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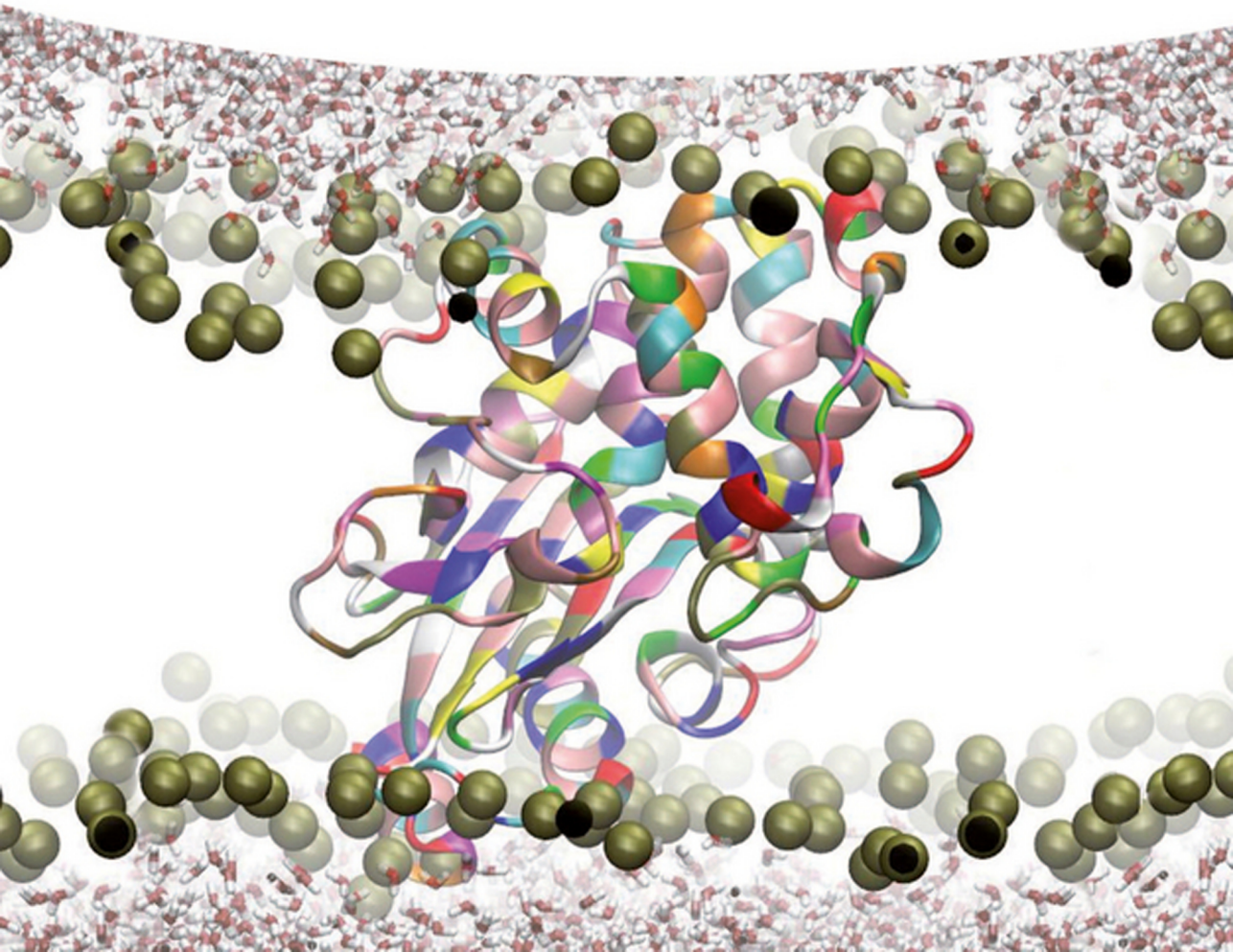

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Cover design

The cover shows beta-lactamase, an enzyme produced by some bacteria, which provide bacterial resistance to beta-lactam antibiotics in the presence of a lipid bilayer. The image was created by Dr. Manuela Mura, University of Central Lancashire, UK.

