Updates in Clinical Dermatology
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Overcoming Antimicrobial Resistance of the Skin

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I would like to dedicate this book to my wonderful wife, Patricia, for her love and patience.

– Stephen K Tyring

I would like to express my boundless appreciation to Dr. Angela Moore whose passion for dermatology has launched me on the trajectory to be a life-long learner of the skin sciences.

I would like to thank Dr. Tyring for his unwavering support and confidence that I could contribute to such a critical work.

– Stephen Andrew Moore

I dedicate this volume to front-line clinicians worldwide who battle microscopic foes and to the next generation who will continue this fight.

– Angela Yen Moore

Preface

Although dermatologists prescribe more antibiotics per provider than any other specialty, *Overcoming Antimicrobial Resistance of the Skin* was written for all healthcare professionals, not just those who prescribe antibiotics. Antimicrobial resistance (AMR) is a public health crisis that existed before the COVID-19 pandemic and unfortunately will continue to be a major problem long after this pandemic has passed. It is an emergent health threat responsible for the death of approximately 35,000 in the United States and approximately 700,000 people globally each year. It is projected that the continued rise in AMR could result in the death of 10,000,000 annually by 2050. In fact, the World Health Organization (WHO) calls AMR one of the most urgent health threats of our time. The AMR crisis does not just involve antibiotic resistance, because similar problems exist for antivirals, e.g., for HIV; antiparasitics, e.g., for malaria; and antifungals, e.g., for *Candida auris.* This book, however, was not written simply to point out the problem, but to focus on possible solutions. In the twenty-first century, it is difficult to imagine the world before antibiotics. In the beginning of the twentieth century, however, dying of sepsis following childbirth or a simple skin infection was common, as were deaths due to pneumonia and meningitis. Although antibiotics only became widely available to the general public in the second half of the twentieth century, as did most currently available vaccines, Dr. Alexander Fleming warned of the potential problem of AMR in his 1954 Nobel Prize address. The solution, however, is not simply new antibiotics nor new methods of killing infectious organisms. The "cure" of an infectious disease is not the global solution, because "it is better to prevent than lament," which means public health measures and vaccines.

The importance of vaccines first gained general knowledge in the late eighteenth century, that is, the smallpox vaccine, and public health measures such as hand washing, clean drinking water, sewage disposal, and pasteurization became more common in the nineteenth century. These measures continue to be of upmost importance in the twenty-first century. Likewise, respiratory precautions, including face masks, quarantines, contact tracing, taking temperatures, asking about symptoms, and social distancing, were advocated and followed during the influenza pandemic of 1918 to 1919. It is sadly ironic that such precautions are not followed more closely during the current COVID-19 pandemic.

The political and economic effects of infectious diseases, like the effects on morbidity and mortality, are striking. Countries undergoing political and economic crisis, for example, Venezuela, often experience collapse of their healthcare systems. The "Black Death" in the form of *Yersinia pestis* not only killed millions of people between 1335 and 1368, it also had disastrous effects on Europe's economy and trade. It also contributed to the collapse of the Chinese, Russian, Persian, and Mongol empires. Like the 1918 to 1919 influenza pandemic, the COVID-19 pandemic has left millions of people globally unemployed. Ironically, the influenza pandemic enabled a presidency, while the COVID-19 pandemic helped destroy a presidency.

The evolution of AMR is now outpacing the development of new countermeasures. This situation threatens patient care, economic growth and security, public health, agriculture, and national security. Agreements and legislation have formed to address the AMR issues, and billions of dollars have been spent. Overcoming AMR is no longer a matter of finding new mechanisms of action. Many other factors to consider include biofilms and the microbiome as well as costs. Phytocompounds are being investigated further. New drug delivery systems are being tested, including use of nanoparticles. Newly discovered cellular pathways, for example, the MHC class II transactivator (CIITA) gene plus CD74, can be explored to block viral infections. Bacteriophages, once the subject of fictional cures, are now being used to overcome AMR.

All of these innovations, however, will be insufficient without public health measures, including vaccines. As the world awaits COVID-19 vaccines, fewer children are being vaccinated against other infections. According to the WHO, >80,000,000 children less than 1 year old could miss routine vaccinations due to the pandemic. Measles deaths worldwide have swelled to their highest level in 23 years. Due to lack of vaccination, 30,000 to 60,000 people, mostly adults, die each year of non-pandemic influenza just in the United States. Lack of vaccination against preventable diseases ultimately leads to further antimicrobial use and accelerates AMR. Antimicrobial overuse during the COVID-19 pandemic could also further AMR. UNICEF and GAVI have found that routine vaccinations are stalled in at least 68 countries. In developed countries, unfounded fear of vaccines by adults will prevent children from receiving available vaccines. The messenger RNA vaccines against COVID-19 are reported to be 95% effective and will be given as two doses. Because not everyone will or can receive these vaccines, 70% to 90% of susceptible individuals will need to be vaccinated to achieve herd immunity. Even then, public health measures, for example, hand washing, face masks, and social distancing, will still need to be maintained to achieve control of the pandemic.

As seen from the COVID-19 pandemic, understanding newly emerging diseases is crucial for all healthcare workers. We have learned many critical lessons: build resilient health systems with trust in science and public health agencies; invest in biomedical research and development; focus on equity and evidence-based facts; and trust and fund global healthcare institutions, because infectious diseases do not respect national borders. The emergence

of novel infectious diseases is a public health threat, further exacerbated by AMR. Antimicrobials have allowed for huge strides in public health over the last century, but danger of resistance is a real and major concern that must be addressed immediately. Therefore, it is imperative that healthcare workers have an understanding of emerging infectious diseases and AMR.

