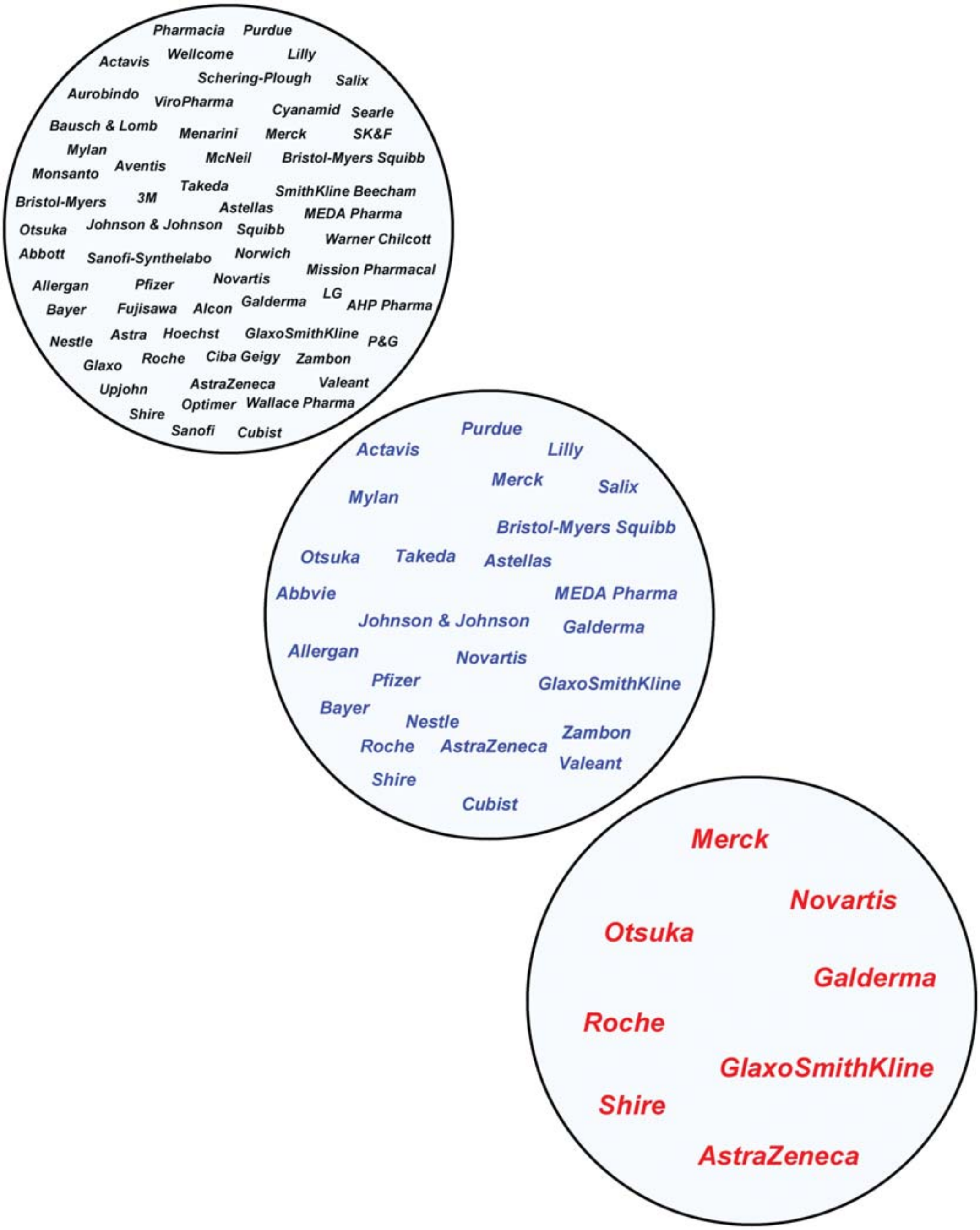
Chapter 1 provides the initial context for the global incidence of life-threatening bacterial illnesses and what kinds of therapeutic agents are antibiotics: small-molecule drugs that either kill bacteria outright (bactericidal) or halt their growth (bacteriostatic) to enable the host immune system to overcome the bacteria that are causing the illness. Empiric therapy, broad-spectrum antibiotics, and differential susceptibility of Gram-positive versus Gram-negative bacteria are introduced as central concepts.

There is an almost inevitable pattern when a new class of antibiotics is approved by health agencies and comes into widespread clinical use. Over time (which can be as short as 1 year), the minimal inhibitory concentration of antibiotic needed to cure a bacterial infection may rise as rare, resistant pathogens take over bacterial populations as sensitive bacteria die off. The percentage of methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant enterococci may rise well into double digits in certain settings, such as hospital intensive care units. This incidence drives the discovery, development, clinical evaluation, and approval of a next-generation antibiotic to treat the resistant pathogens. In turn, a next wave of pathogens resistant to that new antibiotic will be selected. As real examples, we are on fifth-generation β-lactam antibiotics and fourth-generation fluoroquinolones at present. In sum, there is a pressing, constant need for discovery of new antibiotics to meet the successive waves of resistant pathogens.

Chapter 2 introduces the major classes of antibiotics that are in current clinical usage. The most problematic pathogens are also noted. A current mnemonic encompasses "the ESKAPE" pathogens, where the capital letters stand for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species. The first two are Gram-positive pathogens, with an incomplete outer cell wall. The last four are Gram-negative bacteria, in which the intact outer wall is a major permeability barrier and provides intrinsically lower sensitivity to antibiotics of different structural classes.

Over the past century, two lines of investigation have been fruitful for discovery of clinically useful antibiotics. One line was the discovery of molecules from nature—natural products with antibiotic activity. The 2 decades between 1940 and 1960 comprise the golden age of natural antibiotic discovery, resulting in aminoglycosides, tetracyclines, the erythromycin class of macrolides, and the glycopeptide antibiotics of the vancomycin class. The second line of discovery has arisen from the inventions of medicinal and synthetic chemists who have come up with synthetic scaffolds in laboratories. The sulfa drugs, in continuous human use as antibiotics since the 1930s, emerged from chemical investigations of dye molecules. Two additional types of synthetic antibiotics of current clinical importance are the fluoroquinolones from the 1960s and the oxazolidinones from the turn of this century.

