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Insights on the Antimicrobial Resistance Mechanisms of Bacteria

Wanda C. Reygaert*

Department of Biomedical Sciences, William Beaumont School of Medicine,Oakland University, Rochester, MI, USA

***Corresponding Author:** Wanda C Reygaert, Department of Biomedical Sciences, William Beaumont School of Medicine, Oakland University, Rochester, MI, USA, E-mail: reygaert@oakland.edu

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Abstract

Antimicrobial resistance has been on the increase for many years and has become a major source of morbidity and mortality around the globe. When the first antibiotics were introduced, it was thought that the war against microorganisms had been won. Doctors and scientists soon discovered however, that the microorganisms were far more adaptable than was thought, and capable of developing resistance to any drugs. Most pathogenic microorganisms have the ability to develop resistance to one or more antimicrobial agents. The main mechanisms of resistance that bacteria use are: limiting the uptake of a drug, modifying a drug target, inactivating a drug, and actively effluxing a drug. The microorganisms may have innate resistance mechanisms, or may acquire mechanisms from other microorganisms. In order to be better able to treat infectious disease, a better understanding of the mechanisms is necessary. Hopefully this will lead to development of antimicrobial agents that can not only fight the bacteria, but can evade attempts by the microorganisms to become resistant.

Keywords: Antimicrobial; Antimicrobial resistance; Bacteria.

Introduction

With the advent of the use of antibiotics, clinicians thought that the battle against infectious diseases was over. The continued rise in antimicrobial resistance, however, makes it seem as if the war has turned in favor of the microorganisms. Infectious diseases have become a significant cause of morbidity and mortality around the world. A report on these diseases from the World Health Organization (WHO) shows diarrheal diseases, HIV/AIDS, lower respiratory infection, and malaria, are among the top ten contributors to morbidity and mortality [1]. Increased antimicrobial resistance has impacted infectious diseases significantly, in number of infections, recurring as well as chronic infections, and in added healthcare costs. Clinicians have a large repertoire of antimicrobial agents from which to choose for use in infection therapy. Unfortunately, antimicrobial resistance has been seen for all of these, and resistance often occurs shortly after a new drug is developed.

The antimicrobial agents are often characterized in groups based on the mechanism of antimicrobial activity. These groups are agents that: inhibit cell wall synthesis, depolarize the cell membrane, inhibit protein synthesis, inhibit nuclei acid synthesis, and inhibit metabolic pathways in bacteria. Table 1 shows these groups with examples of drugs from each. Even with so many antimicrobial mechanisms available we don't seem to be able to control microorganisms well. It seems that improper stewardship of antimicrobial agents has helped to create the resistance issue that is now facing us. Among the factors that have contributed to the growing resistance problem are: increased use of antimicrobial drugs in general, both by humans and in animals; and improper antimicrobial therapy prescribing. Repeated use of many of the common antimicrobials agents may be a choice based on a combination of cost and possible toxicity [2]. Improper prescribing of antimicrobials drugs may occur with the initial prescription of a broad-spectrum drug that is unnecessary, or later found to be the wrong choice for the causative organism(s) [3]. Excessive use of antibiotics in humans has led to the emergence of some resistant organisms [4,5]. It has also been noted that a patient who has previously been on antimicrobial drugs is at a much higher risk for infection with a drug resistant organism, and those patients with the highest exposure to antimicrobials are most likely to be infected with resistant bacteria [2,6].

The continued increase in antimicrobial resistance has made selecting drugs to treat infections difficult, and has been associated with an increase in both morbidity and mortality. This means that clinicians are now seeing patients with more severe infections who need more extensive treatment. Plus, the resulting longer courses of illness often require extended hospitalization. These developments have greatly increased the cost of healthcare associated with infectious diseases. In a report from the CDC, an estimated more than 2 million people become ill each year with antimicrobial resistant infections in the U.S., with these infections resulting in at least 23,000 deaths [7]. Costs attributed to infections with resistant organisms have ranged from $7000 to over $29,000 per patient [8]. It has been shown that the healthcare costs for methicillin-resistant *Staphylococcus aureus* (MRSA) infections are: in the U.S., over $18,000 per case; in Germany, almost €9,000 per case; and in Switzerland, an average added cost of more than 100,000 Swiss francs [9-11]. Suggestions have been made as to various methods for antimicrobial stewardship that might help to stem the increases in resistance. One suggestion is the use of diversity in antimicrobial selection. This includes the idea of not using a single drug, but instead using two or more drugs, either together or alternatively, and preferably using drugs from different drug classes [12,13].