Houston, TX, USA Stephen K. Tyring

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– Stephen Andrew Moore

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– Angela Yen Moore

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Part I

Emerging Bacterial Resistance to Antibiotics

Mechanisms of Bacterial Resistance

1

Radhika A. Shah

Abbreviations

2-DOS	2-Deoxystreptamine
ABC	ATP-binding cassette
ABC-F	ATP-binding cassette family
AG	Aminoglycoside
AME	Aminoglycoside-modifying enzymes
ARE	Antibiotic resistance
CAMP	Cationic antimicrobial peptide
CAT	Chloramphenicol acetyltransferase
CDC	Centers for Disease Control and Prevention
DAP	Daptomycin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
Erm	Erythromycin ribosomal methylation
LPS	Lipopolysaccharide
MDR	Multidrug-resistant
MEGA	Macrolide efflux genetic assembly
MGE	Mobile genetic elements
MLS_B	Macrolide-lincosamide-streptogramin B
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MurA	UDP-*N*-acetylglucosamine enolpyruvyl transferase
OM	Outer membrane
OptrA	Oxazolidinone and phenicol transferable resistance A
PABA	Para-aminobenzoic acid
PBP	Penicillin-binding protein
PMBN	Polymyxin B nonapeptide
QRDR	Quinolone resistance-determining region
RMTase	RNA methyltransferase
RNAP	RNA polymerase
RPP	Ribosomal protection protein
rRNA	Ribosomal ribonucleic acid
SAM	S-adenosyl-L-methionine
SCC*mec*	Staphylococcal chromosomal cassette *mec*
SSTI	Skin and soft tissue infections
TMP-SMX	Trimethoprim-sulfamethoxazole
UDP-MurNAc	UDP-*N*-acetylmuramic acid
UNAG	UDP-*N*-acetylglucosamine
Vat	Virginiamycin acetyltransferase
Vgb	Virginiamycin gene B
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRE	Vancomycin-resistant *Enterococci*

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Introduction

Antibiotics first achieved widespread use during World War II in the 1940s; however, antibiotic resistance has quickly emerged as a global health crisis over the past several years [1, 2]. The rate at which bacteria are gaining resistance far exceeds that of new drug discovery, placing not only those with infectious diseases at greater risk but also those undergoing immunosuppression by organ transplantation, chemotherapy, or dialysis [3]. Medical and agricultural applications are increasing resistance in both arenas [3]. Eighteen antibiotic-resistant pathogens have recently been identified by the Centers for Disease Control and Prevention (CDC) in their 2019 report as "urgent," "serious," or "concerning" threats to human health (CDC).

Antibiotic resistance is defined as the ability of certain pathogens, including bacteria and fungi, to evade antibiotics designed to kill them (CDC). The number of infections and deaths due to antibiotic-resistant pathogens has fallen since the CDC's Antibiotic Resistance (AR) Threats report was first released in 2013; however, current estimates of almost 2.9 million antibiotic-resistant infections every year prompt investigation into the concept of the antibiotic "resistome" [3]. To begin this investigation, we will discuss resistance in the context of antibiotic targets and biochemical mechanisms of resistance [4].

Origins of Resistance

In 1940, the first antibiotic-resistant bacteria produced penicillinases, which destroyed penicillin [5–7]. Penicillin was first discovered in 1928 by Alexander Fleming, a bacteriologist in London who observed the antibacterial properties of what we now know as penicillin, originally just a fungal contaminant in a petri dish. Years later, scientists could purify the drug and determine its b-lactam structure comprising a four-membered b-lactam ring. The mechanism of action of penicillin antibiotics involves the inhibition of transpeptidase and cross-linking of peptidoglycan via imitation of the last two D-alanine residues [6].

In the decades following the discovery of penicillin, widespread use led to the development of resistant strains of bacteria that produced penicillinases and prompting development of semisynthetic b-lactamase-resistant penicillins [6]. Besides the development of such semisynthetic antimicrobial drugs as methicillin, the discovery of cephalosporin antibiotics in 1945 allowed temporary circumvention around penicillin resistance due to its altered beta-lactam structure [8]. The cephalosporin family of antibiotics includes several generations of drugs, including cephalexin, ceftriaxone, and cefepime, whose spectrum of activity against Gram-negative bacteria increases with each generation.

Resistance to b-lactam antibiotics is mediated by b-lactamase enzymes, which result in the inactivation of cell wall synthesis of bacteria [6]. The enzymes are encoded by genes, known as resistance factors, residing on the bacterial chromosome or plasmids. Specifically, b-lactamases catalyze hydrolysis of the b-lactam bond in the ring structure, producing acidic derivatives that lack antimicrobial properties [9]. Resistance to b-lactam antibiotics will be discussed in further detail later in this chapter.

Mechanisms

The means by which bacteria avoid being targeted by antibiotics comprise an array of simple to complex mechanisms. The simplest and most basic method of resistance involves inherent mutations in the bacterial target gene, preventing binding of the mutant protein by the antibiotic [10]. This type of resistance is inevitable due to intrinsic integrity restrictions of DNA synthesis and can result from just a single gene modification. The acquisition of genes encoding proteins that weaken antibiotic binding to molecular targets can also contribute to de novo bacterial resistance [11]. In addition, molecular targets can be modified by enzymes to block drug binding [12].

Other mechanisms involve lowering an antibiotic's concentration via enzymatic or chemical modification [13]. Efflux pumps along with other transport alterations to decrease permeability can reduce the intracellular concentration of these drugs, to increase resistance to antibiotics [14, 15]. Finally, if an antibiotic target comprises an entity other than a single gene product, resistance to these drugs is attained via retrieval of pre-existing diversity in cell structures and altering their biosynthesis through global cell adaptations (Table 1.1) [16, 17].

