

Antimicrobial resistance: Mechanisms of action of antimicrobial agents

Anthony C. Liwa¹ and Hyasinta Jaka²

¹ Department of Clinical Pharmacology, Catholic University of Health and Allied Sciences, Po Box 1464, Bugando, Mwanza, Tanzania

² Department of Internal Medicine, Catholic University of Health and Allied Sciences, Po Box 1464, Bugando, Mwanza, Tanzania

Microorganisms coexist with other living organisms and exhibit the greatest genetic and metabolic activity. Microbes have evolved various mechanisms to survive pressure exerted by competitive environmental challenges. Infection is the invasion of the host by harmful microorganisms (microbial pathogens), which then multiply in close association with the host's tissues. Infections may differ in severity and may range from in apparent to fulminating.

There has been a continual battle between humans and the multitude of microbial pathogens. Antimicrobial agents are among few drugs that cure by eliminating the infective microorganisms. Development of antimicrobial agents for clinical use has been successful in targeting essential components of general areas of microbial metabolism namely: cell wall synthesis, protein synthesis, ribonucleic acid (RNA) synthesis, deoxyribonucleic acid (DNA) synthesis and intermediary metabolism.

The successful use of antimicrobial agents to inhibit and eliminate the infectious organisms has been facing challenges and difficulties because microbial pathogens are developing various forms of resistance to the drugs and as use of antimicrobial drugs increases, so do the level and complexity of the resistance. Emergence of resistance to multiple antimicrobial agents in pathogens has become an emergency public health problem as there are fewer, or sometimes no, effective antimicrobial agents available for infections caused by these pathogens. Microbial pathogens may manifest resistance to antimicrobial agents through various mechanisms such as by acquiring mutations and selection or acquiring resistance genes from other microicrobial pathogens. Patients infected with resistant pathogens often suffer treatment failure which usually have detrimental outcome especially to those critically ill patients.

Currently most widely used antimicrobial agents are subject to resistance and even some newer agents are facing the same challenge. The resistance has generally been met through the discovery of novel antimicrobial agents and by use of derivatives prepared by semisynthetic methods, which are not affected by existing resistance mechanisms. Understanding the mode of drug action and the how these resistance mechanisms work will highlight the challenges facing the chemotherapy of infectious diseases and the way to tackle these problems.

This chapter reviews the problem of resistance to antimicrobial agents by describing pharmacologic concepts and mechanism of action of selected antimicrobial agents. In particular this chapter focuses on the basis and mode of action of antimicrobial agents, mechanism of acquisition of resistance to antimicrobial agents and ways to prevent emergency and spreading of antimicrobial resistance.

Keywords: Antimicrobial agents; Microbial pathogens; Drug resistance

1. Introduction

The existence of microbial pathogens has been recognized for many years [1], therefore throughout history, there has been a continual battle to eliminate the multitude already existing and emerging pathogens that cause infectious diseases [2]. The discovery of antimicrobial agents resulted to succesful treatment and elimination of infections. Previously fatal diseases were treatable in a significant number of patients [3]. Development of antimicrobial agents for clinical use has been most successful in targeting essential components of 5 general areas of microbial metabolism: cell wall synthesis, protein synthesis, RNA synthesis, DNA synthesis and intermediary metabolism [4]. However, discovery of antimicrobial agents have been tempered by the emergence of microbial pathogens that are resistant to antimicrobial agents where by microbial pathogens have developed numerous resistance mechanisms that enable them to evade the effect of antimicrobial agents. As a result, many have become resistant to almost every available antimicrobial agents. This problem is becoming increasingly acute and it is now clear that a fundamental understanding of the mechanisms that microbial pathogens deploy in the development of resistance is essential to gain new insights into ways to combat this problem [5]. Patients infected with drug resistant microbial pathogens are likely to require hospitalization and to have a prolonged hospital stay. Resistance also compels the need for more toxic or more expensive alternative antimicrobial agents [6]. Drug resistance presents an ever increasing global public health threat that involves all major pathogens and antimicrobial agents [7]. This chapter focuses on pharmacologic basis related to mode of action and mechanisms of acquisition of resistance to antimicrobial agents.

2. Mechanism of action of antimicrobial agents

Microbial cells grow and divide, replicating repeatedly to reach the large numbers present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules. Antimicrobial agents interfere with specific processes that are essential for growth and/or division. Better understanding of how antimicrobial agents induce microbial cell death is centered on the essential microbial cell function that is inhibited by the primary drug-target interaction. Antimicrobial agents can be classified based on the cellular component or system they affect, in addition to whether they induce cell death (bactericidal agents) or merely inhibit cell growth (bacteriostatic agents) [8]. Antimicrobial agents act by targeting specific sites of microbes; therefore antimicrobial agents are often categorized according to their principal mechanism of action. Well known mechanisms of action of antimicrobial agents include; interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of intermediary metabolic pathways and disruption of the cytoplasmic membrane [2].

Table 1 Mechanism of action of antimicrobial agents

Drug target	Antimicrobial agent
Interference with cell wall Synthesis	β -lactams: penicillins, cephalosporins, carbapenems, monobactams Glycopeptides: vancomycin, teicoplanin
Inhibition of protein Synthesis	
50S ribosomal subunit:	Macrolides, chloramphenicol, clindamycin, quinupristin-dalfopristin, linezolid
30S ribosomal subunit:	Aminoglycosides, tetracyclines
Bacterial isoleucyl-tRNA synthetase:	Mupirocin
Interference with nucleic acid Synthesis	Inhibit DNA synthesis: fluoroquinolones Inhibit RNA synthesis: rifamycins
Inhibition of intermediary metabolic pathways:	Sulfonamides, trimethoprim
Disruption and increased permeability of cytoplasmic membrane:	Polymyxins, daptomycin

tRNA refers to transfer RNA.

