Iqbal Ahmad · Shamim Ahmad
Kendra P. Rumbaugh *Editors*

Antibacterial Drug Discovery to Combat MDR

Natural Compounds, Nanotechnology and Novel Synthetic Sources

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Preface

Considering the rapid emergence and spread of multidrug-resistant bacterial pathogens across the globe and the slow discovery of new antibiotics, there is a serious threat to the current availability of bacterial infection chemotherapy. Considerable efforts from academia and industry have been made to develop new and alternative ways to overcome this problem, but the problem remains very serious and warrants immediate attention. In addition to classical approaches, improved and new technologies, including nanomaterials, antipathogenic drugs, and the reemergence of natural products as potential new therapeutics leads, are encouraging developments. There have been several books published covering specific applications; however, this book aims to provide a holistic and comprehensive view of the subject starting with the problem and then discussing classical to modern approaches of drug discovery and alternative ways of combating bacterial infection especially by MDR bacteria. The main areas of interest include various aspects of drug discovery strategies, natural products from various sources (microbes, marine, and lower to higher plants) as novel antibacterials, and the prospects of nanotechnology and nonmaterials in drug discovery and drug delivery.

The concept of this book was developed in India-UK Antimicrobial Resistance Sandpit Meeting 2017, organized by the UK Research Council and DBT, India. The concept was then shared by all the authors and among editors of the book and finally with the Springer Nature publishing team. The editors would like to thank all those who contributed to the discussion, planning, writing, and publishing of this book. They hope that this compilation will provide the most important aspects of antibacterial drug discovery of newer molecules from natural to synthetic sources.

The book chapters are divided into four sections. **Part I: The Challenge of Antibiotic Resistance and Tolerance** covers mechanisms of bacterial resistance and biofilm-related tolerance, impediments to the discovery of new antimicrobials, and developing models with which to test the efficacy of new compounds. **Part II: New Antibiotic Drug Discovery Approaches and Progress** and **Part III: Alternative Antibiotic Resistance Treatment Strategies** discuss a wide array of approaches being explored for anti-infective drugs, from targeting virulence factors, biofilm production, and quorum sensing to using medicinal plants, essential oils, pre- and probiotics, and bacteriophages anti-infective compounds. In silico molecular modelling and computational approaches to rational drug design are also discussed. **Part IV: Prospects of Nanomaterials: Antibacterials and Drug Delivery Agents**

discusses current advances in nanomaterials and nanoparticles for combating MDR bacteria.

We hope that students, teachers, researchers, and companies involved in drug discovery will find ***Antibacterial Drug Discovery to Combat MDR: Natural Compounds, Nanotechnology and Novel Synthetic Sources*** to be a useful resource. With great pleasure, we extend our sincere thanks to all the contributors for their timely response, excellent contributions, and consistent support and cooperation. **We are also grateful to Prof. Tariq Mansoor,** the Vice Chancellor of the Aligarh Muslim University, former Principal of Jawaharlal Nehru Medical College, and Head of the Department of Surgery in the Faculty of Medicine, AMU, for his encouragement and support to the faculty members for Research and Innovation. The cooperation received from postdoctoral researchers, Dr. Mohd. Shavez Khan and Dr. Meenu Maheshwari, and research students, Mr. Faizan Abul Qais and Miss Samreen, in the Department of Agricultural Microbiology, AMU, India, in the book preparation is gratefully acknowledged. We greatly appreciate and thank Dr. Derek Fleming, TTUHSC, Lubbock, USA, for his extensive help in the editing process.

We welcome any suggestions and comments from readers for future improvement of the book in new edition.

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About the Editors

Professor Iqbal Ahmad is currently a Full Professor and Ex-Chairman in the Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India. He is also the Coordinator at the Faculty of Agricultural Sciences, AMU, for BSc (Hons) Agriculture course. He has extensive teaching and research experience in applied and interdisciplinary microbiology and medicinal plant-derived natural products. His present research interest is in the field of AMR in pathogenic bacteria of environmental and clinical origin and conducting research on interference of quorum sensing-linked virulence factors and biofilms by natural products including medicinal plants as possible alternative strategy to combat MDR problem. He has guided several PhDs and MSc students; published more than 150 original research papers, 10 edited books, and 50 book chapters; and completed four extramural research projects. The research work is fairly cited by scientific community. He is an Active Reviewer/Member of editorial board of many high-ranked research journals.

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Prof. (Dr.) Kendra P. Rumbaugh is a Professor in the Department of Surgery, with joint appointments in the Departments of Immunology and Molecular Microbiology and Cell Biology and Biochemistry at Texas Tech University Health Sciences

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Antibacterial Drug Discovery: Perspective Insights

Iqbal Ahmad, Faizan Abul Qais, Samreen, Hussein Hasan Abulreesh, Shamim Ahmad, and Kendra P. Rumbaugh

Abstract

Over the last two decades, the development of new antibacterial drugs has been very limited due to many reasons. In light of the alarming situation of antimicrobial resistance (AMR), it is now vital to act promptly to develop new ways to combat the resistance problem through an integrated approach. Despite the slow progress of drug discovery by pharmaceutical companies, natural products have definitely provided an abundant source of new antibacterial leads. On the other hand, genomics- and proteomics-based drug discovery approaches have been more disappointing when it comes to the discovery of new antibacterials with novel modes of action. In the recent past, improved screening strategies and developments in target identification and validation, combinatorial chemistry, and the use of biochemical synthetic-based approaches have provided hope for the development of new antibacterial leads. Other approaches like novel anti-infective and anti-virulence target-based strategies such as quorum sensing, biofilm, virulence, and pathogenicity inhibitors are gaining popularity among drug discovery researchers. Similarly, nanotechnology-based drug delivery has seemingly unlimited application for improving the efficacy of antibiotics, where metallic and natural nanomaterials with antibacterial efficacy are under scrutiny

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for their possible therapeutic application. In this chapter, we aim to provide a brief overview and discussion of the potential for the various strategies mentioned above to combat drug-resistant bacterial infections.

