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
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].

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

*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., 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].

<table>
<thead>
<tr>
<th>Antimicrobial category</th>
<th>Antimicrobial agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenems</td>
<td>Imipenem</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
</tr>
<tr>
<td></td>
<td>Doripenem</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td>Doxycycline</td>
</tr>
<tr>
<td></td>
<td>Minocycline</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamicin</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
</tr>
<tr>
<td></td>
<td>Netilmicin</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>Colistin</td>
</tr>
<tr>
<td></td>
<td>Polymyxin B</td>
</tr>
<tr>
<td>Extended spectrum cephalosporins third and fourth generation</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Vancomycin</td>
</tr>
<tr>
<td></td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>Phenicols</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Streptomycin</td>
</tr>
</tbody>
</table>

*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].
Bacterial mechanisms of resistance vary. Active resistance can be achieved by three major mechanisms: first, synthesis of specific enzymes that selectively target and inactivate the drug (e.g., β-lactamases, macrolide esterases, epoxidases, or several transferases); second, efflux of the antimicrobial agents from the cell via membrane-associated efflux pumps; third, modification of the antibiotic target sites (alteration of intracellular binding targets such as ribosomal RNA or DNA gyrase involved in DNA replication, or even enzymes involved in the synthesis of bacterial cell wall). The most important example of target change can be seen in MRSA, where the acquisition and expression of meca genes results in resistance to methicillin and most of the β-lactam antibiotics [30–33]. All three mechanisms can make the drug incapable of inhibiting microbial metabolic pathways that are vital for microbial growth and survival [6, 31].

Antimicrobial resistance to a single antimicrobial agent is already problematic, but the emerging multidrug resistance of Gram-negative bacteria is of serious concern and it dramatically limits treatment options [25]. Gram-negative bacterial infections caused by MDR or PDR bacteria (such as E. coli, P. aeruginosa, Klebsiella pneumoniae, and/or A. baumannii) can result in death. In 2013, they were called the nightmare bacteria by the US Centres for Disease Control and Prevention (CDC) and a new coming “red plaque” by Looke et al. [34]. The predominant cause of resistance of Gram-negative bacteria is related to one or more β-lactamases, which can inactivate the β-lactam antibiotic by hydrolyzing the amide bond of the β-lactam ring, leaving β-lactam antibiotics harmless to bacteria [35–37]. In the 1980s, only cephalosporins (e.g., cefotaxime) were less susceptible to β-lactamases; unfortunately, their repetitive use selected resistant strains able to produce plasmid-mediated enzymes such as cefotaximasas (CTX-M) [35]. Research shows that ESBLs carried by E. coli and metallo-β-lactamases (SHV-1, sulhydryl variable) carried by K. pneumoniae and Enterobacter spp can easily destroy the latest generation of penicillins or cephalosporins. They can even inactivate carbapenems, which are often called the last available resort for treatment of serious infections caused by Gram-negative bacteria [13, 35, 36].

Most of the species of E. coli, responsible for urinary tract infections and Gram-negative bacteremia, are antibiotic sensitive, apart from being resistant to ampicillin. However, research showed that up to 60% of E. coli isolates from hospital and non-hospital environments are resistant to ampicillin because of the production of plasmid-mediated TEM-1/2 (Temoniera from whom E. coli TEM was isolated in 1963) β-lactamases [13, 35]. TEM-2 enzyme differs from TEM-1 only by a single amino acid [13]. Microbes producing TEM-1 or TEM-2 are known to be resistant to ampicillin but still are susceptible to the third generation of cephalosporins. However, it has been reported that mutations in TEM-1 and TEM-2 can result in the production of new ESBLs (so far more than 100 of new TEM have been reported). Transferrable plasmids containing genes encoding ESBLs are often associated with aminoglycoside resistance and other resistances [13]. In 1990, the more virulent MDR CTX-M-producing E. coli has emerged, replacing opportunistic hospital outbreaks with SHV- and TEM-type ESBLs-KP. It was established that CTX-M enzymes are encoded in transferrable
plasmids and transposons. These mobile elements have originated from other bacteria such as *Kluyvera* spp. and have spread widely among enterobacteria [13]. Highly transmissible CTX-M-producing *E. coli* can also be resistant to the aminoglycosides and quinolones. As a result of this, MDR in *E. coli* is now increasingly common in the hospital environments and community. It is also known that phages can be involved in bacterial evolution and the creation of new “super bugs” such as the deadly *E. coli* O157:H7 strain [38].