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Preface

The "Golden age of antibiotics" was between 1929 and the 1970s when over 20 antibiotic classes were marketed [1, 2]. Since the 1960s, the rise in the emergence of microbial pathogens with multiple drug resistance (MDR) has led to the realization that the "Golden age" had ended. The pharmaceutical industry has been constantly battling with MDR because of the overprescription and misuse of antibiotics [3–5]. In Chapter 1, Radecka and coworkers give an insight into bacterial resistance being a major threat to public health. They also discuss the implications arising from the threat posed by MDR pathogens in relation to factors such as medical practice and economics, along with an overview of recent practices and measures proposed to contain this threat, such as the introduction of stewardship programs. Concern regarding our future ability to combat infection has been further intensified by the decreasing supply of new agents [3, 6–8], and in the remainder of the book we review approaches being taken to identity and develop the antimicrobials of the future.

In response to the challenges outlined, in this book there has been increasing research into maximizing opportunities to develop and revitalize established classes of antibiotics. Coates and Hu consider this area in Chapter 2 where they look at opportunities to extend the life of old antibiotics such as β-lactams by the addition of agents that can overcome drug resistance factors, such as β-lactamase inhibitors. Identification of new, effective derivatives remains a challenge, and another approach in the search for new antibiotics has been to seek out new targets that would enable new classes of antibacterials to be developed. In Chapter 3, Capasso and Supuran review the cloning and characterization of carbonic anhydrases (CAs). In this chapter, they make reference to the impact of inhibitors that target the α-, β-, and γ-CAs from many pathogenic bacteria and suggest that this provides evidence that these proteins could provide novel antibacterial targets for the development of new antimicrobial compounds.

There remain concerns, though, that only a small number of drugs are currently under research and development as antibacterial agents [9]. It has been suggested that a further approach could be to revisit naturally occurring compounds with antibacterial potential. Due to the arrival of antibiotics, there has been a rapid loss of interest in the therapeutic potential of natural host antibiotics such as

lysozyme [3, 4]. However, more recently, there has been an awakened interest in host defense molecules, such as antimicrobial peptides (AMPs) [10, 11]. Since the early 1990s, the potential of AMPs has been investigated using, for example, magainins isolated from the African clawed frog *Xenopus laevis*, to investigate the effect of the structural and physiochemical properties of these peptides on their antimicrobial action. These AMPs have the potency to target and kill a wide range of Gram-negative and Gram-positive bacteria, fungi, viruses, and some tumor cells [12]. Based on this ability, AMPs are attractive propositions for development as therapeutically useful antimicrobial and anticancer agents [13]. The first clinical trials of these AMPs as potential novel antibiotics have been for topical treatments [14], and Dennison *et al.* review this area in Chapter 4. AMPs are not only produced by eukaryotes but are also generated by prokaryotes, and Lotfipour and coworkers review this class of peptides, generally known as bacteriocins, in Chapter 5. These prokaryotic peptides are produced by gene-encoded or ribosome-independent pathways [15]. Non-ribosomal prokaryotic AMPs generally include examples such as vancomycin and daptomycin, which are assembled by large multifunctional enzyme complexes. Gene-encoded AMPs from prokaryotes include microcins from Gram-negative bacteria, lantibiotics, and nonmodified bacteriocins from Gram-positive bacteria. The potential uses of these molecules are reviewed for their potential in food biopreservation and healthcare. However, both eukaryotic and prokaryotic AMPs have a range of challenges to overcome, such as the cost of production and design complexity of these molecules. For this reason, work has been under way to design mimics and peptidomimetics of these peptides, which is reviewed in Chapter 6 by Cai and coworkers. Major examples of these molecules include : peptoids [16], β-peptides [17], arylamide oligomers [18], AApeptides [19, 20], and other compounds [21–25], which may be considered second-generation AMPs. These molecules are designed to possess properties conducive to therapeutic application and retain key structural characteristics of naturally occurring AMPs, such as positive charge, hydrophobicity, and amphiphilicity, which facilitate their membranolytic and antimicrobial activity. Tuning these properties has led to superior levels of microbial selectivity and antimicrobial activity as compared to both natural AMPs and conventional antibiotics. This Chapter considers the recent development of these synthetic mimics of AMPs based on a variety of peptide backbones other than canonical peptides, including β-peptides, peptoids, and AApeptides.