Keywords
Antimicrobial resistance · AMR · Natural products · Antibacterials · Screening strategies · Target identification · Combinatorial approaches · Efflux pump inhibitors · Anti-infective approaches · Nanoparticles · Drug delivery

1 Introduction

Antimicrobial resistance among clinical and environmental bacteria has become a widespread phenomenon, which has been recognized by most international, national, and local health regulatory agencies (Arya 2002; Smith and Coast 2002). Since their introduction, antibiotics have saved countless lives. However, the development of resistant strains of bacteria was reported soon after the introduction of the first antibiotic, and the rise in resistance has reached a point where medical experts are now warning of a return to the pre-antibiotic era. Many of the pathogenic bacteria associated with human diseases are now multidrug-resistant (MDR) (Perron et al. 2012; Zarrilli et al. 2013), and many Gram-positive and Gram-negative nosocomial pathogens have attained the status of problematic MDR, or "superbug" (Zhang 2010). These MDR pathogens possess a variety of mechanisms that convey drug resistance and the capacity to acquire new genes and/or disseminate resistance genes through various gene exchange mechanisms (Dzidic and Bedeković 2003; Davies and Davies 2010).

Due to the lack of discovery of new antibacterial drugs and the rising AMR problem, scientific and healthcare regulatory bodies have prioritized efforts to immediately address this problem both locally and globally (Projan 2003; Singh and Barrett 2006; Brown and Wright 2016). Various approaches to address antibiotic resistance are discussed by many authors in this book. Here, we aim to provide some perspective insights into these antibacterial drug discovery efforts.

2 Antimicrobial Resistance (AMR): A Global Problem and Threat to Human Health

In the last two decades, the world has witnessed a threatening increase in the absolute number of MDR bacterial pathogens. Major world organizations including the World Health Organization (WHO), European Centre for Disease Prevention and Control (ECDC), and US Centers for Disease Control and Prevention (CDC) now consider antimicrobial resistance as a major and emerging threat to global public health problem (Roca et al. 2015). In the twenty-first century, AMR has become an alarming concern on the forefront of public healthcare problems. In Europe only,

nearly 400,000 people are known to be infected with multidrug-resistant bacteria that cause approximately 25,000 deaths (Prestinaci et al. 2015). Similarly, as per the CDC report in 2013, about 2 million people in the United States were infected with bacterial pathogens that were resistant to at least one conventionally used antibiotic, and nearly 23,000 people died due to infections caused by MDR bacteria (USCDC 2013). Similarly, the emergence of MDR also increased substantially in Asia, Africa, Latin America, the Middle East, and other parts of the world between 2002 and 2011, but exact data is not available (Laxminarayan et al. 2013). This growing, global AMR issue has also considerably contributed to the world's economic health-care burden. It is difficult to assess the total cost of antibiotic resistance worldwide, but undoubtedly, the economic burden due to AMR is substantial (Kaier et al. 2008; Taylor et al. 2014; Tillotson and Zinner 2017).

The development of AMR is due to exposure of pathogens to antimicrobial drugs, which induce a selective pressure resulting in drug-resistant pathogens. The emergence of resistant microorganisms, either by mutations or the acquisition of mobile genetic elements carrying resistance genes, may also occur irrespective of the presence of antibacterial agents (Roca et al. 2015). Hence, the main driving force underlying the prevalence and emergence of AMR is the aggressive and persistent use of antimicrobials both in patients and livestock or release into the environment by other means (Michael et al. 2014). The major drivers of AMR have now been identified to a large extent and are recognized globally (Castro-Sánchez et al. 2016). It is also clear that their management should follow a "one health approach" (Collignon 2012).

3 Approaches for Antimicrobial Drug Discovery

The decade between 1950 and 1960 was considered the golden era of antibiotic discovery, but it was abruptly followed by a gap of almost four decades during which no new antibiotics with novel mechanisms of action were discovered (Fig. 1). This led researchers and pharmaceutical industries to attempt innovative drug discovery approaches. A revolution in computing technology made it possible to combine and analyze larger sets of data, and many new strategies such as genomics- and proteomics-based, high-throughput screening, and synthetic approaches were attempted, albeit without major success (Brown and Wright 2016). Some of the approaches for the discovery of antibiotics, which have been used over the last 80 years, are discussed below:

3.1 Classical Approach for Screening of Antibacterial Drugs

Most of the currently used antibacterial drugs were discovered through the classical approach, used from 1940 to the late 1960s, by which natural products, synthetic or semisynthetic compounds from innumerable sources (mainly microbes), were directly screened for their promising antibacterial activity against a spectrum of