The targets of the major classes of antibiotics are also introduced in chapter 2, delineating five major bacterial processes. DNA and RNA information transfer pathways, bacterial cell wall biosynthesis, bacterial cell membrane integrity, bacterial protein biosynthesis, and the folate pathway for providing the unique DNA building block deoxythymidylate constitute the five canonical targets of broad-spectrum antibiotics. Among the challenges and opportunities are ongoing searches to find new pathways and protein, nucleic acid, or lipid targets in bacterial pathogens that could usher in new classes of antibiotics with novel mechanisms of action.


Pharmacia
Purdue
Actavis
Wellcome
Lilly
Schering-Plough
Salix
Aurobindo
ViroPharma
Cyanamid
Searle
Bausch & Lomb
Menarini
Merck
SK&F
Mylan
McNeil
Bristol-Myers Squibb
Monsanto
Aventis
Takeda
SmithKline Beecham
Bristol-Myers
3M
Astellas
MEDA Pharma
Otsuka
Johnson & Johnson
Squibb
Warner Chilcott
Abbott
Sanofi-Synthelabo
Norwich
Mission Pharmacal
Allergan
Pfizer
Novartis
LG
AHP Pharma
Bayer
Fujisawa
Alcon
Galderma
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Astra
Hoechst
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Shire
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Merck
Novartis
Otsuka
Galderma
Roche
GlaxoSmithKline
Shire
AstraZeneca

Antibiotics: Initial Concepts and Considerations

1

Infectious diseases kill about 14.9 million people a year worldwide, with pneumonia and related lower lung infections responsible for more than 3 million deaths (Fig. 1.1) (Morens et al., 2004). Diarrheal diseases cause about 1.8 million deaths and tuberculosis about 1.5 million deaths. In the United States alone, about 53,000 people succumb to pneumonia (http://www.cdc.gov/nchs/fastats/pneumonia.htm) and more than 130,000 to septicemias (http://www.hcup-us.ahrq.gov/reports/statbriefs/sb122.pdf) each year (http://who.int/mediacentre/factsheets/fs310/en/). While some bacterial diseases are largely prevented by vaccination campaigns, many others require acute treatment once a person is infected. Those acute treatments are courses of antibiotic therapy. Figure 1.2 illustrates graphically that resistant bacterial, viral, and parasitic pathogens are global phenomena. Outbreaks in one part of the world spread to other regions and continents with air and sea travel.

The word "antibiotic" derives from two classical Greek words, *anti* ("against") and *bios* ("life"). Thus, antibiotics are in principle substances "against life." In fact, they are molecules of low molecular weight (typically <1,000 daltons) that are *selectively against bacterial life*, i.e., antibacterial agents. Other kinds of small-molecule drugs target viruses (antiviral agents) or microbial parasites (antiparasitic drugs). They have different molecular targets from antibiotics and are not used to treat bacterial infections. This book does not therefore discuss antiviral and antiparasitic agents.

In one sense, from the perspective of human clinical medicine, the start of the antibiotic era can be ascribed to the introduction in 1935 of the prodrug form of the sulfa drug prontosil, a metabolic precursor to an inhibitor of an enzyme required for bacterial DNA biosynthesis (Trefouel et al., 1935; Scholar and Pratt, 2000) (Fig. 1.3). In another sense, all vertebrates, all macrofauna, even single-celled eukaryotes such as fungi, make a suite of small peptides with antibiotic activity, a class designated as antimicrobial peptides (Zasloff, 2002), which act as part of the first-line innate immune response to invading bacteria. Typically antimicrobial peptides insert selectively into bacterial membranes and disrupt their barrier function.

Farther back in the evolutionary tree of life are the single-celled prokaryotes; we shall spend much of this book in detailing the major classes

Figure 1.0 | Decrease in the number of companies producing antibiotics from 1980s–2010s (Kinch et al., 2014).

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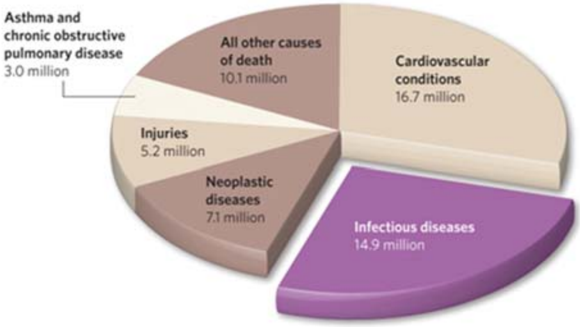


Infectious diseases	Annual deaths (millions)
Respiratory infections	3.96
HIV/AIDS	2.77
Diarrhoeal diseases	1.80
Tuberculosis	1.56
Vaccine-preventable childhood diseases	1.12
Malaria	1.27
STDs (other than HIV)	0.18
Meningitis	0.17
Hepatitis B and C	0.16
Tropical parasitic diseases	0.13
Dengue	0.02
Other infectious diseases	1.76

Figure 1.1 | Infectious diseases remain major killers of humans in the 21st century. STD, sexually transmitted disease. (Reprinted from Morens et al. [2004] with permission.)

of antibiotics made by various types of bacteria. There is continued debate about the physiological role and origins of naturally occurring microbially produced antibiotic molecular scaffolds (Ganz, 2003; Brogden, 2005). Whether they evolved as molecules with signaling functions, e.g., as developmental morphogens for streptomycetes that go through starvation-induced life cycle changes, or whether they have always been elaborated as chemical warfare agents by microbes waging molecular war against their neighbors is not resolved.

