Review Article

MOLECULAR MECHANISMS OF ANTIBIOTICS RESISTANCE

ABSTRACT

Antibiotic resistance has been observed since the discovery of antibiotics, and the indiscriminate use of antibiotics have contributed to the spread of resistance among bacteria species. Antibiotic resistance is encoded by several genes, and can be easily transferred between bacteria; which might be owed to the fact that these resistant genes are mostly carried by mobile genetic elements (such as plasmids, integrons, and transposons). The origin of antibiotic resistance, types of antibiotic resistance, and the molecular mechanisms of resistance are discussed in this article. The resistance to antibiotics mediated by genes encoded on the chromosome, plasmids, integrons, and transposons were highlighted. New improved strategies for sampling and screening microbial population is essential for better understanding of the factors that promotes the dissemination of resistance genes; and to also elucidate the relationships between antibiotic resistance-genes of producer, the environment, and the pathogenic bacteria.

KEYWORDS: Antibiotics, resistance mechanism, plasmids, integrons, transposons, minimum inhibitory concentration (MIC), mutation

INTRODUCTION

The term "antibiotics" according to Davies and Davies [1], refers to chemical compounds that are produced by microorganisms to inhibit or destroy other microbes. Majority of the antibiotics in use today originated from *Actinobacteria* phylum, of which almost 80% were produced by soil-dwelling *Actinobacteria* of the genus *Streptomyces* [2]. Prior to the discovery of natural antibiotics, synthetic chemical compounds such as sulfa drugs, salvarsan, and quinolones were used as chemotherapeutic agents [3]. In the year 1928, the first natural antibiotics "Penicillin" was accidentally discovered by Alexander Fleming; when the fungus *Penicillium* contaminated a culture plate in his laboratory. The antibiotics penicillin was however not developed for use until the late 1930s [4]. Penicillin was found to be effective against Gram-positive bacteria but not Gram-negative bacteria due to its mode of action, inhibiting cell wall synthesis. The Gram-negative bacteria possess an outer membrane aside from the cell wall, limiting the action of penicillin [4].

Following the penicillin discovery, scientists began the deliberate search for antibacterial agents amongst soil microorganisms; bacteria and fungi inclusive. After several researches, it was discovered that

antibacterial activities were predominant in Actinomycete cultures than in other bacteria or fungi [3]. The discovery of Streptomycin from *Streptomyces griseus* in the year 1943, paved the golden age of antibiotics development and discovery (from 1940-1990). Streptomycin was observed to inhibit protein synthesis by binding to the 30S ribosomal subunit of prokaryotes, and thus was effective against both Gram-negative and Gram-positive bacteria [4].

However, the indiscriminate use of antibiotics and the rapid reproduction and transfer of the genetic elements by bacteria lead to the development of antibiotic resistance in bacteria. The ever increasing infections caused by these antibiotic resistant bacteria, poses a threat to the health benefits achieved with antibiotics which have become a global crisis [5]. Antibiotic resistance is encoded by several genes, and can be easily transferred between bacteria; there have been incessant identification of new resistant genes and vectors responsible for gene transmission [6, 7]. At the rate of increase in the global antibiotic resistance epidemic, it is paramount to understand the molecular mechanisms by which bacteria are either intrinsically resistant or acquired resistance to antibiotics. This review is therefore aimed at discussing the molecular mechanisms by which bacteria resist the effects of antibiotics.

ORIGIN OF ANTIBIOTICS RESISTANCE

Bacteria in general are not uniformly susceptible or resistant to any particular antimicrobial agent. The levels of bacteria resistance vary greatly even within related species. Resistance and susceptibility are usually measured based on minimum inhibitory concentration (MIC), which is the minimum concentration of antimicrobial agent that will inhibit the growth of a bacteria [8]. The susceptibility is the range of the average MICs for any drug across the same bacterial species [8]. The origin of antibiotic resistance gene(s) in bacteria can be due to the presence of antibiotics producing bacteria in the soil, the activity of the antibiotics on other bacterial species, natural protection mechanisms in some bacteria, or other factors such as the environment [8]. The prevalence of antibiotics resistance has been fueled by the uncontrolled sale of antibiotics over the counter without prescription (in low and middle income countries), which have resulted in the misuse of antibiotics [9]. The overuse and misuse of antibiotics has become the primary cause of the increase in antibiotics resistance [10]. The use of antibiotics in livestock farm as growth promoters is a common and acceptable practice in many industrialized countries [11]; and have also contributed greatly to the increase in bacteria antibiotics resistance.