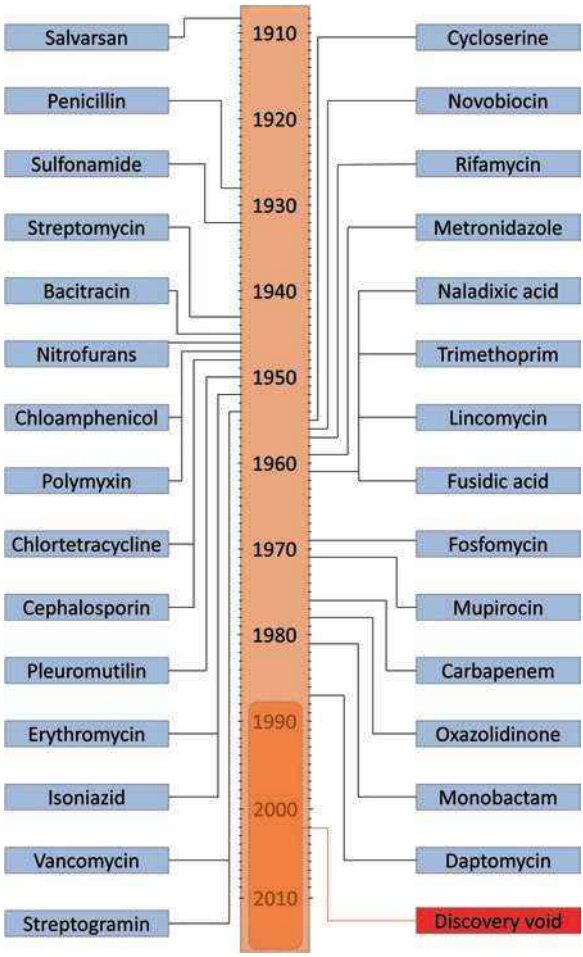

Fig. 1 Timeline of antibiotics discovered or patented (Silver 2011)

bacteria. After this period of "classical antibiotic discovery," there was gap of almost 40 years until the first representative of a new class of antibiotic was released in the market in 2000. One of the reasons for such a prolonged gap in antibiotic discovery is that most of the pharmaceutical industries were engaged in optimizing the already discovered antibiotics to develop their efficacy, spectrum, tolerability, and dosing interval. Moreover, a perception that the problem of bacterial infections had been solved also stalled efforts to develop new drugs. Nevertheless, the availability of scientific literature on antibacterial natural products during that lag period indicates the investment of continuous effort by academic researchers toward the discovery of new antibacterial lead compounds (Newman et al. 2000; Harvey et al. 2015). Such drug development efforts did not prove to be very productive as the compounds discovered were either inferior in their efficacy profile, too complex to be chemically modified, or belonged to already discovered classes of antibiotic (Brötz-Oesterhelt and Sass 2010). The regulations on the safety and efficacy of antibiotics

have substantially increased over time with a parallel improvement in therapeutic standards and technical advancements. Subsequently, the regulatory requirements needed for the approval of a newly discovered antibiotic are much higher today. Many antibiotics that were approved during the golden age of antibiotic discovery might not be able to clear today's regulations (Bax and Green 2015).

3.2 Poor Progress on Genomics- and Proteomics-Based Antibacterial Drug Discovery

The slow progress in the discovery of new antibacterials from microbial extracts and the discovery of a new synthetic quinolone class of antibacterials encouraged researchers to focus on screening novel compounds from natural product libraries and low-molecular-weight synthetic compounds. The availability of sufficient bacterial genomic information in the mid-1990s prompted the development of new screening strategies of antibacterials that led to the beginning of the "genomics era" (Brötz-Oesterhelt and Sass 2010; Lewis 2013). During this time, screening inhibitors against preselected targets were considered more relevant than phenotypic screening. To date, more than one thousand eubacterial genomes have been sequenced that can be exploited for comparative analyses for new antibacterial drug discovery (NCBI 2019). The availability of genomic data supported the idea that there were numerous unidentified targets that could be exploited for antibiotic therapy. The genomes of important bacterial pathogens were compared with available eukaryotic genomes to identify the targets which were conserved among the desired bacterial genera but evolutionary distant in eukaryotes. Using this approach, approximately 150–350 potential targets were assembled by pharmaceutical companies (Freiberg et al. 2004; Payne et al. 2007). The validation of targets crucial for bacterial survival were performed by knockout analyses, mutation studies, and inducible gene expression experiments under in vitro conditions. Such experiments were usually conducted against one Gram-positive and one Gram-negative model species of bacteria only, as it would create massive workload to mutate the target in every species of interest (Brötz-Oesterhelt and Sass 2010). Target proteins were expressed, purified, and screened by high-throughput assays against libraries consisting of a million of synthetic compounds. Extensive effort was put into this approach of antibacterial drug discovery to evaluate the quality of novel targets, and the investment did prove fruitful in identifying suitable leads that were further optimized as potential antibacterial candidates (Fernandes 2006).