It is interesting to note that, in addition to direct action, AMPs are part of more complex innate immune systems and a further approach to developing treatments for the future has involved review of how aspects of such immune systems could be adapted to support treatment of infections. Prior to the discovery and widespread use of antibiotics, it was believed that bacterial infections could be treated by the administration of bacteriophages, which are viruses that infect and kill bacteria via lytic mechanisms but have no effect on humans. With the advent of penicillins and other antibiotics, clinical studies with bacteriophages were not vigorously pursued in the United States and Western Europe, but phage therapy was extensively used in Eastern European countries mainly in the former Soviet Union and Georgia.

However, with the current rise of antibiotic-resistant bacteria, there has been a revitalization of interest in phage therapy in Western countries. In Chapter 7, Lu and coworkers discuss the use of synthetic biology and whether bacteriophages are a re-emerging solution to the current problem of pathogenic microbes. Bacteriophage therapy has a number of potential advantages over the use of conventional antibiotics, such as high bacterial specificity and efficacy against bacteria with MDR, although there are concerns over its use, such as the possibility of inducing immunological responses. Nonetheless, phage therapy is generally regarded as one of the most promising strategies to provide antimicrobial alternatives for fighting antibiotic-resistant bacteria and could lead to the development of new and improved therapies and diagnostics to combat infectious threats of the present and the future.

In addition to the above approaches, there is a wide range of additional natural compounds that have the potential in the treatment of infection. The antimicrobial properties of metals such as copper and silver have been known for centuries especially in use for the treatment of burns and chronic wounds [26]. Recently, the confluence of nanotechnology and the search for new agents in the fight against microbes with MDR has brought metals in the form of nanoparticles to the fore as potential antimicrobial agents. In Chapter 8, Sportelli and coworkers present several examples of nanomaterials based on three of the main inorganic materials with known antimicrobial action (i.e., silver, copper, and zinc oxide) along with the mechanisms underlying their antimicrobial action. The potential applications of these nanoparticles as antimicrobials in areas such as prophylaxis and therapeutics, medical devices, the food industry, and textile fabrics are discussed in more detail. In addition, there are numerous examples of naturally produced organic compounds with antibacterial properties. In the period 2000–2008, over 300 natural metabolites with antimicrobial activity were reported, and in Chapter 9, Saleem reviews these compounds and describes candidates with potentially useful antimicrobial activity with reference to a variety of molecules, including : alkaloids, acetylenes, coumarins, iridoids, terpenoids, and xanthones.

A range of organic compounds with the potential to serve as anti-infectives are those that are known to sequester within bacterial cells and can be light-activated to induce antimicrobial activity. For example, phenothiazinium-based molecules [27, 28], whose antimicrobial properties were first noted in dyes that were used for the histological staining of cellular components, have been shown to be more efficacious than conventional antibiotics [28, 29]. These dyes photoinactivate bacteria, viruses, yeasts, fungi, and protozoa via the production of reactive oxygen species (ROS) such as such as hydroxyl radicals and hydrogen peroxide. Over the last few decades, photosensitizers (PS) have attracted increasing attention as antimicrobial agents with therapeutic potential, and, when applied in this context, the use of PS is known as *photodynamic antimicrobial chemotherapy* (PACT). Phoenix co-workers provide an overview of the photophysics and photochemistry involved in PACT, and illustrate the therapeutic uses of this action with reference to a variety of PACT agents such as methylene blue and 5-aminolevulinic acid. Whilst this area has clear potential, there are also challenges that need to

be overcome if the use of such compounds is to become more widespread. One such limitation is the challenge of ensuring effective light penetration of tissue and in this respect, it has been suggested that ultrasound could be used as part of a new antimicrobial strategy that addresses this limitation based on its superior capacity for tissue penetration. Ultrasound has been shown to have an antibacterial effect comparable to some conventional antibiotics as recently reported in the case of rhinosinusitis. It has also been shown that the application of ultrasound in conjunction with conventional antibiotics such as gentamycin is able to synergize the effects of these drugs when applied to both planktonic and sessile bacteria. More recently, it has been shown that irradiation with ultrasound can activate some PS, which are generally termed *sonosensitizers* (*SS*) in this capacity, and based on these observations it was hypothesized that ultrasound and SS may be exploited for the treatment of infectious diseases. This system has been designated sonodynamic antimicrobial chemotherapy (SACT) and most recently has been shown to be able to eradicate both Gram-positive and Gram-negative bacteria. In Chapter 11, Harris coworkers provides an overview of the impact of SACT.