*K. pneumoniae*, one of the most common clinical pathogen causing sepsis, meningitis, pneumonia, and other diseases, is usually resistant to ampicillin by production of a metallo-β-lactamases (SHV-1), similar to TEM-1 or TEM-2. SHV-1 can be encoded by transferrable plasmid, integrons, or by chromosome mutations. Mutation of SHV-1 results in the production of one or more ESBLs. MDR *K. pneumoniae* is becoming a serious concern worldwide. Carbapenem-resistant organisms can produce several different carbapenemases. Plasmid-mediated *Klebsiella pneumoniae* carbapenemase (KPC) isolates were found to be responsible for many outbreaks worldwide and were associated with a significant mortality rate [36, 39, 40].

Gram-negative *P. aeruginosa*, another well-known opportunistic pathogen [30], is the third most common cause of hospital-acquired Gram-negative bacteremia after *E. coli* and *K. pneumoniae*. Some isolates of *P. aeruginosa* are inherently resistant to most penicillins, cephalosporins, and even to carbapenems [13, 28, 36, 41]. This multidrug resistance was caused by the overexpression of the chromosomally encoded efflux system, which is very common in Gram-negative bacteria such as *A. baumannii* or *P. aeruginosa* [32]. In *A. baumannii* the efflux system is associated with the resistance-nodulation-cell division (RND) family of the transport proteins. These multidrug efflux pumps consist of an efflux membrane transporter (RND) that can interact with an outer membrane factor (OMF), which exports the drug through both membranes [33]. *A. baumannii* shows an extraordinary ability to develop multidrug resistance due to a high level of genomic plasticity and due to mutation of endogenous genes. Alteration of these genes exhibits overexpression of the chromosomally encoded β-lactamases, loss of expression of porins, mutation in *gyrA* and *parC*, and finally overexpression of efflux systems, which is associated with increased drug resistance. There are three types of efflux systems in *A. baumannii*: CraA (resistance to chloramphenicol); AbeM (extrudes several antimicrobials); and AmvA (resistance to several detergents, dyes, disinfectants, and erythromycin). There are also several tetracycline efflux pumps, for example, TetA and TetB. TetA is associated with resistance to tetracycline, while TetB shows resistance to tetracycline, doxycycline, and minocycline [30, 33, 42].

Gram-positive *S. aureus* has a great ability to develop multiple resistances [29]. Reports showed that it can be resistant to penicillin, tetracycline, erythromycin, chloramphenicol, gentamicin, and methicillin. The MRSA, called the superbug, emerged in 1961, only 2 years after methicillin was introduced and since then it has become the most common multiple-antibiotic-resistant pathogen in many parts of the world [27]. In 2011, it was estimated that MRSA was responsible for 171,200 healthcare-associated infections (HAIs) in Europe per year; 5400
Table 1.3  Examples on antibiotics with activity against MRSA [15].

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daptomycin</td>
<td>Causes a calcium ion-dependent disruption of bacterial cell membrane, an efflux of potassium inhibits RNA, DNA, and translation</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Inhibits bacterial protein synthesis by binding to the domain V regions of 23S rRNA</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>Bacteriostatic against most pathogens. Show broad spectrum of antimicrobial activity. Inhibits bacterial protein synthesis by binding to the 30S ribosomal sub-unit blocking binding of amino-acetyl transfer RNA into acceptor side</td>
</tr>
</tbody>
</table>

Deaths, and more than a million extra days of hospitalization [29, 43]. Methicillin resistance developed because of the acquisition of the mecA gene located on a large genetic element called staphylococcal cassette chromosome mec (SCCmec) integrated into the MRSA chromosome [27]. SCCmec has been possibly assimilated by horizontal transfer from an animal coagulase-negative pathogen Staphylococcus sciuri. mecA gene encodes for the production of an abnormal penicillin-binding protein PBP-2a (also called PBP-2'). PBPs are transpeptidases necessary for cell wall peptidoglycan synthesis and are the target for penicillin. PBP-2a is a transpeptidase that does not bind to penicillin so inhibition of cell wall synthesis by penicillin does not occur [13, 31]. Many strains of MDR MRSA remain susceptible only to vancomycin and teicoplanin (glycopeptides). Unfortunately, in recent years some S. aureus isolates have also become glycopeptide tolerant, and even worse, several isolates now show glycopeptide and carbapenems resistance. New antibiotics against MRSA infections such as daptomycin (Cubicin®; Novartis), linezolid, and tigecycline (Tygacil®; Wyeth) have been investigated (Table 1.3). However, a number of novel agents such as a capsular polysaccharide-based vaccine, lipoglycopeptide ortivanacin, or the use of signal molecule-based drugs (quorum sensing inhibitor) or cell wall-anchored adhesions are in different stages of development [14, 29].

Hospital-acquired MRSA (HA-MRSA) have now been found outside the hospitals and spread to other healthcare facilities [27]. There is also massive spread of community-acquired MRSA (CA-MRSA) infections. Some CA-MRSA isolates can produce toxins called Panton-Valentine Leukocidin (PVL), which increases its virulence. Expression of this virulence is controlled by complex staphylococcal regulatory networks including the accessory gene regulator (agr) system. These genes can vary between different strains [29, 43]. PVL is responsible for acute skin infections and pneumonia. CA-MRSA can be easily transmitted from person to person.