For example, GlaxoSmithKline (British pharmaceutical company) selected >350 genes/targets by comparative genome analyses of *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*. Among them, 127 were identified as essential targets that were present in at least one of these test organisms, and finally 67 targets were screened as purified proteins. The high-throughput screening of 260,000–530,000 compounds against these 67 targets only produced 16 hits, in which 5 of them resulted in leads, and ultimately only 1 lead series progressed to development (Brötz-Oesterhelt and Sass 2010). The target only proved to be

suitable for a narrow spectrum, and therefore, the resulting inhibitor was out licensed to Affinium (biotech company). Likewise, Pfizer (American pharmaceutical corporation) found only four leads that were screened from 65 high-throughput screening campaigns in which none of them even reached clinical trials (Miller 2008). Cubist (United States biopharmaceutical company) also tried a somewhat different approach and concentrated on a specific target class, i.e., aminoacyl-tRNA synthetases. All 20 representatives of this target family were essential for bacterial survival. Cubist screened 17 enzymes against a smaller library of 50,000 compounds, with no success (Gallant et al. 2000). Many other pharmaceutical companies such as Bristol Meyers Squibb and Wyeth had similar experiences using high-throughput screening approaches, with many concluding that there was negligible economic or scientific benefit to this method of antibacterial drug discovery (Brötz-Oesterhelt and Sass 2010; Lewis 2013).

3.3 Structure-Based Synthetic Approaches

Recent advancements in nuclear magnetic resonance spectroscopy, X-ray crystallography, and computational tools have created a new direction in the progress of antibacterial drug discovery. Apart from genes, the structures of numerous antibacterial targets have become available, facilitating modeling studies as a screening strategy. Structure-based strategies include virtual screening of new compounds, target-based de novo compound design, fragment-based screening, or determining reaction intermediates. Promising structures that have been studied for antibacterial drug discovery are some topoisomerases, aminoacyl-tRNA synthetases, RNA polymerase, peptide deformylase, certain membrane-bound enzymes required for peptidoglycan biosynthesis, and other diverse groups of metabolic enzymes (Kohanski et al. 2010; Brötz-Oesterhelt and Sass 2010). The structures of substrates, inhibitors, or reaction intermediates have also been solved to generate useful information regarding active site topology, which is employed for the discovery of antibacterial drugs. Recently there have been many examples of structure-based design being used for the identification of new lead structures and the optimization of already discovered antibiotics (Barker 2006; Wimberly 2009).

Iclaprim, a successor of trimethoprim, a diaminopyrimidine antibiotic, reached phase III clinical trials to treat staphylococcal skin infections; however, it did not clear the regulatory standards of the US FDA (Peppard and Schuenke 2008). Trimethoprim competitively inhibits dihydrofolate reductase, an enzyme required for the biosynthesis of tetrahydrofolate. Mutation of one amino acid in the active site of *S. aureus* dihydrofolate reductase alters the trimethoprim-enzyme interaction, creating resistance to the drug (Dale et al. 1997). The mechanism of resistance was understood from the crystal structure of trimethoprim–*S. aureus* dihydrofolate reductase complex. This information was used in modeling studies to design new diaminopyrimidines with enhanced antibacterial activity against the dihydrofolate reductase of Gram-positive bacteria. Iclaprim resulted from such an approach for which the trimethoxyphenyl side chain was replaced by a dimethoxychromene

substituent. This modification increased the hydrophobic interactions in the target protein, resulting in a 20-fold higher affinity compared with unmodified trimethoprim (Schneider et al. 2003).

In another rational design program, high-resolution crystal structures of bacterial ribosome-inhibitor complexes paved the way for the discovery of a new series of m-terphenyls including RX-B72. RX-B72 binds to the A-site of the bacterial ribosome overlapping with the oxazolidinone binding site. The best compounds discovered in this series exhibited very good MIC values against Gram-negative pathogens (Ippolito et al. 2009).

3.4 Revisiting Natural Products for Antimicrobial Drug Discovery

The failure of high-throughput screening assays and small synthetic molecule approaches resulted in an interest among scientists to return to natural products in the search for antimicrobials (Butler and Buss 2006; Baltz 2008; Nicolaou et al. 2009). This is not surprising considering that almost 3/4 of all antibiotic classes are from natural products. Natural antimicrobial products are advantageous over synthetic compounds as natural products have greater structural diversity, unique molecular architectures, and functional complexity (von Nussbaum et al. 2006). Moreover, the antibacterial activity of natural compounds is better due to the fact that antibiotic-producing strains have evolved over longer periods of time in order to compete for ecological niches (Brötz-Oesterhelt and Sass 2010). Researchers agree that only a fraction of the antibacterial agents produced by microbial communities globally have been discovered (Baltz 2006; Clardy et al. 2006). While the majority of antibiotics known today are produced by *Streptomyces* species, it is expected that even more streptomycetes antibiotics are waiting to be discovered (Clardy et al. 2006). Hence, a new strategy for future development of antibiotic drugs is to search for novel natural products with modern technologies. Unexplored natural habitats are being explored to search for new antimicrobials, and improved culture conditions are making previously unculturable microorganisms cultivatable (Nett and König 2007; Muscholl-Silberhorn et al. 2008). For example, a pilot study indicated that previously unculturable microbes could be grown by growing them along with other species from their natural habitat (Kaeberlein 2002). In addition, modern molecular biology tools have made it possible to express foreign biosynthetic gene clusters, and pools of DNA from different environments can be probed by metagenomic techniques (Clardy et al. 2006).