The establishment and mobilization of novel resistance gene(s) is highly determined by environmental factors such as selective pressure, dispersal, and fitness cost [12]. Selection plays a critical role in the maintenance of the resistance genes in the population. Resistance can be selected naturally in a competitive environment, which thereby preserves the resistance gene pool in that niche [13]. The

indiscriminate use of antibiotics by humans, have created a selective pressure that have resulted in the dominance of resistant bacteria and multiplication of the resistant bacteria strain in the environment and among pathogenic bacteria through exchange of resistance genes [14].

TYPES OF ANTIBIOTICS RESISTANCE

In a broad sense, bacterial antibiotics resistance can be either intrinsic or acquired. Antibiotics resistance that occurs naturally in the bacteria are intrinsic resistance mechanism; examples include: The AmpC β-lactamase enzyme of Gram-negative bacteria and the multi-drug resistance (MDR) efflux system [15]. Intrinsic resistance mechanisms may be expressed naturally or induced by exposure to antibiotics [15]. Intrinsic resistance is a trait that is universally shared amongst bacterial species, and is not dependent on previous exposure to antibiotics [16, 7]. The common intrinsic resistance mechanism of bacterial are: reduced permeability of the outer membrane, and the natural activity of efflux pumps [17].

Acquired resistance mechanism involves transfer of resistance gene borne on the plasmids, transposons, and other mobile genetic element; and also mutation in genes targeted by antibiotics [18]. The transfer of genes between bacteria of same genus is very common, but genes can also be transferred between varying genera [18]. Plasmid-mediated gene transmission is the most common route for the acquisition of resistance genes among bacteria [19]. Although some bacteria such as *Acinetobacter spp*. are naturally capable of incorporating genetic material directly from the external environment. Internally, mutation can occur when insertion sequences and integrons move genetic materials around, also stressors such as starvation, UV radiation, chemicals etc. can cause genetic mutations (substitution, deletion etc.) [19]. Bacteria possess an average mutation rate of 1 for every 106 to 109 cell divisions [1], of which some of these mutations may be deleterious to the cell. According to Martinez [15], the mutations that results in antimicrobial resistance usually occur in the following genes:

i. Genes encoding the drug target
ii. Genes encoding the drug transporters
iii. Genes encoding the regulators of the drug transporters
iv. Genes encoding antibiotic-modifying enzymes

In addition to this, Martinez [15] opines that mutations that confers resistance to antimicrobial agents do so at a cost to the organism. An example is the acquisition of methicillin resistance in *Staphylococcus aureus*, which was observed to significantly reduce the growth rate of the bacteria [15].

MOLECULAR MECHANISMS OF ANTIBIOTICS RESISTANCE

Chromosomally-mediated antibiotics resistance

This resistance mechanism usually involves modification of the target site and decrease in drug uptake. Modification of the drug target sites may result from nutation in the genes encoding the target site, enzymatic alteration of the binding sites (e.g. addition of methyl group), and bypass of the original site [20].

Mutation in the genes encoding the target sites: mutation at crucial loci of β-lactamase gene [e.g. gene of Temoneira-1 (TEM 1), sulfihydryl variable (SHV-1)] are mainly responsible for the newly recognized extended spectrum β-lactamases (ESBL) [21]. Also, the alterations in the structure and number of penicillin-binding proteins (PBPs) which are transpeptidases involved in the construction of peptidoglycan in the bacterial cell wall. Mutation in the structure of PBPs (PBP2a in *S. aureus* by acquisition of the *mecA* gene) can limit the ability of β-lactam antibiotics to bind to the PBP [21]. Mutation that results in the decrease in the amount of PBPs also impacts the amount of drugs that can bind to PBPs [21].

