

A Review on Molecular Mechanisms of Bacterial Resistance to Antibiotics

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Abstract: This seminar paper is done with the main objective of describing the molecular mechanisms of bacteria that result in resistance to antibiotics. Antibiotic-resistant bacteria that are difficult to treat are becoming increasingly common and are causing a global health crisis. Widespread use of antibiotics results in the emergence of antibiotic resistance. New resistance mechanisms are constantly being described and new genes and vectors of transmission are identified on a regular basis. The molecular mechanisms are mechanisms by which bacteria are either intrinsically resistant or acquire resistance to antibiotics through mutation and horizontal gene transfer. The major classes of molecular mechanisms of bacterial resistance to antibiotics are enzymatic inactivation of the drug results from the metabolic degradation of the drug, alteration of the drug target which results in the inability of the drug to bind to its target, drug permeability reduction mechanisms prevent cellular entry of drug, active efflux of drugs from bacteria results in the intracellular dilution of drugs, prevention of antimicrobial access to their targets and biofilm formation. Knowledge of the molecular mechanisms of antibiotic resistance is essential for developing new approaches to overcome drug resistance problems. Studies of resistance development and mechanisms of resistance should be known at an early stage of drug development and strategies to improve the delivery or otherwise enhancing the accessibility of antibiotics to their sites of action should be practiced.

Key words: Antibiotic resistance • Bacteria • Enzymatic inactivation • Intrinsic resistance • Molecular mechanisms

INTRODUCTION

Bacteria that are causative agents of infectious disease represent a serious public health concern globally. The discovery and use of antibiotics has been one of the major scientific achievements of the 20th century. Antibiotics were being used to cure potentially lethal infections. Infected cuts and wounds were no longer life threatening and various bacterial diseases, such as syphilis and cholera, were considered on their way to eradication. However, widespread antibiotic use has promoted the emergence of antibiotic-resistant pathogens, including multidrug resistant strains [1].

The history of antibiotic resistance coincides with the history of antibiotics themselves. Ironically, penicillin resistance was discovered even before penicillin was put to clinical use. The first antibiotic resistance mechanism reported in the literature was the production of Penicillinase by pathogenic *Escherichia coli* [2]. Bacteria may be intrinsically resistant to antibacterial agents or acquire resistance by mutation or acquisition of resistance determinants.

Use of antimicrobial agents selects for bacterial Variants within a population that are less susceptible, or resistant, to the antimicrobial agent used, leading to a situation where the resistant variant predominates under such selective pressure [3]. Furthermore, selection of resistance to a single antimicrobial agent often results in bacterial variants that harbor transferable multidrug resistance determinants [4]. These selective pressure phenomena are thought to occur in areas where antimicrobial agents are extensively used, such as in human clinical medicine [5], agriculture and in natural soil and aquatic environments [6]. Therefore, antimicrobial use fosters bacterial drug resistance and dissemination of drug resistance determinants within populations. Multidrug resistant bacteria may be recalcitrant to clinically relevant chemotherapeutic agents, resulting in treatment failures of infectious diseases [7].

The gradual increase in resistance rates of several important pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant Enterococcus (VRE), multidrug-resistant (MDR) *Pseudomonas aeruginosa*, Imipenem-resistant

Acinetobacter baumannii and third-generation Cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae*, poses a serious threat to public health [8]. The frequency of antibiotic resistance in many bacterial pathogens is increasing around the world and resulting in failures of antibiotic therapy that causes hundreds of thousands of deaths annually [9].

Study of these antimicrobial resistance mechanisms in infectious disease causing microorganisms is, therefore, necessary in order to find ways to circumvent conditions that foster such recalcitrant pathogens. Molecular, biochemical, physiological and structural analyses of bacterial multiple drug resistance mechanisms will foster their putative modulation and make possible the restoration of the efficacy of infectious disease chemotherapy [10]. The objectives of this seminar paper are to review the genetics of molecular mechanism of antibiotic resistance and to describe different molecular mechanisms those confer antibiotic resistance.