Table 1.1 Mechanisms of bacterial resistance by class

Antibiotic class	Mechanisms	Resistant bacteria
Tetracyclines	Target protection	*Campylobacter, Staphylococcus, Streptococcus, Enterococcus*
	Efflux pumps	*Staphylococcus, Streptococcus, Enterococcus, Enterobacter*
Macrolides	Target protection	*Staphylococcus*
	Target site mutation	*Mycobacterium avium, Helicobacter pylori, Streptococcus pneumoniae*
	Enzymatic alteration of target	*Staphylococcus, Enterococcus, Bacteroides*
	Destruction of antibiotic	*Staphylococcus, Enterococcus*
	Efflux pumps	*Staphylococcus*, some Gram-negative species
Lincosamides	Target protection	*Staphylococcus*
	Target site mutation	*Mycobacterium avium, Helicobacter pylori, Streptococcus pneumoniae*
	Enzymatic alteration of target	*Staphylococcus, Enterococcus, Bacteroides*
Oxazolidinones	Target protection	*Streptococcus*
	Target site mutation	*Staphylococcus, Streptococcus, Enterococcus*
	Enzymatic alteration of target	*Staphylococcus, Streptococcus*
Phenicols	Target protection	*Enterococcus*
	Target site mutation	*Escherichia coli, Bacillus subtilis*
	Enzymatic alteration of target	*Staphylococcus, Streptococcus, Enterococcus*
	Chemical alteration of antibiotic	*Staphylococcus, Streptococcus, Enterococcus*
Pleuromutilins	Target protection	*Staphylococcus, Streptococcus, Enterococcus*
	Enzymatic alteration of target	*Staphylococcus, Enterococcus*
Streptogramins	Target protection	Group A – *Staphylococcus, Streptococcus, Enterococcus* Group B – *Staphylococcus, Streptococcus, Enterococcus*
	Enzymatic alteration of target	*Staphylococcus, Streptococcus, Enterococcus*
Aminoglycosides	Target site mutation	*Mycobacterium tuberculosis*
	Enzymatic alteration of target	Actinomycetes
	Chemical alteration of antibiotic	*Salmonella enterica, Klebsiella pneumoniae, Legionella pneumophila*
Rifampin	Target site mutation	*Mycobacterium tuberculosis*
Quinolones	Target site mutation	*Staphylococcus, Enterococcus*
	Target protection	
Glycopeptides	Target site mutation	*Staphylococcus, Streptococcus, Enterococcus*
	Global cell adaptations	*Staphylococcus*
Beta-lactams	Complete replacement/bypass of target site	*Staphylococcus*
	Destruction of antibiotic	*Escherichia coli*
	Decreased permeability	*Escherichia coli*
Sulfonamides	Complete replacement/bypass of target site	*Staphylococcus, Escherichia coli*
Epoxides	Destruction of antibiotic	*Escherichia coli, Pseudomonas aeruginosa, Streptococcus*
Lipopeptides	Global cell adaptations	*Staphylococcus, Enterococcus*

Alteration of Bacterial Proteins Serving as Antimicrobial Targets (Changes in Target Sites)

One of the prime targets of antibiotics is the bacterial ribosome [18], a macromolecular machine for manufacturing proteins, that includes several ribosomal proteins along with three ribosomal RNAs (rRNAs) – 16S, 23S, and 5S [19]. Protein synthesis is a three-step process, including initiation, elongation, and termination, of which elongation is most commonly targeted by antibiotics [18]. Elongation involves the translocation of amino acids to the growing peptide across the A-, P-, and E-sites, resulting in the formation of a single polypeptide. When protein synthesis comes to a halt due to targeting by antibiotics, bacterial cells cannot proliferate. For this reason, they possess certain mechanisms, either innate or acquired, against certain classes of antibiotics to evade targeting, including target protection (Table 1.2) or modification of the target site [18].

Target Protection

Tetracyclines

Tetracyclines, a group of antibiotics first introduced in the 1940s, possess a broad spectrum of activity against both Gram-positive and Gram-negative bacteria and can be divided into two groups – typical tetracyclines, such as tetracycline, doxycycline, and minocycline, and atypical tetracyclines. Ribosomal protection via Tet(O) and Tet(M) proteins in these bacteria promotes resistance to the typical tetracyclines, as this group of antibiotics act via binding of the 30S ribosomal subunit and subsequent inhibition of the elongation phase of protein synthesis [20, 21]. These ribosomal protection proteins (RPPs) were initially derived from *Campylobacter jejuni* and *Streptococcus* species. They exhibit their protective function due to their similarity in sequence to ribosomal elongation factors, EF-G and EF-Tu [22]. Since both elongation factors belong to the superfamily of GTPases, the RPPs accordingly possess GTPase activity and can hydrolyze GTP in a ribosome-dependent manner [23, 24]. Two mechanisms may explain Tet(O)-mediated tetracycline resistance – (1) a conformational change induced by tetracycline may lead to the binding of Tet(O) to the ribosome and (2) tetracycline may bind ribosomes with open A-sites, which may be the preferred substrate for Tet(O) as opposed to ribosomes with occupied A-sites [25, 26]. The presence of GTP and its subsequent hydrolysis via Tet(O) and Tet(M) allows these RPPs to dislodge tetracyclines from the 30S subunit, preventing its inhibitory action on protein synthesis and conferring resistance.

Table 1.2 Resistance through target protection

Antibiotic class	Mechanism	Type	Bacteria
Tetracyclines	Tet(O)- and Tet(M)-mediated protection	Ribosomal protection proteins (RPPs)	Gram-positive and Gram-negative species
Macrolides	vga(A)-, msr(A)-, msr(C)-, msr(D)-, and msr(E)-mediated protection	ARE ABC-F proteins	Gram-positive species
Lincosamides	vga(A)-, vga(C)-, vga(E)-, vga(D)-, vga(B)-, sal(A)-, eat(A)-, lsa(A)-, lsa(C)-, lsa(B)-, and lsa(E)-mediated protection	ARE ABC-F proteins	Gram-positive species
Oxazolidinones	optr(A)-mediated protection	ARE ABC-F proteins	Gram-positive species
Phenicols	optr(A)-mediated protection	ARE ABC-F proteins	Gram-positive species
Pleuromutilins	vga(A)-, vga(C)-, vga(E)-, vga(D)-, vga(B)-, sal(A)-, eat(A)-, lsa(A)-, lsa(C)-, lsa(B)-, and lsa(E)-mediated protection	ARE ABC-F proteins	Gram-positive species
Streptogramins (group A)	vga(A)-, vga(C)-, vga(E)-, vga(D)-, vga(B)-, sal(A)-, eat(A)-, lsa(A)-, lsa(C)-, lsa(B)-, and lsa(E)-mediated protection	ARE ABC-F proteins	Gram-positive species
Streptogramins (group B)	msr(A)-, msr(C)-, msr(D)-, and msr(E)-mediated protection	ARE ABC-F proteins	Gram-positive species