In considering approaches to combat growing drug resistance and to identify new means of treatment, the potential of oligonucleotides as antibacterial agents has been investigated. Such molecules are able to act as antisense agents to prevent translation, or, alternatively, can be designed to bind DNA to prevent gene transcription: these approaches are reviewed in Chapter 12 by Beaman coworkers. In this area, a range of new and exciting approaches are being developed. For example, it may be that such agents can inhibit microbial resistance mechanisms by interrupting the expression of resistance genes and hence restore susceptibility to key antibiotics, which would be co-administered with the antisense compound. Such an approach will clearly have significant applications.

Finally, it is worth considering whether antibiotic efficacy can be increased by enhancing the targeting of such molecules to their site of action. In the final chapter, Ehlissi coworkers review an example of such an approach by looking at targeting via the development of antimicrobial agent carrier systems such as the use of nanoparticle constructs. Here, the authors discuss the development of nanostructures for the entrapment and delivery of antimicrobials as an alternative to the direct application of these substances. Specific reference is made to structures formed from liposomes and the effects of the carrier on the activity of the compound are discussed.

In conclusion, it is clear that new approaches are needed if we are to maintain our ability to deal with infection. These approaches have to be holistic and integrated and must involve consideration of stewardship programs as well as the development of new antibiotics and novel approaches to enhancing activity through improved targeting or combination therapies. The need for the development of new antibiotics and antibacterial design strategies has never been greater.

March 2014 *David A. Phoenix, Frederick Harris, and Sarah R. Dennison*

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1
The Problem of Microbial Drug Resistance

Iza Radecka, Claire Martin, and David Hill

1.1
Introduction

Microbial colonization, where it is not wanted, can lead to disease, disability, and death. Therefore, control and/or destruction of pathogenic microorganisms is crucial for the prevention and treatment of disease. Modern medicine is dependent on antimicrobial/chemotherapeutic agents such as antibiotics (Greek *anti,* against, *bios* life). Antibiotics can either destroy pathogens or inhibit their growth and avoid damage to the host. In the nineteenth century, infections such as diarrhea, pneumonia, or post-surgical infections were the main causes of death. Therefore, the discovery of antibiotics was of great importance to society and impacted on the prevention and treatment of disease. Antibiotics can be defined as compounds produced by microorganisms that are effective against other microorganisms but nowadays also include microbial compounds that have been synthetically altered. The classification of antibiotics is based not only on the cellular components or systems they affect but also on whether they inhibit cell growth (bacteriostatic drug) or kill the cells (bactericidal drug) [1]. Other chemotherapeutic synthetic drugs, not originating from microbes, such as sulfonamides, are also sometimes called *antibiotics* [2].

1.2
History of the Origins, Development, and Use of Conventional Antibiotics

The modern era of antimicrobial agents began with the work of the German scientist Paul Ehrlich (1854–1915), who, together with a Japanese scientist Sahachiro Hata (1873–1938), discovered in 1909 the first sulfa drug called *arsphenamine* – initially known as *compound "606"* (the 606th compound tested). This new drug was available for treatment in 1910 under the trade name Salvarsan. Arsphenamine, considered as a "magic bullet" with selective toxicity, was used in the treatment of syphilis and sleeping sickness. Despite the fact that

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the mode of action of arsphenamine remained unclear, it was the most popular antimicrobial drug successfully used until the 1940s [2, 3].