The glycopeptides (e.g. vancomycin) which inhibits bacterial cell wall synthesis by depolarizing the cell membrane have been resisted by *Enterococci* (vancomycin-resistant enterococci VRE) and *S. aureus* (MRSA) through the acquisition of *van* genes which result in the structural change of peptidoglycan precursors leading to decrease in the binding ability of vancomycin [21]. The antibiotics Daptomycin requires the presence of calcium for binding; mutation in the *mprF* genes changes the charge of the cell membrane surface to positive, inhibiting the binding of calcium thus inhibiting daptomycin [21].

Enzymatic alteration of the binding sites: an example of this, is the methylation of ribosome (catalyzed by an enzyme encoded by the *erm* genes) resulting in the resistance to macrolide antibiotics [22]. The enzyme introduces one or two methyl group (-CH_3) to Adenine residue at the 23rRNA of the 50S ribosomal subunit (Figure 1).

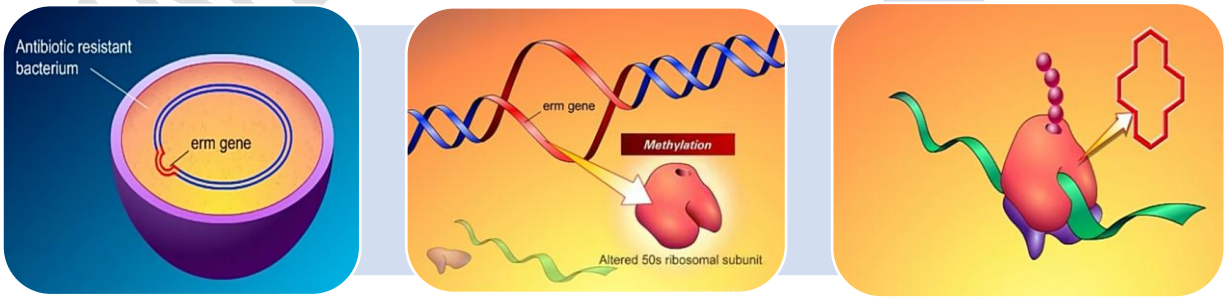

Figure 1: Enzymatic alteration of the antibiotics binding site. The resistant bacteria encode an enzyme in the *erm* gene which alters the 50S ribosomal subunit, which is the target site for macrolide antibiotics (in order to prevent DNA translation and protein synthesis within the bacteria cell). This alteration of the 50S ribosomal subunit inhibits the binding of macrolide to the ribosome (Source: Mechanisms in Medicine https://youtu.be/qBdYnRhdWcQ)

Replacement or Bypass of the original site: target bypass involves the production of additional antibiotics targets that are not susceptible to binding of the antibiotics. This mechanism of resistance can be utilized by bacteria to resist several classes of antibiotics, which includes; glycopeptides, β-lactams, lincosamides, macrolides, streptogramins, and aminoglycosides [23]. *Streptomyces spp*. have been observed to be resistant to β-lactam antibiotics by producing alternative low-affinity PBPs or through the overproduction of PBPs [24]. Three classes of PBPs (A, B, and C) have been identified in bacteria, of which analysis have shown that some of these PBPs indeed have low affinity for β-lactams [likely due to the absence of a serine/threonine protein kinase domain (STPK renamed PASTA)] [24].

Glycopeptides (such as vancomycin and teicoplanin) which inhibits cell wall transpeptidation and transglycosylation in bacteria by associating with peptidoglycan precursors (D-Ala-D-Ala) [25] have been resisted by some bacteria that alters the peptidoglycan precursor from D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser which have a very reduced affinity for the glycopeptides [26]. Other examples include; the synthesis of additional B subunit of DNA gyrase for novobiocin resistance, alternate fatty acid synthase for platensimycin resistance, and alternate resistant RNA polymerase for rifamycin resistance [27].

