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REVIEW ARTICLE

Antibiotic Resistance and Efflux Pumps

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HIGHLIGHTS

- The wide consumption of antibiotics; the over prescription of antimicrobial drugs by medical doctors; unnecessary, incorrect and inadequate self-medication by the patient and use of several antimicrobial agents either to support a healthy growth or therapeutic purposes in animals consumed as food triggered severe antibiotic resistance.
- One of the mechanism of action, which leads to antibiotic resistance, is efflux pumps.
- Understanding efflux pumps and discovering new inhibitors against these pumps could probably save the future of human beings.

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ABSTRACT

The main purpose of this manuscript is to review the resistance against antibiotics and efflux pumps, one of the mechanisms important in resistance against antibiotics. As a definition, the resistance against antibiotics is accepted as the capability of a microorganism to resist the activity of antimicrobials, which were successfully used to kill the microorganism once. Antibiotic resistance is characterized by several antibiotic susceptibility tests. The wide consumption of antibiotics; the over prescription of antimicrobial drugs by medical doctors; unnecessary, incorrect and inadequate self-medication by the patient and use of several antimicrobial agents either to support a healthy growth or therapeutic purposes in animals consumed as food triggered severe antibiotic resistance. Therefore, the resistance against antimicrobials became a considerable, wide-spread issue in all around the world and the studies have been initiated to overcome the resistance against antibiotics. There are several different mechanisms, which could lead bacteria to be resistant overtime. One of the mechanism of action, which leads to antibiotic resistance, is efflux pumps. Several efflux pump inhibitors were discovered until now, but since some of them are highly cytotoxic, they have very limited use. Understanding efflux pumps and discovering new inhibitors against these pumps could probably save the future of human beings.

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1. Introduction

Infectious diseases are diseases, which are persistent for long years and even more cause high morbidity and mortality throughout the world [1]. With an historical perspective, smallpox, tuberculosis, cholera and plague can be given as instances of diseases, which are infectious that transmitted worldwide with catastrophic effects [2]. Throughout the history, several actions were taken against these diseases and not only natural remedies, such as plants, natural paints and incenses, but also synthetic therapeutics were invented [3, 4]. By using disinfectants and antiseptic substances, some surgical infections and childbed fever (puerperal fever) have been diminished to a certain degree. However, antibiotics are known to be invented in the late 19^{th} and early 20^{th} century. Penicillin is the first antibiotic known to be invented [3]. Antibiotics are the substances either existing in natural resources, such as plants and fungi, or are produced artificially to inhibit and/or destroy the development of microorganisms. The word "antibiotics" is originated by two words from Ancient Greek, namely "anti" and "bios", which mean "against" and "life" respectively [5].

With the invention of the first antibiotic, "The Era of Antibiotics" has started and a great number of synthetic, semi-synthetic and natural antimicrobial drugs were developed and used against infectious diseases [3]. About the mid of 1900's in addition to penicillin, several new antibiotic classes were discovered [6]. These new antibiotics took great attention between the late 1960s and 1970s, even some scientists started to believe that such diseases could defeated forever. But sadly, in the beginning of 1990s, scientists realized a new challenge of an exceptional number of infectious diseases either new or raised again, although previously defeated [7]. The very first data, which clearly present the resistance to antimicrobial agents were collected by Paul Ehrlich, who is accepted as the father of modern chemotherapy [8].

There are several factors, which are the reasons of emerging resistance against antimicrobials. Probably the most remarkable factor is either the over prescription of antimicrobial drugs by medical doctors as a result of misevaluation of susceptibility tests or without any significant indications, or unnecessary, incorrect and inadequate self-medication by the patient, which may usually noncompliant with proper treatments. Other important factor regarding medication is failing in full-course therapy by discontinuing the medication right after feeling better due to a decrease in the symptoms. In addition, some common practices applied in the hospitals against nosocomial infections are also known contribute to the antimicrobial resistance too. An additional factor, which is also responsible in resistance against antibiotics is the usage of several antimicrobials either to support a healthy growth or therapeutic purposes in animals consumed as food [9].

2. Mechanism of Action in Antibiotics

Antibiotics act against bacteria in two different ways, namely as a bacteriostatic or a bactericidal agent. The meaning of being bacteriostatic or bactericidal agent seem to be very clear to microbiologists. The agents, which prevent the bacterial growth, in other words that keep bacteria in the stationary phase of growth, known as bacteriostatic, where the one kill the bacteria stated as bactericidal [10]. The reaction of antibiotics killing the bacterial cells mainly based on inhibiting some cellular functions through a target-drug reaction. The main specific targets of antibiotics are the synthesis of the cell wall, cell membrane, nucleic acid (DNA and RNA), protein and folate synthesis [11–13].

2.1. Antibiotics Targeting Cell Wall Synthesis

Bacteria are enclosed by a cell wall, which protects them from harsh and unpredictable environmental changes. Bacteria containing these structures are categorized as gram-positive and gram-negative [14]. The cell walls of gram-negative bacteria are built by a thin layer of peptidoglycan, and an outer membrane surrounds this structure too. But outer membranes are not observed in gram-positive bacteria and contain only a thicker peptidoglycan layer than the one found in the gram-negative bacteria [15, 16]. Peptidoglycan is a long sugar polymer, which presents cross-linking between glycan strands and the peptide chains projecting from the sugars form cross-links from one peptide to another

[17]. These cross-links, which support the cell wall, are formed between the D-alanyl-alanine section of peptides and glycine residues as penicillin binding proteins (PBP) are present [18].

B-lactams and the glycopeptides, cause an inhibition in synthesis of the cell walls. Key target of the β-lactams, such as cephalosporin, penicillin, monobactam and carbapenem, are the PBP. Since the β-lactam ring have similar structure with the D-alanyl D-alanine section of peptides it can easily bind to PBP, so that PBP can't be available for a new peptidoglycan synthesis. Thus the disorder in the peptidoglycan layer causes the lysis of bacteria [19]. In addition to β-lactams, glycopeptides (vancomycin, bacitracin and etc.) also prevent synthesis of the cell wall [12]. It is known that the glycopeptides bind to D-alanyl D-alanine section of peptide side chain of the precursor peptidoglycan subunit. As a result, a large antibiotic agent vancomycin inhibits forming of a bond between the PBP and D-alanyl subunit, therefore synthesis of the cell wall is also inhibited [20].

2.2. Antibiotics Targeting Cell Membrane

Polymyxins disrupt the structure of either outer or inner bacterial cell membrane by interacting with lipopolysaccharide (LPS) or phospholipids respectively. As polymyxins bind to LPS or phospholipids, they modify the membrane structure, so the membrane become more permeable. As a result, osmotic balance is disrupted, cellular molecules are leaked, respiration is inhibited and water uptake is increased, which induce cell death [21].