Waves of Resistant Bacterial Infections

Currently there is widespread concern that, again from the anthropocentric perspective, we may be approaching the end of the antibiotic era and returning to a pre-20th century existence in which even minor scratches could lead to life-threatening infections and death (World Health Organization, 2014). The 80 years of widespread clinical use of antibiotics since 1935 have been spent treating waves of infections by

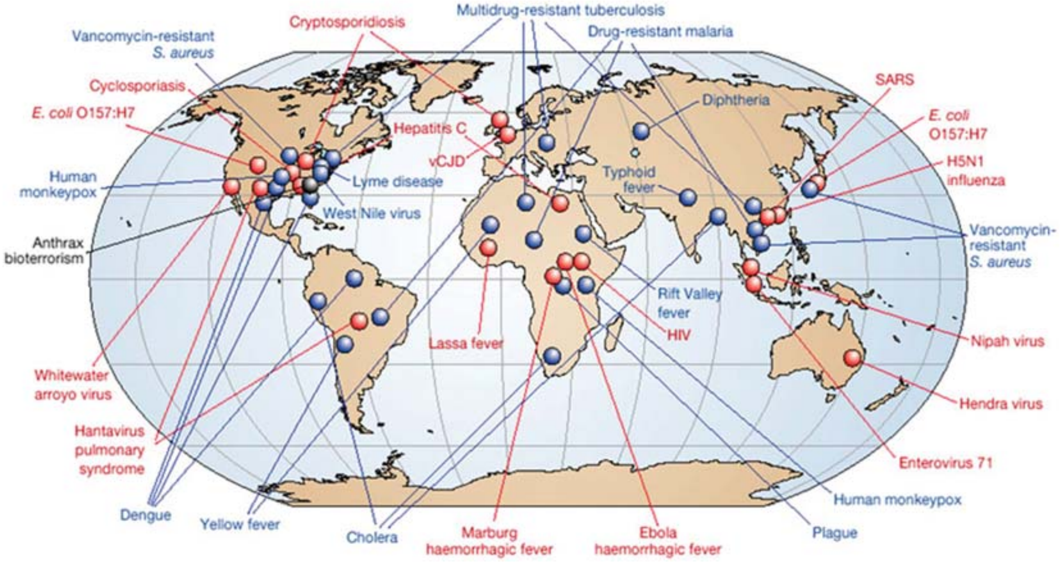

Multidrug-resistant tuberculosis
Cryptosporidiosis
Drug-resistant malaria
Vancomycin-resistant S. aureus
Cyclosporiasis
E. coli O157:H7
Hepatitis C
vCJD
Diphtheria
SARS
E. coli O157:H7
H5N1 influenza
Human monkeypox
Lyme disease
Typhoid fever
West Nile virus
Anthrax bioterrorism
Vancomycin-resistant S. aureus
Rift Valley fever
HIV
Lassa fever
Nipah virus
Whitewater arroyo virus
Hendra virus
Hantavirus pulmonary syndrome
Enterovirus 71
Human monkeypox
Dengue
Yellow fever
Cholera
Marburg haemorrhagic fever
Ebola haemorrhagic fever
Plague

Figure 1.2 | Infectious disease outbreaks occur in all parts of the globe, including reports of drug-resistant pathogens. SARS, severe acute respiratory syndrome; vCJD, variant Creutzfeldt-Jakob disease. (Reprinted from Morens et al. [2004] with permission.)

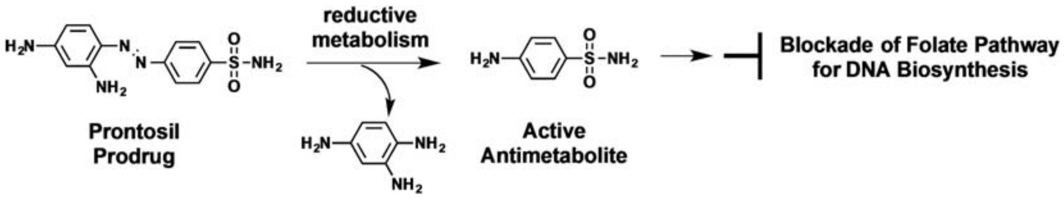


Figure 1.3 | The sulfonamide antibacterial drugs have been in continuous use for 80 years. The founding member of the class is *para*-aminobenzenesulfonamide, arising from metabolic reduction of the prodrug prontosil. The active drug blocks bacterial DNA synthesis by denying access to deoxythymidylate, a key building block for DNA replication.

bacterial pathogens, each showing resistance to previous generations of antibiotics.