Table 1. Antimicrobial Groups Based on Mechanism of Action

Inhibit Cell Wall Synthesis	β-Lactams Carbapenems Cephalosporins Monobactams Penicillins Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit Aminoglycosides Tetracyclines Bind to 50S Ribosomal Subunit Chloramphenicol Lincosamides Macrolides Oxazolidinones Streptogramins
Inhibit Nucleic Acid Synthesis	Quinolones Fluoroquinolones
Inhibit Metabolic Pathways	Sulfonamides Trimethoprim

Resistance Origins

Members of a bacterial group or species are not necessarily all susceptible or resistant to any given antimicrobial agent. The levels of resistance may be varied in related groups of bacteria. The susceptibility and resistance to drugs are measured as minimum inhibitory concentration (MIC), which is the minimal concentration of a drug that will inhibit the growth a specific bacterium. The susceptibility actually includes a range of the average MICs assessed across the same bacterial species for a given drug. If the average MIC for a species falls within the resistant area of the range, that species is considered to have intrinsic resistance to that drug. Alternatively, bacteria may acquire resistance genes from other related microorganisms, and then the level of resistance will vary depending on the species and the genes acquired [14,15].

Innate resistance may either be intrinsic (always expressed in the species), or induced (genes are naturally occurring in the bacteria, but are expressed to resistance levels only after exposure to a specific antibiotic). Intrinsic resistance has been defined as a trait that is shared universally within a bacterial species, is independent of previous antibiotic exposure, and not related to horizontal gene transfer [15,16]. The most common mechanisms that are involved in intrinsic resistance are: reduced permeability of the outer cell wall (most specifically the lipopolysaccharide, LPS, in gram negative bacteria), and the natural activity of efflux pumps. Multidrug-efflux pumps are a common mechanism of induced resistance[16,17].

Bacteria may acquire genetic material that confers resistance through all of the normal routes that bacteria use to acquire any genetic material: transformation, transposition, and conjugation (all termed horizontal gene transfer – HGT). In addition, the bacteria may experience mutations to its own chromosomal DNA. The acquisition of outside genetic material may be temporary or permanent. Plasmid-mediated transmission of resistance genes is the most commonly seen route for the acquisition of outside genetic material; bacteriophage-borne transmission is fairly rare. In the bacterial genome, insertion sequences and integrins may move genetic material around, and stress (starvation, UV radiation, chemicals, etc.) on the bacteria are common causes of genetic mutations (substitutions, deletions etc.). Bacteria have an average mutation rate of 1 for every 10^6 to 10^9 cell divisions, but most of these mutations will be deadly to the organism [14,18]. Mutations that confer antimicrobial resistance usually occur in only a few types of genes: those encoding drug targets, those encoding drug transporters, those encoding regulators that control drug transporters, and those encoding antibiotic-modifying enzymes [15]. In addition, many mutations that confer antimicrobial resistance do so at a cost to the organism. For example, in the acquisition of resistance to methicillin in *Staphylococcus aureus*, the growth rate of the bacteria is decreased [19].

Resistance Mechanisms

Antimicrobial resistance mechanisms in bacteria are of four main types: 1) limiting the uptake of a drug, 2) modifying a drug target, 3) inactivating a drug, 4) active drug efflux. The types of mechanisms which might be seen in intrinsic resistance are limiting uptake, drug inactivation, and drug efflux; those mechanisms usually seen in acquired resistance are drug target modification, drug inactivation, and drug efflux. There is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria, usually because of differences in structure, etc. Gram negative bacteria are more likely to make use of all four main mechanisms, whereas gram positive bacteria sometimes use limiting the uptake of a drug (no LPS), and don't have the capacity for certain types of drug efflux mechanisms (refer to the drug efflux pumps later in this paper) [20,21]. Figure 1 illustrates the general antimicrobial resistance mechanism

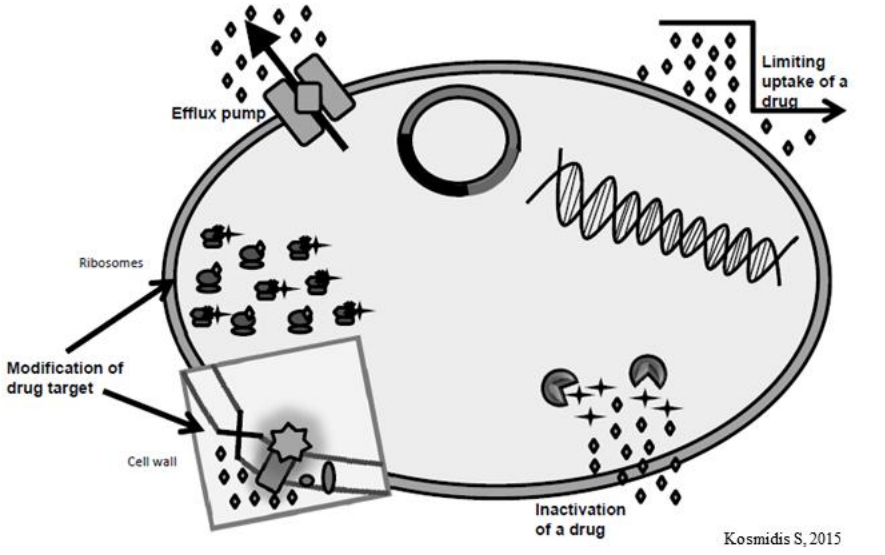

Fig. 1 General antimicrobial resistance mechanisms.