Decrease of drug uptake: the structure and functions of the LPS layer in Gram negative bacteria have provided an innate resistance to certain groups of antimicrobial agents; in the sense that, this layer provides barrier to certain types of molecules [19]. The outer membrane of the mycobacteria contains high lipid which enables easy access of hydrophobic drugs (such as rifamipicin and the fluoroquinolones) into the cell, but limits the access of hydrophilic drugs [19]. Naturally, bacteria that lack cell walls (such as the Mycoplasma and other related species), are intrinsically resistant to antibiotics that targets cell wall (β-lactams and glycopeptides). In *Enterococci*, polar molecules encounter difficulties penetrating the cell, this gives an intrinsic resistance to aminoglycosides. It has been observed that substance can enter a bacteria cell through the porin channels. The two major ways in which changes in porin can limit drug uptake, are: mutation that changes the selectivity of the porin channel, and the decrease in the number of porins present [19]. Members of the *Enterobacteriaceae* family have been observed to become resistant to certain antibiotics as a result of decrease in the number of porins. This resistance has been observed for carbapenems (example as seen in *E. aerogenes*), tetracycline and β-lactams (example as seen in *Neisseria gonorrhoeae*) [19].

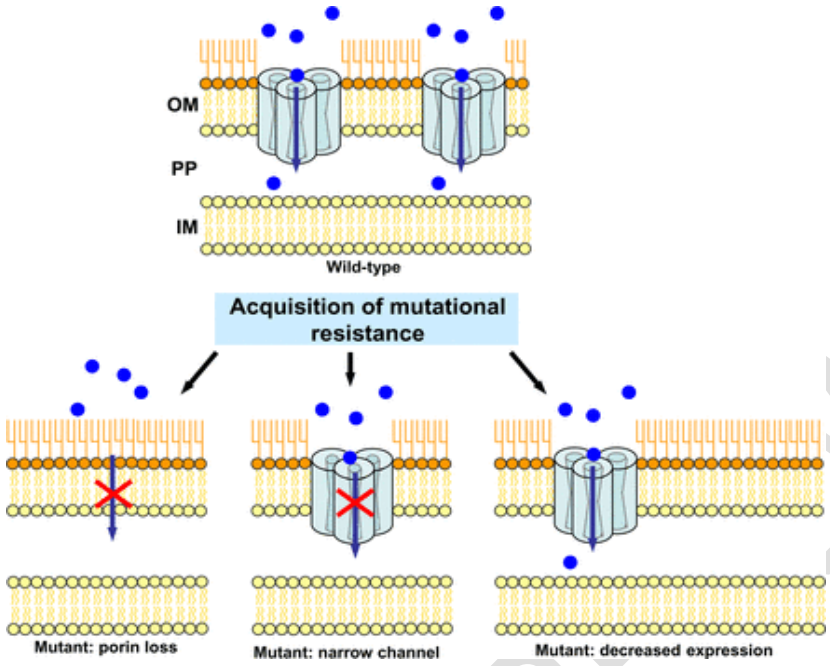

Figure 2: different mechanisms of acquired mutational resistance associated with porin. The blue circles represent antibiotics; the red crossed lines indicate the inhibition of antibiotics passages across the outer membrane; IM-inner membrane; OM-outer membrane; PP-periplasmic space [19]

Plasmid Mediated Resistance

According to Schultsz and Geerlings [28], "plasmid is a small DNA molecule within a cell, which is capable of replicating independently of the host cell, and often contain multiple antibiotics resistance genes contributing to the spread of multidrug resistance (MDR)". Plasmids are generally, closed circular double-stranded DNA molecules whose size ranges from less than 10 kilobase (kb) pairs to greater than 400kb. They are very common within bacterial cells, and multiple copies of a specific plasmid, or multiple different plasmids or both may be identified in a single bacterial cell [29]. Closely related plasmids cannot coexist in the same cell [29]. The transfer of plasmid DNA between bacterial species involves a complex process with the presence of the *tra* genes (genes needed for transfer), although some small plasmids can be able to transfer to other bacteria through conjugation apparatus provided by a co-resident conjugative plasmid [29].