2.3. Antibiotics Targeting Nucleic Acid Synthesis

During processes called transcription or replication DNA separation is essential, in which bacterial DNA gyrase has an important role. It is known that this enzyme is inhibited by fluoroquinolones [22–24].

Rifampicin, one of the rifamycins, inhibits the initiation of RNA synthesis by blocking bacterial RNA polymerase [25].

As DNA gyrase and RNA polymerase inhibited, nucleic acid synthesis is blocked.

2.4. Antibiotics Targeting Protein Synthesis

Ribosomes take an important role in protein synthesis. The bacterial 70S ribosome is composed of 30S and 50S subunits [22]. Antimicrobials, which target the 30S or 50S subunit, inhibit protein biosynthesis [26, 27]. Tetracyclines and aminoglycosides are known to target 30 S, where macrolides, clindamycin, linezolid, chloramphenicol and streptogramin are targeting 50S subunit [12]. Thus, antibiotics targeting either 30S or 50S subunits inhibit protein synthesis.

2.5. Antibiotics Targeting Folic Acid Metabolism

Sulfonamides and trimethoprim inhibits different steps in folic acid metabolism [12].

3. Antibacterial Drug Resistance

As a definition, the resistance against antibiotics is accepted as the capability of a microorganism to resist the activity of antimicrobials, which were successfully used to kill the microorganism once [28]. As it was mentioned previously, the extensive use, especially the misuse of antibacterial drugs will cause antibacterial drug resistance, so that conventional treatments are failed to be successful against that resistant microorganism [29]. On the other hand, using an incomplete or a low dose of antibiotic will led to a slow selection of high level resistance to antibiotics, where the regular dose used before cannot be sufficient later [30].

Drug resistance may occur through several mechanisms, such as intrinsic resistance, mutation, enzymatic damaging of the drugs having antimicrobial properties through enzyme catalyzed reactions, modifications in the proteins that are key targets of antimicrobials, horizontal gene transfer, efflux pumps, an alteration of membrane permeability for antimicrobial agents, biofilm resistance and quorum sensing [31, 32].

As it was mentioned previously β-lactams inhibit the synthesis of the bacterial cell wall. Bacterial resistance against β-lactams can be generated due to acquiring plasmids, which encode β-lactamases. As a result, the resistance occurs through modification in porins, which are barriers for the permeability. It is known that β-lactamases cut antibiotics' β-lactam rings. Modification of the target for the drug by the production of β-lactamases and inhibiting the release of autolytic enzymes, causes lower attraction of PBP for β-lactams. Thus, this cause them to be inactive [33, 34].

Researchers produced carbapenems and cephalosporins, which are based on the β-lactams' structure. But it was observed later that carbapenemases cleaved carbapenems and extended spectrum β-lactamases (ESBL) cleaved cephalosporins [34–36]. Moreover, efflux pump overexpression such as in Pseudomonas aeruginosa and Escherichia coli with MexA-MexB-OprM and AcrA-AcrB-TolC pumps respectively caused resistance against cephalosporins and β-lactams [36]. Overexpression of these efflux pumps also causes a multidrug resistance against tetracyclines, rifamycin, oxazolidinones, chloramphenicol, macrolides and fluoroquinolones [37].

As it was mentioned previously, glycopeptides prevent transpeptidases to recognise their substrate as they bind to the peptidoglycan chain by D-alanyl-D-alanine terminal [38]. The resistance against glycopeptides is developed by changing the terminal D-alanyl-D-alanine of the peptidoglycan chain either to D-alanyl-D-lactic acid or D-alanyl D-serine. However, glycopeptides can still bind to D-alanyl-D-lactic acid and D-alanyl D-serine, but the affinity is not as much as the one against D-alanyl-D-alanine [34, 39].

Lipopeptides are antibiotics, which are targeting especially the membranes of gram-positive bacteria. It is proposed that these antibiotics are inserted into the membrane irreversibly, so that they create pores in the bacterial membrane through oligomerisation, which causes leakage of the cellular biomolecules that will disrupt bacterial homeostasis [40]. An antibacterial resistance has been proposed against lipopeptides and the mechanism of resistance is suggested to

be due to ring-opening esterases, removing fatty acyl tails by lipases or proteases [41].

Quinolones and fluoroquinolones are antibiotics, which target DNA synthesis. The resistance mechanisms against quinolones and fluoroquinolones are proposed to be grouped into three main groups, namely target-mediated, plasmid-mediated and chromosome-mediated resistance [42]. The type of resistance, which is target-mediated is the generally well-known resistance observed against quinolones and fluoroquinolones. This type of resistance is appeared as a result of alterations in DNA gyrase and topoisomerase IV, which are the target enzymes and due to the alterations in the efflux and entry of the drug [43]. Due to mutations in the enzymes, the interactions between these enzymes and quinolones were deteriorated. Extrachromosomal elements affect another type of resistance, which is plasmid-mediated. This type of resistance is end up with encoding several proteins those can block the interactions between the enzyme and quinolone, which may increase quinolone efflux or modify drug metabolism. Chromosome-mediated resistance may be observed due to overexpression of efflux pumps or under expression of porins, which lowers the quinolone concentration within the bacterial cell [42].

As it was mentioned previously, rifamycin inhibits the initiation of RNA synthesis by blocking bacterial RNA polymerase. It was shown that rifamycin tightly binds to RNA polymerase from its β-subunit. A mutation observed in a gene, namely rpoB, which is responsible to encode RNA polymerase's β-subunit causes a reduced affinity between rifamycin and the subunit itself [34].

The resistance against aminoglycosides, which target protein synthesis can be achieved by aminoglycoside-modifying enzymes [34], where the resistance for tetracycline, another antibiotic targeting 30S subunit of ribosome, is achieved by tetracycline efflux [34, 44].

Chloramphenicol and macrolides also target protein synthesis by preventing elongation of peptide chains [34]. There are several mechanisms observed in the resistance against chloramphenicol, which are mutations in the 50S subunit of ribosomes and reduction in membrane permeability [45]. Chloramphenicol efflux is also effective and mainly chloramphenicol acetyltransferases (CAT) plays an important role in chloramphenicol resistance by attaching an acetyl group to chloramphenicol, which could affect the antibiotic to bind to 50S ribosomal subunit [34]. The resistance against macrolides is observed due to peptide-mediated resistance and inducible expression of Erm methyltransferases [46]. Also, an efflux pump for macrolide, which is encoded by the mef genes are responsible in macrolide resistance too [47].

As it was mentioned previously sulfonamides and trimethoprim inhibits different steps in folic acid metabolism. It is known that bacteria need to synthesise folic acid to grow. They convert folic acid to tetrahydrofolate, which is required for nucleotide biosynthesis. Sulfonamides and trimethoprim block tetrahydrofolate synthesis together. Sul1 and sul2, which are drug-resistant dihydropteroate synthase genes, accepted as the reason of most sulfonamide resistance, where several dfr genes are the reason of trimethoprim resistance [34, 48].