Due to the gap in the discovery of new antimicrobials in the late twentieth century, many pharmaceutical companies decided to revisit already available natural product libraries. Wyeth (pharmaceutical company) initiated a project to reinvestigate fractions of their natural product collection they had previously discarded due to their narrow spectrums of activity. For example, a glycopeptide class of mannopeptimycins was obtained from a fraction of *Streptomyces hygroscopicus* LL-AC98. This antibiotic complex was known to Wyeth since the 1950s, but they didn't

perform structural studies until the beginning of the twenty-first century (CORDWINDER 1862; He et al. 2002). To date, natural product complexes have shown antibacterial activity against penicillin-resistant streptococci, methicillin-resistant *S. aureus*, and vancomycin-resistant *Enterococcus* (Singh et al. 2003). Mannopeptimycins also inhibit peptidoglycan synthesis, but they have other binding sites than that of vancomycin, which explains their activity against vancomycin-resistant *Enterococcus* (Ruzin et al. 2004). Another novel antibiotic obtained from natural products is plectasin (a peptide antibiotic) that was isolated from *Pseudoplectania nigrella* by Novozymes (global biotechnology company) (Mygind et al. 2005). Plectasin is a 40-amino-acid-long oligopeptide that closely resembles the defensins of invertebrates (Mygind et al. 2005). NZ2114, a new derivative of plectasin, exhibited enhanced activity against staphylococci and streptococci (Andes et al. 2009) in comparison to naturally occurring plectasin.

Similarly, Merck (American pharmaceutical company) ventured to rescreen its culture extract collection for novel inhibitors of selected targets. They discovered platensimycin by expressing FabF (the ketoacyl-ACP synthase II) in *S. aureus* (Young et al. 2006; Wang et al. 2006). Platensimycin inhibited FabF with an IC50 of 48 nM. The MICs were in the μg/ml range for streptococci, staphylococci, and enterococci. In vivo efficacy against disseminating *S. aureus* infection was also demonstrated in mice (Lee et al. 2006). For Gram-negative bacteria, Cubist has developed a lipopeptide (CB-182804) exhibiting bactericidal activity against *Acinetobacter*, *Pseudomonas*, *Escherichia*, and *Klebsiella*. The peptide is currently in phase I clinical trials against MDR Gram-negative bacteria (Brötz-Oesterhelt and Sass 2010). The company has not yet disclosed the structural details and profile of this antibiotic.

Simultaneously, academic researches continue to screen microbes from various extreme environments including the deep ocean. These academic efforts have resulted in the discovery of novel compounds, which might be developed into new antibiotics in the future (Butler and Buss 2006). These efforts have successfully demonstrated that exploring microbial diversity from culturable and nonculturable microbes can result in the discovery of new compounds that can refill the dry pipeline of drug candidates.

Scientists at pharmaceutical companies and universities have invested innumerable efforts to screen and identify potent broad-spectrum antibiotics from plants but have failed. One possible reason for this failure is that plants may use different chemical strategies to manage microbial infections that aim to reduce the selective pressure for the development of resistance (Lewis and Ausubel 2006). For instance, certain plant-derived antibacterials show potent activity in combinations while exhibiting limited efficacy alone. A classic example is the combination of berberine and 5′-methoxyhydnocarpin. Berberine, commonly present in barberry plants, is a DNA intercalator and increases membrane permeability (Amin et al. 1969). Additionally, the positive charge on berberine enables it to accumulate in bacterial cells (Severina et al. 2001). Considering it has such a broad target, berberine should be a perfect antibacterial (Lewis and Ausubel 2006). However, berberine alone is ineffective because it is easily pumped out by pathogen-encoded multidrug resistance pumps (Hsieh et al. 1998). In barberry plants, another compound, 5′-methoxyhydnocarpin, was isolated that is

potent in blocking the efflux pumps that expel berberine. The combination of berberine and 5′-methoxyhydnocarpin acts as an effective antibacterial; however, neither compound is very effective alone (Stermitz et al. 2000). Similar is the case with many other phytocompounds such as rhein, plumbagin, resveratrol, gossypol, and coumestrol where the antibacterial activity is enhanced up to 100-fold by disabling efflux pumps (Lewis and Ausubel 2006).

Another chemical strategy that plants use to overcome bacterial infections is the production of compounds that selectively target bacterial virulence but not bacterial growth. Although there is a lack of abundant literature on the specific mechanisms, many plant extracts and phytocompounds, such as *Hibiscus sabdariffa*, *Momordica charantia*, *Forsythia suspense*, and green tea, have been reported to inhibit bacterial virulence (Kalia 2013; Khan et al. 2018; Qais et al. 2019). Research is still ongoing to find plant-based novel antibacterials with multiple targets and broad-spectrum activity.

4 Alternative Approaches for Targeting Bacterial Pathogens

Apart from conventional antibiotics, there are other approaches that may reduce the selective pressure of developing AMR. The most useful replacements for antibiotics are bacteriocins, bacteriophages, and predatory bacteria or other natural compounds that inhibit bacterial growth. Each of these approaches has its own pros and cons in terms of efficacy, benefits, health risks, and costs (Allen et al. 2013). One common pro is that these alternative strategies can be used to target a specific group of bacteria, a desirable trait to reduce the selection of resistance among nontargeted bacteria (Allen et al. 2014). Some of these approaches are discussed below:

4.1 Bacteriocins

Antimicrobial peptides are alternative agents for conventional antimicrobials. A group of antimicrobial peptides, which are nontoxic to mammalian cells, are bacteriocins (Allen et al. 2014). These are small ribosomally synthesized peptides, secreted by bacteria to inhibit the growth of other closely related bacterial species. Bacteriocins form pores by inserting into the plasma membrane of target bacteria, causing lysis of the cell. It has been found that almost all major lineages of bacteria produce bacteriocins. According to some estimates, approximately 99% of all bacteria secrete at least one bacteriocin. Thus, there is an immense diversity of such compounds that can be potentially exploited for therapeutic purposes (Snyder and Worobo 2014). Many commensal bacteria produce bacteriocins that could potentially be exploited (Cotter et al. 2013). For instance, lactic acid bacteria produce a bacteriocin called nisin A; it is currently used as a food preservative in many countries due to its bacteriodical activity. Bacteriocins produced from other food-grade

microbes could also be adapted considering their long historical use in food products such as cheese or yogurt (Vidhyasagar and Jeevaratnam 2013).