Plasmids are vital vehicle for the carriage of mobile genetic elements and acquired resistance genes associated with these elements in both Gram positive and negative bacteria [30]. In resistance plasmids, aside from the core genes that form the backbone of the plasmid (genes responsible for vertical and/or horizontal transfer), the accessory region is made up of one or more resistance genes [30].

Transposon Mediated Resistance

Generally, transposon are pieces of DNA sequence that contain terminal regions (which participates in recombination) and specifies a protein or proteins (e.g. recombinase or transposase) that enables their incorporation into and from specific genomic regions [31]. According to Archana *et al*. [31], "they are mobile genetic element that are proficient in altering their position in the Loci". Transposon mediate antibiotic resistance by facilitating the destruction of the antibiotics. According to the transposon role in identification and recombination of particular sequences, they can be categorized into two classes. Class I; holds a range of resistance genes which are identical structurally and functionally, but small DNA homology, while class II; constitute three dissimilar but interrelated families (Tn3, Tn21, and Tn2501) [32]. The class I or retro-transposons works by copying RNA from DNA (transcription) and then copying the RNA back to DNA by reverse transcription, thus can be inserted into diverse locations in the genome [33]. The class II works by cut and paste mechanism and does not involve RNA intermediate [34]. An example of transposon mediated resistance is the production of β-Lactamase which is capable of destroying the amide bond of the lactam ring of beta-lactam antibiotics, making it ineffective [35]. The *bla* gene which encodes β-lactamase have been observed to be transposons, and its transcription can be constructive or induced [35]. Also, it has been observed that antibiotic resistance genes are frequently associated with the Tn3 family transposon, which are broadly characterized by approximately 38 bp terminal inverted repeats (IR) [36].

Two types of transposable genetic elements; transposons and insertion sequence (IS) exists with almost similar characteristics; these mobile sequences probably influence the genetic variation and evolution in prokaryotes [30]. The difference between transposon and insertion sequence is that, transposons encodes functional genes that effect a recognizable phenotypic characteristic. Their similarities include; they can both translocate as an independent unit, they are both flanked by short identical sequences of DNA in reverse order (inverted repeats) [30]. These mobile elements (transposon and IS) depends on chromosome, plasmid or bacteriophage for replication, in that they are incapable of autonomous self-replication [30].

Integron Mediated Antibiotic Resistance

Integrons are genetic elements that can acquire and carry genes (antibiotic resistance gene included). They depend on transposons for their translocation [37]. Integrons contains a collection of genes (gene cassettes) that can be grouped based on the protein (integrase) that results from the recombination [38]. Integrons carry resistance genes for numerous antibiotics at the same time, causing multi drug resistance and can integrate into regions of other DNAs where they supply multiple new genes (in a single exchange) [38]. They have been observed to possess five or more antibiotic reistance genes arranged in a tandem sequence along a single integrin [37]. Integrons are prevalent in bacterial population, playing a

part in the dissemination of antibiotic-resistance genes. They do not transpose independently as a unit from one sequence of DNA to another [30]. They may become flanked by transposable elements or integrated into a transposon [38]. Integrons function primarily in providing a convenient insertion site for antibiotic-resistance genes from foreign DNA sources [38]. Although, five classes of integrons encode antibiotic-resistance gene, but type I integrins are the most common in pathogenic microorganisms [38].

Integron can serve as expression cassette for antibiotic-resistance genes due to the fact that an efficient promoter site resides closely to the 5' end of the newly inserted DNA sequence. The frequency of expression of a resistance gene diminishes as the distance of the promoter (from the resistance gene) increases [38].

Prevention of Antibiotics Resistance

Antibiotic resistance can be prevented through the prudent use of antibiotics, such as the administration of antibiotics only when needed and narrow spectrum antibiotics should be more preferably used over broad-spectrum antibiotics (drug specificity) [39]. Individuals should be well educated on the proper use of antibiotics in under developed and developing countries. According to Gerber *et al*. [39], antibiotic-resistance dissemination can be mitigated through proper sanitation and disinfection of medical care centers, patients, visitors, and staffs. Also researches have shown that effective communication, and training aimed towards strengthening the knowledge of antibiotic-resistance reduced the incidence of infection [39].