Molecular Mechanism of Bacterial Resistance to Antibiotics: Understanding the molecular mechanisms underlying antibiotic resistance requires an understanding of bacterial structure and function [11]. The molecular mechanism of antibiotics resistance genetically divided into two categories: intrinsic resistance and acquired resistance. The former refers to the situation where a bacterial species is unaffected by an antibiotic due to its fundamental physiological properties like β -lactam resistance in *Mycoplasma* species due to lack of cell wall and Vancomycin resistance in Enterobacteriaceae due to the outer membrane of Gram-negative species. In contrast, acquired resistance refers to the situation where a bacterium that used to be susceptible to an antibiotic at a given concentration is no longer inhibited at that concentration [12].

For all practical purposes, only the relative form of antibiotic resistance, i.e. acquired resistance, has important clinical implications. This is because the emergence of resistance renders previously effective treatments useless, resulting in increased morbidity and mortality, especially in the transition phase where resistance is too low to motivate a change in the empirical treatment. Acquired resistance can be further divided into horizontally acquired resistance and mutational acquired resistance. Horizontally acquired resistance refers to the situation where resistance emerges as a result of horizontal gene transfer (HGT), commonly in the plasmid conjugation, phage transduction or non-specific DNA uptake. In contrast, mutational acquired resistance occurs when the bacterial genome mutates to overcome the effect

of an antibiotic and normally only involves the change of one or a few nucleotides. Both horizontally and mutational acquired resistance play a major role in clinical relevant resistance [13].

Intrinsic Resistance: Is the innate ability of a bacterial species to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics, which allow tolerance of a particular drug or antimicrobial class. This can also be called “insensitivity” since it occurs in organisms that have never been susceptible to that particular drug. Such natural insensitivity can be due to: lack of affinity of the drug for the bacterial target, inaccessibility of the drug into the bacterial cell, extrusion of the drug by chromosomally encoded active exporters, innate production of enzymes that inactivate the drug [14].

Intrinsic mechanism are those specified by naturally occurring gene found on host chromosome such as β -lactamase of gram negative bacteria and many MDR efflux system. Efflux systems in Gram-positive bacteria always comprise a single polypeptide located in the cytoplasmic membrane. The cell envelope of Gram-negative bacteria is a major barrier for antibiotics and consists of the plasma membrane, the periplasm and the outer membrane. The outer membrane is the major barrier for antibiotics penetrate through porins or by passive diffusion through the outer membrane phospholipids (inner leaflet) -lipid A (outer leaflet) bilayer. The lipopolysaccharide (LPS) forms another barrier for many antibiotics but poly cationic compounds such as Gentamicin and Colistin are being transported through the outer membrane via interaction with LPS in a process called self-promoted uptake. Efflux pumps of the resistance nodulation cell division (RND) superfamily are major players in antibiotic resistance of Gram-negative bacteria [15].

It has been proposed that intrinsic resistance is mainly the consequence of the impermeability of cellular envelopes, the activity of multidrug efflux pumps or the lack of appropriate targets for a given family of drugs. However, recently published articles indicate that the characteristic phenotype of susceptibility to antibiotics of a given bacterial species depends on the concerted activity of several elements, what has been named as intrinsic resistome. These determinants comprise not just classical resistance genes. Other elements, several of them involved in basic bacterial metabolic processes, are of relevance for the intrinsic resistance of bacterial pathogens. Three are the most relevant causes of this intrinsic resistance: lack of the target, activity of