In this section you should present the conclusion of the paper. Conclusions must focus on the novelty and exceptional results you acquired. Allow a sufficient space in the article for conclusions. Do not repeat the contents of Introduction or the Abstract. Focus on the essential things of your article.

4. Efflux Pumps

Efflux pumps are known to be transport proteins, which are active pump systems those are important in discharging of toxic substances from cells to extracellular environment. These pumps exist not only in gram-negative and gram-positive bacteria, but in eukaryotic cells too [49]. Overexpression in these pumps are accepted to be linked to a resistance against drugs [50]. Efflux pumps reduces the drug concentration without modification of the antibiotic itself [51]. A decrease in the permeability of the outer membrane cause a decrease in influx of antimicrobial agents. Therefore, this causes resistance in several important clinical microorganisms [52].

The first discovered efflux pump system was tetracycline efflux pump by Stuart Levy et al. in Escherichia coli [44, 53–55]. The tetracycline pump is a secondary active transporter that is activated by membrane proton gradient [55, 56]. This type of resistance is controlled by plasmids or chromosomes [57].

Efflux pumps are the resistance mechanisms used in several bacteria for some antibiotics from different classes, such as tetracyclines, β-lactams, macrolides, aminoglycosides, streptogramins, lincosamides, phenicols, oxazolidinones, pyrimidines, quinolones, rifamycins, sulphonamides and cationic peptides [58].

For example, the multidrug efflux pump NorA that was firstly recognised in Staphylococcus aureus isolates, which are fluoroquinolone-resistant, isolated from a hospital in Japan in 1986 and is known to export several antimicrobials, such as acriflavine, benzalkonium chloride, tetraphenylphosphonium bromide, cetrimide, ethidium bromide and fluoroquinolones [59–65]. With the same mechanism Escherichia coli exhibited resistance against the hydrophilic quinolone norfloxacin [65, 66].

Tetracycline resistance is achieved by several types of tetracycline resistance genes, such as tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO, tetS, tetA(P), tetQ and tetX, which are exist in gram-negative and gram-positive bacteria [67, 68]. MarRAB, another tetracycline resistance related operon was observed to be widely distributed among enteric bacteria, such as Salmonella, Shigella and *Escherichia coli* [69].

Although the resistance to a range of antimicrobial agents in gram-negative bacteria was previously attributed to their outer membrane structure and function [70], today it is clear that efflux pumps have a vital position for antimicrobial resistance in these microorganisms [71–73].

Efflux pumps are known to be specialised for merely to one compound or lead resistance to a wide-ranging chemicals, for example antibiotics, antimicrobial peptides, biocides, detergents, cancer chemotherapeutic agents, colourants and

heavy metals by discharging them from the bacterial cell, which could lead to a multiple drug resistance (MDR) [74, 75].

The efflux pump mechanisms are triggered by mutations in the regulatory genes or environmental signals and this requires energy [75, 76]. The resistant cells use ATP-driven transporters and/or proton-driven antiporters to discharge toxic compounds, which can generally flow into the cell by passive diffusion [77].

One of the reasons that cause bacterial resistance is the low concentration of antibiotics in the cell, which may arise the probability of resistance mutations [78]. There are two major types of mechanisms that cause low antibiotic concentration in the cell, which are due to the efflux pumps and modifications in the surfaces of the cells such as reduction in the number of the entry channels, like porins, namely, adaptive and mutational types of resistance. These two factors have a great importance in the acceleration of the resistance against antimicrobials in microorganisms, which are pathogenic [79].

The influx and efflux of endogenous or exogenous compounds are regulated by the membrane transporter proteins [80]. Approximately 5-10% of all bacterial genes are related to the transport, where a majority of these genes code efflux pumps [81–83].

4.1. The Classification of Efflux Pump Systems

Efflux proteins defined until now have been classified into five different superfamilies: Major Facilitator (MF), Multidrug and Toxic Compound Extrusion (MATE), Resistance-Nodulation-Division (RND), Small Multidrug Resistance (SMR) and ATP Binding Cassette (ABC) [84].

4.1.1. Major Facilitator (MF) Superfamily

Major facilitator superfamily (MFS) is one of the two largest families of membrane transporter proteins [85]. MF transmitters contain approximately 400 amino acids [77]. Typically MFS permeases have either 12 or 14 transmembrane α-helical segment [85] with a large cytoplasmic loop between helices six and seven. The MFS and ATP-binding cassette (ABC) superfamily [86–89] are two superfamilies, which are found universally in all living organisms. They regulate uniport, symport and antiport processes [90–95]. MFS transport sugar [96, 97], drugs and Krebs cycle metabolites [93, 98]. This type of efflux pumps transport aminoglycosides, tetracyclines, rifampicin, fluoroquinolone, macrolides, chloramphenicols, lincosamides and pristinamycins to the outside of the cell [99].

4.1.2. Multidrug and Toxic Compound Extrusion (MATE) Superfamily

MATE transporters have quite similar size to MFS transporters, which contain approximately 450 amino acids and have 12 α-helical segment [77]. Firstly they defined as a bacterial drug transporter family, but today it is known that they are found nearly in all eukaryotes and prokaryotes [100]. MATE family cause multidrug resistance by carrying wide-ranging therapeutic compounds across the membrane [101].

4.1.3. Resistance-Nodulation-Division (RND) Superfamily

Resistance-Nodulation-Division (RND) transporters have larger size than MFS transporters, which contain approximately 1000 amino acids and have 12 α-helical segment [77]. RND pumps are key factors for resistance against multidrug especially in gram-negative bacteria [84].

The first inhibitor discovered, which inhibits efflux pumps of RND types was phenylalanine-arginine β-naphthylamide (PAβN) [102–104]. These types of efflux pumps transport β-lactams, fucidic acid and sulphonamide to the outside of the cell [99].

4.1.4. Small Multidrug Resistance (SMR) Superfamily

SMR protein family is composed of proteins, which are bacterial multidrug transporters. As their name implies they are small proteins, which contain about 100 to 140 amino acids and have 4 transmembrane α-helical segment. The best defined SMR pump is EmrE, which exists in *E coli* that contributes resistance against EtBr (Ethidium Bromide) and methyl viologen [103]. This type of efflux pumps transport tetracycline, sulfadiazine and erythromycin to the outside of the cell [99].