A novel tetracycline antibiotic, sarecycline (Seysara), has achieved widespread use and recognition in recent years to treat moderate-to-severe acne via a narrow spectrum of antimicrobial activity targeting *C. acnes* and clinically relevant Gram-positive bacteria, including organisms with high-level resistance to the macrolide erythromycin, while having a limited activity against enteric Gram-negative bacteria, a major constituent of the gut microflora [27]. In addition to its anti-inflammatory and anti-bacterial efficacy, sarecycline boasts an improved safety profile, causing less nausea, diarrhea, dizziness, vertigo, and photosensitivity compared to tetracycline, doxycycline, and minocycline [28]. Its mechanism of action involves extension of the C7 group of the sarecycline into the mRNA channel on the small ribosomal subunit, giving way for the drug to interact with the A-site codon in mRNA [29]. This interaction leads to additional stabilization, greater affinity, and increased inhibitory effect of the antibiotic. Due to its narrow spectrum of activity and rational structural design, resistance is less likely to be encountered [27, 30]. It is currently the only antibiotic used in the treatment of acne with a low resistance claim on its label; *Cutibacterium acnes* displays a low propensity for the development of resistance to sarecycline, with spontaneous mutation frequencies being 10^{-10} at 4-8 x MIC. The main mechanism by which bacteria develop resistance against tetracycline-class drugs is ribosomal protection and efflux pump [31]. The hydrolytic activity of the Tet(M) protein in bacteria causes tetracyclines to display an elevated MIC, resulting in decreased susceptibility and ultimately, resistance. An association between broad-spectrum tetracycline antibiotics, especially doxycycline, and gastrointestinal disorders, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), has been reported in the literature [32–34]. The etiology of this association is still unclear, but it is reported that the broad-spectrum antibiotics' effects may alter the human microbiome to the extent of causing disease. It is important to consider this possibility when prescribing broad-spectrum tetracycline-class antibiotics, especially in long-term treatment of acne, for which doxycycline and minocycline are commonly used.

Macrolides

Macrolides have been used clinically since the 1950s, as the first-generation erythromycin was discovered around that time [35]. Second-generation macrolides, which include clarithromycin and azithromycin, showcased superior pharmacological properties and were introduced later in the 1980s [35, 36]. The emergence of resistance further provoked the development of ketolides, a newer generation of macrolides [37]. Their mechanism of action is similar to that of the tetracyclines; however, rather than binding the 30S subunit of the ribosome, the macrolides bind the 23S rRNA of the 50S subunit of the ribosome to block protein synthesis [38]. The ATP-binding cassette (ABC) family of proteins plays a role in resistance to macrolides by Gram-positive bacteria via ribosomal protection [39]. The ABC-F proteins consist of a single polypeptide grouped together with two ABC domains and are involved in a variety of functions within the cell, including DNA repair, enzyme regulation, and translational control [40]. The particular subgroup of the ABC-F proteins possessed by Gram-positive bacteria responsible for mediating resistance to macrolides and other antibiotics that act on the 50S ribosomal subunit are known as the antibiotic resistance (ARE) ABC-F proteins. The mechanism by which resistance against macrolides is conferred was recently discovered when studying the *vga*(A) determinant of the ARE ABC-F protein class found in *Staphylococcus* species. It was found that this determinant, in addition to msr(A), msr(C), msr(D), and msr(E) found in other species, reduced susceptibility to various classes of antibiotics, including macrolides, though vga(A) has only been previously associated with lincosamide, pleuromutilins, and group A streptogramin resistance [39, 41]. Based on the protein's ability to trigger dissociation of several structurally different classes of antibiotics, its mechanism was determined to be ribosomal protection [39].

Lincosamides

The lincosamide class of antibiotics is structurally composed of L-proline substituted by a 4′-alkyl chain connected to a lincosamine by an amide bond [42, 43]. Lincomycin and clindamycin are

two antibiotics in this class, which target anaerobic bacteria, streptococci, and staphylococci [44]. Lincomycin was first isolated from *Streptomyces lincolnensis*, and its chlorinated derivative, clindamycin, has shown superior antibacterial activity, making it a viable option for clinical application [45]. The mechanism of action of these antibiotics, just like macrolides, involves the inhibition of protein synthesis by binding the 23S rRNA of the 50S ribosomal subunit and inhibiting translocation [45]. To evade this, bacteria employ a ribosomal protection mechanism similar to that of macrolides, which involves the ARE ABC-F protein class. The specific determinants conferring resistance to the lincosamides include the Vga, Lsa, Sal, and Vsl homologues [46]. These homologues protect the ribosome via the displacement of the antibiotic [39, 46, 47].

Oxazolidinones

Oxazolidinones, particularly linezolid, were first introduced in 1996 and approved by the Food and Drug Administration in 2000 after their antibacterial effects had been studied [48]. Linezolid, particularly, has since been identified as a lead compound, exhibiting pharmacological parameters proposing its value as a starting point for therapeutics development [48, 49]. It is commonly used in the treatment of diseases caused by various Gram-positive bacteria, including vancomycin-resistant *Enterococci* (VRE) species, such as *Enterococcus faecium*, hospital-acquired pneumonia caused by *Staphylococcus aureus*, and community-acquired pneumonia caused by *Streptococcus pneumoniae* [49]. Unlike the antibiotic classes already discussed, this class attacks bacteria by binding both the 30S and 50S ribosomal subunits, preventing the formation of the initiation complex and ultimately decreasing the rate of translation [49, 50]. Although linezolid has been utilized successfully in the treatment of several multidrug-resistant (MDR) organisms, resistance to oxazolidinones is concerning. Resistance through ribosomal protection occurs by dissociation of the antibiotic due to the oxazolidinone and phenicol transferable resistance A (OptrA) determinant of the ARE ABC-F class of proteins via binding of the peptidyl transferase A site [47, 51, 52].