Limiting the Uptake of a Drug

There is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. In the gram negative bacteria the structure and functions of the LPS cell wall layer provide a barrier to certain molecules. Those bacteria, then, have innate resistance to certain of the antimicrobial agents groups [22]. The outer membrane of the mycobacteria has a high lipid content, which allows easier cell access for hydrophobic drugs such as rifampicin and the fluoroquinolones; hydrophilic drugs, however, have limited access [23,24].

Certain species of bacteria, such as the mycoplasmas, because they lack an outer cell wall, are intrinsically resistant to drugs such as the β-lactams and glycopeptides which target the cell wall [25]. Since gram positive bacteria do not possess an outer cell wall, restricting drug access is not as prevalent in that group of bacteria. Intrinsic resistance to aminoglycosides occurs in the enterococci due to the fact that polar molecules have difficulty penetrating the enterococci cell wall. Fairly recently, another gram positive bacteria, *Staphylococcus aureus*, has acquired resistance to vancomycin. There are two mechanisms that *S. aureus* uses against vancomycin. One of those mechanisms, which are not yet understood, allows the bacteria to produce a thickened cell wall which makes it difficult for vancomycin to enter the cell, and provides an intermediate resistance to vancomycin. These strains are designated as VISA (vancomycin-intermediate *Staphylococcus aureus*) strains [24,26].

Bacteria with large outer cell walls often allow substances to enter the cell through porin channels. In gram negative bacteria, the porin channels generally allow access to hydrophilic molecules [22,27]. There are two main ways in which drug uptake can be limited by changes in porins: a decrease in the number of porins, and changes in the selectivity of the porin channel, usually through mutations which affect porin channel structure or charge [23]. One bacterial group that uses reduction in porin number for resistance is the *Enterobacteriaceae*. Sometimes this involves stopping the production of certain porins. This group of bacteria is known to reduce porin number as a mechanism for resistance to carbapenems [28,29]. *Enterobacter aerogenes* which becomes resistant to imipenem and certain cephalosporins often uses mutations that cause changes in the porin channel, as do *Neisseria gonorrhoeae* which becomes resistant to β-lactams and tetracycline [27,30].

Modifying Drug Targets

The bacterial cell contains many components that may be potential targets for antimicrobial agents; and the bacteria is capable of modifying any or all of these targets to enable resistance to those drugs. Resistance to the β-lactam drugs is commonly achieved via alterations in the structure and/or number of penicillin-binding proteins (PBPs), which are transpeptidases involved in the construction of peptidoglycan in the cell wall (a mechanism used almost exclusively by gram positive bacteria). A change in the number of PBPs impacts the amount of drug that can bind to that target. Changes in the PBPs may include an increase in the number of PBPs that have a decreased drug binding, or a decrease in PBPs with normal drug binding. A change in PBP structure may decrease the ability of the drug to bind, or stop the ability of a drug to bind [19,31].

The glycopeptide drugs, such as vancomycin, also inhibit cell wall synthesis. Lipopeptide drugs, such as daptomycin, depolarizing the bacterial cell membrane. Many gram negative bacteria have intrinsic resistance to these drugs [32]. Resistance to vancomycin has become a major issue. In the VRE (vancomycin-resistant enterococci) and in *Staphylococcus aureus* (MRSA), resistance is mediated through acquisition of *van* genes. Resultant changes in the structure of peptidoglycan precursors cause a decrease in the ability of vancomycin to bind [19,31]. In order to have the ability to depolarize the bacterial cell membrane, the presence of calcium is required for daptomycin to be able to bind. Mutations in genes, such as *mprF*, change the charge of the cell membrane surface to positive. This change in charge inhibits the binding of calcium, and therefore, daptomycin [33-35].

The bacterial ribosome is often a target for drugs (inhibition of protein synthesis). The bacteria have several mechanisms for protecting the ribosomal subunits from drug attack. These mechanisms are usually via ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal methylation (aminoglycosides, macrolides – gram positive bacteria, oxazolidinones, and streptogramins), most commonly involving *erm* genes, or ribosomal protection (tetracyclines). All of these mechanisms interfere with the ability of any given drug to bind to the ribosome, and the level of drug interference varies greatly among these mechanisms [36-38].

Another important drug target is bacterial DNA. Drugs that target nucleic acid synthesis, such as the fluoroquinolones, are resisted via modifications in DNA gyrase (gram negative bacteria – e.g. *gyrA*) or topoisomerase IV (gram positive bacteria – e.g. *grlA*). The mutations in those genes cause changes in the structure of gyrase or topoisomerase. Those changes decrease or eliminate the ability of the drug to bind to these components [39,40].