2.1 Interference with microbial cell wall synthesis

The cell wall is an essential microbial structure responsible for the cell shape. In addition, the cell wall prevents cell lysis due to the high cytoplasmic osmotic pressure and allows the anchoring of membrane components and extracellular proteins, such as adhesins [9]. On the basis of the number of antimicrobial drugs in clinical use, bacterial cell wall synthesis has been perhaps the target area most extensively exploited for antimicrobial development. The components of the cell wall synthesis machinery are appealing antimicrobial targets because of the absence of counterparts in human biology, thereby providing intrinsic target selectivity. The sequential late steps in cell wall synthesis include the cytoplasmic synthesis of building blocks composed of *N*-acetyl muramic acid (M) linked to *N*-acetyl glucosamine (G) with an attached pentapeptide (P) side chain (referred to as MGP subunits). Linkage of an MGP subunit to a lipid II molecule allows subsequent translocation across the cytoplasmic membrane to the cell exterior or periplasmic space. Transglycosylase enzymes then assemble the MGP subunits into a linear backbone by catalyzing glycosidic linkages between the M and G components of the MGP subunits. Linearly linked MGP subunits constitute an immature peptidoglycan structure. Transpeptidase enzymes then act to cross-link the peptide side chains with pentaglycine bridges, cleaving the terminal 2 D-alanines of the peptide side chain in the process, thereby producing the mature, lattice-like peptidoglycan that provides the bacterium with its shape and osmotic stability [4].

The most commonly used antimicrobial agents that inhibit cell wall biosynthesis include β -lactam antibiotics such as penicillins and cephalosporins [10]. These β -lactam antibiotics interact and efficiently inhibit the bacterial transpeptidases directly. The β -lactams as transpeptidase inhibitors thus block the conversion of immature to mature peptidoglycan therefore these enzymes are often termed penicillin-binding proteins (PBPs). They are able to do this owing to the stereochemical similarity of the β -lactam moiety with the D-alanine–D-alanine substrate. In the presence of the drug, the transpeptidases form a lethal covalent penicilloyl enzyme complex that serves to block the normal transpeptidation reaction. This results in weakly cross-linked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death [11].

The other class of antimicrobial agents that inhibit cell wall synthesis is glycopeptides. The mechanism of action common to all members of the glycopeptide class, including vancomycin, is the inhibition of peptidoglycan synthesis. Vancomycin and other glycopeptides bind to the carboxyl terminal acyl-D-alanyl-D-alanine (acyl-D-Ala-D-Ala) residues of the pentapeptide moiety of lipid II. This specific binding, which is referred to as the primary binding site, sterically hinders the transglycosylase enzyme from incorporating the disaccharide-pentapeptide monomer into nascent peptidoglycan. This inhibition of peptidoglycan synthesis by vancomycin results in a relatively slow inhibition compared with β -lactams, and its bactericidal activity does not depend on concentration. Vancomycin forms a stoichiometric 1:1 complex with the peptidoglycan precursor UDP-N-acetylmuramylpentapeptide by forming hydrogen bonds. The transglycosylase enzyme that transfers the disaccharide of the peptidoglycan precursor to the growing glycan polymer of the cell wall peptidoglycan is inhibited, presumably due to the steric bulkiness of the glycopeptide peptidoglycan precursor. Both the transglycosylase and transpeptidase enzyme reactions that complete the synthesis of the rigid cell wall peptidoglycan are inhibited by the glycopeptides [12].

2.2 Inhibition of microbial protein synthesis

Several classes of antimicrobial agents act by inhibiting bacterial protein synthesis (ribosome function). These include aminoglycosides, macrolides, tetracyclines, lincosamides, ketolides, streptogramins, oxazolidinones and chloramphenicol [4,13]. Microbial protein synthesis is directed by ribosomes in conjunction with cytoplasmic factors, which transiently bind to particles during the initiation phase, elongation phase and termination phases. Microbial ribosomes contain 70S particles comprising two subunits, of 50S and 30S, which join at the initiation step of protein synthesis and separate at the termination step. Antimicrobial agents block different steps in bacterial protein synthesis by interfering with the function of either the cytoplasmic factors or the ribosomes. Inhibitors that bind to the 30S ribosomal subunit interfere primarily with initiation, although some interfere with pairing of the mRNA codon with the AA-tRNA anticodon, so impairing elongation. Inhibitors that bind to the 50S ribosomal subunit or to the elongation factors, which are transiently linked to ribosomes at certain steps of the cycle, interfere with steps involved in the elongation process [14].

Aminoglycosides act by binding to specific ribosomal subunits. The aminoglycoside-type drugs can combine with other binding sites on 30S ribosomes and they kill bacteria by inducing the formation of aberrant, non-functional complexes as well as by causing misreading. Spectinomycin is an aminocyclitol antimicrobial agent that is closely related to the aminoglycosides. It binds to a different protein in the ribosome and is bacteriostatic but not bactericidal. Other agents that bind to 30S ribosomes are the tetracyclines. These agents appear to inhibit the binding of aminoacyl-tRNA into the A site of the bacteria] ribosome. Tetracycline binding is transient, so these agents are bacteriostatic. Nonetheless, they inhibit a wide variety of bacteria, *chlamydias* and *mycoplasmas* and are extremely useful agents [15].