Table 1: Examples of intrinsic resistance and their respective mechanisms

Organisms	Natural resistance against	Mechanism
Anaerobic bacteria	Aminoglycosides	Lack of oxidative metabolism to drive uptake of aminoglycosides
Aerobic bacteria	Metronidazole	Inability to anaerobically reduce drug to its active form
Gram-positive bacteria	Aztreonam (a beta-lactam)	Lack of penicillin binding proteins (PBPs) that bind and are inhibited by this beta lactam antibiotic
Gram-negative bacteria	Vancomycin	Lack of uptake resulting from inability of vancomycin to penetrate outer membrane
Klebsiella species	Ampicillin (a beta-lactam)	Production of enzymes (beta-lactamases) that destroy ampicillin before the drug can reach the PBP targets
<i>Stenotrophomonasmaltophilia</i>	Imipenem (a beta-lactam)	Production of enzymes (beta lactamases) that destroy imipenem before the drug can reach the PBP targets.
Lactobacilli and Leuconostoc	Vancomycin	Lack of appropriate cell wall precursor target to allow Vancomycin to bind and inhibit cell wall synthesis
<i>Pseudomonas aeruginosa</i>	Sulfonamides, trimethoprim, tetracycline, or chloramphenicol	Lack of uptake resulting from inability of antibiotics to achieve effective intracellular concentrations
Enterococci	Aminoglycosides	Lack of sufficient oxidative metabolism to drive uptake of aminoglycosides
	All cephalosporins	Lack of PBPs that effectively bind and are inhibited by these beta lactam antibiotics

Source: [14]

chromosomally encoded antibiotic-inactivating enzymes and reduced uptake of the antibiotic, the later includes reduced permeability of the cellular envelopes and activity of efflux pumps [16].

Acquired Resistance: Changes in bacterial genome through mutation or horizontal gene acquisition, on the other hand, may consequently lead to a change in the nature of proteins expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic. This is referred to as acquired resistance, which is limited to selected isolates of that particular species or group of microorganisms. For example, we know that methicillin resistance of *Staphylococcus aureus* is primarily due to changes that occur in the penicillin binding protein (PBP), which is the protein which beta-lactam antibiotics bind and inactivate to consequently inhibit cell wall synthesis. This change is actually rendered by the expression of a certain gene in some strains of these bacteria, which is hypothesized to have been induced by the excessive use of penicillin. Expression of this gene results in an alternative PBP (PBP2a) that has a low affinity for most β -lactam antibiotics, thereby allowing these strains to replicate in the presence of Methicillin and related antibiotics. Some antimicrobial resistance is brought about by multiple changes in the bacterial genome [17].

Mutation: Is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait which it codes for. Any change in a single base pair may lead to a corresponding change in one or more of the

amino acids for which it codes, which can then change the enzyme or cell structure that consequently changes the affinity or effective activity of the targeted antimicrobials. In prokaryotic genomes, mutations frequently occur due to base changes caused by exogenous agents, DNA polymerase errors, deletions, insertions and duplications. For prokaryotes, there is a constant rate of spontaneous mutation of about 0.0033 mutations per DNA replication that is relatively uniform for a diverse spectrum of organisms. The mutation rate for individual genes varies significantly among and within genes [18].

Horizontal Gene Transfer: A principal mechanism for the spread of antibiotic resistance is by horizontal transfer of genetic material. Antibiotic resistance genes may be transferred by different mechanisms. Resistance genes can be further incorporated into the recipient chromosome by recombination. These genes may contain single mutations or more severe sequence changes. Tetracycline resistance in most bacteria is due to the acquisition of new genes often associated with mobile elements. These genes are usually associated with plasmids and/or transposons and are often conjugative [19].

Horizontal transfer of resistance genes is a mechanism for the dissemination of multiple drug resistance because resistance genes can be found in clusters and transferred together to the recipient. This is enabled by the existence of specific DNA structures called integrons [20]. Integrons are DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site-specific recombination. Studies about horizontal gene transfer-emerging multidrug resistance in hospital bacteria have demonstrated that the transfer of antibiotic resistance genes can take place in

Table 2: Examples of acquired resistance through mutation and horizontal gene transfer