There are specific types of drugs that target bacterial metabolic pathways. Resistance to these drugs is via mutations in specific enzymes (DHPS – dihydropteroate synthase, DHFR – dihydrofolate reductase) that are involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes. The sulfonamides (target - DHPS) and trimethoprim (target - DHFR) are able to bind to their respective enzymes because they are structural analogs of the natural substrates. Some of the sulfonamides are analogs for *p*-amino-benzoic acid, and trimethoprim is an analog for dihydrofolate. The drugs competitively inhibit folate production by binding in the active site of the enzymes. Mutations in these enzymes are often located in or near the active site. The resulting structural changes in the enzyme interfere with drug binding while still allowing the natural substrate to bind [41,42].

Inactivation of Drugs

Bacteria inactivate drugs by using two main mechanisms: by actual degradation of the drug, or by transfer of a chemical group to the drug. One very large group of drug hydrolyzing enzymes is the β-lactamases. The common structure of all of the β-lactam drugs is a four-sided ring (the β-lactam ring). The process by which the β-lactamases inactivate a drug is though hydrolyzation of a site in the ring structure. This action breaks the ring and inactivates the drug. Any of the β-lactam drugs can be inactivated by at least one of the many β-lactamases. Some of the earliest discovered β-lactamases were those that were active against the penicillins (penicillinases) and many of the cephalosporins (cephalosporinases). More recently, some strains of the enterobacteriaceae have been found that produce extended-spectrum β-lactamases (ESBLs). These enzymes provide protection against all of the cephalosporins. Very recently, β-lactamases have been identified that are active against the carbapenems. Examples of these are the KPCs (*Klebsiella pneumoniae* carbapenemases) and *ndm* encoded carbapenemases which are found in many gram negative pathogens [31,43]. Tetracycline is another drug that can be inactivated by hydrolyzation, via the *tetX* gene [36].

Drug inactivation may also be accomplished by the transfer of a chemical group to the drug. The most commonly transferred chemical groups are acetyl, adenyl, or phosphoryl groups. A large number of transferase enzymes have been identified. The most diversely used transfer mechanism is acetylation. This is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolone drugs. Adenylation and phosphorylation are primarily used against the aminoglycoside drugs [43-46].

Active Efflux of Drugs

Most bacteria have genes for efflux pumps that are chromosomally encoded. Some of the genes for these pumps are expressed constitutively while other pumps are only induced (or sometimes overexpressed) by certain environmental stimuli or presence of a suitable substrate. High level drug resistance by efflux pumps usually occurs via a mutation that modifies the inside of the transport channel of the pump. The main function of efflux pumps is to protect the bacteria from toxic substances. Many of these pumps are capable of transporting a wide variety of compounds, and are known as multi-drug (MDR) efflux pumps [22]. The presence of specific carbon sources influences the resistance capability of many of these pumps [47].

The average bacteria may express many efflux pumps; and many different types of pumps as well. The efflux pumps belong to five main family groups. These groups are categorized based on energy source and structure. The groups are: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family. The majority of the efflux pumps are single-component pumps which simply transport substances across the cytoplasmic membrane. The RND pump families are always multi-component pumps, efflux substances across the entire cell envelope, and are found almost exclusively in gram negative bacteria. These pumps function in association with an outer membrane protein (porin – OMP) and during active transport the structure is held stably in place by periplasmic fusion proteins (MFPs) [22,23,48,49].

The efflux pumps that are found chromosomally encoded in gram positive bacteria may confer intrinsic resistance. These pumps may include members of the MFS and MATE families and generally efflux fluoroquinolone drugs. Gram positive bacteria may also carry efflux pumps on plasmids, which currently are known to be MFS pumps [50-53]. The efflux pumps that are found in the gram negative bacteria are widely distributed and have been shown to come from all five of the pump families. The most clinically significant pumps in gram negative bacteria are members of the RND pump family [22,50]. Figure 2 illustrates the general structure of the major efflux pump families.