There are three important classes of drugs that inhibit the 50S ribosomal subunit. Chloramphenicol is a bacteriostatic agent that inhibits both Gram-positive and Gram-negative bacteria. It inhibits peptide bond formation by binding to a peptidyltransferase enzyme on the 50S ribosome. Macrolides are large lactone ring compounds that bind to 50S ribosomes and appear to impair a peptidyltransferase reaction or translocation, or both. The most important macrolide is erythromycin, which inhibits Gram-positive species and a few Gram-negative species such as *haemophilus*, *mycoplasma*, *chlamydia*, and *legionella*. New molecules such as azithromycin and clarithromycin have greater activity than erythromycin against many of these pathogens. Lincinoids, of which the most important is clindamycin, have a similar site of activity. Both macrolides and lincinoids are generally bacteriostatic, inhibiting only the formation of new peptide chains [15].

2.3 Interference with nucleic acid synthesis

2.3.1 Inhibitors of DNA topoisomerases

DNA synthesis, mRNA transcription and cell division require the modulation of chromosomal supercoiling through topoisomerase-catalyzed strand breakage and rejoining reactions [8]. DNA topoisomerase enzymes are divided into two types, I and II, depending on whether they catalyze reactions involving the transient breakage of one (type I) or both (type II) strands of DNA [16]. Topoisomerases control the topological state of DNA within cells and are critical for the essential processes of protein translation and cell replication [17]. DNA gyrase, a type II DNA topoisomerase, is the enzyme that negatively supercoils DNA in the presence of ATP. In addition, this enzyme plays a role in catenation and decatenation reaction of a double-stranded DNA circles, resolves knots in DNA and also relaxes negatively supercoiled DNA in the absence of ATP. As a result, the enzyme is vital for almost all cellular processes that involve duplex DNA, namely replication, recombination and transcription. It is exclusive to the prokaryotic kingdom and is essentially crucial for the survival of the organism. Thus, DNA gyrase remain an ideal and attractive target for antibacterial drugs [18].

Quinolones are the most successful antimicrobial agents targeting to DNA gyrase. The compounds originated from nalidixic acid, a naphthyridone discovered by accident as a by-product during the synthesis of chloroquine [19]. Quinolones are specific inhibitors of DNA gyrase. Quinolones inhibit reactions of DNA gyrase such as supercoiling and relaxation that require DNA breakage and reunion, specifically they interfere with the breakage-re-union reaction of

DNA gyrase by interacting with subunit A (GyrA) [20]. The first-generation quinolones, nalidixic acid and oxolinic acid have relatively weak antimicrobial activity. However, the synthesis of fluoroquinolones and their improvement over several generations, e.g., norfloxacin and ciprofloxacin (second gen.), levofloxacin (third gen.) and moxifloxacin and gemifloxacin (fourth gen.), has led to a range of potent antimicrobial agents [19]. Besides the type II topoisomerases, most bacterial pathogens possess an additional essential topoisomerase, topoisomerase I (Topo I). Topo I is architecturally and mechanistically distinct from gyrase and topoisomerase IV and as such represents an attractive target for the discovery of novel antibacterial chemotypes [17].

2.3.2 Inhibitors of microbial RNA synthesis

Rifamycins inhibit DNA-dependent transcription by binding with high affinity to the β -subunit (encoded by *rpoB*) of a DNA-bound and actively transcribing RNA polymerase. The β -subunit is located in the channel that is formed by the RNA polymerase-DNA complex, from which the newly synthesized RNA strand emerges. Rifamycins uniquely require RNA synthesis to not have progressed beyond the addition of two ribonucleotides; this is attributed to the ability of the drug molecule to sterically inhibit nascent RNA strand initialization. It is worth noting that rifamycins are not thought to act by blocking the elongation step of RNA synthesis, although a recently discovered class of RNA polymerase inhibitors (based on the compound CBR703) could inhibit elongation by allosterically modifying the enzyme [8].

2.4 Inhibition of microbial metabolic pathways

Trimethoprim and sulfonamides interfere with folic acid metabolism in the microbial cell by competitively blocking the biosynthesis of tetrahydrofolate, which acts as a carrier of one-carbon fragments and is necessary for the ultimate synthesis of DNA, RNA and bacterial cell wall proteins. Unlike mammals, bacteria and protozoan parasites usually lack a transport system to take up preformed folic acid from their environment. Most of these organisms must synthesize folic acid, although some are capable of using exogenous thymidine, circumventing the need for folic acid metabolism [15].

Sulfonamides competitively inhibit the conversion of pteridine and *p*-aminobenzoic acid (PABA) to dihydrofolic acid by the enzyme pteridine synthetase. Sulfonamides have a greater affinity than PABA for pteridine synthetase. Trimethoprim has a tremendous affinity for bacterial dihydrofolate reductase (10,000 to 100,000 times higher than for the mammalian enzyme); when bound to this enzyme, it inhibits the synthesis of tetrahydrofolate [15].

2.5 Disruption and increased permeability of cytoplasmic membrane

Biologic membranes are composed basically of lipids, proteins and lipoproteins. The cytoplasmic membrane acts as a diffusion barrier for water, ions, nutrients and transport systems. Most health workers now believe that membranes are a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bilayer. A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic, and neutral agents. The best-known compounds are polymyxin B and colistimethate (polymyxin E) [15].