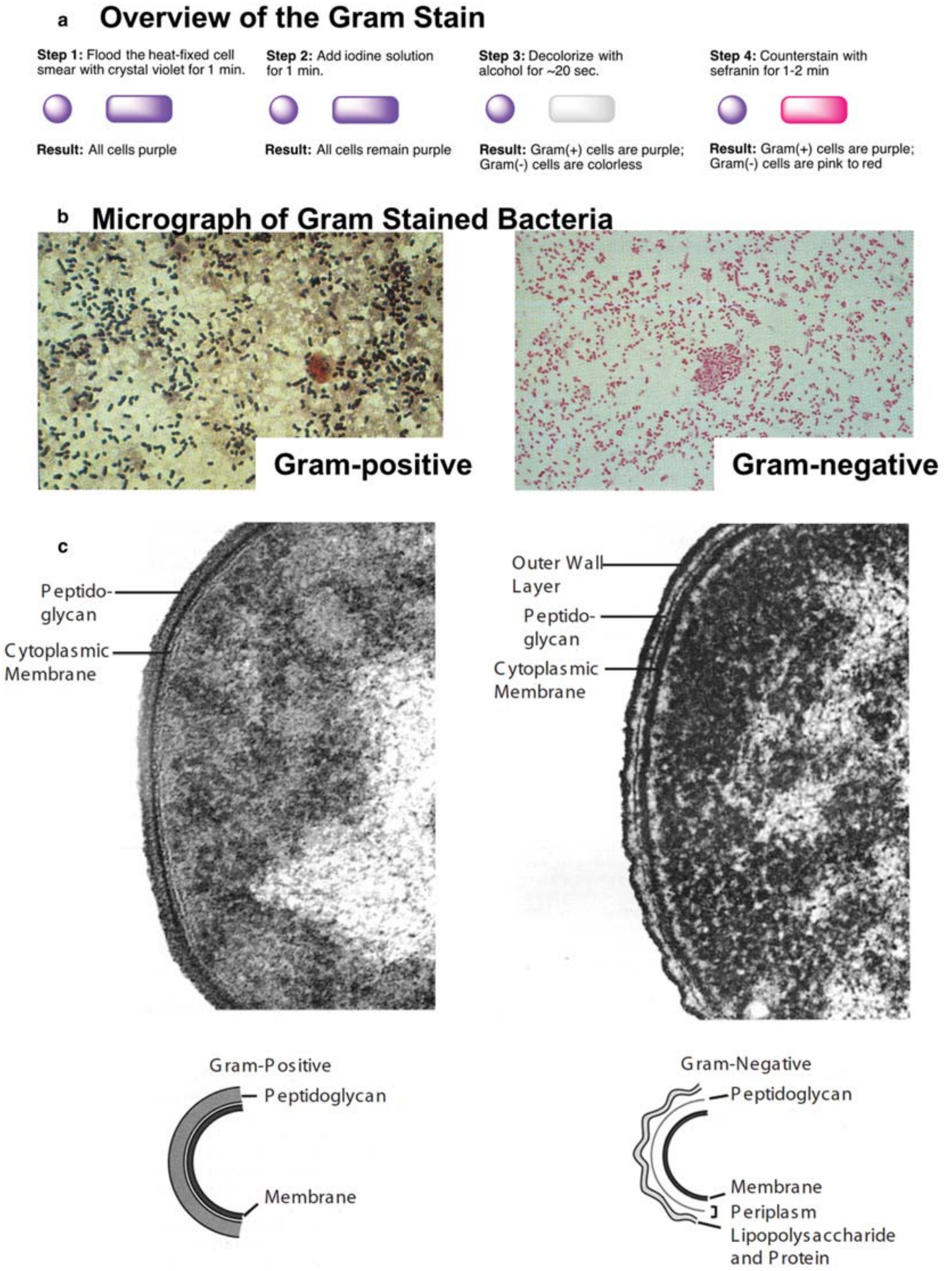
One truism in the antibiotic arena is that resistance is (almost) inevitable. The dynamic works as follows. All animals, humans included, go through life with a large cadre of associated bacteria, the great majority of which are beneficial to harmless, known as commensals. A small fraction of these are potentially problematic depending on what niche they occupy in or on their host. The global population of bacteria is estimated to be $\sim 5 \times 10^{30}$ (Whitman et al., 1998), which converts to about 50 tons of bacteria for each person on the planet (Woolhouse and Farrar, 2014)!

It has been estimated that humans comprise $\sim 10^{10}$ human cells and an associated $\sim 10^{11}$ bacterial cells. Bacteria can replicate their DNA and divide quickly: under certain nutrient conditions, they can double every 20 minutes in the gastrointestinal tract. The bacterial DNA polymerases make about 1 error in every 10^6 base pairs of DNA replicated. For genomes of 3 to 10 megabases, that means 3 to 10 mutants per generation, so bacterial populations of 10^8 in a given niche might have 300 to 1,000 mutants in the population. Perhaps 1 mutation confers resistance to the mechanism of action of a particular antibiotic, e.g., a penicillin. In the presence of killing concentrations of that antibiotic, the sensitive bacteria will die. The resistant clone, previously an extremely minor variant in the population, will now have space and nutrients released from dying bacterial neighbors and will grow up and take over the population. It has been estimated that, taken as a group, the total genes for soil bacteria on the planet experience 4 independent mutations every 3.4 hours. Referring back to the global bacterial population of $\sim 5 \times 10^{30}$ organisms, mutants are clearly abundant.

In this sense, antibiotic use selects for resistant bacterial strains. As will be noted in a later chapter, this is a generalized phenomenon for all the antibiotics widely used in human medicine. Two consequences emerge. The first is that the lifetime for any antibiotic widely introduced to treat human infections will tick down with some rate constant. Resistance is not a matter of if but only of when. Second, there is a constant need for new or improved antibiotics to treat the bacterial populations that come back in resistant form after the first wave of antibiotic treatments (Fischbach and Walsh, 2009; Walsh and Fischbach, 2009; Walsh and Wencewicz, 2014).

Differential Susceptibility to Antibiotics

An early step in microscopic identification of bacteria typically involves staining with crystal violet in a protocol originated in the 19th century by a Danish microbiologist named Gram. If the bacteria retain the stain, they are designated Gram positive; if not, they are Gram negative (Brown and Hopps, 1973; Brock et al., 1994) (Fig. 1.4). The staining outcome depends on morphology differences: Gram-negative bacteria have both an inner membrane and outer membrane that serve as permeability barriers, while Gram-positive organisms lack the outer membrane and its lipopolysaccharide



a Overview of the Gram Stain

Step 1: Flood the heat-fixed cell smear with crystal violet for 1 min.
Result: All cells purple
Step 2: Add iodine solution for 1 min.
Result: All cells remain purple
Step 3: Decolorize with alcohol for ~20 sec.
Result: Gram(+) cells are purple; Gram(-) cells are colorless
Step 4: Counterstain with sefranin for 1-2 min
Result: Gram(+) cells are purple; Gram(-) cells are pink to red

b Micrograph of Gram Stained Bacteria

Gram-positive
Gram-negative
c
Peptido-glycan
Cytoplasmic Membrane
Outer Wall Layer
Peptido-glycan
Cytoplasmic Membrane
Gram-Positive
Peptidoglycan
Membrane
Gram-Negative
Peptidoglycan
Membrane
Periplasm
Lipopolysaccharide and Protein

Figure 1.4 | The Gram stain, a venerable cytological test, groups bacteria into two large classes, Gram negative and Gram positive, based on the structures of the cell walls and the resultant permeability barriers. (a) Schematic of staining procedure; (b) micrograph of stained Gram negative and Gram positive bacteria; (c) electron micrographs showing the distinct layers of cell wall architecture of Gram-negative and Gram-positive bacteria.

constituent (Fig. 1.4c). Infections caused by Gram-negative bacteria are typically harder to treat than those caused by Gram-positive bacteria because fewer antibiotic classes can penetrate through both membrane barriers to reach intracellular bacterial targets. Fabbretti et al. (2011) note that screening of an unbiased library produces 10 to 100 times more hits against *Staphylococcus aureus* than against *Escherichia coli* and even more compared with nonfermenters such as *Pseudomonas aeruginosa*.