Acquired resistance through:	Resistance observed	Mechanism involved
Mutations	<i>Mycobacterium tuberculosis</i> resistance to rifamycins	Point mutations in the rifampin-binding region
	Resistance of many clinical isolates to fluoroquinolones	Predominantly mutation of the quinolone-resistance-determining-region (QRDR)
Horizontal gene transfer	<i>Escherichiacoli, Hemophilus influenzae</i> resistance to trimethoprim	Mutations in the chromosomal gene specifying dihydrofolate reductase
	<i>Staphylococcus aureus</i> resistance to methicillin (MRSA)	Via acquisition of <i>mecA</i> genes which is on a mobile genetic element called "staphylococcal cassette chromosome" (SCCmec) which codes for penicillin binding proteins (PBPs) that are not sensitive to β -lactam inhibition.
	Resistance of many pathogenic bacteria against sulfonamides	Mediated by the horizontal transfer of foreign genes or parts of it.
	<i>Enterococcus faecium</i> and <i>Echinococcus faecalis</i> resistance to vancomycin	Via acquisition of one of two related gene clusters VancomycinA and Vancomycin B, which code for enzymes that modify peptidoglycan precursor, reducing affinity to vancomycin.

Source: [17]

the intestine between Gram-positive or Gram-negative bacilli in bacteria is often the result of the acquisition of mobile genetic elements [21].

Multidrug-resistance (MDR) contains multiple resistance genes. Nucleotide sequence analysis of multi resistant integrons shows that the inserted resistance gene cassettes differ markedly in codon usage, indicating that the antibiotic resistance determinants are of diverse origins [22].

Many of the antibiotic resistance genes are carried on plasmids, transposons or integrons that can act as vectors that transfer these genes to other members of the same bacterial species, as well as to bacteria in another genus or species. Horizontal gene transfer may occur via three main mechanisms: transformation, transduction or conjugation. Transformation involves uptake of short fragments of naked DNA by naturally transformable bacteria. Transduction involves transfer of DNA from one bacterium into another via bacteriophages. Conjugation involves transfer of DNA via sexual pilus and requires cell-to-cell contact. DNA fragments that contain resistance genes from resistant donors can then make previously susceptible bacteria express resistance as coded by these newly acquired resistance genes [23].

Major Molecular Mechanisms of Bacterial Resistance Prevention of Antimicrobial Access to Their Targets:

Antimicrobial compounds almost always require access into the bacterial cell to reach their target site where they can interfere with the normal function of the bacterial organism. Porin channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls [24].

There are several physical restrictions that limit antibiotic access to a bacterial target. Several of these have been mentioned already, to include the thick mycolic acid layer of protection produced by mycobacteria and an outer lipid membrane produced by gram-negative bacteria. Such structures limit a large variety of antibiotics from reaching their targets. For gram-negative bacteria in particular glycopeptides are specifically limited. Glycopeptides have limited access to their peptidoglycan precursor targets in gram-negative bacteria because glycopeptides have large hydrophobic structures in their molecular makeup that cannot readily cross the gram-negative cell's outer membrane [25].

The cytoplasmic membrane is a barrier to hydrophilic compounds. Entry of cytoplasmically targeted compounds is usually through carrier-mediated transport mechanisms or via channels in the outer membrane of Gram-negative bacteria formed by porins. Antibacterial compounds transported in this way may be subject to resistance by loss of non-essential transporters, by lack of porins or by mutations that are able to modify the structure of these channels and thus decreasing the influx [26]. Some microbes possess impermeable cell membranes that prevent drug influx as exemplified by *Pseudomonas aeruginosa*. Furthermore, many large molecule antimicrobials are naturally inactive against certain groups of bacteria because they simply cannot pass into the bacterial cell [24].

Efflux Pumps: Bacterial efflux pumps actively transport many antibiotics out of the cell and are major contributors to the intrinsic resistance of Gram-negative bacteria to many of the drugs that can be used to treat Gram-positive bacterial infections. When over expressed, efflux pumps can also confer high levels of resistance to previously

clinically useful antibiotics. Some efflux pumps have narrow substrate specificity (for example, the Tetracycline pumps), but many transport a wide range of structurally dissimilar substrates and are known as multidrug resistance (MDR) efflux pumps. Bacteria that over express efflux pumps, including Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, have been isolated from patients since the 1990s [27].