Cytoplasmic membrane forms an effective barrier to many antimicrobial agents. The mode of action of some antimicrobial agents may lie in the ability of certain drugs to increase the permeability of the membrane, facilitating the entry of themselves and other compounds. Cationic antibacterial agents, such as polymyxin B have been reported to increase the permeability of the outer membrane to lysozyme and hydrophobic compounds. The initial action of these antimicrobial agents is to disrupt outer membrane structure, allowing themselves and other compounds to enter the cell and inhibit specific metabolic processes [21]. Polymyxin B has several cell-damaging properties: (i) it disturbs the surface charge, lipid composition and structure of the membranes; (ii) it dissipates the K^+ gradient on the cytoplasmic membrane; and (iii) it depolarizes the cytoplasmic membrane. The permeability of the outer membrane to lipophilic compounds is one of the main factors controlling bacterial sensitivity to polymyxin B. Since polymyxin B is bulkier than the inorganic divalent cations it displaces, the packing order of lipopolysaccharide (LPS) is altered in the presence of polymyxin B. This results in increased permeability of the outer membrane to a variety of molecules and also facilitates the uptake of polymyxin B ("self-promoted" uptake) [22].

3. Mechanism of acquisition of resistance to antimicrobial agents

Resistance to antimicrobial agents has become important in clinical management and control of many diseases and deserves scientific intervention to bring about some control measures [23]. Microbes are known of its versatility towards drugs; however, they have a limited number of mechanisms of acquired antimicrobial resistance [6]. The main mechanisms for survival of a threatened microbial population are genetic mutation, expression of a latent resistance gene and acquisition of genes with resistance determinants [24]. Microbial pathogens have evolved genetic and biochemical ways of resisting antimicrobial agents. Microbial pathogens may be innate resistant or may acquire resistance to one or few classes of antimicrobial agents. The main genetic mechanisms leading to antimicrobial resistance are genetic mutation (single point mutations or major deletions or rearrangements), expression of a latent

resistance gene and acquisition of genes or DNA segments with resistance determinants. Some of the genes are inherited, some emerge through random mutations in microbial DNA and some are imported from other organisms. These genetic changes code for changes in binding proteins (a), ribosomes (b) membrane structure (c) or inactivating enzymes. After a pathogen gains resistance genes to protect itself from various antimicrobial agents, pathogens use several biochemical types of resistance mechanisms [25]. Pathogens resist antimicrobial agents biochemically by inactivating the drugs with β -lactamases, acetylases, adenylases and phosphorylases; reducing drug access sites of action by virtue of membrane characteristics; altering the drug target so that the antimicrobials no longer binds to it; by passing the drug's metabolism; and develop tolerance [26]. The mechanism behind the development and spread of resistance to antimicrobial agents are reviewed in the following section.

Table 2 Mechanism of acquisition of resistance to antimicrobial agents

Antimicrobial class	Mechanism of resistance
Target modification	
Aminoglycosides	Altered ribosomal protein
β -lactam antibiotics	Altered or new penicillin-binding proteins
Erythromycin, clindamycin and Streptogramin B	Methylation of the bacteria ribosome producing resistance.
Quinolones	Alterations in DNA topoisomerase
Rifampin	Altered RNA polymerase
Sulfonamides	New drug-insensitive dihydropteroate synthase
Tetracycline	Ribosomal protection
Trimethoprim	New drug-insensitive dihydrofolate reductase
Vancomycin	Altered cell-wall precursors with decreased affinity
Detoxifying enzymes	
Aminoglycosides	Aminoglycoside-modifying enzymes: acetyltransferase, nucleotidyl-transferase, phosphotransferase
β -lactam antibiotics	β -lactamases
Chloramphenicol	Acetyltransferase
Trimethoprim-sulfamethoxazole	Resistant enzymes in folate-synthesis pathway
Decreased drug uptake	
Diminished permeability; β -lactam antibiotics, chloramphenicol, quinolone, tetracycline, trimethoprim	Alteration in outer-membrane proteins
Active efflux; erythromycin, tetracycline	New membrane transport system

3.1 Drug resistance by target site modification

An interaction between an antimicrobial agent and a target molecule is very specific so even small changes in a target molecule can influence antibiotic binding to a target [25]. Antimicrobial agents act at targets that are present in microbial cells but differ sufficiently to mammalian cells to allow for selective inhibition of the bacterial counterparts. Because of the vital cellular functions of the target sites, organisms cannot evade antimicrobial action by dispensing with them entirely. However, microbes have been acquiring some mutational changes in the target that reduce susceptibility to inhibition whilst retaining their cellular function [27]. Examples of resistance due to target alterations are discussed below.

3.1.1 Alterations of penicillin binding proteins

Mosaic PBPs in Streptococcus pneumoniae; Resistance to β -lactam antimicrobial agents in *S. pneumoniae* is due to the development of penicillin binding proteins (PBPs) with decreased affinity for the drugs. Resistance to third-generation cephalosporins is due to the presence of altered forms of PBP1a and 2x, whereas penicillin resistance also involves alterations in PBP2b [28, 29, 30]. The altered PBPs are generated by recombinational events between the PBP genes of *S. pneumoniae* and related PBP genes from closely related streptococcal species acquired by transformation of this naturally competent organism [31].

Altered PBPs in *Neisseria gonorrhoeae*; *N. gonorrhoeae* has four PBPs, designated PBP 1, 2, 3 and 4. Of these, only PBP1 and 2 are essential for cell viability and are, thus, potential antibiotic killing targets in *N. gonorrhoeae*. Because penicillin G has an approximately 10-fold higher rate of acylation of PBP 2 than of PBP 1, it kills *N. gonorrhoeae* at its MIC by inactivation of PBP 2. Penicillin-resistant strains of *N. gonorrhoeae* are capable of transferring their resistance genes to susceptible strains via transformation and homologous recombination. Susceptible strains of *N. gonorrhoeae*

become resistant to penicillin by acquiring multiple resistance genes in a stepwise fashion. Transformation to high-level penicillin resistance is mediated by the *penA* gene, encoding altered forms of PBP 2 that display 5- to 10-fold decreases in their rate of acylation by penicillin [32].

