Mechanisms of antimicrobial resistance

Lee Xianhao Song *

Redmond High School, Washington State, United Sates

* Corresponding Author Email: leesongguo@outlook.com

Abstract. Antimicrobial resistance or AMR is a phenomenon that is characterized by a resistance towards antibiotics found in bacteria. It has become especially prevalent in the last 5 decades due along with development of antibiotics which are often misused. Bacteria obtain these resistant characteristics from mutation and all other mechanisms of acquiring new genes. A variety of resistant mechanisms are present in bacteria, each of which are better suited for certain antibiotics which include: Inactivation of the drug, alteration of target sites, alteration of cell permeability, and efflux pumps. There are two main ways which drugs are inactivated. Firstly, the drug is completely degraded and second, the drug is inactivated by binding to a chemical group. Both are done by enzymes within the bacteria. Alteration of drug sites involves the mutation of potential targets within the cell for antibiotics. This often takes the form of changing its chemical structure, inhibiting the target, or increasing in potential target sites. Alteration of permeability are often mutations in the cell membrane so that antibiotics are unable to enter the cell. This often involves the alteration of porins which are channels that allow foreign substance into the cell. The mechanism of efflux pumps involves the action of removing antibitics that are already in the cell. Efflux pumps are believed to be an innate factor of bacteria originally being used to remove potentially harmful but natural substances.

Keywords: Antimicrobial Resistance (AMR), Antimicrobial agents, β-lactam drugs, Efflux Pumps, Tetracycline.

1. Introduction

Antimicrobial medicine has been an effective way to fight off life threatening bacteria and fungi ever since its development. However, like any living organism, the target of these microbial has found a way to fight back against the antimicrobial medicine designed to kill them. Whether through mutation or gene transference, the target of these antimicrobials has acquired sufficient genes to defend against a wide variety of currently used antimicrobials. The issue of antimicrobial resistance has especially risen over the last five decades. This sudden rise is likely due to the overuse and misuse of common antibiotics giving bacteria sufficient exposure to develop resistant mutations. The rising resistance is an issue of great concern because it limits the options of treatment for certain diseases. The result is that harmful bacteria become more dangerous and more impactful. Growing antimicrobial resistance also has far reaching effects beyond that of those who are directly infected. Animals have also developed a reliance on antimicrobial agents to protect them from potentially harmful bacteria. Similarly, this rise of resistance also affects livestock wellness and mortality which in turn brings negative impact to the economy.

Antimicrobial agents are grouped based on their mechanism of killing bacteria. These include the inhibition of wall synthesis, the inhibition of protein synthesis, the inhibition of metabolic pathways, and the inhibition of nucleic acid synthesis.

Cell wall synthesis inhibitors have historically been very effective and include B-Lactam antibiotics. The cell wall is a vital part of the survival of bacteria cells. It provides the cell with a rigid structure which in turn allows the cell to carry out vital functions. The rigidness of the cell wall is due to a polymer called peptidoglycan, which attaches itself to the cell wall. Before mentioned **β**-Lactam antibiotics actively inhibit the synthesis of peptidoglycan. Another mechanism for these drugs is that they bind to amino acids within the cell wall which prevents the peptidoglycan from binding to them.

Proteins are an essential component to every living organism therefore, protein synthesis is a vital process. The translation of RNA to functional proteins is carried out by the ribosomes. Protein

synthesis inhibiting drugs bind to these ribosomes preventing the synthesis of vital proteins killing the bacteria.

Metabolic pathways are the pathways in which metabolites are created. Dihydrofolic acid is a metabolite which is essential for the synthesis if thymine, nucleotides, and other amino acids. Metabolic pathways inhibitors inhibit the process that produces dihydrofolic acid which deprives the cell of essential biological molecules resulting in death of the cell.

Nucliec acids are building blocks of the genetic material of any living organism. Synthesis of such genetic material requires many components and enzymes which nucleic acid inhibitors attack. DNA topoisomerase, responsible for releasing the strains of DNA for DNA synthesis, is a common target of nucleic acid synthesis inhibitors. When topoisomerase is inhibited, DNA synthesis is blocked.

2. Mechanisms of antimicrobial resistance

Resistance against antimicrobials in bacteria acquired through mutation or other bacteria are categorized into four main categories: inactivation of the drug, alteration of targeted areas, alteration of cell permeability, and efflux pumps (shown in figure 1).