Infections in different body tissues can provide distinct challenges to antibiotic therapy, based on access and presumably the participation of the host immune system. Miller and Miller (2011) note that a single antibiotic dose can be curative for gonorrhea. On the other hand, urinary tract infections can be simple to complicated and be cured by an antibiotic course of 3 to 7 days. Infection of the pharynx, pharyngitis, by staphylococcal strains may require 10 days of treatment. When the inner lining of the heart is colonized by staphylococcal pathogens, curative antibiotic treatment may require 4 to 6 weeks. Front-line therapy of both sensitive and drug-resistant tuberculosis may involve three to five drugs taken as a daily regimen for 6 to 12 months. Finally, a venous leg ulcer may require systemic antibiotic treatment for 1 to 12 months.

In all cases, the state of the host immune system is crucial to therapeutic outcome. Patients who are immunocompromised have a much harder time staving off infections and may be colonized with opportunistic organisms. Immunocompetent patients may fight off the opportunistic pathogens, such as enterococci and *Acinetobacter* strains, but have a harder time with “professional” pathogens, such as *S. aureus*, that make a more challenging array of toxins and acquire resistance genes rapidly.

Empiric Therapy and Broad-spectrum Antibiotics

Historically, bacterial infections have been treated empirically, based on symptoms such as bloodstream infections (bacteremias), skin and soft tissue infections, pneumonia, meningitis, intra-abdominal infections, and so on, rather than on upfront identification of the causative bacterial pathogen. This lack of pathogen identification at the time of treatment reinforced a desire to develop broad-spectrum antibiotics, ones that would be “-cidal” for a diverse set of potential pathogens. (“Broad spectrum” means activity against a wide range of pathogens; a narrow-spectrum drug, on the other hand, might be active against only *S. aureus* infections.)

Current views suggest limitations and negative consequences to the broad-spectrum approach. One is the likely development of resistance across many bacterial species to a broad-spectrum antibiotic (later chapters will note mechanisms of resistance gene spread). The second goes back to the recently appreciated view of humans as a superorganism (Sleator, 2010) and the beneficial roles of their various microbiomes. *A priori*, one would like only to eradicate the pathogens and leave the great bulk of the commensal bacteria undisturbed and in equilibrium.

This concern would argue for narrow-spectrum antibiotics, but against that are economic consequences of small, niche markets that would stifle development and the concomitant need for real-time diagnostic tests to identify the (likely) pathogens in a particular patient. This potential approach also assumes that effective narrow-spectrum antibiotics (e.g., against methicillin-resistant *S. aureus* or quinolone-resistant *P. aeruginosa*) are actually available.

A second historical trend in the treatment of infectious diseases of note is the tendency to use monotherapy for bacterial infections but combination therapy as the standard of care for viral diseases such as HIV and in the treatment of cancers. In the latter two therapeutic arenas, there is the same concern as in bacterial infections that rapidly dividing, resistant organisms or tumor cells will break through monotherapy regimens. Thus, triple therapy, with distinct inhibitors of different targets in viral replication and/or entry, is the standard of care in AIDS. Similarly, combination therapy is standard in most cancer chemotherapeutic regimens. We will note the success of some combination antibiotic regimens, and perhaps these successes will be transitional to more widespread use of antibiotic combinations in the future.

Antibiotic Flow Chart

A typical path for antibiotic discovery and development of a novel antibiotic might involve the steps shown in Fig. 1.5. Sample acquisition constitutes an initial step. This may be a set of synthetic chemicals made for the specific purpose or a prior collection of molecules made for some other purpose, such as a different therapeutic area or an agricultural program. (The antibiotic linezolid derives from an initial synthesis in an agricultural inhibitor program [Brickner, 1996]). A second major source historically and contemporaneously is samples of microbes, such as spore-forming actinomycete bacteria and filamentous fungi such as the *Penicillium* molds that have provided many classes of useful natural products (Clardy et al., 2006). The microbes are grown up and then extracted with organic solvents to solubilize molecules such as antibiotics.

Next, the chemical matter is screened against a set of bacterial pathogens. In the past, known bacterial enzyme targets have been assayed for inhibition, but often the molecules that are potent in those *in vitro* assays are not active against the intact bacteria due to the membrane barriers that block penetration. Thus, most current screening is conducted against whole bacteria. An active molecule can then be compared in various profiles to known antibiotic classes to give an indication of the mechanism of action and potential target being hit.