3.1.2 Modification of peptidoglycan precursors

The cause of resistance to the glycopeptide antibacterial agents in *E. faecium* and *E. faecalis* is the acquisition of one of two related gene clusters, known as VanA and VanB. These gene clusters encode enzymes that produce a modified peptidoglycan precursor terminating in D-Alanyl-D-Lactate (D-Ala-D-Lac) instead of D-Ala-D-Ala [33]. The glycopeptides bind with much lower affinity to D-Ala-D-Lac than to D-Ala-D-Ala [34]. The low affinity binding of glycopeptides to D-Ala-D-Lac results from an altered pattern of intermolecular hydrogen bonding responsible for the normally high affinity binding between drug and its substrate [35].

3.1.3 Modification by ribosomal methylation

So far, ribosomal methylation remains the most widespread mode of resistance to macrolides and lincosamides. In pathogenic organisms, Erm proteins dimethylate a single adenine in nascent 23S rRNA, which is part of the large (50S) ribosomal subunit [36]. The A2058 residue is located within a conserved region of domain V of 23S ribosomal RNA, which plays a key role in the binding of macrolides, lincosamides and streptogramins B antimicrobials. As a consequence of methylation, binding of erythromycin to its target is impaired. The overlapping binding sites of macrolides, lincosamides and streptogramins B in 23S rRNA, account for cross-resistance to the 3 classes of drugs. A wide range of microbial pathogens that are targets for macrolides and lincosamides, including gram-positive species, spirochetes and anaerobes, express Erm methylases. Nearly 40 *erm* genes have been reported so far [37].

3.1.4 Interference with DNA synthesis

The mechanism of resistance is a modification of two enzymes: DNA gyrase (also known as topoisomerase II) (genes *gyrA* and *gyrB*) [38] and topoisomerase IV (*parC* and *parE*). Mutations in genes *gyrA* and *parC* are followed by replication failure, and then quinolones/fluoroquinolones cannot bind. The most common mutation in *E. coli gyrA* causes a reduced drug affinity for modified-DNA complex, and MIC is higher. Quinolones (ciprofloxacin) bind to DNA gyrase A subunit. Usually resistance to quinolones is associated with mutations in chromosomes, but plasmid-mediated and point mutation-related (in genes *gyrA* and *parC*) resistance to quinolones was reported as well [25].

3.2 Drug inactivation

Inherent to this mechanism of resistance, and unlike other mechanisms, inactivation of antimicrobial agents confers resistance to structurally related drugs. The major enzymes that inactivate antimicrobial agents include; β -lactamases, aminoglycoside-modifying enzymes and chloramphenicol acetyltransferases [25].

3.2.1 Antimicrobial modification by hydrolysis

β -Lactamases are broadly prevalent enzymes that are classified using two main classification systems: Ambler and Bush-Jacoby-Medeiros [39]. It is known about 300 different β -lactamases. The most clinically important resistance are produced by gram negative organisms [40] and are coded on chromosomes and plasmids. Genes that encode β -lactamases are transferred by transposons but also they may be found in the composition of integrons [41]. β -Lactamases hydrolyze nearly all β -lactams that have ester and amide bond, e.g., penicillins, cephalosporins, monobactams, and carbapenems. Serine β -lactamases cephalosporinases, e.g. AmpC enzyme are found in *Enterobacter* spp. and *P. aeruginosa* and penicillases in *S. aureus*. Metallo- β -lactamases (MBLs) found in *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *Proteus mirabilis* (*P. mirabilis*), *Enterobacter* spp. have the same role as serine β -lactamases and are responsible for resistance to imipenem, new generation cephalosporins and penicillins. MBLs are resistant to inhibitors of β -lactamases but sensitive to aztreonam. Specific *A. baumannii* carbapenem-hydrolyzing oxacillinase (OXA) enzymes that have low catalytic efficiency together with porin deletion and other antibiotic resistance mechanisms can cause high resistance to carbapenems [42]. The resistance of *K. pneumoniae* carbapenamases (KPC-1) to imipenem, meropenem, amoxicillin/clavulanate, piperacillin/ tazobactam, ceftazidime, aztreonam, and ceftriaxone is associated with the non-conjugative plasmid-coded *bla* gene [43].

Extended spectrum β -lactamases (ESBL)-TEM, SHV, OXA, PER, VEB-1, BES-1, GES, IBC, SFO and CTX mainly are encoded in large plasmids. They can be transferred in connection of two plasmids or by transposon insertion. ESBL are resistant to penicillins (except temocillin), third-generation oxyiminocephalosporins (e.g., ceftazidime, cefotaxime, ceftriaxone), aztreonam, cefamandole, cefoperazone, but they are sensitive to methoxycephalosporins, e.g., cephamycins and carbapenems, and can be inhibited by inhibitors of β -lactamases, e.g., clavulanic acid, sulbactam, or tazobactam [42]. Strains producing ESBL are commonly resistant to quinolones but their resistance depends not on multiple resistance plasmids but on mutations in *gyrA* and *parC* genes. Such strains are found among *E. coli*, *K.*

pneumoniae, and *P. mirabilis* [44]. The number of known ESBLs reaches 200 [45]. Hydrolysis of antimicrobials can be run by other enzymes, e.g., esterases. *E. coli* gene *ereB* encodes erythromycin esterase II that hydrolyzes a lactone ring of erythromycin A and oleandomycin. *ereB* gene is prevalent in *Enterobacteriaceae* strain and is responsible for resistance to erythromycin A and oleandomycin. Ring-opening epoxidases cause resistance of bacteria to fosfomycin [44].