4.1.5. ATP Binding Cassette (ABC) Superfamily

ABC type efflux pumps composed of proteins, which uses substrates, such as various drugs, xenobiotics (including dietary toxins and drugs) and endogenous compounds to transport them through the membranes [105]. While ABC superfamilies of membrane transporters are pumping their substrates through cell membrane, since they are primary active transporters, they supply the energy required for transportation from the hydrolysis of ATP [77, 106]. As it is true for microorganisms, ABC efflux transporters, which facilitate transportation of both endogenous and exogenous compounds through membranes are commonly expressed in membranes of several organs of the human body, such as testis, mammary gland, uterus, placenta, lungs, heart, brain, intestine, kidney and liver too [107]. Some important members of ABC superfamily, such as breast cancer resistance protein (BCRP), multidrug resistance associated proteins (MRPs) and P-glycoprotein (P-gp), have an important function in detoxification and pharmacokinetics of drugs and drug metabolites promoting excretion of drugs into urine in kidneys and intestinal secretion into the bile in liver [108]. ABC proteins are expressed both in healthy cells and cancer cells. Since ABC type efflux pumps transport drugs through the membranes, they not only support the cancer cell survival, but also the cancer progression [109]. The transported compounds are either antibiotics or cancer drugs, the resistance shown against multiple drugs is known as multidrug resistance (MDR) [37, 109]. This type of efflux pumps transport aminoglycosides, tetracyclines, rifampicin, fluoroquinolone, macrolides, chloramphenicols and lincosamides to extracellular environment [99].

4.2. The Structure of Efflux Pumps

Transporters can be categorized according to the substrate specificity, the phylogenic relationship and the energy source. The primary active transporters are the drug efflux pumps, which use energy produced by the hydrolysis of ATP. They belong to ABC superfamily. The secondary active transporters, are the drug pumps, which employ the proton motive force (PMF) or sodium motive force (SMF) in

order to discharge drugs. This system work as antiporters of H+/drug or Na+/drug. Secondary active transporters belong to several families, such as MF, SMR, RND and MATE superfamily [110–113].

Structures for efflux systems present differences as a result of the bacterial cell wall type. While a single pump protein facilitates efflux in gram-positive bacteria, in gram-negative bacteria efflux is facilitated either by a single pump protein or a system of a pump composed of three protein parts [99, 114, 115]. This three-part system consists of a transport efflux pump protein located in the cell membrane; a channel protein as an outer membrane factor (OMF) or outer membrane channel (OMC) and a membrane fusion protein (MFP) that provides continuous connection between these two proteins [99, 116].

4.3. Some Bacteria Having Clinical Importance with Antibiotic Resistance

It is a well-known issue that gram-negative bacteria present more resistance compared to gram-positive [117]. Efflux systems, which cause resistance against antimicrobials were defined in various types of bacteria having clinical importance, such as *Compylobacter jejuni* (CmeABC), *Pseudomonas aeruginosa* (MexAB-OprM, Mex-CD-Oprj, MexXY-OprM), *Streptococcus pneumoniae* (PrmA), *Staphylococcus aureus* (NorA), *Escherichia coli* (AcrAB-TolC, AcrEF-TolC, EmrB, EmrD) and *Salmonella typhimurium* (AcrB) [49].

4.4. Some Efflux Pump Inhibitors

There are several compounds discovered, which could inhibit efflux pumps, known as efflux pump inhibitors, where phenyl-arginine, INF271, β-naphthylamide, carbonyl cyanide m-chlorophenyl hydrozon, bicodar (incel), reserpine, timkodar, milbemycin, verapamil, paroxetine, chlorpromazine and omeprazole can be given as examples. However, pump inhibitors with clinical importance have very limited use due to their toxicity problems [99].

Efflux pump inhibitors can be classified under several classes.

4.4.1. Peptidomimetics

As a result of the studies regarding the efflux systems acting on gram-negative resistant *Pseudomonas aeruginosa* several efflux pump inhibitors (EPI) was discovered and classified as peptidomimetics. PAβN (phenylalanine arginyl β-naphthylamide) (MC-207 110), INF271 (BLT-4) and INF55 are examples of peptidomimetic EPIs. Substrates are determined as quinolones, chloramphenicol, macrolides, carbenicillin, tetracycline and they can potentially be used in several microorganisms, such as *P. aeruginosa*, *Campylobacter jejuni*, *K. pneumoniae*, *E. aerogenes*, *E. coli* and *Acinetobacter baumannii* [118, 119]. PAβN, INF271 and INF55 affect efflux pumps such as gram-negative (RND), gram-positive (MFS) and gram-positive (MFS) respectively [99].

4.4.2. Ionophore and Proton Motive Force Uncouplers

These compounds have serious effects on the bacterial membrane energy level. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) is an example for this type of EPIs. Since it causes damage in the PMF of the membrane, it leads to cell death. There are still debates regarding the activity of CCCP, whether it acts as EPI to kill the bacteria or the alteration of in the PMF. Several ionophore and proton motive force uncouplers, for example chlorophenylhydrazone (CCCP), are accepted as extremely harmful and having high cytotoxicity. Because of that they have nearly no clinical use [118–122]. CCCP affects pumps found in gram-negative bacteria, such as MFS, RND and MATE and mycobacteria [99].

4.4.3. Alkaloids

Reserpine is an example for this type of EPIs. Reserpine is known to inhibit efflux pumps, such as Bmr and NorA, present in gram-positive bacteria. It changes the generation of the membrane PMF, which is essential for the activity of MDR efflux pumps. It can also inhibit the ABC efflux pumps, but the concentration needed to block this efflux pump was founded to be neurotoxic [116–118]. Reserpine affects pumps found in gram-positive bacteria, such as ABC and MFS [99].

4.4.4. Piperazine Derivatives

1-(1-Naphthylmethyl)-piperazine (NMP) is an example for this type of EPIs. It was shown that could reverse the MDR, especially found in E. coli, by affecting pumps of RND type [99, 118, 123].

4.4.5. Calcium Ion (Ca^+) Antagonists

These types of efflux pump inhibitors are blockers of transmembrane Ca^+ influx, which are also known as Ca^+ antagonists. Verapamil is an example for this type of EPIs. Verapamil affects pumps, such as ABC and MFS found in gram-negative bacteria [99, 118].

4.4.6. Phenothiazines

Phenothiazines proved to block several energy dependent systems in bacteria, such as the function of some MDR efflux pumps [124]. Chlorpromazine is an example for this type of EPIs. Chlorpromazine is known to affect potassium flux across the membrane in the yeast *Saccharomyces cerevisiae* and *S. aureus* [125–127]. ABC and SMR types of pumps, which are found in gram-positive bacteria are affected by chlorpromazine [99].

4.4.7. Phenylpiperidine Selective Serotonin Re-uptake Inhibitors

Phenylpiperidine selective serotonin re-uptake inhibitors are proved to inhibit the function of two *S. aureus* multidrug efflux pumps and also affect moderately the activity of the AcrAB-TolC pump in *E. coli* [127]. Paroxetine is an example for this type of EPIs. It was one of the first defined phenylpiperidine selective serotonin re-uptake inhibitor that inhibits MepA (MATE) and NorA (MFS) efflux pumps [128]. Paroxetine affects MFS and RND efflux pumps in gram-positive bacteria [99].