Bacteriocins can also be used to treat bacterial infections, including those that are MDR. A bacteriocin produced by *Enterococcus faecium* was active against 29 different vancomycin-resistant *Enterococcus* strains; however, it did not inhibit the growth of other pathogens such as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, or *E. coli* (Shokri et al. 2014). Another bacteriocin, thuricin CD, killed *Clostridioides difficile* without disturbing the normal microbiota (Zendo 2013). Low-molecular-weight bacteriocins are documented to be more resilient; however, high-molecular-weight bacteriocins are more prone to degradation by intestinal proteases or heat (Bastos et al. 2010; Allen et al. 2014; Shokri et al. 2014). Due to their narrow spectrum of antibacterial action, bacteriocins should exert selective pressure only on the species they target. For example, nisin A has been used extensively but no resistance has been reported (Zendo 2013). On the contrary, *E. coli* and *Listeria monocytogenes* have developed resistance to bacteriocins under in vitro conditions by long-term exposure at progressively increasing concentrations (Naghmouchi et al. 2011).

4.2 Phage Therapy

Phage are viruses that infect bacteria and cause lysis. Therapeutic application of phage to kill pathogenic bacteria is called phage therapy, and it has been used to treat infections in humans as well as animals (Johnson et al. 2008; Abedon et al. 2011). Phage therapy was developed to be used topically to treat infections such as skin infections or paranasal sinus infections (Chan et al. 2013). However, there is evidence suggesting phage are effective against systemic infections as well (Smith and Huggins 1982; Biswas 2002; Międzybrodzki et al. 2012). Phage have a very narrow spectrum of bacteria they can infect. So, unlike antibiotics, they do not harm nontarget bacteria (Allen et al. 2014). Studies have suggested that phage specificity depends on the phage titer that may either be narrow or broad (Koskella and Meaden 2013). Currently, the use of phage therapy for human infections is mainly limited to Eastern European countries (Międzybrodzki et al. 2012). In the United States, phage therapy is used for biocontrol of plant pathogens and foodborne pathogens in animals (Brussow 2007; Balogh et al. 2010; Goodridge and Bisha 2011). The application of phage therapy to treat human infections in Western countries is significantly restricted by regulatory agencies.

4.3 Predatory Bacteria

The use of predatory bacteria for treatment of infections is unconventional compared to phages and bacteriocins, but it presents a fascinating possibility as an alternative for antibiotics. Different types of predatory bacteria have been isolated, but the *Bdellovibrio* and like organisms (BALOs) exhibit particular potential. BALOs mainly prey on

Gram-negative bacteria for nutrients and energy (Dwidar et al. 2012). The genomes of BALOs encode numerous hydrolases, DNases, and proteases presumably used to digest prey or to attack bacterial biofilms (Lambert and Sockett 2013; Pasternak et al. 2013). BALOs can potentially be useful against complex microbial communities dwelling in biofilms where antibiotics have limited access (Sockett and Lambert 2004; Van Essche et al. 2011). Predatory bacteria have been investigated in clinical settings to target multidrug-resistant pathogens including *Acinetobacter baumannii*, *K. pneumonia*, *E. coli*, *Pseudomonas putida*, and *P. aeruginosa* (Kadouri et al. 2013). There are reports that BALOs can colonize the host's intestinal tract and serve as both a probiotic and antibiotic (Dwidar et al. 2012). In one study, BALOs were orally administered to *Salmonella enterica*-challenged chickens, resulting in a reduction of inflammation (Atterbury et al. 2011). The collateral effects of BALO administration to nontarget bacteria have yet to be explored in detail; however, some evidence suggests that BALOs do not colonize in vivo (Allen et al. 2014). Thus, despite some promising preliminary data, more extensive research is needed to validate the safety of BALOs.

4.4 Combinatorial/Synergistic Approaches

Another approach to manage MDR pathogens is to use a combination of drugs. In doing so, the toxic effects of antibiotics can be reduced and their potency enhanced (Khameneh et al. 2016). A combination of antibiotics and non-antibiotics can also be exploited to target resistance mechanisms and interfere with bacterial signaling pathways (Worthington and Melander 2013a). One such well-known strategy is to combine β-lactam antibiotics with β-lactamase inhibitors (Worthington and Melander 2013b). Plant extracts, phytocompounds, essential oils, as well as nanoparticles have exhibited synergistic interactions with different classes of antibiotics against microorganisms, including drug-resistant strains (Wolska et al. 2012; Langeveld et al. 2014). In clinical settings, the combination of two or more antimicrobial drugs is used to treat MDR infections, including those caused by bacteria and fungi. For instance, the combination of four drugs is used to treat *Mycobacterium tuberculosis* infections (Mitchison and Davies 2012). The widespread emergence of MDR pathogens has demonstrated that the use of single antibiotics often poses more selective pressure, and hence combination therapy should be utilized to reduce the further emergence of drug-resistant pathogens (Tamma et al. 2012). For example, the combination of amoxicillin and clavulanic acid is used; whereby, β-lactamase production is inhibited by clavulanic acid, and amoxicillin inhibits cell wall biosynthesis. This combination has allowed the continued use of amoxicillin to treat infections caused by pathogens that may have developed resistance to β-lactam antibiotics (Ball 2007). Reserpine, a MDR pump inhibitor, is used in combination with ciprofloxacin to suppress resistance in *S. aureus* and *Streptococcus pneumoniae* strains (Lomovskaya et al. 2001). Likewise, celecoxib, another MDR efflux pump inhibitor, improves the sensitivity of *S. aureus* to many antibiotics such as ampicillin, chloramphenicol, kanamycin, and ciprofloxacin (Kalle and Rizvi 2011). In addition, phytocompounds