Antibiotic resistance infections can be contained through the following means:

- Increased investment in new medicine, vaccine, diagnostic tools, and other interventions.
- Optimized use of antimicrobials in medicine and agriculture
- Constant research in the development of new antimicrobials so as to maintain a pool of effective antimicrobials in the market against the resistant bacteria increase.

There is need for global coalition in order to address the threats posed by antibiotics resistance, in that such a system will provide insight to areas of prevalent resistance and information that are necessary for evaluation of strategies already in place to curb antibiotic resistance. The idea of global tracking system has not been fully implemented; and more information and attention is still needed to understand and measure trends in antibiotics resistance on the global scale.

CONCLUSION

The mechanisms adopted by microbes to evade antibiotics predates and outnumbers the therapeutic interventions available. New improved strategies for sampling and screening microbial population is

essential for better understanding of the factors that promotes the dissemination of resistance genes; and to also elucidate the relationships between antibiotic resistance-genes of producer, the environment, and the pathogenic bacteria. The use of bioinformatics and algorithms in determining the relationship between resistance determinants of several ecological niche have been beneficial because they provide computational tools for rapid prediction of antibiotic resistance genes; their target in newly sequenced genomes, and their phylogenetic relationships. Antibiotics resistance mitigation strategy should also focus on limiting selective pressure by reducing irrelevant use of antibiotics and avoiding settings which select for resistance.

REFERENCES

1. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, 74(3), 417-433.
2. Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H., Clement, C., Ouhdouch, Y., & van Wezel, G.P. (2016). Taxonomy, physiology, and natural products of *Actinobacteria*. *Microbiology and Molecular Biology Reviews*, 80, 1-43.
3. Aminov, R.I. (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in Microbiology*, 1, 130-134.
4. Hopwood, D.A. (2007). How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? *Molecular Microbiology*, 63, 937-940.
5. Ventola, C.L. (2015). The antibiotic resistance crisis. *Pharmacy and Therapeutics*, 40(4), 277-283.
6. Surette, M.D., & Wright, G.D. (2017). Lessons from the environmental antibiotic resistome. *Annual Reviews in Microbiology*, 71, 309-329.
7. Martinez, J. L. (2018). Ecology and evolution of chromosomal gene transfer between environmental microorganisms and pathogens. *Microbiology Spectrum*, 6, 1–16.
8. Iredell, J., Brown, J., & Tagg, K. (2016). Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications. *BMJ*, 352, 6418-6420.
9. Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., & Sumpradit, N. (2013). Antibiotic resistance – the need for global solutions. *The Lancet Infectious Diseases Commission*, 13(12), 1-7.
10. Andersson, D. I., & Hughes, D. (2017). Selection and transmission of antibiotic-resistant bacteria. *Microbiology Spectrum*, 5, 1–17.
11. Matthew, A.G., Cissell, R., & Liamthong, S. (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathogen and Diseases*, 4(2), 115-133.
12. Bengtsson-Palme, J., Kristiansson, E., & Larsson, D.G.J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Review*, 42(1), 21-25.
13. Laskaris, P., Tolba, S., Caluo-Bado, L., & Wellington, L. (2010). Coevolution of antibiotic production and counter-resistance in soil bacteria. *Environmental Microbiology*, 12(7), 783-796.