4.4.8. Proton Pump Inhibitors

Proton pump inhibitors, such as pantoprazole, esomeprazole and omeprazole are known to be P-glycoprotein inhibitors [129]. Omeprazole affects MFS efflux pumps in gram-positive bacteria [99].

4.4.9. Macrolide Analogs

Milbemycin is an example for this type of EPIs. Milbemycin affects ABC efflux pumps in gram-positive and gram-

negative bacteria and bricodar affects MFS and ABC efflux pumps in gram-positive and gram-negative bacteria [99].

4.4.10. Piperidine-carboxylic Acid Derivatives

Timcodar and bricodar are two examples for this type of EPIs. Both timcodar and bricodar affect MFS and ABC efflux pumps in gram-positive and gram-negative bacteria [99].

5. Conclusion

The wide consumption of antibiotics; the over prescription of antimicrobial drugs by medical doctors; unnecessary, incorrect and inadequate self-medication by the patient and use of several antimicrobial agents either to support a healthy growth or therapeutic purposes in animals consumed as food triggered severe antibiotic resistance. Therefore, the antibiotic resistance has become a major, wide-spread issue in all around the world and the studies have been initiated to overcome the resistance against antibiotics.

There are several different mechanisms, which could lead bacteria to be resistant overtime. One of the mechanism of action, which leads to antibiotic resistance is efflux pumps. Several efflux pump inhibitors were discovered until now, but since some of them are highly cytotoxic they have very limited use. Understanding efflux pumps and discovering new efflux pump inhibitors could probably save the future of human beings.

References

[1] Drexler M. *What You Need to Know about Infectious Disease. Institute of Medicine (US)*: National Academies Press (US) (2010).

[2] Epstein PR. Commentary: Pestilence and Poverty—Historical Transitions and the Great Pandemics. *American Journal of Preventive Medicine* (1992) **8**(4):263–265.

[3] Töreci K. Antibiyotik kullanimi ve direnç iliskisi [The relationship between antibiotic use and resistance]. *Flora* (2003) **8**(2):89–110.

[4] Çiftçi A, Aksoy A. Acquired Resistance Mechanisms Against Antibiotics. *Turkiye Klinikleri Journal of Veterinary Sciences-Pharmacology and Toxicology-Special Topics* (2015) **1**(2):1–10.

[5] Gökçe T. *Birinci basamak saglik kurulusuna basvuran hastalarin antibiyotik kullanimi konusundaki davranis ve bilgi düzeylerinin arastirilmasi [Research on habits and awareness levels on the antibiotic use of the patients who consult primary care health services]*. Pamukkale University.

[6] Infectious Diseases Society of America. *Bad Drugs, No Drugs*: Infectious Diseases Society of America (2010).

[7] Conly JM, Johnston BL. Where are all the new antibiotics? The new antibiotic paradox. *Canadian Journal of Infectious Diseases and Medical Microbiology* (2005) **16**(3):159–160.

[8] Jacoby G. History of Drug-Resistant Microbes. In: Mayers D, Sobel J, Ouellette M, Marchaim D, editors. *Antimicrobial Drug Resistance*: Humana Press (2009).

[9] Knobler SL, Lemon SM, Najafi M, Burroughs T. *The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment: Workshop Summary*: The National Academies Press (2003).

[10] Pankey G, Sabath L. Clinical Relevance of Bacteriostatic versus Bactericidal Activity in the Treatment of Gram-Positive Bacterial Infections. *Clinical Infectious Diseases* (2004) **38**(6):864–870.

[11] Kohanski M, Dwyer D, Collins J. How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology* (2010) **8**(6):423–435.

[12] Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology* (2017) **33**(3):300–305.

[13] Walsh C. *Antibiotics: Actions, Origins, Resistance*: ASM Press (2003).

[14] Silhavy T, Kahne D, Walker S. The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives in Biology* (2010) **2**(5):a000414-a000414.

[15] Vollmer W, Blanot D, Pedro M de. Peptidoglycan structure and architecture. *FEMS Microbiology Reviews* (2008) **32**(2):149–167.

[16] Gan L, Chen S, Jensen G. Molecular organization of Gram-negative peptidoglycan. *Proceedings of the National Academy of Sciences* (2008) **105**(48):18953–18957.

[17] Kahne D, Leimkuhler C, Lu W, Walsh C. Glycopeptide and Lipoglycopeptide Antibiotics. *Chemical Reviews* (2005) **105**(2):425–448.

[18] Reynolds P. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *European Journal of Clinical Microbiology & Infectious Diseases* (1989) **8**(11):943–950.

[19] Dzidic S, Šuškovic J, Kos B. Antibiotic Resistance Mechanisms in Bacteria: Biochemical and Genetic Aspects. *Food Technology & Biotechnology* (2008) **46**(1):11–21.

[20] Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. *The Lancet* (2006) **368**(9538):874–885.

[21] Trimble M, Mlynárcik P, Kolár, Mand Hancock, RE. Polymyxin: Alternative Mechanisms of Action and Resistance. *Cold Spring Harbor Perspectives in Medicine* (2016) **6**(10):a025288.

[22] YONEYAMA H, KATSUMATA R. Antibiotic Resistance in Bacteria and Its Future for Novel Antibiotic Development. *Bioscience, Biotechnology, and Biochemistry* (2006) **70**(5):1060–1075.

[23] Wise R. A Review of the Mechanisms of Action and Resistance of Antimicrobial Agents. *Canadian Respiratory Journal* (1999) **6**(SupplA):20A-2A.

[24] Higgins P, Fluit A, Schmitz FJ. Fluoroquinolones: Structure and Target Sites. *Current Drug Targets* (2003) **4**(2):181–190.

[25] Clancy C, Yu Y, Lewin A, Nguyen MH. Inhibition of RNA Synthesis as a Therapeutic Strategy against Aspergillus and Fusarium: Demonstration of In Vitro Synergy between Rifabutin and Amphotericin B. *Antimicrobial Agents and Chemotherapy* (1998) **42**(3):509–513.

[26] Vannuffel P, Cocito C. Mechanism of Action of Streptogramins and Macrolides. *Drugs* (1996) **51**(Supplement 1):20–30.

[27] Johnston N, Mukhtar T, Wright G. Streptogramin Antibiotics: Mode of Action and Resistance. *Current Drug Targets* (2002) **3**(4):335–344.

[28] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health* (2015) **109**(7):309–318.

[29] Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *Journal of Orthopaedic Research* (2017) **36**(1):22–32.

[30] Bhattacharjee MK. Development of Resistance to Antibiotics. In: Bhattacharjee MK, editor. *Chemistry of Antibiotics and Related Drugs*: Springer (2016).