Multidrug efflux transporters are normal constituents of bacterial cells. These transporters are major contributors to intrinsic resistance of bacteria to many antimicrobial agents [28]. Multidrug resistance proteins (MDRs) or multidrug efflux pumps are widespread in bacteria [29]. Such drug resistant bacteria harbor energy-driven drug efflux pumps which extrude antimicrobial agents thus reducing their intracellular concentrations to sub- or non-inhibitory levels. There are two main types of active efflux pumps. The first type, called primary active transport, uses the hydrolysis of ATP to actively efflux drugs from cells, while the second type, called secondary active transport, uses an ion gradient for active drug efflux from cells [30].

A mutation resulting in over expression of a multidrug efflux pump leads to resistance to a wide variety of structurally unrelated antimicrobials [26]. The ATP driven transporters are also known as ABC (ATP-binding cassette) or Poly glycoprotein transporters. Both active transport systems are used by bacteria to resist the inhibitory effects of antimicrobial agents and are often referred to as efflux pumps [31]. These efflux pumps function by using the energy of the cation gradient generated by cellular respiration to catalyze the “uphill” transport of solute (e.g., drug substrate) across the membrane by translocation of the cation down its concentration gradient in a process called antiport, where cation moves in one direction across the membrane and drug (substrate) moves in the opposite direction [32]. Efflux pumps affect all classes of antibiotics, especially the macrolides, tetracyclines and fluoroquinolones because these antibiotics inhibit different aspects of protein and DNA biosynthesis and therefore must be intracellular to exert their effect [33].

Ribosome Protection: Ribosomal protection mechanism is an important defense strategy found both in gram negative and gram positive bacteria against tetracycline [34]. The most prevalent and best characterized ribosome protection proteins (RPPs) are TetO and TetM [35]. The TetO and TetM catalyze the release of tetracycline from the ribosome [36].

Certain bacteria have developed resistance mechanisms that protect the antimicrobial target. For example, in the case of bacterial protein synthesis inhibitors, such as tetracycline, the bacteria have the ability to produce ribosome protection proteins that bind to the ribosomal target thus preventing the binding of tetracycline to the ribosome [19]. Such ribosome protected bacteria will be able to grow in the presence of tetracycline as protein synthesis will be possible. Disease-causing bacteria harboring such ribosome protection mechanisms have been demonstrated to be clinically important and these resistance determinants have been discussed extensively elsewhere [37].

Biofilm Formation: Biofilm is a structured population of bacteria embedded in a matrix, which is composed by polysaccharides, proteins and extracellular DNA. It has been shown that cells growing in biofilms are less susceptible to antibiotics than those growing planktonically [38].

The antibiotic resistance associated with biofilms depends on several causes, some due to the structure of the extracellular matrix, some other to the physiological state of biofilm-growing bacteria; which is different to that of planktonic cells. Even inside the biofilm, bacteria show different metabolic states, because there is a gradient of nutrients and oxygen between the surface of the biofilm and its deeper region. The extracellular matrix may change the activity of the antibiotics by two different reasons; by diminishing the diffusion of the antibiotic or by sequestering it through its binding to the matrix. This is not a general trend, since in several occasions; slow diffusion of the antibiotic is not the most important element in the phenotypic resistance displayed by biofilms [39].

Biofilm production occurs in many loci, including teeth plaque, water environments, medical catheters, trauma wounds, etc [40]. As such, microorganisms that are found in biofilms are protected from the entry of multiple antimicrobial agents [41].

Target Alteration (Modification): Natural variations or acquired changes in the target sites of antimicrobials that prevent drug binding or action is a common mechanism of resistance. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antimicrobial. This type of resistance mechanism is shown by both Gram negative as well as positive bacteria. The mechanism of action for most of the antibacterial

antibiotics involves interaction between the drug and intracellular enzyme/protein. The development of drug resistance for these antibiotics requires the reduction in affinities to their enzymatic targets. Altering an antibiotic's target protein directly at the DNA level is a common mechanism of target modification [24].