After Ehrlich's success, many more compounds were tested for their possible antimicrobial properties. In the 1930s, Gerard Domagk (1895–1964) tested a number of leather, nontoxic (for animals) dyes for their antimicrobial activity. His work led to the discovery of Prontosil Red (1932), the first sulfa antimicrobial agent effective against pathogenic streptococci and staphylococci. This discovery was so important that in 1939 he received the Nobel Prize for its discovery. However, it was the discovery of the first antibiotic called *penicillin* that revolutionized the treatment of infectious diseases and initiated the new antibiotic era. Although penicillin was first discovered by a French medical student Ernest Duchesne in 1896, it was Alexander Fleming (1881–1955) who first observed the lethal/antimicrobial activity of the substance, which he later named penicillin, against *Staphylococcus aureus.* He reported (1928) the inhibition of the growth of pathogenic bacteria contaminated with *Penicillium notatum* spores. Fleming published several papers about penicillin production and began efforts to characterize penicillin. Unfortunately, he stopped his research with penicillin at this stage as he was not able to demonstrate the stability of penicillin within the body. In 1930, Fleming's paper about penicillin produced by *P. notatum* was again an object of great interest to Professor Howard Florey (1898–1968) and his coworker Ernest Chain (1906–1979) who were investigating the antimicrobial properties of many substances including Fleming's penicillin. Crude penicillin produced by *P. notatum* (Fleming's strain) was purified and successfully tested against staphylococci and streptococci. In March 1942, the first adult patient was successfully treated with penicillin, which led to both scientists receiving the Nobel Prize in 1945. In 1943, a new strain of *Penicillium chrysogenum* was isolated from a moldy cantaloupe by Mary Hount from the Horthen Regional Research Laboratory, Illinois, US, and the mass production of penicillin began [3]. In 1944, Selman Waksman, after screening about 10 000 strains of soil bacteria and fungi, discovered a new antibiotic produced by *Streptomyces griseus* called *streptomycin*. For his success, he received the Nobel Prize in 1952. By 1953, production of chloramphenicol, neomycin, tobramycin, and tetracycline was also possible [2].

Cephalosporins are the second class of antibiotics following penicillins. In 1945, Giuseppe Brotzu (1895–1955) isolated *Cephalosporium acremonium* from sewage water in Sardina, Italy. Brotzu observed great antimicrobial activity against some Gram-negative bacteria. Unable to proceed with his research, Brotzu sent his cultures to Edward Abraham (Oxford University) who, together with Guy Newton, isolated cephalosporin P, active only against Gram-positive bacteria. Shortly after, cephalosporin N and cephalosporin C were discovered (paper published in 1961). Cephalosporin N was later identified to be penicillin N – active against both Gram-negative and Gram-positive bacteria.

Modern antibiotics used today are, or derive from, natural molecules isolated during the "golden age" of antibiotic era (1940–1970) mostly from *Streptomyces* species, a few from Gram-positive *Bacillus* species, and some from strains of

Table 1.1 Examples of natural, semi-synthetic and synthetic antibiotics and their mode of action [1, 3, 4, 6].

Group of antibiotics	Mode of action	Primary target	Derivation	Organisms
β-lactams	Inhibition of cell wall synthesis	Penicillin binding protein	Natural and semi-synthetic	Gram-positive and Gram-negative bacteria
Glycopeptides and glycolipopeptides	Inhibition of cell wall synthesis	Peptidoglycan units	Natural and semi-synthetic	Gram-positive bacteria
Rifamycins	Inhibition of RNA synthesis	RNA polymerase	Natural and semi synthetic	Gram-positive and Gram-negative bacteria, *M. tuberculosis*
Lipopeptides	Inhibition of cell wall synthesis	Cell membrane	Natural and semi synthetic	Gram-positive and Gram-negative bacteria
Aminoglycosides	Inhibition of protein synthesis	30S ribosome	Natural and semi synthetic	Aerobic Gram-positive and Gram-negative bacteria, *M. tuberculosis*
Tetracyclines	Inhibition of protein synthesis	30S ribosome	Natural and semi synthetic	Aerobic Gram-positive and Gram-negative bacteria
Macrolides	Inhibition of protein synthesis	50S ribosome	Natural and semi synthetic	Aerobic and anaerobic Gram-positive and Gram-negative bacteria
Streptogramins	Inhibition of protein synthesis	50S ribosome	Natural and semi synthetic	Aerobic and anaerobic Gram-positive and Gram-negative bacteria
Phenicols	Inhibition of protein synthesis	50S ribosome	Natural and semi synthetic	Some Gram-positive and Gram-negative bacteria
Trimethoprim-sulfamethoxazole	Inhibition of DNA synthesis	Inhibition of synthesis of tetrahydrofolic acid	Synthetic	Gram-positive and Gram-negative bacteria
Fluoroquinolones	Inhibition of DNA synthesis	Topoisomerase II and IV	Synthetic	Aerobic Gram-positive and Gram-negative bacteria; some anaerobic Gram-negative bacteria and *M. tuberculosis*