3.2.2 Drug inactivation by group transfer

The group of enzymes inactivating aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin is called transferases. Inactivation is made by binding adenylyl, phosphoryl, or acetyl groups to the periphery of the drug molecule. These modifications are achieved in the process of transport across the cytoplasmic membrane (co-substrate ATP, acetyl-CoA, NAD⁺, UDP-glucose, or glutathione) [44]. Aminoglycosides are neutralized by specific enzymes: phosphoryltransferases (APHs), nucleotidyltransferases or adenylyltransferases (ANTs), and acetyltransferases (AACs). These aminoglycoside modifying enzymes (AMEs) reduce affinity of a modified molecule, impede binding to the 30S ribosomal subunit and provide extended spectrum resistance to aminoglycosides and fluoroquinolones [46]. AMEs are identified in *S. aureus*, *Enterococcus faecalis* (*E. faecalis*), and *S. pneumoniae* strains. Presumably, they evolved from actinomycetes (*Streptomyces* spp. and *Micromonospora* spp.) that produce AMEs. Most AMEs are transferred by transposons [47].

Gram-positive and gram-negative bacteria and some of *H. influenzae* strains are resistant to chloramphenicol and they have an enzyme chloramphenicol transacetylase that acetylates hydroxyl groups of chloramphenicol. Modified chloramphenicol is unable to bind to a ribosomal 50S subunit properly [48].

3.2.3 Drug inactivation by redox process

Oxidation and reduction reactions are used by pathogenic organisms as a resistance mechanism against antimicrobials. *Streptomyces virginiae* produces type A antibiotic virginiamycin M1 and protects itself from its own antimicrobial agents by substituting a ketone group to an alcohol residue at position 16 [44].

3.3 Ribosome protection

The mode of action of macrolide, lincosamide and streptogramin B group of antibiotics is to block protein synthesis in bacteria by binding to the 50S ribosomal subunit [49]. Resistance to these drugs is referred to as MLS(B) type resistance and occurs in a wide range of Gram-positive and Gram-negative bacteria [50]. Resistance results from a post-transcriptional modification of the 23S rRNA component of the 50S ribosomal subunit involving methylation or dimethylation of key adenine bases in the peptidyl transferase functional domain. E.g. in *E. coli*, base A2058 of the 23S rRNA is the target of methylation. Methylation is catalyzed by adenine-specific *N*-methyltransferases specified by the *erm* class of genes (erythromycin ribosome methylation), present in a wide range of organisms and frequently plasmid-encoded. Mutations in 23S rRNA close to the sites of methylation have also been associated with resistance to the macrolides in a range of organisms [51, 52]. In addition to multiple mutations in the 23S rRNA, alterations in the L4 and L22 proteins of the 50S subunit have been reported in macrolide-resistant *S. pneumoniae* [53].

3.4 Reduced membrane permeability

The outer membrane in gram-negative organisms contains an inner layer that has phospholipids and an outer layer that has the lipid A. Such composition reduces drug uptake to a cell and transfer through the outer membrane (through porin proteins, e.g., OmpF in *E. coli* and OprD in *P. aeruginosa*). Drug molecules to a cell can be transferred by the following mechanisms: (i) diffusion through porins, (ii) diffusion through the bilayer, and (iii) by self-promoted uptake. Acquired resistance to all antimicrobial classes in *P. aeruginosa* is due to low outer membrane permeability. Small hydrophilic molecules (β -lactams and quinolones) can cross the outer membrane only through porins. Aminoglycosides and colistin cannot be transferred to the cell through porins; therefore, self-promoted uptake to the cell is initiated by binding to lipopolysaccharides of the outer side of the outer membrane [54]. Acquired resistance is characteristic of high resistance to almost all aminoglycosides (especially to tobramycin, netilmicin, and gentamicin) [55].

3.5 Active drug efflux

Efflux pumps are energy-dependent transporters that extrude toxic compounds, including antimicrobials, being one of the major mechanisms by which microbial pathogens resist to different classes of antimicrobial agents [9]. The efflux systems are composed of three protein components, an energy-dependent pump located in the cytoplasmic membrane, an outer membrane porin and a linker protein which couples the two membrane components together [56]. Efflux pumps can be specific to antimicrobials or may be capable to pump a wide range of unrelated agents e.g. macrolides, tetracyclines and fluoroquinolones and thus significantly contribute to multidrug resistance (MDR) [25]. Active efflux of drugs from the cell is one of the common mechanisms of antimicrobials resistance in bacteria, with resistance

developing when the rate of drug efflux across the membrane exceeds that of drug influx, bacterial genomes encode several membrane-bound multidrug efflux systems. These systems are usually under the control of an intricate regulatory network, which, in response to the presence of drug and other stress molecules, increases the overall efflux activity and decreases influx capacity [57]. Microbial pathogens resistant to tetracyclines often produce increased amounts of membrane proteins that are used as export or efflux pumps of antimicrobial agents. To eliminate toxic compounds from the cytoplasm and periplasm, *P. aeruginosa* uses more than four powerful MDR efflux pumps. MexV-MexW-OprM MDR efflux pumps are responsible for resistance to fluoroquinolones, tetracyclines, chloramphenicol, erythromycin, ethidium bromide and acriflavine. Increased expression of MexAB-OprM efflux pumps results in higher inhibitory concentration against penicillins, broad-spectrum cephalosporins, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline and trimethoprim [25].