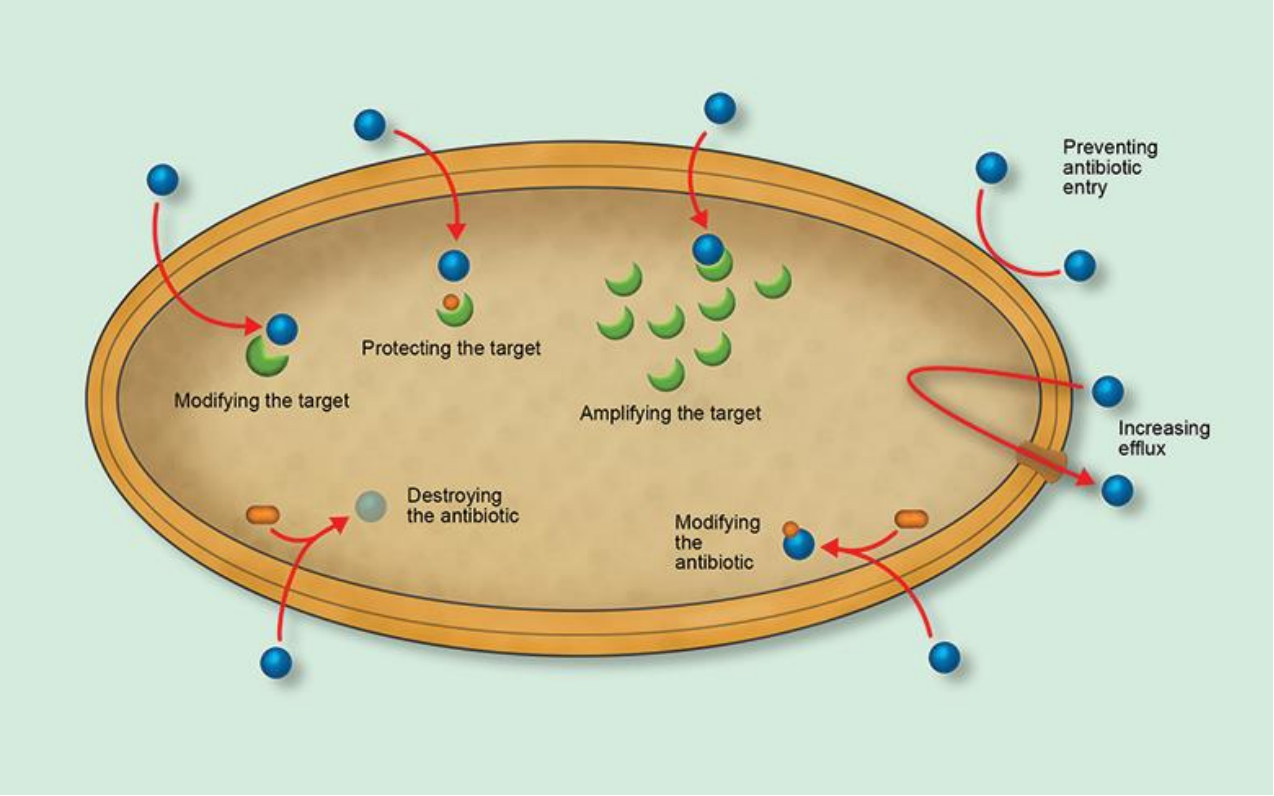

Fig. 1 Mechanisms of antimicrobial resistance [1].

The blue circular objects are respresentations of antibiotics, the green objects respresent target proteins, the orange objects represent enzymes. The figure above the text “modifying the target“ displays a modified target protein so that the antibiotic may no longer fit. It is followed by a visual representation of a protective enzyme competing for the target site above the text “protecting the target“. On the right of the protected target displays the situation where the target is amplified so that it will require more antibiotics to be effective. On the top right where the antibiotic is unable to enter the cell represents when the membrane of the cell prevents entry of the antibiotic. On the right the figure displays an antibiotic entering then leaving the cell which visualizes the function of efflux pumps removing antibiotics. The two situations near the bottom shows how an enzyme may degrade an antibiotic or modify it by attaching a group to the anitbiotic.

2.1. Inactivation of the drug

Bacteria have developed the mechanism to inactivate drugs in two ways, complete degradation of the drug, or transference of a chemical group to the drug. Penicillin is well-known and was deemed a miracle upon discovery. It is part of the **β**-Lactam drug class which is characterized by the presence of a β-lactam ring and its ability to inhibit cell wall synthesis. Target sites are called penicillin binding proteins or PBPs. Over the years, the targeted organisms have developed a defense mechanism of producing β-lactamases which are able to hydrolyze β-lactam class drugs. **β**-lactamases hydrolyze specific sites of the drug which opens the ring. With the ring opened, the drug is unable to bind to its target PBP. **β**-lactamases are categorized into three different functional groupings: the cephalosporinases, the serine β-lactamases, and the metallo β-lactamase. They are also categorized into four structural groups: A, B, C, and D. There is a relation in the functional grouping and functional groupings as they can be mutually classified. Structural Class C β-lactamases correspond with cephalosporinases, class A and D β-lactamases correspond with serine β-lactamases, and class B β-lactamases correspond with metallo β-lactamases. Classes perform better against certain types of antibiotics. Cephalosporinases are characterized by its effectiveness against cephalosporins, a type of β-lactam antimicrobial, and benzylpenicillin and resistant to inhibition of clavulanic acid. Serine β-lactamases are the most common group of β-lactamases. They are effective against benzylpenicillin and other derivatives of penicillin but less effective against cephalosporins. The third class of β-lactam antibiotics is the most unique since there is a zinc ion present at its binding site. They are recognized for their effectiveness against carbapenems which serine β-lactam are usually not effective.