Phenicols

Chloramphenicol was first isolated in 1947, claiming its title as the first phenicol antibiotic and first natural product containing a nitro group [53]. Other phenicols, including thiamphenicol and florfenicol, are rarely used in humans but are sometimes employed in veterinary medicine [53]. Chloramphenicol's spectrum of activity ranges across various classes of Gram-positive and Gram-negative bacteria, but the serious adverse effects associated with its use, such as dose-independent aplastic anemia, dose-dependent bone marrow suppression, and gray baby syndrome in neonates and infants, have downgraded its status as a promising antimicrobial agent [54, 55]. Due to this, actual clinical use to treat infections is very limited [53]. Its mechanism of action is like that of many other antibiotics, through binding of the 50S ribosomal unit to inhibit the elongation step of translation. Just like the antibiotic classes already discussed, one mechanism by which bacteria evade the actions of phenicols is via ribosomal protection. The same determinant of the ARE ABC-F class of proteins which confers resistance to oxazolidinones, OptrA, also confers resistance to the phenicol class of antibiotics by dissociating the antibiotic from its ribosomal target [46].

Pleuromutilins

Like phenicols, pleuromutilins were discovered as natural antimicrobial agents in the early 1950s [56]. From these, tiamulin and valnemulin, two semisynthetic pleuromutilins, were created. The pleuromutilins possess activity against anaerobic Gram-negative and Gram-positive bacteria in particular. Although tiamulin and valnemulin are exclusively utilized in veterinary medicine, retapamulin was the first pleuromutilin approved for human use as a topical treatment in 2007 [53, 56–59]. Furthermore, lefamulin was the first pleuromutilin developed for use in the intravenous and oral forms to treat systemic infections [58]. The mechanism by which pleuromutilins exhibit their bacteriostatic activity is via inhibition of peptide bond formation through binding of the V domain of the 50S ribosomal subunit, thereby interfering with

proper positioning of the CCA ends of tRNAs for peptide transfer in the A- and P-sites [58, 60]. It has also been postulated that the pleuromutilins may also act via inhibition of the initiation step of translation [58, 61]. Although resistance is rarely a concern in this class of antibiotics, it does exist. The manner by which the target classes of bacteria evade the pleuromutilins is via ribosomal protection. Vga/Lsa/Sal/Vml, the same ARE ABC-F protein homologues that confer resistance to lincosamides, confer resistance to pleuromutilin antibiotics through interaction with the ribosome and displacement of the bound drug [46, 47].

Quinolones

The quinolones are a synthetic class of antibiotics rather than being isolated from living organisms. The first quinolone, nalidixic acid, was derived from chloroquine, an anti-malarial drug, and through further manipulation and addition of a fluorine atom, fluoroquinolones were developed [62, 63]. Newer-generation fluoroquinolones exhibit improved coverage against Gram-positive and Gram-negative organisms, and they include ciprofloxacin, levofloxacin, and moxifloxacin [62]. These antibiotics exert their bactericidal effects via inhibition of the bacterial DNA gyrase and topoisomerase IV, which, in turn, inhibits DNA replication [62]. Their extensive Gram-positive and Gram-negative coverage makes them a desirable treatment option for many infectious processes. Although their effects as antibiotics are outstanding, they may still succumb to bacterial resistance via two mechanisms, one of which is target protection.

Target protection is plasmid-mediated and was first reported in 1998. The responsible gene, *qnrA*, was identified by PCR in 2002 and found at low frequency on plasmids in Gram-negative isolates [64]. This gene encodes a pentapeptide repeat protein (PRP), QnrA1, which binds to topoisomerase II and competes with DNA by protecting DNA gyrase and topoisomerase IV from inhibitory quinolone activity [65]. Other PRPs responsible for quinolone resistance include QnrB1 and QnrS1. PRPs contain domains composed of tandem repeats of amino acid sequences. In a study involving Qnr-type determinants from *Vibrio parahaemolyticus*, it was shown that a single amino acid substitution significantly enhanced resistance to quinolone antibiotics when the gene was cloned and expressed in *Escherichia coli* [65, 66].

Streptogramins

The streptogramin family of antibiotics consists of two substances which are chemically unrelated: streptogramin A and streptogramin B. The A group are polyunsaturated mactolactones, and they belong to the polyketide family of antibiotics. The B group, on the other hand, are cyclic hexadepsipeptides of the nonribosomal peptide antibiotic family [67, 68]. This family of antibiotics, which was patented by Merck in 1957, gets its name from the strain from which it was isolated, *Streptomyces graminofaciens* [67, 69]. Although initially targeted for use in animal production, the streptogramin family of antibiotics, particularly pristinamycin, was finally introduced into human therapy. Pristinamycin covers a wide range of Gram-positive pathogens and a few Gram-negative pathogens, including drug-resistant organisms [67, 70]. Streptogramin A and streptogramin B have moderate bacteriostatic activity through inhibition of protein synthesis, and they both act on the 50S subunit of the ribosome, and while the A type prevents binding of the amino acyl-tRNA, the B type inhibits peptide elongation by releasing the peptidyl-tRNA [67, 71]. Several mechanisms of resistance to streptogramins have been described in the literature (Fig. 1.1); however, not much is known about ribosomal protection. Different transporter genes that code for ABC transporters, such as varL, varM, and varS, have been implicated in this particular mechanism and confer resistance to streptogramin antibiotics [67, 72, 73].