If the original chemical hit has promising activity, either in terms of potency or, more importantly, against a broad range of clinically

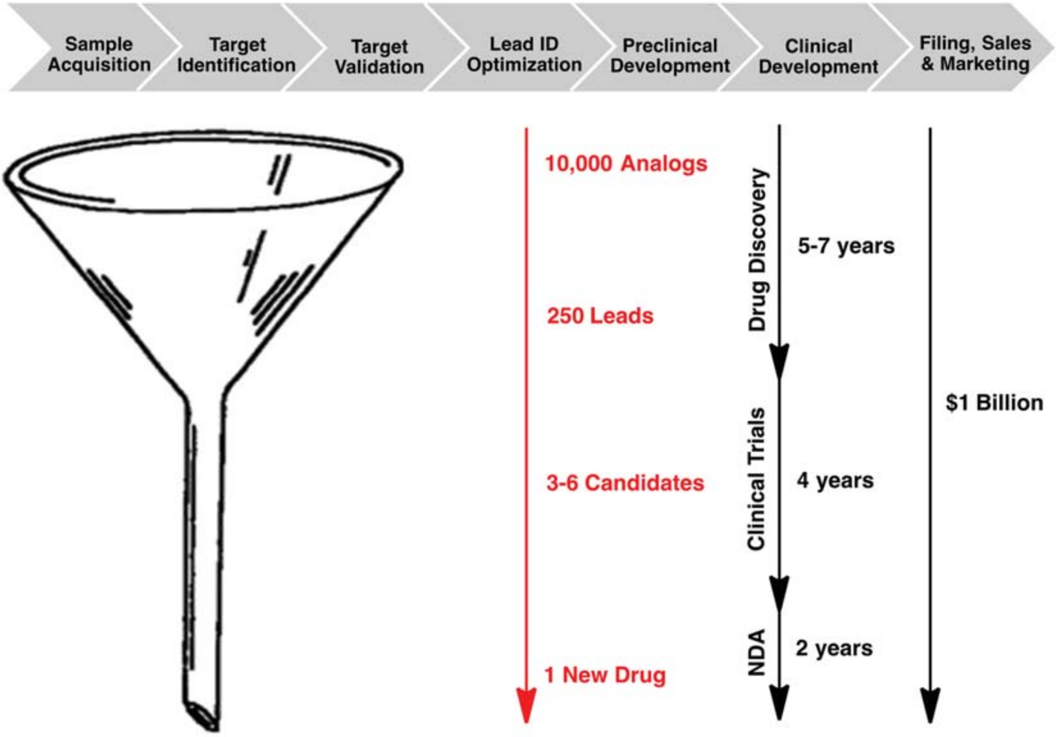

Sample Acquisition
Target Identification
Target Validation
Lead ID Optimization
Preclinical Development
Clinical Development
Filing, Sales & Marketing
10,000 Analogs
250 Leads
3-6 Candidates
1 New Drug
Drug Discovery
Clinical Trials
NDA
5-7 years
4 years
2 years
$1 Billion

Figure 1.5 | Flow chart for discovery and development of a prototypical antibiotic. Timelines and projected costs are estimates in line with industry standards. NDA, New Drug Application. (Ryan and Patterson, 2002) relevant bacterial pathogens, a variety of chemical modifications, from semisynthetic variations of the periphery or even the core of the molecule to total synthesis of analogs, proceeds in a lead optimization campaign. This may involve hundreds to thousands of analogs. With an optimized lead structure, the molecule may be declared a therapeutic candidate and be scaled up to kilogram quantities in order to undergo extensive tests in a battery of acute toxicology and chronic safety models that could take a year.

At this point, the molecule may enter clinical trials. Classically there are three phases of such trials. Phase 1 is a first-in-human trial, conducted in healthy volunteers with single dose escalation and then multiple ascending doses to evaluate safety. In phase 2, the candidate antibiotic drug is given to patients who have infections to get a sense of therapeutic dose amounts and scheduling. If one or more phase 3 trials are warranted, they may be conducted in patients with different infections, such as a trial for skin and soft tissue infections, a trial for intra-abdominal infections, or a trial for hospital-acquired pneumonia. A sufficient number of patients (usually several hundred) are enrolled to allow statistical significance in a blinded fashion and comparison with an existing standard of care (e.g., vancomycin in methicillin-resistant *S. aureus* infections). If the new antibiotic candidate performs as well as or better than the standard of care, it will likely be brought forward by the sponsoring organization for regulatory approval, before the Food and Drug Administration (FDA) in the United States and the European Medicines Agency in Europe.

If approved, the new antibiotic can be marketed only for the specified indications. The timeline for discovery and development may be upwards of 10 years and the cost in the range of $800 million to $1 billion.

Recent Approvals and the Current Antibiotic Pipeline

Figure 1.6 shows a timeline for new antibiotic approvals by the FDA in the United States over the past half century (Fischbach and Walsh, 2009; Walsh and Wencewicz, 2014). Between

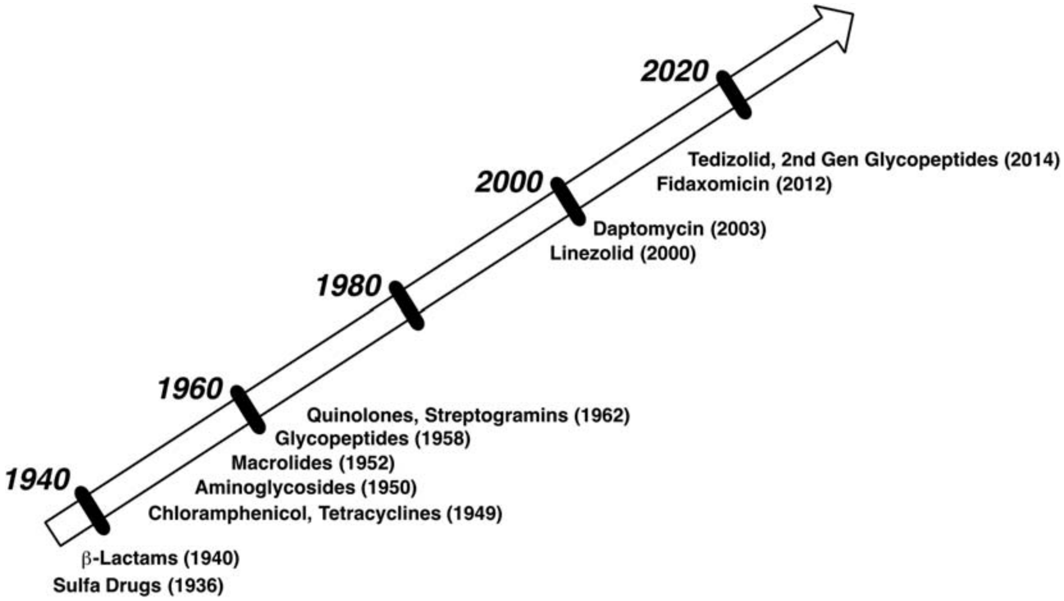

2020
Tedizolid, 2nd Gen Glycopeptides (2014)
Fidaxomicin (2012)
2000
Daptomycin (2003)
Linezolid (2000)
1980
1960
Quinolones, Streptogramins (1962)
Glycopeptides (1958)
Macrolides (1952)
Aminoglycosides (1950)
Chloramphenicol, Tetracyclines (1949)
1940
β-Lactams (1940)
Sulfa Drugs (1936)

Figure 1.6 | Timeline for discovery and commercialization of new antibiotic classes in human clinical use from 1940 to 2014. An innovation gap from 1960 to 2000 is shown.