Penicillium and *Cephalosporium* [4, 5]. Most bactericidal antibiotics kill the cell by interfering with the essential cellular processes (Table 1.1). They inhibit DNA, RNA, cell wall, or protein synthesis [1, 3, 4, 6].

Interestingly, it was also Fleming who, in his Nobel lecture, stated that bacteria can develop resistance to penicillin if exposed to low doses and that negligent use could encourage resistance. Sadly, he was right, and soon after penicillin G was introduced to hospitals (1940s) the problem of antibiotic-resistant bacteria

emerged [7]. Only 3 years after his warning, 38% of *S. aureus* strains in only one London hospital were penicillin resistant. Currently, around 90% of strains in the United Kingdom and nearly all in the United States show penicillin resistance [8].

Antibiotic resistance (AR) is driven by the misuse of antibiotics due to selective pressure. Moreover, unprecedented human air travel allows bacterial mobile resistance genes to be transported between continents. So the fact that bacteria and their resistance genes can travel faster and further than ever before creates serious risk to human health and development on a global scale [9, 10]. At the moment, in Europe at least 25 000 patients die every year because of bacterial infections, which cannot be treated with the available antibiotics [11]. Therefore, the development of new antimicrobial drugs with new modes of action and the preservation of the agents "in hand" are essential steps for the foreseeable future [7]. Great efforts have also been made to understand the mechanisms by which currently available antibiotics affect microbial cells. Antibiotic-facilitated cell death is very complex and involves many genetic and biochemical pathways. It is essential to understand the multilayered mechanisms by which currently available antibiotics kill bacteria, and also create new alternative antimicrobial therapies [1].

1.3 Problems of Antibiotic Resistance

Unquestionably, the discovery of antibiotics was one of the most important medical achievements in modern medicine and their introduction represents a remarkable success story for society. However, the widespread use and misuse of antibiotics for both clinical and nonclinical settings has resulted in the emergence (selection) of a number of multiresistant bacteria called *superbugs* such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA) [12], vancomycin-resistant *Enterococcus* spp., [10] carbapenem-resistant *Mycobacterium tuberculosis* [5], extended spectrum β-lactamase-producing *Escherichia coli*, or the highly virulent antibiotic-resistant *Clostridium difficile* [11, 13]. The emergence of antibiotic resistance in bacteria, selected by negligent antibiotic usage, provides the most dramatic demonstration of Darwinian selection as a result of a specific evolutionary pressure to adapt to the presence of antimicrobials [14]. It has been reported that the consumption of antimicrobials by food-producing animals around the world is also a powerful driver of antibiotic multidrug resistance (AMR) in both humans and animals [8]. These activities also clearly create an ongoing explosion of antibiotic-resistant infections generating a significant risk to public health on a global scale, as there are very few or sometimes no effective antimicrobial agents available to treat infections caused by both Gram-positive and Gram-negative pathogenic bacteria [15, 16]. The problem of ever-increasing bacterial multiresistance is even more alarming when we consider the diminishing number of new antimicrobials entering clinical practice [17, 18]. There is clearly an urgent need for the development of new antibiotics or new alternatives to conventional antimicrobial

agents with novel mechanisms of antimicrobial action as even some common infections are becoming increasingly difficult to treat. It is also very important to stress that antimicrobial resistance is not only found in bacteria – that there is a growing number of other pathogens such as viruses (that cause chronic hepatitis B (CHB) or influenza), parasites (cause malaria), and fungi (*Candida* infections) resistant to the antimicrobial agents [6, 19, 20]. Resistance to all classes of antimalarial drugs has been well documented including artemisinin derivatives and chloroquine. Moreover, resistance rates (10–20%) to anti-HIV drug regimens have been reported in the United States and Europe. Many people around the world suffer because of antimicrobial resistance.