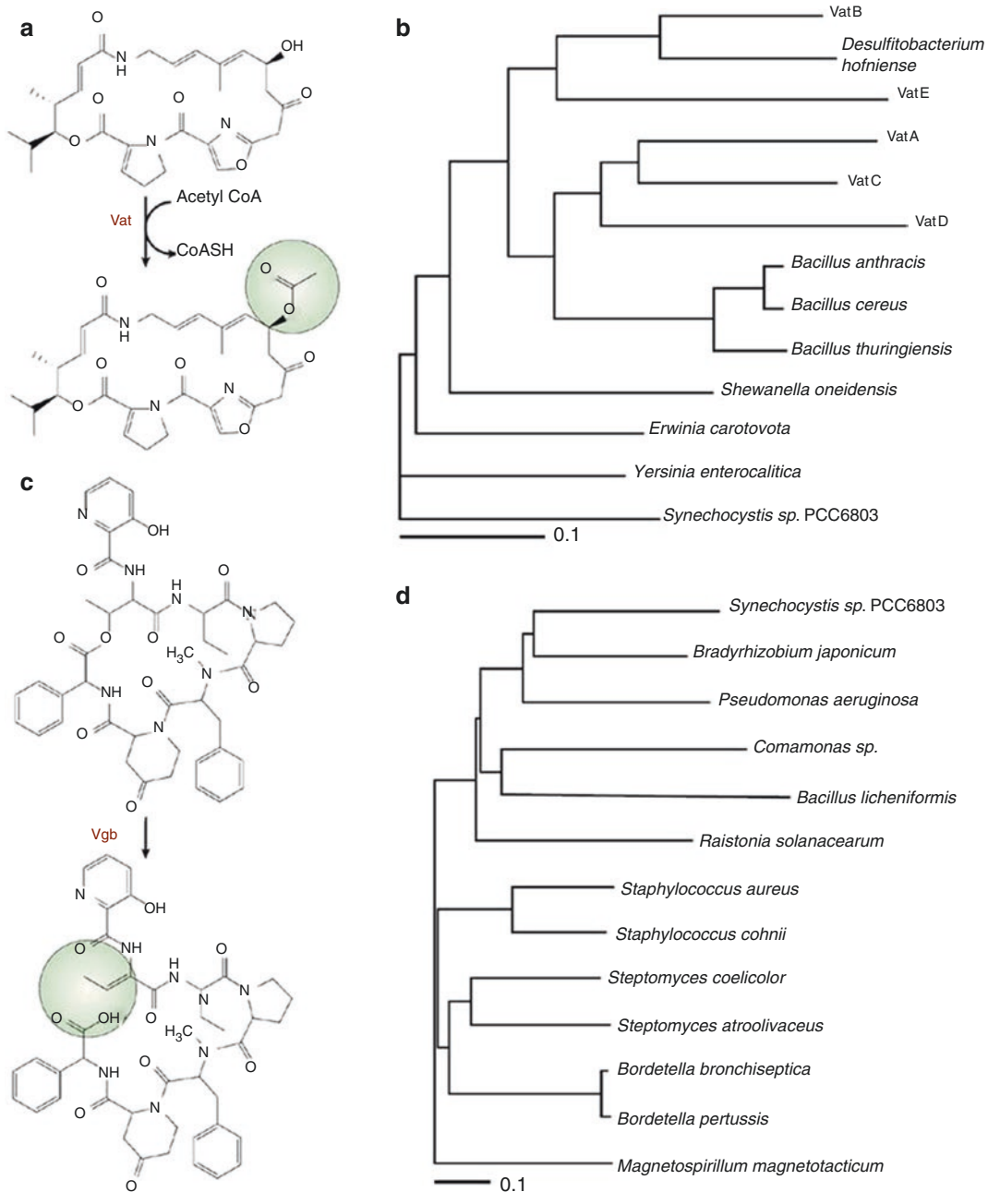

Fig. 1.1 Resistance to streptogramin A antibiotics occurs via acetylation of a hydroxyl group. (a) Chemical modification of pristinamycin is catalyzed by virginiamycin acetyltransferase (Vat) enzymes. (b) Various homologues and orthologues of Vat enzymes, which are found in clinically resistant strains of bacteria, are also widely distributed in several environmental bacterial species. (c) Resistance to streptogramin B antibiotics is catalyzed by virginiamycin resistance gene B (Vgb) enzymes through cleavage of the cyclic depsipeptide of pristinamycin IA. (d) Various homologues and orthologues of Vgb genes are found in the genomes of environmental bacteria. The sequence alignments of amino acids were constructed using Clustal W. The trees do not represent a phylogenetic analysis, but they convey the sequence relationship among the enzymes [74].

Modification of Target Site

More commonly than ribosomal protection, modification of target sites is employed by many pathogens to evade the bacteriostatic or bactericidal effects of antibiotics. Since antibiotics typically bind their targets with high affinity, any changes to the target structure that prevent binding by the antibiotic but preserve function can confer resistance [75]. These changes in the target structure can be achieved in several ways, including point mutations in genes encoding target sites, enzymatic alterations of binding sites, and replacement or bypass of target sites [76].

Mutations

Aminoglycosides

The aminoglycoside (AG) class of antibiotics was introduced in the clinical setting in the 1940s. The first AG, streptomycin, was isolated from *Streptomyces* griseus, and it was the first antibiotic successfully used to treat tuberculosis [77]. Due to its success, several other AGs were subsequently discovered and used in clinical practice to target Gram-positive and Gram-negative pathogens, including neomycin, gentamicin, and tobramycin. In addition to severe adverse effects related to these drugs, including ototoxicity and nephrotoxicity, widespread use inevitably led to the development of resistance against these antibiotics, leading to attempts to counter this through the development of semi-synthetic second-generation AGs, such as amikacin. Like many other classes of antibiotics, AGs act via inhibition of protein synthesis. These drugs bind to the 16S rRNA of the 30S ribosomal subunit with high affinity, altering the structure and ultimately promoting mistranslation and error-prone protein synthesis [78, 79]. Other ways by which AGs inhibit protein synthesis is through inhibition of initiation and elongation [76, 77]. Resistance to these drugs is achieved most commonly via modification of the bacterial target site through mutations [79, 80]. These mutations can occur in the *rrs* gene, which codes for the 16S rRNA, hindering AG binding [80]. Many of the mutations, however, are lethal and not very common. A viable mutant, A1408G, disrupts the hydrogen bonding interaction between 2-deoxystreptamine (2-DOS) AGs, such as neomycin B and gentamicin, and the helix 44 (h44) nucleotide A1408. This mutation has been found in some resistant strains of *Mycobacterium tuberculosis* [80, 81]. Another mutation leading to resistance in *M. tuberculosis* is the rspL mutation, which affects the S12 protein and leads to high-level resistance to streptomycin. This mutation interferes with tRNA selection through conformational distortions of the decoding site, impairing GTPase activation of Ef-Tu [82].