2000 and 2014, several new antibiotics were approved. These include two fully synthetic molecular classes: the oxazolidinones, which inhibit bacterial protein synthesis; and a diarylquinoline, which disrupts energy metabolism in the tuberculosis pathogen. They also include the lipopeptide daptomycin and the polyketide fidaxomicin. A semisynthetic version of the natural product pleuromutilin has also been approved to target bacterial protein synthesis. In 2014 alone, a second-generation glycopeptide, dalbavancin, and second-generation oxazolidinone, tedizolid, with optimized scheduling or spectrum of action were approved by the FDA.

Most notable is the 40-year period between 1960 and 2000 during which no new classes of antibiotics were approved. There were molecules advanced in this 4-decade interval, but they were close relatives of existing antibiotic scaffolds (Wright et al., 2014). These included third-, fourth-, and fifth-generation β-lactam antibiotics and second- and third-generation versions of erythromycin, effective against new waves of existing bacteria but not adding to the inventory of novel molecular classes for the future (Fig. 1.6).

There are several explanations for the 40-year innovation gap, including de-emphasis of antibiotic discovery efforts across the pharmaceutical industry. On the one hand, there may have been some complacency that enough antibiotics had been discovered in the 20-year golden age of discovery from 1940 to 1960 to take care of future needs. On the other hand, the classical methods of finding new antibiotic classes from nature by existing microbial culture and isolation methods reached saturation with respect to discovery of new molecular structures with robust broad-spectrum antibiotic activity. Figure 1.6 indicates that we are making a comeback after the 40-year innovation gap.

An analysis of 21 antibiotics launched between 2000 and 2011, broken into natural product-derived and synthetically-derived categories, shows that half of the drugs in the natural product category are β-lactam antibiotics that have been tweaked in their periphery around a β-lactam core that has been in use for three quarters of a century (Butler and Cooper, 2011). In the synthetic category, eight of the nine antibiotics are variants of the fluoroquinolone scaffold, discussed in chapter 6. Analogously, five compounds that were in the final phase of clinical trials during 2011 were second and third generations of existing antibiotic scaffolds, both natural (tetracycline, glycopeptides [approved by the FDA in 2014], and macrolide) and synthetic (oxazolidinone, approved by the FDA in June 2014). This concentration in a small set of old molecular classes emphasizes the “too many eggs in one basket” vulnerability and therefore the need for more structural diversity in next-generation antibiotics.

Recognition of Pressing Need for New Antibiotics: “The End is Near” Scenarios

For much of the first decade and a half of the 21st century, there has been a rising awareness of the coupled phenomena of the dramatic increase in life-threatening bacterial infections and the paucity of effective new antibiotics (President’s Council of Advisors on Science and Technology, 2014; Boucher et al., 2009). There are many anecdotes—from “flesh-eating” bacteria, to killer *E. coli* contamination of bean sprouts, to recalls of hamburger meat contaminated with pathogenic *E. coli* strains, to celebrities dying from bacterial pneumonia—that have been noted in the popular press. This has been paralleled by reports showing antibiotic resistance on the rise and antibiotic discovery and approval on the decline. Figure 1.7 shows a 25-year trend for increases in the incidence of methicillin-resistant *S. aureus*, vancomycin-resistant enterococci, and fluoroquinolone-resistant pseudomonal infections (Infectious Diseases Society of America, 2004). This has led to the mantra “bad bugs, no drugs” and to gloomy predictions that if we don’t reverse the trends shown in Fig. 1.7 we could pass into a “post-antibiotic era.”

In such an era, radical changes to medical care would ensue: common surgeries would

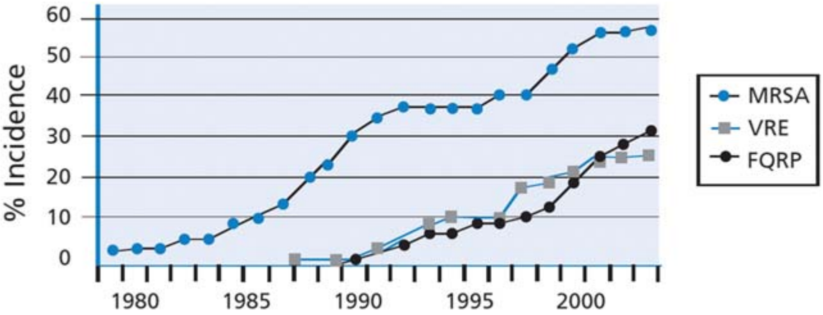


Figure 1.7 | Chart showing the growing incidence of three drug-resistant bacterial pathogens over the 25-year period from 1980 to 2005. MRSA, methicillin-resistant *S. aureus*; VRE, vancomycin-resistant enterococci; FQRP, fluoroquinolone-resistant pseudomonal infections. (Reprinted from Infectious Diseases Society of America [2004] with permission.)

carry the risk of life-threatening infections, as would cancer chemotherapy regimens, since they typically have the side effects of wiping out host white cells (Woolhouse and Farrar, 2014). Analogously, organ transplant procedures would fail from postoperative infections. Even intensive agricultural practices have come to depend on antibiotic usage. As just one example, the Lancet Infectious Diseases Commission in 2013 (Laxminarayan et al., 2013) noted that without effective antibiotics, about 30 to 40% of hip transplant patients would suffer postoperative infections. Of those, 30% would be likely to die from those infections, for an overall fatality rate of 1 in 10.