[31] Ali J, Rafiq Q, Ratcliffe E. Antimicrobial resistance mechanisms and potential synthetic treatments. *Future Science OA* (2018) **4**(4):FSO290.

[32] Dever L, Dermody T. Mechanisms of bacterial resistance to antibiotics. *Archives of Internal Medicine* (1991) **151**(5):886–895.

[33] Heesemann J. Mechanisms of Resistance to Beta-Lactam Antibiotics. *Infection* (1993) **21**(Suppement 1):S4-S9.

[34] Carlson-Banning KM, Zechiedrich L. Antibiotic Classes and Mechanisms of Resistance. In: Highlander SK, Rodriguez-Valera F, White BA, editors. *Encyclopedia of Metagenomics, Environmental Metagenomics*: Springer (2015).

[35] Boucher H, Talbot G, Bradley J, Edwards J, Gilbert D, Rice L, et al. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clinical Infectious Diseases* (2009) **48**(1):1–12.

[36] Wilke MS, Lovering AL, Strynadka NC. ß-Lactam antibiotic resistance: a current structural perspective. *Current Opinion in Microbiology* (2005) **8**(5):525–533.

[37] Nikaido H. Multidrug Resistance in Bacteria. *Annual Review of Biochemistry* (2009) **78**(1):119–146.

[38] Pootoolal J, Neu J, Wright GD. Glycopeptide Antibiotic Resistance. *Annual Review of Pharmacology and Toxicology* (2002) **42**(1):381–408.

[39] Méndez-Álvarez S, Pérez-Hernández X, Claverie-Martín F. Glycopeptide Resistance in Enterococci. *International Microbiology* (2000) **3**(2):71–80.

[40] Beiras-Fernandez A, Vogt F, Sodian R, Weis F. Daptomycin: a novel lipopeptide antibiotic against Gram-positive pathogens. *Infection and Drug Resistance* (2010) **3**:95–101.

[41] D'Costa V, Mukhtar T, Patel T, Koteva K, Waglechner N, Hughes D, et al. Inactivation of the Lipopeptide Antibiotic Daptomycin by Hydrolytic Mechanisms. *Antimicrobial Agents and Chemotherapy* (2012) **56**(2):757–764.

[42] Aldred K, Kerns R, Osheroff N. Mechanism of Quinolone Action and Resistance. *Biochemistry* (2014) **53**(10):1565–1574.

[43] Jacoby G. Mechanisms of Resistance to Quinolones. *Clinical Infectious Diseases* (2005) **41**(Supplement_2):S120-S126.

[44] Nelson M, Levy S. The history of the tetracyclines. *Annals of the New York Academy of Sciences* (2011) **1241**(1):17–32.

[45] Das B, Patra S. Nanostructures for Antimicrobial Therapy. In: Anton F, Grumezescu A, editors. *Antimicrobials: Meeting the Challenges of Antibiotic Resistance through Nanotechnology*: Elsevier (2017).

[46] Gaynor M, Mankin A. Macrolide Antibiotics: Binding Site, Mechanism of Action, Resistance. *Frontiers in Medicinal Chemistry - Online* (2005) **2**(1):21–35.

[47] Schlünzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, et al. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* (2001) **413**(6858):814–821.

[48] Sköld O. Resistance to trimethoprim and sulfonamides. *Veterinary Research* (2001) **32**(3/4):261–273.

[49] Webber MA, Piddock LJV. The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy* (2003) **51**(1):9–11.

[50] Sun, Jand Deng, Zand Yan, A. Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochemical and Biophysical Research Communications* (2014) **453**(2):254–267.

[51] Fraqueza MJ. Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. *International Journal of Food Microbiology* (2015) **212**:76–88.

[52] Denyer S, Maillard J. Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. *Journal of Applied Microbiology* (2002) **92**(s1):35S-45S.

[53] McMurry L, Petrucci RE, Levy SB. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in Escherichia coli. *Proceedings of the National Academy of Sciences* (1980) **77**(7):3974–3977.

[54] McMurry L, Levy S. Two Transport Systems for Tetracycline in Sensitive Escherichia coli: Critical Role for an Initial Rapid Uptake System Insensitive to Energy Inhibitors. *Antimicrobial Agents and Chemotherapy* (1978) **14**(2):201–209.

[55] Kumar S, Varela MF. Molecular mechanisms of bacterial resistance to antimicrobial agents. In: Méndez-Vilas A, editor. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*: Formatex Research Center (2013). p. 522–534.

[56] McMurry LM, Cullinane JC, Petrucci RE, Levy SB. Active uptake of tetracycline by membrane vesicles from susceptible Escherichia coli. *Antimicrobial Agents and Chemotherapy* (1981) **20**(3):307–313.

[57] Balassiano IT, Bastos M, Madureira DJ, Silva I, Freitas-Almeida Â, Oliveira S. The involvement of tetA and tetE tetracycline resistance genes in plasmid and chromosomal resistance of Aeromonas in Brazilian strains. *Memórias do Instituto Oswaldo Cruz* (2007) **102**(7):861–866.

[58] Sikri N, Dalal S, Taneja R. Efflux Pumps: An Overview. *International Journal of Pharmaceutical Sciences and Research* (2018) **9**(3):854–861.

[59] Deng X, Sun F, Ji Q, Liang H, Missiakas D, Lan L, et al. Expression of Multidrug Resistance Efflux Pump Gene norA Is Iron Responsive in Staphylococcus aureus. *Journal of Bacteriology* (2012) **194**(7):1753–1762.

[60] Costa SS, Viveiros M, Amaral L, Couto I. Multidrug Efflux Pumps in Staphylococcus aureus: an Update. *The Open Microbiology Journal* (2013) **7**(1):59–71.

[61] Ubukata K, Itoh-Yamashita N, Konno M. Cloning and expression of the norA gene for fluoroquinolone resistance in Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy* (1989) **33**(9):1535–1539.

[62] Hsieh P, Siegel S, Rogers B, Davis D, Lewis K. Bacteria lacking a multidrug pump: A sensitive tool for drug discovery. *Proceedings of the National Academy of Sciences* (1998) **95**(12):6602–6606.

[63] Kaatz G, Seo S. Inducible NorA-mediated multidrug resistance in Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy* (1995) **39**(12):2650–2655.

[64] Neyfakh AA, Borsch CM, Kaatz GW. Fluoroquinolone resistance protein NorA of Staphylococcus aureus is a multidrug efflux transporter. *Antimicrobial Agents and Chemotherapy* (1993) **37**(1):128–129.

[65] Ng EY, Trucksis M, Hooper DC. Quinolone resistance mediated by norA: physiologic characterization and relationship to flqB, a quinolone resistance locus on the Staphylococcus aureus chromosome. *Antimicrobial Agents and Chemotherapy* (1994) **38**(6):1345–1355.