Macrolides

In addition to ribosomal protection, bacteria can confer resistance to macrolides via mutations in their target sites. As mentioned earlier, macrolides act on the 23S rRNA of the 50S ribosomal subunit, so mutations altering this part of the ribosome can lead to resistance to these antibiotics. Mutants of the ribosome observed in macrolide-lincosamide-streptogramin B (MLS_B) antibiotics include base substitutions in domain II or V of 23S rRNA and ribosomal proteins, such as L4 and L22 [83–85]. Since macrolides primarily interact with A2058 and A2059 of the 23S rRNA, mutations in these nucleotides confer resistance to these antibiotics. In addition, insertion, deletion, and missense mutations in genes encoding L4 and L22 proteins of the ribosome can lead to resistance to macrolide antibiotics.

The L4 and L22 proteins consist of globular surface domains and elongated "tentacles," which are able to extend into the large ribosomal subunit's core and line part of the peptide exit tunnel. As a result of these mutations, rRNA processing and ribosome assembly are affected, making the bacterial ribosome a nonviable target for macrolide antibiotics [86–88].

Phenicols

Unlike resistance to macrolides via target mutation, resistance to phenicols is rarely achieved through this mechanism; however, it has been reported in the literature. Mutations in major ribosomal protein gene clusters have been observed in *Escherichia coli* and *Bacillus subtilis*, resulting in resistance to phenicol antibiotics [54, 89]. In addition, in a similar mechanism of resistance to that affecting macrolide antibiotics, mutations in the gene coding for 23S rRNA can also confer resistance to phenicols [54, 90, 91]. An explanation why this type of resistance is rarely seen against phenicol antibiotics is the lethality of the mutations themselves, rendering the ribosomes nonfunctional [54].

Rifampin

Rifampin, discovered in Italy in 1965 and applied in clinical practice in the United States in 1971, is an established first-line drug utilized in the treatment of tuberculosis. The drug is derived from rifamycin SV, which itself is semisynthetically derived from rifamycin B, a complex macrocyclic antibiotic [92]. Through binding and inactivation of bacterial DNA-dependent RNA polymerase (RNAP) paired with intracellular penetration, rifampin is able to execute its bactericidal effects against a wide spectrum of pathogens, including Gram-positive and Gram-negative species as well as *Chlamydia* and *Legionella* species [92, 93]. Resistance to rifampin occurs primarily through target mutation involving the *rpoB* gene coding for the b-subunit of the RNAP. The region affected is known as the *Rif* site and resides between amino acid positions 500 and 575. As a result of this mutation, rifampin's binding affinity for RNAP decreases, ultimately leading to resistance [93, 94]. Although binding affinity of rifampin for RNAP is decreased, catalytic activity of the RNAP is preserved, allowing transcription to occur normally [95].

Lincosamides

Lincosamides, particularly clindamycin, interact with the 23S rRNA of the 50S ribosomal subunit primarily at the A2058 and A2059 sites. Mutant strains of *Mycobacterium smegmatis* were created via transformation with plasmid pMV361 to observe and detail the exact mechanism of resistance conferred to clindamycin. Susceptibility of these mutant strains were subsequently tested, and it was found that an A-to-G mutation at site 2058 conferred a high level of resistance to clindamycin, whereas an A-to-G mutation at site 2059 conferred a lower level of resistance [96].

Quinolones

The quinolones are a synthetic class of antibiotics rather than being isolated from living organisms. The first quinolone, nalidixic acid, was derived from chloroquine, an anti-malarial drug, and through further manipulation and addition of a fluorine atom, fluoroquinolones were developed [97]. Newer-generation fluoroquinolones exhibit improved coverage against Gram-positive and Gram-negative organisms, and they include ciprofloxacin, levofloxacin, and moxifloxacin [97]. These antibiotics exert their bactericidal effects via inhibition of the bacterial DNA gyrase and topoisomerase IV, which, in turn, inhibits DNA replication [97, 98]. Their extensive Gram-positive and Gram-negative coverage makes them a desirable treatment option for many infectious processes. Although their effects as antibiotics are outstanding, they may still succumb to bacterial resistance via two mechanisms, one of which is target mutation. Amino acid substitu-

tions at the quinolone resistance-determining regions (QRDR) of corresponding genes account for mutations in the bacterial DNA replication enzymes. In mutations affecting DNA gyrase, substitutions occur at the *gyrA* and *gyrB* genes, while substitutions occur at *parC* and *parE* in mutations affecting DNA topoisomerase IV [99]. In Gram-positive organisms, *parC* is usually the first gene to undergo mutation to target topoisomerase IV; however, in Gram-negative organisms, mutations in *gyrA* confer protection for the bacteria through DNA gyrase [99, 100]. The QRDR corresponds to particular regions on the DNA-binding surfaces of affected enzymes, and mutations here reduce the antibiotic's binding affinity for the enzymes, thereby conferring resistance [98]. It has been found that resistance to fluoroquinolones occurs in a stepwise fashion with progressively more mutations, increasing resistance [100].

Oxazolidinones

Since oxazolidinones, such as linezolid and tedizolid, interact with the 23S rRNA, mutations here lead to resistance of several Gram-positive organisms, such as enterococci, to these drugs by decreasing binding affinity [101] (Fig. 1.2). As mentioned earlier, oxazolidinones exert their bacteriostatic effects by inhibiting the initiation of translation and translocation of the peptidyl-tRNA from the A-site to the P-site [102]. Most mutations in the 23S rRNA involve G to U substitutions in the peptidyl-transferase region at position 2576, affecting the P-site. This particular mutation has been observed in vancomycin-resistant enterococci, resulting in a decrease in linezolid sensitivity [103]. Other 23S rRNA modifications have been observed in *E. coli*, including mutations closer to the A-site at positions 2032 and 2447 [104].