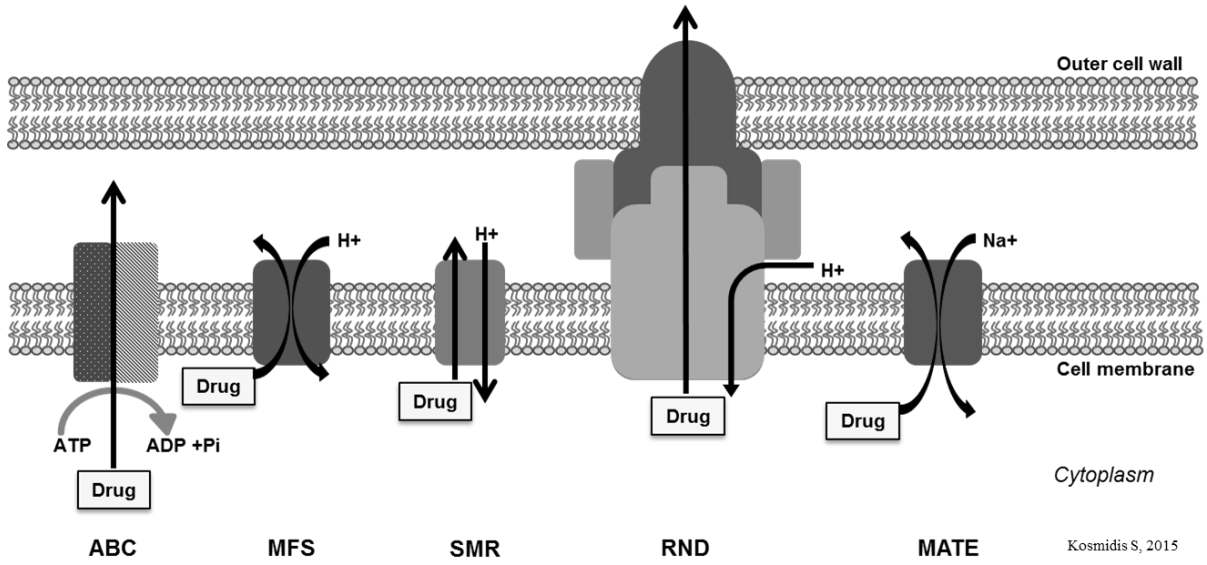

Fig. 2 General structure of the main efflux pump families: ABC – ATP-binding cassette; MATE – multidrug and toxic compound extrusion; MFS – major facilitator superfamily; RND – resistance-nodulation=cell division; SMR – small multidrug resistance.

The ABC Transporter Family

The members of the ABC efflux family may be involved in uptake or efflux transport systems. This pump family is unique in the energy source used, which is derived from ATP hydrolysis. These pumps are known to be transporters of amino acids, drugs, ions, polysaccharides, and proteins. The bacterial ABC transporters usually consist of six transmembrane segments (TMS), which are α-helices. These transporters work in conjunction with cytoplasmic ATPases, and function as either homodimers or heterodimers in the cell membrane. The ABC pumps have fairly specific substrates, and very few are known to be found in clinically significant bacteria. An example of a significant ABC pump is the VcaM pump found in *Vibrio cholerae*. This pump transports tetracycline and fluoroquinolone drugs [23,54,55].

The MATE Transporter Family

The members of the MATE efflux family use a Na^+ gradient as energy source. These pumps efflux cationic dyes and most are capable of transporting fluoroquinolone drugs. A small number of MATE pumps are capable of transporting some aminoglycoside drugs. Other substances that are transported by these pumps may be unrelated in chemical structure. The pumps are usually made up of twelve TMS. These pumps have not been well characterized in bacteria, and have been found primarily in gram negative bacteria. One of the first MATE pumps discovered was the NorM pump found on the chromosome in *Vibrio parahaemolyticus*. This pump is also of clinical significance in *Neisseria gonorrhoeae* and *N. meningitidis* [48,56,57].

The MFS Transporter Family

The members of the MFS efflux family catalyze transport via solute/cation (H^+ or Na^+) symport, or solute/H^+antiport. These pumps are involved in the transport of anions, drugs (e.g. tetracycline and macrolides), metabolites such as bile salts, and sugars. As a group, the MFS pumps have the largest substrate diversity, and yet the individual pumps tend to be substrate specific. In *Acinetobacter baumannii*, for example, there are separate MFS pumps for chloramphenicol (the CraA and CmlA pumps) and erythromycin (the SmvA pump). In *Escherichia coli* there are separate pumps for fluoroquinolones (QepA), macrolides (MefB), and trimethoprim (Fsr). There are rare instances where MFS pumps have a broader substrate range. Examples of this are the NorA pump in *Staphylococcus aureus* which transports chloramphenicol and fluoroquinolones (chloramphenicol and fluoroquinolones are the most commonly transported drugs in MFS members), and the LmrS pump in *S. aureus*, which transports chloramphenicol, erythromycin, linezolid, and trimethoprim. The structure of the MFS pumps may contain twelve or fourteen TMS, and over 1000 from bacteria have been sequenced. Most MFS pumps are located on bacterial chromosomes; and in *E. coli*, nearly half of the efflux pumps are from this family [22,23,36,58].

The SMR Transporter Family

The members of the SMR efflux family are energized via the proton-motive (H^+) force. These pumps are usually hydrophobic in nature and mainly efflux lipophilic cations (a very narrow substrate range). These pumps have been found to be encoded on chromosomes, plasmids and transposable elements. The pump basic structural unit contains four TMS, and these pumps often function as asymmetrical homotetramers. Only a few of the SMR pumps are known to transport drugs; most commonly β-lactams and some of the aminoglycosides. Examples of SMR pumps found in bacteria include the SMR pump in *Staphylococcus epidermidis*, which transports ampicillin, erythromycin, and tetracycline; and the EmeR pump in *E. coli*, which transports erythromycin, tetracycline, and vancomycin [22,23,59,60].