[66] Yu J, Grinius L, Hooper D. NorA Functions as a Multidrug Efflux Protein in both Cytoplasmic Membrane Vesicles and Reconstituted Proteoliposomes. *Journal of Bacteriology* (2002) **184**(5):1370–1377.

[67] Bryan A, Shapir N, Sadowsky M. Frequency and Distribution of Tetracycline Resistance Genes in Genetically Diverse, Nonselected, and Nonclinical Escherichia coli Strains Isolated from Diverse Human and Animal Sources. *Applied and Environmental Microbiology* (2004) **70**(4):2503–2507.

[68] Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews* (1996) **19**(1):1–24.

[69] Cohen S, Yan W, Levy S. A Multidrug Resistance Regulatory Chromosomal Locus Is Widespread among Enteric Bacteria. *Journal of Infectious Diseases* (1993) **168**(2):484–488.

[70] Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. *Antimicrobial Agents and Chemotherapy* (1989) **33**(11):1831–1836.

[71] Ma D, Cook D, Hearst J, Nikaido H. Efflux pumps and drug resistance in Gram-negative bacteria. *Trends in Microbiology* (1994) **2**(12):489–493.

[72] Poole K, Krebes K, McNally C, Neshat S. Multiple antibiotic resistance in Pseudomonas aeruginosa: evidence for involvement of an efflux operon. *Journal of Bacteriology* (1993) **175**(22):7363–7372.

[73] Zhao Q, Li X-Z, Srikumar R, Poole K. Contribution of Outer Membrane Efflux Protein OprM to Antibiotic Resistance in Pseudomonas aeruginosa Independent of MexAB. *Antimicrobial Agents and Chemotherapy* (1998) **42**(7):1682–1688.

[74] Anes J, McCusker M, Fanning S, Martins M. The ins and outs of RND efflux pumps in Escherichia coli. *Frontiers in Microbiology* (2015) **6**:587.

[75] Zarakolu P. Mikroorganizmalarda direnç mekanizmasi olarak aktif pompa sistemleri [Active Pump Systems as Resistance Mechanism in Microorganisms]. *Hastane Enfeksiyonlari Dergisi [Turkish Journal of Hospital Infections]* (2003) **7**(3):131–136.

[76] Blanco P, Hernando-Amado S, Reales-Calderon J, Corona F, Lira F, Alcalde-Rico M, et al. Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants. *Microorganisms* (2016) **4**(1):14.

[77] BORGES-WALMSLEY MI, McKEEGAN K, WALMSLEY A. Structure and function of efflux pumps that confer resistance to drugs. *Biochemical Journal* (2003) **376**(2):313–338.

[78] Sandegren L. Selection of antibiotic resistance at very low antibiotic concentrations. *Upsala Journal of Medical Sciences* (2014) **119**(2):103–107.

[79] Fernández L, Hancock R. Adaptive and Mutational Resistance: Role of Porins and Efflux Pumps in Drug Resistance. *Clinical Microbiology Reviews* (2012) **25**(4):661–681.

[80] Girardin F. Membrane Transporter Proteins: A Challenge for CNS Drug Development. *Dialogues in Clinical Neuroscience* (2006) **8**(3):311–321.

[81] Piddock L. Clinically Relevant Chromosomally Encoded Multidrug Resistance Efflux Pumps in Bacteria. *Clinical Microbiology Reviews* (2006) **19**(2):382–402.

[82] Lomovskaya O, Warren M, Lee A, Galazzo J, Fronko R, Lee M, et al. Identification and Characterization of Inhibitors of Multidrug Resistance Efflux Pumps in Pseudomonas aeruginosa: Novel Agents for Combination Therapy. *Antimicrobial Agents and Chemotherapy* (2001) **45**(1):105–116.

[83] Saier, Jr, Milton H, Paulsen IT. Phylogeny of multidrug transporters. *Seminars in Cell & Developmental Biology* (2001) **12**(3):205–213.

[84] Fernando D, Kumar A. Resistance-Nodulation-Division Multidrug Efflux Pumps in Gram-Negative Bacteria: Role in Virulence. *Antibiotics* (2013) **2**(1):163–181.

[85] Pao SS, Paulsen IT, Saier MH. Major Facilitator Superfamily. *Microbiology and Molecular Biology Reviews* (1998) **62**(1):1–34.

[86] Dean M, Allikmets R. Evolution of ATP-binding cassette transporter genes. *Current Opinion in Genetics & Development* (1995) **5**(6):779–785.

[87] Fath MJ, Kolter R. ABC Transporters: Bacterial Exporters. *Microbiology and Molecular Biology Reviews* (1993) **8**(1):67–113.

[88] Higgins CF. ABC Transporters: From Microorganisms to Man. *Annual Review of Cell Biology* (1992) **8**(1):67–113.

[89] Kuan G, Dassa E, Saurin W, Hofnung M, Saier MH. Phylogenetic analyses of the ATP-binding constituents of bacterial extracytoplasmic receptor-dependent ABC-type nutrient uptake permeases. *Research in Microbiology* (1995) **146**(4):271–278.

[90] Saier Jr MH, Beatty JT, Goffeau A, Harley KT, Heijne WH, Huang SC, et al. The Major Facilitator Superfamily. *Journal of Molecular Microbiology and Biotechnology* (1999) **1**(2):257–279.

[91] Baldwin SA. Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. *Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes* (1993) **1154**(1):17–49.

[92] Goswitz VC, Brooker RJ. Structural Features of the Uniporter/Symporter/Antiporter Superfamily. *Protein Science* (1995) **4**(3):534–537.

[93] Griffith J, Baker M, Rouch D, Page M, Skurray R, Paulsen I, et al. Membrane transport proteins: implications of sequence comparisons. *Current Opinion in Cell Biology* (1992) **4**(4):684–695.

[94] Henderson P. Sugar transport proteins. *Current Opinion in Structural Biology* (1991) **1**(4):590–601.

[95] Marger MD, Saier MH. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. *Trends in Biochemical Sciences* (1993) **18**(1):13–20.

[96] Henderson P, Maiden M. Homologous Sugar Transport Proteins in Escherichia coli and Their Relatives in Both Prokaryotes and Eukaryotes. *Philosophical Transactions of the Royal Society B: Biological Sciences* (1990) **326**(1236):391–410.

[97] Maiden MC, Davis E, Baldwin S, Moore D, Henderson P. Mammalian and bacterial sugar transport proteins are homologous. *Nature* (1987) **325**(6105):641–643.

[98] Paulsen I, Skurray R. The POT family of transport proteins. *Trends in Biochemical Sciences* (1994) **19**(10):404.

[99] Aygül A. Antibiyotik Direncinde Disa Atim Sistemlerinin ve Dirençle Mücadelede Disa Atim Pompa Inhibitörlerinin Önemi [The Importance of Efflux Systems in Antibiotic Resistance and Efflux Pump Inhibitors in the Management of Resistance]. *Mikrobiyoloji Bülteni [Bulletin of Microbiology]* (2015) **49**(2):278–291.