2.2. Alteration of targeted areas

Alteration of targeted areas is a common strategy among bacteria against antibiotics. Tetracycline is a drug that targets the ribosome and is commonly protected by altering drug targets. Tetracycline resistance genes, Tet(M) and Tet(O), are genes that code for certain proteins that are resistant to tetracycline. They are commonly found throughout the chromosomal DNA of different bacteria likely due to conjugation. Tet(O) and Tet(M) coded proteins are able to remove already attached tetracycline from the ribosomes. Tet(M) may directly remove tetracycline molecules from domain IV of the 16S rRNA tetracycline binding site. When this interaction happens, the binding site of the ribosome so that the same tetracycline is unable to bind (as shown in figure 2). Tet(O) has been observed to undergo a similar interaction where it competes with tetracycline over the same binding space. This alters the binding site of the ribosome to displace the tetracycline [2].

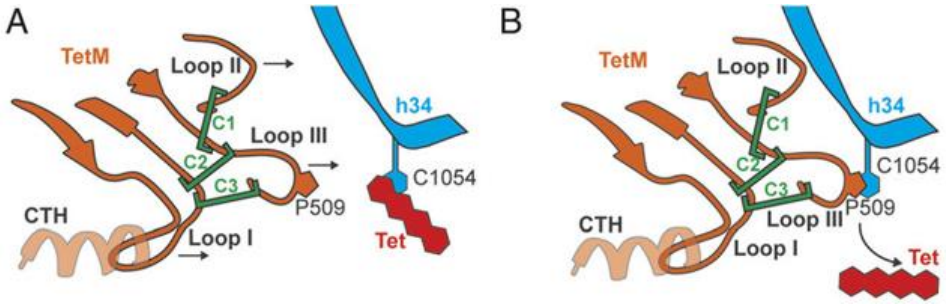

Fig. 2 The mechanism of action of the TetM protein [3].

In figure A, the tetracycline is binded to the proline residue while the TetM is moving towards the tetracycline bonding site. In figure B, the TetM competes for the bonding site and dislodged the tetracycline from the bonding site. In both figures, the TetM is binded together by green objects lables C1-3 which are intermolecular interactions that stabilize the protein.

There is also mechanisms of target alteration of defense against β-lactam drugs almost exclusive to gram positive bacteria. The resistance is developed through alteration of the number or structure of penicillin-binding proteins. Transpeptidases are a common penicillin binding protein which is responsible for the construction of peptidoglycan, the polymer responsible for making the cell wall rigid. Bacteria may change the number of transpeptidases to decrease the number of drugs that could

potentially bind. It may also alter the structure of transpeptidase to decrease the ability of drugs to bind to it or completely remove the ability to bind [4].

Resistance to another cell wall synthesis inhibiting drug known as vancomycin has become especially problematic in enterococci. Acquisition of the *van* genes which changes the structure of the peptidoglycan precursor decreases the ability of vancomycin to bind to it. Another example, daptomycin works by depolarizing the cell. It requires the presence of calcium to bind to. Mutations in certain bacteria have changed the charge of the membrane which prevents the binding of calcium making daptomycin ineffective.

Every component of the bacteria that can be targeted by a drug has some present form of alteration in response to the drug [4].

2.3. Alteration of cell permeability

Gram negative bacteria possess an innate resistance to certain antibiotics due to the characteristics of its lipopolysaccharides (LPS) layer. The surface of the LPS layer is largely hydrophilic due to the prevalence of sugar and phosphate groups. This results in the decreased permeability of hydrophobic substances like aminoglycosides and macrolides. Membrane permeabilizers such as polymyxin have been used to increase the susceptibility of certain gram-negative bacteria. They cause the release of LPS from the outer layer making the cells outer layer lose its protective properties [5].

Certain bacteria such as *S. typhimurium* and *E. coli* have been found to posses mutations resistant to polymyxin. The LPS derived from these resistant bacteria have found to possess 4 to 6 times more 4-aminoarabinose, a sugar commonly found in the construction of the LPS layer, and phosphorylethanolamine, a compound linking a phosphate to the second carbon of an ethanolamine. Subsequently, the bacteria have a lower charge and decrease the distance between LPS molecules which leads the layer being tighter and less susceptible to polymyxin [5].