and biosurfactants are considered safe and have been approved by the FDA for use in pharmaceuticals and food (Joshi-Navare and Prabhune 2013).

Combination approaches can be subcategorized into three categories based on the drug target (Worthington and Melander 2013b; Hamoud et al. 2014): combining antibiotics that target different pathways, combining antibiotics that target different parts of the same pathway, and combining antibiotics that attack the same target by multiple mechanisms. The success of combination therapy against infection depends on the ability to kill bacteria, avoid resistance, minimize host toxicity, and not disturb the natural microflora. To further boost the efficacy of combination therapy, drug delivery is also important. Overall, the key features of a combination treatment include (Hagihara et al. 2012) enhancement of antibiotic activity by synergistic effect(s), prevention of resistance emergence, possession of anti-biofilm activity, improvement of antibiotic penetration to cells and tissues, and inhibition of virulence factors, such as toxin or enzyme production in pathogens.

4.5 Use of Nanoparticles as Nanomedicine

Many alternative strategies that have been proposed to combat MDR bacteria use nanotechnology to develop novel nanomaterials that possess broad-spectrum antimicrobial action (Baptista et al. 2018). Nanoparticles (NPs) are promising because they possess bactericidal action and also have the capacity to deliver conventional antibiotics (Wang et al. 2017). A wide range of nanomaterials have been developed and tested, including liposomes, metallic vectors, polymer-based nano-drug carriers, and gold NPs (Burygin et al. 2009). An important aspect of nanomedicine is the delivery of drugs to the site of infection by either attaching the drugs to the large NP surface area or by encapsulating antibiotics within a nanostructure (Gholipourmalekabadi et al. 2017). Nanomaterials typically range from 0.2 to 100 nm in at least one dimension and exhibit high surface-to-volume ratios. Nanomaterials can have different chemical, mechanical, electrical, optical, magnetic, and electro-potential properties compared to their bulk materials (Hajipour et al. 2012; Rudramurthy et al. 2016). NPs can enhance the solubility, stability, and biocompatibility of drugs, giving them an advantage over conventional therapies for the treatment of infections caused by drug-resistant bacteria (Rudramurthy et al. 2016; Gholipourmalekabadi et al. 2017). Among metal nanoparticles, silver nanoparticles are the most studied and effective nanomaterial against pathogenic bacteria; however, other metal and metal oxide nanoparticles such as zinc, copper, titanium, tin, and iron also exhibit antibacterial potential (Hemeg 2017; Qais et al. 2018).

While conventional antibiotics have limited membrane permeability, thereby reducing their potency (Andrade et al. 2013), NPs can penetrate the bacterial membrane either by endocytosis or through interactions with surface lipids (Huang et al. 2010; Wang et al. 2017). Moreover, multiple drug combinations can be loaded into or onto NPs to reduce the possibility of developing bacterial resistance (Huh and Kwon 2011). Some NPs also demonstrate broad-spectrum bactericidal activity against Gram-positive and Gram-negative pathogens (Rai et al. 2016; Zaidi et al. 2017). When used as drug carriers, NPs can protect

antimicrobial agents from degrading or inactivating enzymes while effectively delivering the drug to the target site (Huh and Kwon 2011; Wang et al. 2017). Clearly, NPs have significant potential to improve antibiotic therapy, but the systemic use of nanomedicine against drug-resistant bacteria is under scrutiny.

4.6 Anti-virulence Strategies Against MDR Pathogens

In 2014, the WHO declared the beginning of a post-antibiotic era and considered AMR a public health priority demanding global action. It is expected that by 2050 AMR will become a major killer, surpassing cancer, if no action is taken. New antibiotic discovery has been essentially nonexistent over the last several decades, with the exception of teixobactin, and new strategies to combat MDR bacteria must be developed (Ahmad et al. 2009; Totsika 2016). Targeting the virulence and pathogenicity of bacteria has been considered a promising strategy (Ahmad and Husain 2014). In theory, anti-virulence drugs should inhibit bacterial virulence, but not kill bacteria, thus lessening the emergence of resistance. One major anti-virulence strategy that has been pursued is to neutralize or inactivate bacterial toxins, which has been successful to prevent or relieve acute disease symptoms (Adalja and Kellum 2010; Lopez et al. 2010; Chen et al. 2011; Bender et al. 2015). In addition to bacterial toxins, other virulence mechanisms have been identified as potential drug targets (Rasko and Sperandio 2010; Ahmad and Husain 2014; Anthouard and DiRita 2015; Heras et al. 2015) such as bacterial adhesion and colonization (Steadman et al. 2014; Cascioferro et al. 2014), cell-to-cell communication (quorum sensing), secretion systems, and biofilm formation.