The RND Transporter Family

The members of the RND efflux family catalyze substrate efflux using a substrate/H^+ antiport system. These pumps are frequently found in gram negative bacteria. The RND pumps are often considered to be multi-drug transporters. These pumps efflux detergents, dyes, heavy metals, and solvents, as well as many other substances. Certain of the RND pumps drug or drug class specific (Mef pumps – macrolides, Tet pumps – tetracycline), while others are capable of transporting a variety of drugs (MexAB-OprM in *Pseudomonas aeruginosa* – β-lactams, chloramphenicol, some fluoroquinolones, tetracycline, sulfamethoxazole, trimethoprim). The RND pumps are the complex multi-component pumps. The cell membrane pump consists of twelve TMS, and contains two large periplasmic loops between TMS 1 and 2, and TMS 7 and 8. These pumps work in association with an OMP, and MFPs help stabilize the multi-component structure. The genes that encode RND pumps are generally organized as an operon. The organization often is: first a gene that encodes a regulator, then, adjacent to that gene is one encoding the MFP gene, next is the gene encoding the cellular membrane pump, and next the gene encoding the OMP gene. One of the best characterized RND pumps is the AcrAB-TolC pump found in *E. coli*. This pump confers resistance to chloramphenicol, fluoroquinolones, macrolides, penicillins, and tetracycline. The *E. coli*AcrB cellular membrane pump contains two binding pockets which allow the binding of substrates with varying chemical properties and size range [22,23,43,48-50,55,61].

Conclusion

Antimicrobial resistance is not likely to be conquered anytime in the near future. The reality is that bacteria are ultimate survivors. They have to fend off attacks from other organisms as well as deal with toxic waste products in the environment. Scientists should not be surprised that bacteria are so capable of defending themselves against antimicrobial drugs. Antimicrobial resistance is continuing to increase, so we need to quickly find new or different methods for fighting these organisms. Coming up with new antimicrobial drugs is not a cheap or simple process, and perhaps we need to start thinking differently about combating bacteria, by approaching antimicrobial design creatively.

A huge part of the problem is that the antimicrobial mechanisms used by bacteria are seemingly infinitely varied. As new antimicrobial agents are discovered and put into use, the bacteria come up with ways to combat them. This has left physicians with few resources to fight some of the worst pathogens. Table 2 gives a summary of the known mechanisms used against the various drug groups.

It would not come as a surprise if there are more resistance mechanisms out there in the bacteria that have not yet been discovered. We don't yet have a good idea as to how many different antimicrobial mechanisms each individual bacteria might possess. If we consider a single bacterium such as *Staphylococcus aureus*, we know that this bacterium possesses multiple virulence factors. If we add in antimicrobial mechanisms, such as in MRSA strains, we are looking at an extremely dangerous organism [62]. Other bacteria that are becoming more and more dangerous are members of the enterobacteriaceae, especially *E. coli* and *Klebsiella pneumonia*, which are now resistant to most of the antimicrobial drugs available for gram negative bacteria. Scientists will need to keep up with antimicrobial information while working hard to find answers to this alarming scenario.

Table 2. Known Antimicrobial Resistance Mechanisms

Drug	Limiting Drug Uptake	Modifying Drug Target	Drug Inactivation	Efflux Pumps
β-Lactams	X	X	X	X
Carbapenems				
Cephalosporins				
Monobactams				
Penicillins				
Glycopeptides	X	X		
Lipopeptides		X		
Aminoglycosides	X	X	X	X
Tetracyclines	X	X	X	X
Chloramphenicol		X		X
Lincosamides		X		X
Macrolides		X		X
Oxazolidinones		X		X
Streptogramins				X
Fluoroquinolones		X	X	X
Sulfonamides		X		X
Trimethoprim		X		X

References

[1] World Health Organization (2014). World Health Statistics.

[2] Griffith M, Postelnick M, Scheetz M. (2012). Antimicrobial stewardship programs: methods of Operation and suggested outcomes. Expert Rev Anti Infect Ther 10:63-73.

[3] Yu VL (2011). Guidelines for hospital-acquired pneumonia and health-care-associated pneumonia: Vulnerability, a pitfall, and a fatal flaw. Lancet Infect Dis. 11:248-252.

[4] Goossens H (2009). Antibiotic consumption and link to resistance. ClinMicrobiol Infect. 15 Suppl 3:12-15.

[5] Pakyz AL, MacDougall C, Oinonen M, Polk RE. (2008a). Trends in antibacterial use in US academic health centers: 2002 to 2006. Arch Intern Med. 168:2254-2260.

[6] Tacconelli E. (2009). Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. CurrOpin Infect Dis. 22:352-358.

[7] Centers for Disease Control and Prevention (CDC) (2014). Antibiotic resistance threats in the United States, 2013. US Department of Health and Human Services. CS239559-B.

[8] Maragakis LL, Perencevich EN, Cosgrove SE. (2008). Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther 6:751-763.

[9] Filice GA, Nyman JA, Lexau C, Lees CH, Bockstedt LA, et al. (2010). Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. Infect Control HospEpidemiol., 31:365-373.