As one thread in the tapestry of "bad bugs, no drugs," during the 1990s and early 2000s, many of the large pharmaceutical companies closed their discovery research efforts in the antibacterial therapeutics space. In part, this was driven by pharmacoeconomic issues (Laxminarayan et al., 2013, and references therein). Americans treating acute bacterial infections take antibiotics 14 days a year on average. For many chronic diseases and even lifestyle drugs, days of therapy may be well over 150 to 200 days per year. In part, the decision to exit antibiotics research and development was also a tacit recognition that it had been extremely difficult to come up with new chemical matter that would become widely used broad-spectrum antibiotics. However, the growing realization of the life-threatening rise of many multidrug-resistant bacterial pathogens has spurred pharmaceutical, biotech, and academic research groups to use the new approaches in genomics, analytical and synthetic chemistry, and DNA-based diagnostics to refocus on the challenges of finding new antibiotics with clinical utility.

The global antibiotic resistance problem (Bush et al., 2011) has been compared to the global climate change crisis in terms of consequences and impact on human life on earth. The following quote emphasizes the perceived dilemmas: "As a result, the world is left with a decreasing stock of effective antibiotics, an inadequate pipeline of new classes and analogues, a broken antibiotic market, a paucity of antibiotic discovery infrastructure in academia, and insufficient infrastructure in industry" (Laxminarayan et al., 2013).

Other threads of the resistance tapestry include the fact that of the 100,000 to 200,000 tons of antibiotics manufactured and dispensed each year, most goes to agricultural, horticultural, and veterinary sectors (Laxminarayan et al., 2013). This adds to the resistome reservoirs (Wright, 2007), and any solution to safeguarding the utility and efficacy of current antibiotics must be transnational and global. One recent thread has been the dramatic rise (3- to 4-fold) in over-the-counter/nonprescription use of carbapenem antibiotics from 2005 to 2010 in countries such as India, Pakistan, and Egypt (Laxminarayan et al., 2013); the irrational use of antibiotics of uncertain quality and failure to complete courses of casual therapy add to the resistance problems.

Approach and Organization of This Volume

This book provides some context to aid efforts in antibiotic discovery. The complex issues of return on investment, market failure, and the possible need for different kinds of incentives, given the crucial public health role played by antibiotics, are beyond the scope of this book and are treated elsewhere (see, e.g., Boucher et al., 2009; Laxminarayan et al., 2013; President's Council of Advisors on Science and Technology, 2014; World Health Organization, 2014). However, throughout the many layers of complexity that range from economics to pricing strategies to differential access to antibiotics, one constant **challenge** is new antibiotic discovery. Unless this challenge is continuously met going forward, waves of resistant bacterial pathogens will be extremely difficult to combat.

This book examines several of the issues raised in the earlier sections of this opening chapter by addressing the origins of antibiotics, synthetic and natural, that have comprised the front-line agents for the treatment of human bacterial infections.

The **mechanisms** of action of the major antibiotic classes are examined in three stages. First is an analysis of how particular classes of antibiotic scaffolds interdict functions of their bacterial targets to produce bacteriostatic or bactericidal effects. A second major aspect of antibiotic mechanisms is why and how they stop working. This means a close examination of antibiotic resistance mechanisms, both intrinsic and acquired, and includes exploration of the concept of the resistome, the reservoir of resistance genes that exist in bacterial populations. The third aspect of mechanisms delineates the biosynthetic strategies used by microbes to generate the major natural antibiotic classes. Both the molecular logic and enzymatic machinery for assembly of polyketide, peptide, terpene, and carbohydrate scaffolds are explored, with the ultimate aim of pathway engineering.

The last section of the book examines the opportunities that may be achievable in the near term. One approach is to reexamine targets that have been underexplored historically despite reasonable rationales for efficacy. A complementary approach is to ask where new antibiotics will come from. What are the prospects for finding new molecular scaffolds, both from natural niches and from new synthetic chemical strategies, for next-generation antibiotics?

The main focus of the book is **chemocentric**, i.e., about the antibiotic molecules themselves: where they come from, how they act, and how bacteria defend themselves. The central premise is that **new chemical matter is needed** to be turned into next generations of effective antibiotic agents to function as lifesaving drugs. We argue that the chances for finding those next molecules are improved by understanding as much as possible about how antibiotics interact with bacterial physiology and pathology.

This volume does not venture into clinical microbiology, for example, the detection, identification, and classification of bacterial pathogens. Reference works for those important activities exist, such as *Manual of Clinical Microbiology*, 11th edition (J.H. Jorgensen and M.A. Pfaller [eds], ASM Press, 2015). Nor is this book a guide to clinical dispensing of antibiotics, including such topics as antibiotic formulations, dosing schedules for adult and pediatric patients, pharmacokinetics, pharmacodynamics, and metabolism and excretion routes of clinically approved antibiotics. For such a reference, readers might consult Kucers, *The Use of Antibiotics*, 6th edition (M.L. Grayson [ed], CRC Press, 2010). Also, interested readers are referred to the volume compiled by Dougherty and Pucci (*Antibiotic Discovery and Development*, Springer, 2012) for criteria used to evaluate antibiotics as preclinical and clinical candidates. One additional reference volume, *Antibacterial Agents: Chemistry, Mode of Action, Mechanisms of Resistance, and Clinical Applications* (R.J. Anderson, P.W. Groundwater, A. Todd, A.J. Worsley, John Wiley & Sons, 2012), is a good complement to the approach in this book. In particular, this text notes clinical applications and adverse drug reactions for the major classes of antibiotics used to treat human infections.

Finally, the exigencies posed by the global spread of multidrug-resistant bacterial