The development of antiviral drug resistance also represents serious complications. CHB virus is an example of antiviral drug resistance. The development of resistance in hepatitis B virus (HBV) is related to the lack of proofreading function in the DNA polymerase and its high replication rate [19], which, in the presence of
the antiviral drug, result in specific DNA mutations (during replication process). Clinically, antiviral drug resistance is first exhibited in higher levels of HBV DNA (virological breakthrough), followed by increased levels of alanine aminotransferase (biochemical breakthrough). Although the DNA mutations developed can affect the “fitness” of the viruses, they will also help the virus to survive the presence of the drug and develop a high level of antiviral drug resistance. In addition, compensatory additional DNA mutations help restore viral “fitness,” leading to viral rebound. HBV shows a high level of resistance to antiviral drugs such as lamivudine, telbivudine, and adeovir. Rapid development of antiviral drug resistance has also been seen for influenza viruses A and B. There are two classes of antiviral drugs approved in many countries: the adamantanes (active only against influenza virus A) and the neuraminidase inhibitors (NAIs). However, due to the rapid emergence of viral resistance, only NAIs are recommended by WHO (since 2010) for the treatment or prophylaxis of influenza A and B infections. At present, only two NAIs are licensed worldwide for therapeutic and prophylactic uses: oseltamivir, commercially available as Tamiflu® (F. Hoffmann-La Roche), and zanamivir, commercially available as Relenza® (GlaxoSmithKline). In 2009, influenza pandemic patients with suspected or known influenza A (H1N1)pdm09 were treated with a new drug peramvir (BioCryst). The mechanism of resistance is also linked to DNA mutations. Influenza viruses showing reduced sensitivity to NAIs contain mutations, which directly or indirectly change the shape of the influenza surface antigen—neuraminidase (NA) catalytic sites (made of 8 functional and 11 framework residues). The NA surface antigen exhibits two important functions: first, it releases progeny virions, and second, it facilitates viral spread. Any alterations to NA catalytic sites reduce the inhibitor binding of the drug and therefore lower the efficiency of Tamiflu. In 2007, H1N1 influenza strains in Europe and North America were reported resistant to NAI Tamiflu owing to the H274Y mutation [44]. Rapid evolution of influenza surface genes can create more worldwide dissemination of drug-resistant influenza infections caused by A(H1N1) variants; therefore, the development of new antiviral drugs and surveillance of viral infections is extremely important [45].

Pathogenic fungi such as MDR Candida spp. or MDR Candida krusei are known to be responsible for life-threatening infections. They are called hidden killers resulting in 46–75% mortality [46]. The multidrug resistance in Candida spp. is related to low accumulation of drugs caused by genes encoding drug transporters. ATP-binding cassette (ABC) transporters are encoded by Candida drug resistance (CDR1 and CDR2) and a major facilitator superfamily (MFS) transporter encoded by MDR1 genes. Overexpression of MDR1, which encodes the MDR efflux pump of the MFS often, increases resistance toazole antifungal drugs. Long-term therapies with fluconazole (antifungal drug) have led to the emergence of fluconazole-resistant Candida albicans and C. krusei strains, which can also be resistant to other drugs. C. krusei also showed decreased susceptibility to flucytosine and amphotericin [47]. Novel antimicrobial peptides that can target the mitochondria and DNA of MDR Candida spp. are being developed in order to fight microbial resistance [48].
Antimicrobial resistance has been recognized as a major global threat. Globalization of the world results in population movement, which favors the rapid spread of new MRD organisms and infectious diseases [16]. The dramatic increase in antibiotic-resistant infections leads to higher mortality, longer hospital stays, and unavoidably increased treatment costs [49]. It can be said that the gene pool for antimicrobial resistance has never been so big nor its selection pressure so strong [1]. There was a time when antimicrobial agents were highly successful in treating infections caused by pathogenic microbes; however, their uncontrolled use in human clinical therapy, aquaculture, and food animal production has triggered rapid development of antimicrobial resistance, especially in the developing world [34, 38]. In recent years, the scientific community has raised serious concerns about the fact that drug development will not be able to address the problem posed by drug or multidrug resistance. So what do we do next? How do we fight this multiresistance problem? Recognizing the serious global problem, several nations, international health agencies, and many other organizations worldwide have taken actions to counteract microbial resistance through the application of novel strategies/initiatives. For example, the ARTEMIS Antifungal Surveillance Program (2001–2005) was created to increase our understanding and to monitor the spread of the uncommon but MDR fungal pathogen *C. krusei* [47]. In 2001, a WHO global strategy was introduced in order to slow down and reduce the spread of antimicrobial-resistant organisms. The strategy included better access to appropriate antimicrobial agents, better use of antimicrobials, better surveillance of antimicrobial resistance by strengthening health systems, and enforcing of regulations and legislation. The strategy also included the development of new drugs and vaccines [24]. In 2008, to avoid the spread of resistance, virological monitoring of HIV patients was required. To facilitate this, the WHO developed a Global Strategy for Prevention of HIV Drug Resistance and established the HIVResNet network of experts and laboratories in order to reduce the spread of HIV infection due to resistance to anti-HIV drugs [18]. In 2011, during the World Health Day, the WHO urged the world for a political commitment and the creation of a comprehensive plan that may help fight antimicrobial resistance. Following advice, hospitals implemented novel initiatives such as antimicrobial stewardships. The antimicrobial stewardship is a combined set of strategies/guidelines created to reduce microbial resistance [50–52]. The mission of antimicrobial stewardship program is to reduce inappropriate use of antimicrobial agents (dose, duration, route of administration) and improve patient outcomes [53]. Antimicrobial stewardship also aims to reduce the spread of infections and the development of antimicrobial resistance [52]. Several studies showed that antibiotic resistance can be reduced by shortening the length of antibiotic courses [49, 54, 55]. An antimicrobial stewardship program is multidisciplinary, and brings together key healthcare professionals (nurses, general practitioners, pharmacists, clinical microbiologists, infectious
diseases physicians) and hospital management. The program cannot be just limited to large hospitals or academic centers only; it needs to include small regional facilities in both developed and developing healthcare systems around the world. Adopting novel stewardship strategies in the hospitals and community can provide a systematic approach to the growing threat of antibiotic resistance.