Bacteria have found ways to alter the molecular targets of antimicrobial agents. Altered targets may include, for example, DNA gyrase, a target of quinolone antimicrobials [42]. RNA polymerase, a target of rifampin [43], the prokaryotic ribosome, a target of tetracycline and other protein synthesis inhibitors [44] and targets of antimetabolite drugs, such as the sulfonamides and related drugs [45]. One classical example of drug target modification is the staphylococcal mechanism of variously altering the penicillin binding protein (PBP) which is the target of β -lactam antibiotics. *Staphylococcus aureus*, the causative agent of serious infectious disease, becomes resistant to these antibiotics by any one of the several mechanisms such as mutation in PBP or acquisition of new PBP with reduced affinity to penicillins, over expression of PBP, etc [46]. Another example of an altered target mechanism includes substitution of amino acids in the quinolone-resistance determining region (QRDR) of DNA gyrase and topoisomerase IV resulting in less efficient binding of quinolone antibiotics [47].

This mechanism has been responsible for widespread quinolone resistance among the Enterobacteriaceae. Methylation of drug binding targets on 16S rRNA by rRNA methyl transferases is responsible for aminoglycoside resistance in several bacterial species [48]. Methylation of the 23S rRNA component of 50S ribosomal subunit by adenine-specific N-methyltransferases is a common mechanism of macrolide resistance in many Gram-positive and -negative bacteria [24]. Also, mutations around the methylated sites have also been responsible for additional macrolide resistance. Modification of the drug target site which involves a Guanine to Adenine substitution at position 2, 032 in the peptidyltransferase center of 23S rRNA results in reduced affinity of linezolid to the 50S subunit [49].

On the other hand, mutations in genes encoding ribosomal subunits. Microbial pathogens and strategies for combating them: science, technology and education lead to altered ribosomal protein targets which resist aminoglycoside binding, a mechanism responsible for streptomycin resistance in *Mycobacterium tuberculosis*, the causative agent of tuberculosis and other infections [50].

The vancomycin resistant enterococci (VRE) have evolved a unique mechanism of synthesizing peptidoglycan using alternate pathway thereby producing the peptidoglycan precursors instead of the vancomycin target [51].

Some examples of bacterial resistance due to target site modification are alteration in penicillin-binding protein (PBPs) leading to reduced affinity of beta-lactam antibiotics (Methicillin-Resistant *Staphylococcus aureus*, *streptococcus pneumoniae*, *Neisseria gonorrhoeae* and *Listeria monocytogenes*), changes in peptidoglycan layer and cell wall thickness resulting to reduced activity of vancomycin (Vancomycin-resistant *Staphylococcus aureus*), changes in vancomycin precursors reducing activity of vancomycin (*Enterococcus faecium* and *Enterococcus faecalis*), alterations in subunits of DNA gyrase reducing activity of fluoroquinolones (Many Gram-negative bacteria), alteration in subunits of topoisomerase IV leading to reduced activity of fluoroquinolones (Many Gram positive bacteria, particularly *Staphylococcus aureus* and *Streptococcus pneumoniae*) and changes in RNA polymerase leading to reduced activity of rifampicin (*Mycobacterium tuberculosis*) [24].

Reduced Permeability: Acquired resistance to antibiotics through a decrease in the permeability of the cell membrane requires major structural changes in the membrane [52]. Compared with Gram-positive species, Gram-negative bacteria are intrinsically less permeable to many antibiotics as their outer membrane forms a permeability barrier [53].

One mechanism that results in reduced drug permeability in bacteria is the cell wall's lipopolysaccharide (LPS), which consists of lipid A, a core consisting of polysaccharide and O-antigen [54]. Bacteria that harbor LPS moieties show resistance to erythromycin, roxithromycin, clarithromycin and azithromycin in Gram-negative bacteria such as strains of *Pseudomonas aeruginosa* and *Salmonella enterica*, all of which are serious pathogens, especially in immune-compromised patients [55].

Another mechanism that confers reduced permeability involves the porin channels that reside in the outer membrane and allow small molecular weight molecules, such as antimicrobial agents, to gain cellular entry. Drug resistant bacteria alter the expression of these outer membrane proteins such that they fail to integrate into the outer membrane or are functionally defective, thus preventing the entrance of growth-inhibitory molecules ([56].