1.4 Multiple Drug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pan-Drug-Resistant (PDR) Organisms

There are many definitions in the medical literature used to characterize different patterns of bacterial multiresistance. International organizations such as the European Centre for Disease Prevention and Control (ECDC), the Clinical Laboratory Standards Institute (CLSI), the European Committee and Antimicrobial Susceptibility Testing (EUCAST), and the United States Food and Drug Administration (FDA) have made a combined effort to create standardized terminology that can be applied to all bacteria responsible for infections associated with multidrug resistance [18, 21]. Consequently, "antimicrobial categories" were created (for each specific organism or group), each category containing the related antimicrobial agents (Table 1.2). The term *multiple drug resistance* (MDR) refers to organisms non-susceptible to at least one agent in three or more antimicrobial categories. Extensively (extreme) drug resistant (XRD) means the organism shows non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan-drug resistant (PDR) refers to an organism that shows non-susceptibility against all (or nearly all) of the antimicrobial agents within the antimicrobial categories.

1.5 MDR Mechanisms of Major Pathogens

At present, the treatment of bacterial infections is severely affected by the emergence of antibiotic-resistant infections and epidemic increases of multidrug resistant (MDR), XRD, or increasingly PDR microorganisms [22] such as vancomycin-resistant *Enterococcus faecium* (VRE), *Enterobacter cloacae,* MRSA), XRD carbapenem-resistant *Acinetobacter baumannii* [8], third generation cephalosporin-resistant *E. coli,* third generation cephalosporin-resistant, extended spectrum β-lactamase producing *Klebsiella pneumonia* (ESBL-KP), carbapenem-resistant *Klebsiella pneumoniae* (CRKP) [8], carbapenem-resistant

Table 1.2 Examples of antimicrobial categories and antimicrobial agents used to define MDR, XDR and PDR [18].

Antimicrobial category	Antimicrobial agent
Carbapenems	Imipenen
	Meropenem
	Doripenem
Tetracyclines	Tetracycline
	Doxycycline
	Minocycline
Aminoglycosides	Gentamicin
	Tobramycin
	Amikacin
	Netilmicin
Polymyxins	Colistin
	Polymyxin B
Extended spectrum cephalosporins	Cefotaxime
third and fourth generation	Ceftriaxone
	Ceftazidime
Glycopeptides	Vancomycin
	Teicoplanin
Phenicols	Chloramphenicol
Streptomycin	Streptomycin

Pseudomonas aeruginosa, multidrug resistant *Mycobacterium tuberculosis* (MDR-TB) [23], and *C. difficile* [6, 13, 15, 24–29].

Drug resistance can be caused by mobile genes or, in the absence of mobile genetic elements, by sequential mutations in the microbial chromosome. Mobile genes can be transferred between different bacteria by mobile genetic elements such as plasmids, naked DNA, transposons, or bacteriophages. These genes code for information against a particular antibiotic. In some microbes, multiple genes can be present, resulting in MDR. Alternatively, resistance or MDR can also be caused by sequential mutation in chromosomal DNA, which can result in mutation in the antibiotic target enzymes (topoisomerases) or/and in the overexpression of efflux pumps that expel structurally unrelated drugs [6, 30]. Chromosomal genes can also be transferred. They can be acquired by one bacterium through the uptake of naked DNA released from another microorganism by the process called *transformation* (an introduction of an exogenous DNA into a cell, resulting in a new phenotype). For example, emergence of high-level resistant *S. aureus* to vancomycin, caused by a mobile element – transposon from enterococci – first appeared in response to an intermediate dose of vancomycin. Bacteria are also mobile and can easily travel from person to person, from continent to continent, spreading the problem of microbial resistance [10].