1.7 Discussion

Multidrug resistance is a serious danger to the future of humans (and animals) and could result in the development of untreatable diseases and death. Immediate measures must be taken worldwide to safeguard the remaining antimicrobials and to facilitate the development of new antimicrobial agents. Numerous papers have been published about microbial multidrug-resistant pathogens created by intensive use of antibiotics in human and animal therapy, and food animal production. As a consequence, AR and MDR bacteria have been found in hospitals, healthcare centers, community, and various food products and even in the environments without a history of direct exposure to antibiotics [38]. Enteric bacteria such as *E. coli* or *Enterococcus* spp. have been extensively investigated and the impact of these commensal bacteria on the emergence of MDR gene pool has been recognized by the scientific community [38]. In addition, recent reports, released by the WHO, have raised concerns about MDR tuberculosis (MDR)-TB. In January 2010, 58 countries reported cases of XDR-TB [56].

It can be said that the problem of bacterial resistance or multidrug resistance in the ecosystem is a serious and complex issue. How should we manage and prevent multidrug resistance? First, detailed knowledge of the nature of AR and MDR pathogens is required in order to implement new successful strategies to control the transmission of multidrug resistance within hospital/healthcare environments and the community. Investigations of historic strain and events that have led to the origin of resistant strains such as mechanisms involved in the emergence and dissemination are essential. Secondly, the rate of MDR microbes can be reduced by the implementation of different intervention strategies relevant to the control of antibiotic use and control of hospital infections [18, 41, 57]. The control of antibiotic use focuses on factors such as choice of antibiotic or combination of antibiotics for treatment, duration of the therapy, monitoring and feedback on antimicrobial resistance, rational antibiotic usage, and regulations. ESBL-producing *E. coli* was reported in 79% of surgical wound infections in India, but only less than 5% in New Zealand [11, 57]. MRSA are highly dominant in the United States (34%) while in the Netherlands the prevalence is ≤2%. These massive differences could be associated to the local differences in antibiotic policies [57].

The second essential point is the control of hospital infection (to control cross-infections within hospitals and within community) through rapid detection/diagnostic tests, prevention, and control of antimicrobial resistance, and also
plans for patients (admitted, re-admitted, discharged, or transferred) colonized with resistant microbes. For example, to prevent further spread of MRSA the “Search-and-Destroy” policy was implemented in Denmark in 1983, when prevalence of MRSA was 30%; since then, the MRSA prevalence decreased to less than 1% [27]. Nemeth et al. [57] reported that a substantial proportion of the patients transferred to the hospital (Zurich University Hospital) from abroad are colonized with MDR organisms. They concluded that the rigors of infection control in the hospital are important and every patient admitted to the hospital should be screened for MDR colonization. Reducing the spread of MDR by using existing antibiotics and developing new antibiotics or novel alternative therapies should be seen as a collective responsibility [8]. Worldwide surveillance systems of resistance and reduction of transmission of resistant organisms are required to generate valuable data important for research and development. Managing the problem of bacterial resistance requires researchers, community, and politicians working together to promote research and implement global strategies.

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References