[100] Moriyama Y, Hiasa M, Matsumoto T, Omote H. Multidrug and toxic compound extrusion (MATE)-type proteins as anchor transporters for the excretion of metabolic waste products and xenobiotics. *Xenobiotica* (2008) **38**(7-8):1107–1118.

[101] Lu M. Structures of multidrug and toxic compound extrusion transporters and their mechanistic implications. *Channels* (2016) **10**(2):88–100.

[102] Bay D, Rommens K, Turner R. Small multidrug resistance proteins: A multidrug transporter family that continues to grow. *Biochimica et Biophysica Acta (BBA) - Biomembranes* (2008) **1778**(9):1814–1838.

[103] Renau T, Léger R, Flamme E, Sangalang J, She M, Yen R, et al. Inhibitors of Efflux Pumps inPseudomonasaeruginosaPotentiate the Activity of the Fluoroquinolone Antibacterial Levofloxacin. *Journal of Medicinal Chemistry* (1999) **42**(24):4928–4931.

[104] Opperman T, St. Nguyen. Recent advances toward a molecular mechanism of efflux pump inhibition. *Frontiers in Microbiology* (2015) **6**:421.

[105] Kettenmann H, Ransom BR. The Concept of Neuroglia: A Historical Perspective. In: Kettenmann H, Ransom BR, editors. *Neuroglia*: Oxford University Press (2005). p. 1–16.

[106] Venter H, Shilling RA, Velamakanni S, Balakrishnan L, van Veen HW. An ABC transporter with a secondary-active multidrug translocator domain. *Nature* (2003) **426**(6968):866–870.

[107] Xie W. *Drug Metabolism in Diseases*: Academic Press (2016).

[108] Kara ZP, Öztürk N, Öztürk D, Okyar A. ABC Tasiyici Proteinleri: Sirkadiyan Ritimler vee Cinsiyete Bagli Farkliliklar [ABC Carrier Proteins: Circadian Rhythms and Gender Differences]. *MÜSBED* (2013) **3**(1):1–13.

[109] Begicevic RR, Falasca M. ABC Transporters in Cancer Stem Cells: Beyond Chemoresistance. *International Journal of Molecular Sciences* (2017) **18**(11):2362.

[110] van Bambeke F, Balzi E, Tulkens PM. Antibiotic efflux pumps. *Biochemical Pharmacology* (2000) **60**(4):457–470.

[111] Paulsen IT, Sliwinski MK, Nelissen B, Goffeau A, Saier MH. Unified inventory of established and putative transporters encoded within the complete genome of Saccharomyces cerevisiae. *FEBS Letters* (1998) **430**(1-2):116–125.

[112] Paulsen IT, Sliwinski MK, Saier MH. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities 1 1Edited by G. Von Heijne. *Journal of Molecular Biology* (1998) **277**(3):573–592.

[113] Lubelski J, Konings WN, Driessen AJM. Distribution and Physiology of ABC-Type Transporters Contributing to Multidrug Resistance in Bacteria. *Microbiology and Molecular Biology Reviews* (2007) **71**(3):463–476.

[114] Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *Journal of Applied Microbiology* (2002) **92**(s1):65S-71S.

[115] Hasdemir U. Çoklu Ilaç Direncinde Bakteri Hücre Duvari Organizasyonu ve Aktif Pompa Sistemlerinin Rolü [The Role of Cell Wall Organization and Active Efflux Pump Systems in Multidrug Re-sistance of Bacteria]. *Mikrobiyoloji Bülteni [Bulletin of Microbiology]* (2007) **41**:309–327.

[116] Li XZ, Nikaido H. Efflux-Mediated Drug Resistance in Bacteria. *Drugs* (2009) **69**(12):1555–1623.

[117] Wada A, Kono M, Kawauchi S, Takagi Y, Morikawa T, Funakoshi K. Rapid Discrimination of Gram-Positive and Gram-Negative Bacteria in Liquid Samples by Using NaOH-Sodium Dodecyl Sulfate Solution and Flow Cytometry. *PLoS ONE* (2012) **7**(10):e47093.

[118] Mahamoud A, Chevalier J, Alibert-Franco S, Kern W, Pagès JM. Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy. *Journal of Antimicrobial Chemotherapy* (2007) **59**(6):1223–1229.

[119] Lomovskaya O, Bostian K. Practical applications and feasibility of efflux pump inhibitors in the clinic—A vision for applied use. *Biochemical Pharmacology* (2006) **71**(7):910–918.

[120] Pagès JM, Masi M, Barbe J. Inhibitors of efflux pumps in Gram-negative bacteria. *Trends in Molecular Medicine* (2005) **11**(8):382–389.

[121] Mallea M, Chevalier J, Bornet C, Eyraud A, Davin-Regli A, Bollet C, et al. Porin alteration and active efflux: two in vivo drug resistance strategies used by Enterobacter aerogenes. *Microbiology* (1998) **144**(11):3003–3009.

[122] Thanassi DG, Cheng LW, Nikaido H. Active efflux of bile salts by Escherichia coli. *Journal of Bacteriology* (1997) **179**(8):2512–2518.

[123] Kern W, Steinke P, Schumacher A, Schuster S, Baum H, Bohnert J. Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of Escherichia coli. *Journal of Antimicrobial Chemotherapy* (2005) **57**(2):339–343.

[124] Amaral L, Martins A, Spengler G, Molnar J. Efflux pumps of Gram-negative bacteria: what they do, how they do it, with what and how to deal with them. *Frontiers in Pharmacology* (2014) **4**:168.

[125] Eilam Y. Membrane effects of phenothiazines in yeasts. I. Stimulation of calcium and potassium fluxes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* (1983) **733**(2):242–248.

[126] Kristiansen J, Mortensen I, Nissen B. Membrane stabilizers inhibit potassium efflux from Staphylococcus aureus strain No. U2275. *Biochimica et Biophysica Acta (BBA) - Biomembranes* (1982) **685**(3):379–382.

[127] Kaatz G, Moudgal V, Seo S, Kristiansen J. Phenothiazines and Thioxanthenes Inhibit Multidrug Efflux Pump Activity in Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy* (2003) **47**(2):719–726.

[128] Handzlik J, Matys A, Kiec-Kononowicz K. Recent Advances in Multi-Drug Resistance (MDR) Efflux Pump Inhibitors of Gram-Positive Bacteria S. aureus. *Antibiotics* (2013) **2**(1):28–45.

[129] Omar MS, Damanhuri NS, Kumolosasi E. Influences of proton pump inhibitor on Helicobacter pylori adherence to the gastrointestinal cell lines. *The Turkish Journal of Gastroenterology* (2017) **28**(1):53–59.