4. Control and prevention of emergency and spreading of antimicrobial resistance

Controlling microbial resistance to antimicrobial agents requires a multifaceted approach. Essential components the approach must include reducing inappropriate prescribing not only for humans but also animals, reducing transmission of resistant organisms through enhanced infection control and environmental hygiene, and identifying trends in resistance through surveillance. This approach fits neatly within the classic bug-drug-host paradigm. The overuse of antibiotics is considered the main factor in the emergence and dissemination of antimicrobial resistance. Many factors lead to inappropriate antimicrobial prescribing practices, including patient expectations and demands, desire of the physician to give the best possible treatment regardless of cost or subsequent effects, failure to consider alternative treatments, inappropriate use of diagnostic laboratory studies, inadequacy of the physician's knowledge and management of patients with infectious diseases, medico-legal considerations and the belief that the newer and broad-spectrum agents represent the most effective treatment [24]. Special vigilance must now be paid to appropriate selection and timing of antimicrobial agents as a major force in reducing the development of antimicrobial resistance. Proper hygiene practices will help to reduce plasmid transfer and the establishment of multiple drug-resistant microbial pathogens in the hospital and will delay the appearance of such species in the community. The health care provider must be continually alert to the appearance of drug resistance within the hospital and community [15].

References

- [1] Morse SS. Factors in the Emergence of Infectious Diseases. *Emerging Infectious Diseases*. 1995;1(1):7-15.
- [2] Tenover FC. Mechanisms of Antimicrobial Resistance in Bacteria. *The American Journal of Medicine* 2006, 119: S3-S10.
- [3] Bonafede M, Rice LB. Emerging antibiotic resistance. *Journal of Laboratory and Clinical Medicine*. 1997;130:558-66.
- [4] Hooper DC. Mechanisms of Action of Antimicrobials: Focus on Fluoroquinolones. *Clinical Infectious Diseases* 2001, 32(Suppl 1): S9-15.
- [5] McKeegan KS, Borges-Walmsley MI, Walmsley AR. Microbial and viral drug resistance mechanisms. *Trends in Microbiology*. 2002;10:8-14.
- [6] Jacoby GA, Archer GL. New mechanisms of bacterial resistance to antimicrobial agents. *The New England Journal of Medicine*. 1991;324(9):60-612.
- [7] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*. 2004; 10:122-129.
- [8] Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, 2010;8:423-435.
- [9] Guilhelmelli F, Vilela N, [Albuquerque P](#), [Derengowski LS](#), [Silva-Pereira S](#), Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Frontiers of Microbiology*. 2013;4:353.
- [10] Kotra LP, Mobashery S. β -lactam antibiotics, β -lactamases and bacterial resistance. *Bulletin of Institute Pasteur*. 1998; 96:139-150.
- [11] Wilke MS, Lovering AL, Strynadka NCJ. β -Lactam antibiotic resistance: a current structural perspective. *Current Opinion in Microbiology*. 2005;8:525-533.
- [12] Nagarajan R. Antibacterial Activities and Modes of Action of Vancomycin and Related Glycopeptides. *Antimicrobial Agents and Chemotherapy*. 1991;35(4):605-609.
- [13] McKee EE, Ferguson M, Bentley AT, Marks TA. Inhibition of Mammalian Mitochondrial Protein Synthesis by Oxazolidinones. *Antimicrobial Agents and Chemotherapy*, 2006, 50(6): 2042-2049.
- [14] Cocito C, Di Giambattista M, Nyssen E, Vannuffel P. Inhibition of protein synthesis by streptogramins and related antibiotics. *Journal of Antimicrobial Chemotherapy*. 1997;39:7-13 .
- [15] Neu HC, Gootz TD. Baron S, editor. *Medical Microbiology*. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
- [16] Liu LF, Liu C-C, Alberts BM. Type II DNA topoisomerases: enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. *Cell*. 1980;19:697-707.
- [17] Ehmman DE, Lahiri SD. Novel compounds targeting bacterial DNA topoisomerase/DNA gyrase. *Current Opinion in Pharmacology* 2014;18:76-83.