[10] Hübner C, Hübner NO, Hopert K, Maletzki S, Flessa, S. (2014). Analysis of MRSA-attributed costs of hospitalized patients in Germany. Eur J ClinMicrobiol Infect Dis 33:1817-1822.

[11] Macedo-Viñas M, De Angelis G, Rohner P, Safran E, Stewardson A, et al. (2013). Burden of methicillin-resistant *Staphylococcus aureus* infections at a Swiss University hospital: excess length of stay and costs. J Hosp Infect 84:132-137.

[12] Pakyz A, Powell JP, Harpe SE, Johnson C, Edmond M, Polk RE. (2008b). Diversity of antimicrobial use and resistance in 42 hospitals in the United States. Pharmacotherapy 28:906-912.

[13] Sandiumenge A, Diaz E, Rodriguez A, Vidaur L, Canadell L, el al. (2006). Impact of diversity of antibiotic use on the development of antimicrobial resistance. J AntimicrobChemother57:1197-1204.

[14] Coculescu BI. (2009). Antimicrobial resistance induced by genetic changes. J Med Life 2:114-123.

[15] Martinez JL. (2014). General principles of antibiotic resistance in bacteria. Drug Discov Today Technol 11:33-39.

[16] Cox G, Wright GD. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. Int J Med Microbiol 303:287-292.

[17] Fajardo A, Martinez-Martin N, Mercadillo M, Galan JC, Ghysels B, et al. (2008). The neglected intrinsic resistome of bacterial pathogens. PloS One 3:e1619.

[18] Davies J, Davies D. (2010). Origins and evolution of antibiotic resistance. MicrobiolMolBiol Rev 74:417-433.

[19] Reygaert WC. (2009). Methicillin-resistant *Staphylococcus aureus* (MRSA): molecular aspects of antimicrobial resistance and virulence. Clin Lab Sci 22:115-119.

[20] Chancey ST, Zähner D, Stephens DS. (2012). Acquired inducible antimicrobial resistance in Gram-positive bacteria. Future Microbol 7:959-978.

[21] Mahon CR, Lehman DC, Manuselis G. (2014). Antimicrobial agent mechanisms of action and resistance. In: Textbook of Diagnostic Microbiology. St. Louis: Saunders, p. 254-273.

[22] Blair JM, Richmond GE, Piddock LJ. (2014). Multidrug efflux pumps in Gram-negative bacteriaand their role in antibiotic resistance. Future Microbiol 9:1165-1177.

[23] Kumar A, Schweizer HP. (2005). Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv Drug Deliv Rev 57:1486-1513.

[24] Lambert PA. (2002). Cellular impermeability and uptake of biocides and antibiotics in gram-positive bacteria and mycobacteria. J ApplMicrobiol 92 Suppl: 46S-54S.

[25] Bébéar CM, Pereyre S. (2005). Mechanisms of drug resistance in *Mycoplasma pneumoniae*. CurrDrug Targets Infect Disord 5:263-271.

[26] Miller WR, Munita JM, Arias CA. (2014). Mechanisms of antibiotic resistance in enterococci. Expert Rev Anti Infect Ther 12:1221-1236.

[27] GillMJ, Simjee S, Al-Hattawi K, Robertson BD, Easmon CS, Ison CA. (1998). Gonococcal resistance to β-lactams and tetracycline involves mutation in loop 3 of the porin encoded at the *penB* locus. Antimicrob Agents Chemother 42:2799-2803.

[28] Cornaglia G, Mazzariol A, Fontana R, Satta G. (1996). Diffusion of carbapenems through the outer membrane of enterobacteriaceae and correlation of their activities with their periplasmic concentrations. Microb Drug Resist 2:273-276.

[29] Chow JW, Shlaes DM. (1991). Imipenem resistance associated with the loss of a 40 kDa outer membrane protein in *Enterobacteraerogenes*. J AntimicrobChemother 28:499-504.

[30] Thiolas A, Bornet C, Davin-Régli A, Pagès JM, Bollet C. (2004). Resistance to imipenem, cefepime, and cefpirome associated with mutation in Omp36 osmoporin of *Enterobacteraerogenes*. BiochemBiophys Res Commun 317:851-856.

[31] Beceiro A, Tomás M, Bou G. (2013). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? ClinMicrobiol Rev 26:185-230.

[32] Randall CP, Mariner KR, Chopra I, O'Neill AJ. (2013). The target of daptomycin is absent from *Escherichia coli* and other gram-negative pathogens. Antimicrob Agents Chemother 57:637-639.

[33] Yang SJ, Kreiswirth BN, Sakoulas G, Yeaman MR, Xiong YQ, et al. (2009). Enhancedexpression of *dltABCD* is associated with development of daptomycin nonsusceptibility in a clinical endocarditis isolate of *Staphylococcus aureus*. J Infect Dis 200:1916-1920.