Clinically important bacterial pathogens like *Serratia marcescens*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, have utilized this reduced drug uptake system to resist important antimicrobial agents, such as the beta-lactams, fluoroquinolones, aminoglycosides, as well as chloramphenicol [57].

Antibiotic Inactivation: Some bacteria produce modifying enzymes that reside within or near the cell surface, which selectively target and inactivate the drug. Enzymatic inactivation either by hydrolysis or by modification (group transfer and redox mechanisms) is a major mechanism of resistance to natural antibiotics in pathogenic bacteria. The resistant isolates in most cases inherit the antibiotic resistance genes on resistance plasmids. These resistance determinants are most probably acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera, including antibiotic producing organisms [26].

Many antibiotics possess hydrolytically susceptible chemical bonds (e.g. esters and amides) whose integrity is central to biological activity. When these vulnerable bonds are cleaved, the antibiotic activity is destroyed. The most diverse and largest family of resistance enzymes is the group transferases. Those enzymes covalently modify antibiotics leading to structural alterations that impair target binding [58].

β -Lactamases are hydrolytic enzymes that disrupt the amide bond of the characteristic β -lactam ring, before the antibiotic can get to the site of cell wall synthesis, rendering the antimicrobial ineffective [59]. Lyases are enzymes that cleave carbon-carbon, carbon-oxygen, carbon-nitrogen and carbon-sulfur bonds by non-hydrolytic or non-oxidative routes. These reactions frequently result in double bond formation or ring closure [58]. In 1984, the first erythromycin esterase was reported from a macrolide-resistant isolate of *Escherichia coli* [60]. Acyl transfer and specifically acetyl transfer, is a common mechanism of antibiotic inactivation employed by bacteria. Covalent modification of vulnerable hydroxyl and/or amine groups on antibiotics results in compounds that lose their ability to bind target and, therefore, become inactive [61].

Kinases are ubiquitous enzymes that catalyze phosphate transfer from a nucleoside trinucleotide, typically ATP, to a diverse set of substrates. The known antibiotic kinases involved in resistance are exclusively O-phosphotransferases and many share structural and mechanistic details with other kinases such as the protein kinases [62].

CONCLUSION AND RECOMMENDATIONS

Antibiotic resistance is the ability of bacteria to survive and multiply in the presence of antibiotic agent that would normally kill these species of bacteria. Antibiotic resistance traits are genetically coded and can either be intrinsic or acquired. Bacteria are able to resist molecularly the effect of antibiotics through preventing intracellular access, immediately removing antibiotics through efflux pumps, inactivation of the antibiotic, reducing permeability to antibiotics, biofilm formation and target modification. We now have the ability to rapidly evaluate the potential for the emergence of resistance to novel drugs, identify where and when this might occur and determine the mechanisms responsible. The early identification of naturally occurring resistance mechanisms and targets that can accommodate numerous structural changes should lead to the discontinuation of the development of agents that are likely to fail in the clinic as a result of resistance. Knowledge about how and when resistance occurs and potential synergies with combinations of agents will also facilitate the development of dosing regimens that can help to minimize the emergence of resistance to current and new antibiotics, enabling these drugs to be used to best effect. All the strategies to overcome resistance require expanded knowledge of the molecular mechanisms of antibiotic resistance, their origins and evolution and their distribution throughout bacterial populations and genomes. Based on the above conclusion the following recommendations are forwarded:

- Studies of resistance development and mechanisms of resistance must be a mandatory requirement at an early stage of drug development; such studies will enable academic institutions and industry to work together.
- Strategies towards treatment options should be developed to target virulence factors of pathogens instead of whole bacteria, for example, develop drugs that target the plasmids containing resistance genes or drugs that target the adhesion of virulent bacteria to a tissue and promote appropriate drug use policies and public health education on appropriate antibiotic drug use.
- Other strategies to enhance the accessibility of antibiotics to their sites of action, for example, liposomal preparations of hydrophobic antibiotics, such as ethambutol for treatment of mycobacterial infections, should be practiced.

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