14. Larsson, D.G.J. (2014). Pollution from drug manufacturing: review and perspectives. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*,369, 11-18.
15. Martinez, J. L. (2014). General principles of antibiotic resistance in bacteria. *Drug Discovery Today*, 11, 33–39.
16. Cox, G., & Wright, G.D. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *International Journal of Medicine and Microbiology*, 303, 287–292
17. Fajardo, P., Pastrana, L., Mendez, J., Rodriguez, I., Fucinos, C., & Guerra, N.P. (2012). Effects of feeding of two potentially probiotic preparations from lactic acid bacteria on the performance and faecal microflora of broiler chickens. *Scientific World Journal*, 5-12.
18. Courvalin, P. (2008). Predictable and unpredictable evolution of antibiotic resistance. *Journal of Internal Medicine*, 264(1), 1-10.
19. Fernandez, L., & Hancock, R.E. (2012). Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clinical Microbiology Reviews*, 6-13.
20. Jose, M.M., & Cesar, A.A. (2016). Mechanism of antibiotics resistance. *Microbiology Spectrum*, 4(2), 10-15.
21. Shen, Z., Wang, Y., Zhang, Q., & Shen, J. (2018). Antimicrobial resistance in *Campylobacter Spp. Microbiology Spectrum*, 6(2), 4-10.
22. Almutairi, M. M., Park, S. R., Rose, S., Hansen, D. A., Vazquez-Laslop, N., & Douthwaite, S. (2015). Resistance to ketolide antibiotics by coordinated expression of rRNA methyltransferases in a bacterial producer of natural ketolides. *Proceedings of National Academy of Science U.S.A,* (112), 12956–12961.
23. Yeats, C., Finn, R.D., & Bateman, A. (2002). The PASTS domain: a beta-lactam-binding domain. *Trends in Biochemistry and Sciences*, 27, 432-438.
24. Ogawara, H. (2015). Penicillin-binding proteins in *Actinobacteria*. *Journal of Antibiotics*, 68, 223-245.
25. Binda, E., Marinelli, F., & Maroone, G.L. (2014). Old and new glycopeptide antibiotics: action and resistance. *Antibiotics (Basel)*, 3, 572-594.
26. Frasch, H.J., Kalan, L., Kilian, R., Martin, T., Wright, G.D., & Stegmann, E. (2015). Alternative pathway to a glycopeptide-resistant cell wall in the balhimycin producer *Amycolatopsis balhimycina*. *ACS Infectious Diseases*, 1, 243-252.
27. Peterson, R.M., Huang, Rudolf, J.D., Smanski, M.J., & Shen, B. (2014). Mechanism of self-resistance in the platensimycin-and platencin-producing *Streptomyces platensis* MA7327 and MA7339 strains. *Chemistry and Biology*, 21, 389-397.
28. Schultsz, C., & Geerlings, S. (2012). Plasmid-mediated resistance in *Enterobacteriaceae*. *Drugs*, 72, 1–16.
29. Nikaido, H. (2009). Multidrug resistance in bacteria. *Annual Reviews in Biochemistry*, 78, 119-146.
30. Partridge, S.R., Kwong, S.M., Firth, N., & Jensen, S.O. (2018). Mobile genetic element associated with antimicrobial resistance. *Clinical Microbiology Review*, 31(4), 1-45.

31. Archana, I., Elie, B., Esam, I.A., Alaaeddin, A.E.S., Hani Mutlak, A.H., & Ishtiaq, Q. (2013). Transposable elements in *Escherichia coli* antimicrobial resistance. *Advance Bioscience and Biotechnology*, 4, 415–423.
32. Bennett, P.M. (2010). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, 153, 347–357.
33. Kapitonov, V.V., & Jurka, J. (2008). A universal classification of eukaryotic transposable elements implemented in rephrase. *Nature and Reviews in Genetics*, 9, 411-412.
34. Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J.L., Capy, P., & Chalhoub, B. (2017). A unified classification system for eukaryotic transposable elements. *Natural Review on Genetics*, 8, 973–982
35. Bush, K. (2013). Proliferation and significance of clinically relevant β-lactamases. *Annuals of New York Academy of Science*, 1277, 84–90
36. Nicolas, I., Bordeau, V., Bondon, A., Baudy-Floc'h, M., & Felden, B. (2019). Novel antibiotics effective against Gram-positive and negative multi-resistant bacteria with limited resistance. *PLOS Biology*, 17(7), 5-13.
37. Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O., & Piddock, L.J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42-51.
38. Mazel, D. (2006). Integrons: agents of bacterial evolution. *Nature Reviews Microbiology*, 4(8), 608-620.
39. Gerber, J.S., Hersh, A.L., Kronman, M.P., Newland, J.G., Ross, R.K., & Metjian, T.A. (2017). Development and application of an antibiotic spectrum index for benchmarking antibiotic selection patterns across hospitals. *Infection Control and Hospital Epidemiology*, 38(8), 993-997.