[34] Mishra NN, Bayer AS, Weidenmaier C, Grau T, Wanner S, et al. (2014). Phenotypic and genotypiccharacterization of daptomycin-resistant methicillin-resistant *Staphylococcus aureus* strains: relative roles of *mprF* and *dlt* operons. PloS One 9:e107426. Doi: 10.1371/journal.pone.0107426.

[35] Stefani S, Campanile F, Santagati M, Mezzatesta ML, Cafiso V, Pacini G. (2015). Insights andclinical perspectives of daptomycin resistance in *Staphylococcus aureus*: a review of the available evidence. Int J Antimicrob Agents.doi: 10.1016/j.ijantimicag.2015.05.008.

[36] Kumar S, Mukherjee MM, Varela MF. (2013). Modulation of bacterial multidrug resistance efflux pumps of the major facilitator superfamily. Int J Bacteriolpii:204141.

[37] Roberts MC. (2003). Tetracycline therapy: update. Clin Infect Dis., 36:462-467.

[38] Roberts MC. (2004). Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. MolBiotechnol 28:47-62.

[39] Hawkey PM. (2003). Mechanisms of quinolone action and microbial response. J AntimicrobChemother,Suppl 1:28-35.

[40] Redgrave LS, Sutton SB, Webber MA, Piddock LJ. (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol 22:438-445.

[41] Huovinen P, Sundström L, Swedberg G, Sköld O. (1995). Trimethoprim and sulfonamide resistance. Antimicrob Agents Chemother 39:279-289.

[42] Vedantam G, Guay GG, Austria NE, Doktor SZ, Nichols BP. (1998). Characterization of mutationscontributing to sulfathiazole resistance in *Escherichia coli*. Antimicrob Agents Chemother42:88-93.

[43] Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. (2015). Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42-51.

[44] Ramirez MS, Tolmasky ME. (2010). Aminoglycoside modifying enzymes. Drug Resist Updat13:151-171.

[45] Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, et al. (2006). Fluoroquinolone- modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 12:83-88.

[46] Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 28:519-542.

[47] Villagra NA, Fuentes JA, Jofré MR, Hidalgo AA, García P, Mora GC. (2012). The carbon source influences the efflux pump-mediated antimicrobial resistance in clinically important Gram-negativebacteria. 67:921-927.

[48] Piddock LJ. (2006). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. ClinMicrobiol Rev 19:382-402.

[49] Poole K. (2007). Efflux pumps as antimicrobial resistance mechanisms. Ann Med 39:162-176.

[50] Kourtesi C, Ball AR, Huang YY, Jachak SM, Khondar P, et al. (2013). Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. Open Microbiol J 7:34-52.

[51] Costa SS, Viveiros M, Amaral L, Couto I. (2013). Multidrug efflux pumps in *Staphylococcus aureus*: an update. Open Microbiol J 7:59-71.

[52] Truong-Bolduc QC, Dunman PM, Strahilevitz J, Projan SJ, Hooper DC. (2005). MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. J Bacteriol 187:2395-2405.

[53] Jonas BM, Murray BE, Weinstock GM. (2001). Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob Agents Chemother45:3574-3579.

[54] Lubelski J, Konings WN, Driessen AJ. (2007). Distribution and physiology of ABC-typetransporters contributing to multidrug resistance in bacteria. MicrobiolMolBiol Rev 71:463-476.

[55] Putman M, van Veen HW, Konings WN. (2000). Molecular properties of bacterial multidrugtransporters. MicrobiolMolBiol Rev 64:672-693.

[56] Kuroda T, Tsuchiya T. (2009). Multidrug efflux transporters in the MATE family. BiochimBiophysActa., 1794:763-768.

[57] Rouquette-Loughlin C, Dunham SA, Kuhn M, Balthazar JT, Shafer WM. (2003). The NorM efflux pump of *Neisseria gonorrhoeae* and *Neisseria meningitidis* recognizes antimicrobial cationic compounds. J Bacteriol 185:1101-1106.

[58] Collu F, Cascella M. (2013). Multidrug resistance and efflux pumps: insights from molecular dynamics simulations. Curr Top Med Chem 13:3165-3183.

[59] Bay DC, Rommens KL, Turner RJ. (2008). Small multidrug resistance proteins: a multidrug transporter family that continues to grow. BiochimBiophysActa 1778:1814-1838.

[60] Yerushalmi H, Lebendiker M, Schuldiner S. (1995). EmrE, an *Escherichia coli* 12-kDa multidrugtransporter, exchanges toxic cations and H^+ and is soluble in organic solvents. J BiolChem270:6856-6863.

[61] Martinez JL, Sánchez MB, Martinez-Solano L, Hernandez A, Garmendia L, et al. (2009). Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS Microbiol Rev 33:430-449.

[62] Reygaert WC. (2013) Antimicrobial resistance mechanisms of *Staphylococcus aureus*. In: Microbial pathogens and strategies for combating them: science, technology and education. Spain:Formatex; p. 297-310.