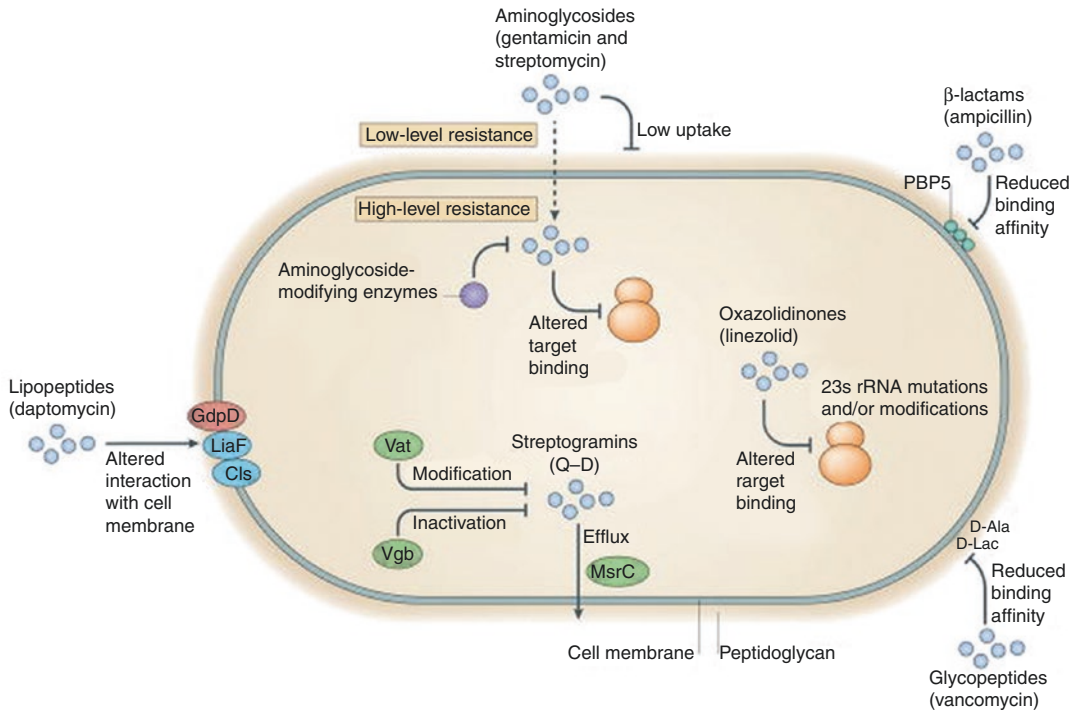

Fig. 1.2 Main mechanisms of enterococcal antibiotic resistance

Enterococci possess both intrinsic and extrinsic antibiotic resistance mechanisms, which are shown here. Resistance to ampicillin in *Enterococcus faecium* occurs through the production of penicillin-binding protein 5 (PBP5), which has a low affinity for b-lactam antibiotics. In addition, these bacteria exhibit low-level resistance to aminoglycoside antibiotics due to decreased uptake of the polar molecules. On the other hand, high-level resistance to aminoglycosides can also occur through the acquisition of modifying enzymes, leading to altered target binding. The peptidoglycan synthesis pathway is affected in resistance to glycopeptide antibiotics, such as vancomycin. In resistance to streptogramin quinupristin-dalfopristin (Q-D) antibiotics, several pathways are implicated, including drug modification via Vat, drug inactivation via Vgb, and drug efflux via ABC proteins [102]. Although rare, resistance to the oxazolidinone antibiotic, linezolid, involves 23S rRNA-binding site mutations. Daptomycin resistance involves altered cell membrane interactions [103].

Glycopeptides

The glycopeptide antibiotics consist of a group of cyclic or polycyclic non-ribosomal peptides, which include vancomycin, teicoplanin, and other semisynthetic lipoglycopeptide derivatives, such as dalbavancin and oritavancin. These drugs act as substrate binders of cell-wall precursors and achieve their bactericidal effects via inhibition of cell-wall synthesis. Specifically, glycopeptides prevent cross-linking of the bacterial cell wall by binding the D-alanyl-D-alanine (D-Ala-D-Ala) terminus of the lipid II cell wall precursor. Their spectrum of activity is limited to Gram-positive bacteria due to their inability to traverse through the outer membrane in Gram-negative species [104]. Of significance, vancomycin was first used clinically in 1955 to treat infections caused by penicillin-resistant staphylococci [104]. It was not until 1987 that the first case of a vancomycin-resistant strain was reported [105, 106]. While Gram-negative species of bacteria are intrinsically resistant to the action of glycopeptides due to the outer membrane's capacity to block the passages of large, complex molecules, Gram-positive species possess different mechanisms to evade death, including target mutation. Several mutations in genetic loci contribute to a thickened cell wall, which serves as the target of glycopeptide antibiotics.

Enzymatic Alteration

Macrolides, Lincosamides, and Streptogramin B

A well-known mechanism of resistance employed by some bacterial species is an erythromycin ribosomal methylation (*erm*) gene-encoded enzymatic methylation of the ribosome. Methylation occurs on an adenine residue in position A2058 of domain V of the 23S rRNA of the 50S ribosomal subunit, thus impairing binding of the antibiotic molecule to its target and ultimately leading to resistance. Since macrolides, lincosamides, and streptogramin B interact with the same binding site on the ribosome, *erm* gene expression confers cross-resistance across antibiotic classes through plasmids and transposons in pathogenic bacteria [76, 107]. These genes, specifically *erm(A)* and *erm(C)*, can be found in staphylococci. While *erm(A)*is predominantly found in methicillin-resistant *Staphylococcus aureus* (MRSA), *erm(C)*is found in methicillin-susceptible *Staphylococcus aureus* (MSSA). In streptococci and enterococci, *erm(B)* plays a role in resistance through methylation. The specific antibiotics in these classes induce the *erm* family of genes, leading to the production of Erm and methylase and subsequent methylation of 23S rRNA [107]. In the absence of an inducer, a mRNA transcript is generated along with a secondary structure that works to conceal the upstream *erm* binding site. Translation then proceeds normally, preventing the production of Erm [76].

Aminoglycosides

Aminoglycosides (AGs) can also succumb to bacterial resistance via enzymatic alteration. The 16S ribosomal subunit contains RNA methyl-