Despite their potential, the development of anti-virulence agents has primarily been pursued within the confines of academia and a few small biotech companies. The lack of interest by big pharmaceutical companies is likely due to an increase in development costs, with poor projected profits. However, with the increasing AMR problem and lack of available new antibiotics, the exploration and development anti-infective/anti-virulence drugs is becoming more attractive. Quorum sensing inhibitors or quorum quenching compounds are being developed as anti-virulence drugs against specific MDR bacteria such as *P. aeruginosa* (Kalia et al. 2014). Similarly, inhibition of fimbrial adhesion, a well-known virulence factor in *E. coli*, has shown promise against urinary tract infection in vivo (Guiton et al. 2012), and inhibition of type three secretion system (TTSS) (e.g., salicylidene acylhydrazides) has shown promising results against several pathogenic species (Baron 2010).

Biofilm formation by pathogenic bacteria is a common strategy used to establish infection and persist in the harsh host environment. Biofilm inhibition or eradication is an effective strategy to prevent and treat infection. Anti-biofilm drugs hold significant potential to enhance the efficacy of antibiotics, increase drug penetration, and reduce tolerance to antibiotics. While there are many types of anti-biofilm agents being explored, biofilm-degrading enzymes have shown particular efficacy in vitro and in vivo (Fleming et al. 2016; Fleming and Rumbaugh 2017, 2018). Although there are several challenges in evaluating and developing anti-virulence drugs

(Totsika 2016), these efforts are critical and may hopefully result in the discovery of new approaches (Allen et al. 2014).

5 Conclusions

The discovery of new antibacterial drugs with new modes of action has stalled, and AMR has now become a global problem and major threat to mankind. Developing new strategies to combat the MDR problem is now a priority. A number of conventional and modern approaches have been identified to reduce the emergence of drug resistance and develop new drugs. In consideration of the progress made so far on various fronts, we can conclude that:

(a) Natural products are still a major source for the discovery of new antibacterial leads.
(b) New molecular and bioinformatics approaches can be useful in obtaining new compounds.
(c) Alternative strategies such as combination drugs and antimicrobial peptides have potential in combating the MDR problem.
(d) Nanotechnological advances can be effectively harnessed to improve antibiotic performance.
(e) Anti-infective approaches should be more thoroughly explored and integrated into therapy when possible.

Lastly, concerted efforts by academia, industry, government, and the public are greatly needed to develop new antibiotics that will protect human and animal health in the future.

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Part I

The Challenge of Antibiotic Resistance and Tolerance

Problematic Groups of Multidrug-Resistant Bacteria and Their Resistance Mechanisms

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Abstract

The occurrence of multidrug-resistant pathogenic bacteria is steadily increasing, not only in medical centers but also in food, animals and the environment, which is of primordial concern for health authorities worldwide. The World Health Organization (WHO) published a global pathogen priority list to encourage international interdisciplinary research initiatives on the occurrence, dissemination, and epidemiology of the most dangerous multiresistant pathogens with the aim to develop effective prevention strategies against the spread of these bugs and new therapeutic approaches to treat infections in agreement with the One Health concept. According to the WHO global pathogen priority list, the most critical resistant pathogens include carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and carbapenem-resistant as well as third-generation cephalosporin-resistant *Enterobacteriaceae.* This critical group is followed by pathogens of high priority including vancomycin-resistant *Enterococcus faecium*, methicillin- and vancomycin-resistant *Staphylococcus aureus*, and clarithromycin-resistant *Helicobacter pylori*. Here, we summarize recent data on the occurrence and spread of these and other harmful resistant pathogens, on their resistance mechanisms as well as on the modes of resistance spread, as far as is known. We finish the chapter with an outlook on promising innovative strategies to treat infectious diseases caused by multiresistant pathogens – in combination with antibiotic therapy – as well as on approaches to combat the antibiotic resistance spread.

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Keywords
Antibiotic resistance · Bacterial pathogen · Biofilm · Horizontal gene transfer · Multidrug resistance · WHO pathogen priority list

Abbreviations

Agr	accessory gene regulator
BLNAR	ß-lactamase-negative ampicillin resistant
CDC	Centers for Disease Control and Prevention
COPD	chronic obstructive pulmonary disease
CRAb	carbapenem-resistant *Acinetobacter baumannii*
CRE	carbapenem-resistant *Enterobacteriaceae*
CRPa	carbapenem-resistant *Pseudomonas aeruginosa*
ESBL	extended spectrum ß-lactamase
EU	European Union
FDA	Food and Drug Administration
G-	Gram-negative
HGT	horizontal gene transfer
ICU	intensive care unit
IMP	active on imipenem
IS	insertion sequence
KPC	*Klebsiella pneumoniae* carbapenemase
MBL	metallo-ß-lactamase
MDR	multidrug resistant
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *S. aureus*
NDM	New Delhi MBL
OMP	outer membrane protein
OXA	oxacillinase
PBP	penicillin-binding protein
PMQR	plasmid-mediated quinolone resistance
PNSP	penicillin-non-susceptible *Streptococcus pneumoniae*
RND	resistance-nodulation-cell division
SCC*mec*	staphylococcal chromosome cassette *mec*
VIM	Verona integron-encoded MBL
VRE	vancomycin-resistant *Enterococci*
VREfm	vancomycin-resistant *E. faecium*
VRSA	vancomycin-resistant *S. aureus*
WHO	World Health Organization
XDR	extremely multidrug resistant