- [18] Chatterji, Unniraman MS, Mahadevan S, Nagaraja V. Effect of different classes of inhibitors on DNA gyrase from *Mycobacterium smegmatis*. *Journal of Antimicrobial Chemotherapy*. 2001;48:479-485.
- [19] Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Applied Microbiology and Biotechnology*. 2011;92:479-497.
- [20] Chen AY, Liu LF. DNA Topoisomerases: Essential Enzymes and Lethal Targets. *Annual Review in Pharmacology and Toxicology*. 1994;34:191-218.
- [21] Peterson JW. Baron S, editor. *Bacterial Pathogenesis. Medical Microbiology*. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
- [22] Krupovič M, Daugelavičius R, Bamford DH. Polymyxin B Induces Lysis of Marine Pseudoalteromonads. *Antimicrobial Agents and Chemotherapy*. 2007, 51(11): 3908-3914.
- [23] Soyege AO, Ajayi AO, Ngwenya E, Basson AK, Okoh AI. Vancomycin and Oxacillin Co-Resistance of Commensal Staphylococci. *Jundishapur Journal of Microbiology*. 2014;7(4):e9310.
- [24] Conly J. Antimicrobial resistance in Canada. *Canadian Medical Association Journal*. 2002;167(8):885-891.
- [25] Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic Resistance Mechanisms of Clinically Important Bacteria *Medicina (Kaunas)*. 2011;47(3):137-46.
- [26] McManus MC. Mechanisms of bacterial resistance to antimicrobial agents. *American Journal of Health-System Pharmacy*. 1997;54 (12):1420-1433.
- [27] Lambert PA. Bacterial resistance to antibiotics: Modified target sites. *Advanced Drug Delivery Reviews*. 2005;57:1471-1485.
- [28] Grebe T, Hakenbeck R. Penicillin-binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of β -lactam antibiotics. *Antimicrobial Agents and Chemotherapy*. 1996;40:829-834.
- [29] Nagai K, Davies TA, Jacobs MR, Appelbaum PC. Effects of amino acid alterations in penicillin-binding proteins (PBPs) 1a, 2b, and 2x on PBP affinities of penicillin, ampicillin, amoxicillin, cefditoren, cefuroxime, cefprozil, and cefaclor in 18 clinical isolates of penicillin-susceptible, Qintermediate, and -resistant pneumococci. *Antimicrobial Agents and Chemotherapy*. 2002;46:1273-1280.
- [30] Kosowska K, Jacobs MR, Bajaksouzian S, Koeth L, Appelbaum PC. Alterations of penicillin-binding proteins 1A, 2X, and 2B in *Streptococcus pneumoniae* isolates for which amoxicillin MICs are higher than penicillin MICs. *Antimicrobial Agents and Chemotherapy*. 2004;48:4020-4022.
- [31] Coffey TJ, Dowson CG, Daniels M, Spratt BG. Genetics and molecular biology of β -lactam-resistant pneumococci. *Microbiology and Drug Resistance*. 1995;1:29-34.
- [32] Spratt BG. Hybrid penicillin-binding proteins in penicillin-resistant strains of *Neisseria gonorrhoeae*. *Nature*. 1988;332:173-176.
- [33] Bugg TDH, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry*. 1991;30:10408-10415.
- [34] Cooper MA, Fiorini MT, Abell C, Williams DH. Binding of vancomycin group antibiotics to d-alanine and d-lactate presenting self-assembled monolayers. *Bioorganic and Medicinal Chemistry*. 2000;8:2609-2616.
- [35] Williams DH, Maguire AJ, Tsuzuki W, Westwell MS. An analysis of the origins of a cooperative binding energy of dimerization. *Science*. 1998;280:711-714.
- [36] Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrobial Agents and Chemotherapy*. 1995;39:577-585.
- [37] Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrobial Agents and Chemotherapy*. 1999;43: 2823-30.
- [38] Kim YK, Cha C-J, Cerniglia CE. Purification and characterization of an erythromycin esterase from an erythromycin-resistant *Pseudomonas* sp. *FEMS Microbiology Letters*. 2002;210:239-44.
- [39] Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. *Cell*. 2007;128:1037-50.
- [40] Wickens H, Wade P. Understanding antibiotic resistance. *Pharmaceutical Journal*. 2005;274:501-4.
- [41] Jacoby GA, Munoz-Price LS. The new β -lactamases. *New England Journal of Medicine*. 2005, 352: 380-91.
- [42] Thomson JM, Bonomo R. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: β -lactams in peril! *Current Opinion in Microbiology*. 2005;8:518-24.
- [43] Babic M, Hujer AM, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resistant Update*. 2006;9:142-56.
- [44] Džidic S, Šuškovac J, Kos B. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology and Biotechnology*. 2008;46:11-21.
- [45] Govinden U, Mocktar C, Moodley P, Sturm AW, Essack SY. Geographical evolution of the CTX-M β -lactamase: an update. *African Journal of Biotechnology*. 2007;6:831-9.
- [46] Strateva T, Yordanov D. *Pseudomonas aeruginosa* a phenomenon of bacterial resistance. *Journal of Medical Microbiology*. 2009;58:1133-48.
- [47] Martínez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemics and antibiotic resistance. *Clinical Microbiology Review*. 2002;15(4):647-79.
- [48] Tolmasky ME. Bacterial resistance to aminoglycosides and beta-lactams: the Tn1331 transposon paradigm. *Frontiers of Bioscience*. 2000;5:D20-9.
- [49] Vannuffel P and Cocito C. Mechanism of action of streptogramins and macrolides. *Drugs*. 1996, 51: S20-S3.
- [50] Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrobial Agents Chemotherapy*. 1995, 39: 577-85.
- [51] Vannuffel P, M. Di Giambattista, Morgan EA, Cocito C. Identification of a single base change in ribosomal RNA leading to erythromycin resistance. *Journal of Biological Chemistry*. 1992;267:8377-8382.
- [52] Wang G, Taylor DE. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. *Antimicrobial Agents and Chemotherapy*. 1998;42:1952-1958.

- [53] Reinert RR, Wild A, Appelbaum P, Lutticken R, Cil MY, Al-Lahham A. Ribosomal mutations conferring resistance to macrolides in *Streptococcus pneumoniae* clinical strains isolated in Germany. *Antimicrobial Agents and Chemotherapy*. 2003;47:2319-2322.
- [54] Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of Royal Society of Medicine*. 2002;95 Suppl 41:22-6.
- [55] Ferguson D, Cahill OJ, Quilty B. Phenotypic, molecular and antibiotic resistance profiling of nosocomial *Pseudomonas aeruginosa* strains isolated from two Irish hospitals. *Journal of Medicine*. 2007, 1(1):1-14.
- [56] Nikaido H. Multiple antibiotic resistance and efflux. *Current Opinion in Microbiology*. 1998;1:516-23.
- [57] Misra R, Morrison KD, Cho HJ, and Khuu T. Importance of Real-time Assays to Distinguish Multidrug Efflux Pump Inhibiting and Outer Membrane Destabilizing Activities in *Escherichia coli*, *Journal of Bacteriology*. 2015, doi:10.1128/JB.02456-14.