The way in which foreign compounds can enter bacterial cells are through surface proteins called porins. They are present in all types of bacteria which leaves them open to antimicrobial resistance mutations [6]. Resistance to certain drugs would result in the presence of different types of porins and number of porins themselves. Three major mutations of porins can be found to increase resistance of the bacteria, as shown in figure 3, which are: porin-loss, narrower porin channels, and decreased porin expression [6].

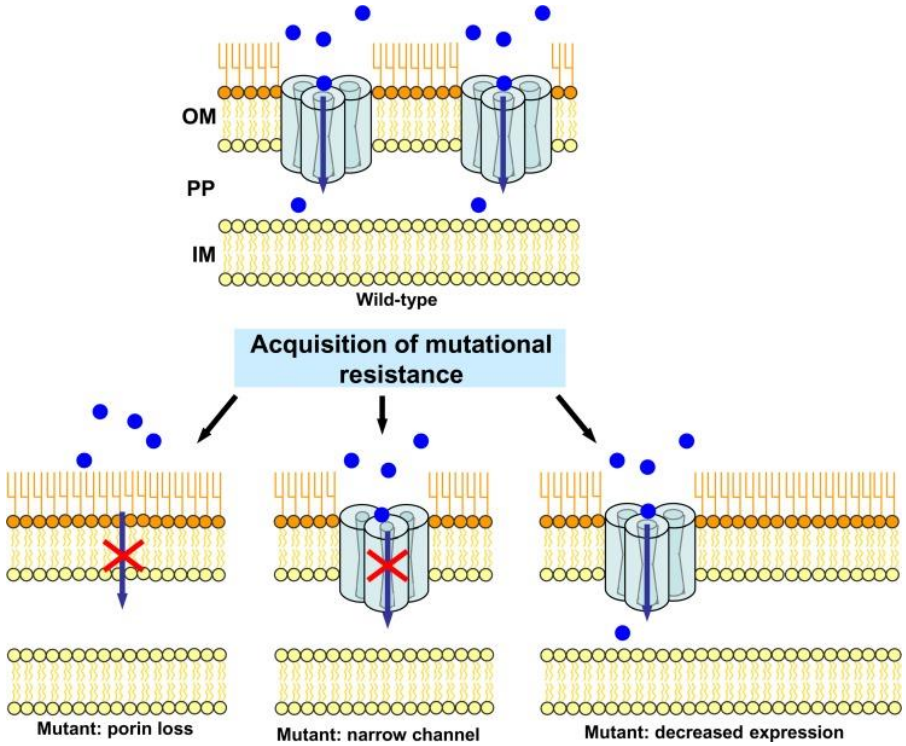

Fig. 3 A visual representation of the different mutations that lead to AMR via porins [5].

The blue circular objects are representitive of antibiotic molecules. The top of the figure displays the regular passage of antibiotics without any mutations as the antibiotic passes through the porin challen and into through the outer membrane. Then it displays the certain mutations that could arise to prevent the entry of antibiotics. First the right displays a cell membrane with no prorins leaving no possible entrance for antibiotics. In the middle displays the mutation of a narrow channel where the middle of the channel is the narrowest part and prevents any antibiotics from entering. On the right, the figure displays decreased expression where there is only one porin which leaves less possible passsages of entrance for the anitbiotics.

Some bacteria have become resistant by a complete loss of certain types of porins or reduction of their porins. Some examples include: the decrease of OprD in the bacteria species *P. aeruginosa* allowing it to be resistant to imipenem, loss of CarO in *A. baumanii* allowing the resistance of carbapenem [7].

Resistant mutations can also affect the function of the porins. Loop three of the porin is the area of the channel that tightens into the porin as shown in the figure. An example of alteration in this area comes from the mutation of the OmpF protein of the *Entrerobacter aerogenes* which leads to a loss of conductance of the porins. This decreases the susceptibility of β-lactam type antibiotics [5].

2.4. Efflux pumps

The final major mechanism resistance is the presence of multidrug effective efflux pumps. Efflux pumps are pumps with the function of displacing harmful substances from the cytoplasm to either outside the cell membrane or the outer membrane. The genes that code for efflux pumps are present in all bacteria, likely indicating that efflux pumps are an intrinsic characteristic of bacteria [8]. Bacteria often possess many different types of efflux pumps. There are five different types of efflux pumps based on their energy source and its selectivity of drugs: ATP-binding cassette, the multidrug and toxic compound extrusion family, the small multidrug resistance family the major facilitator superfamily, and resistance-nodulation-cell division family.

3. Conclusion

The growing antimicrobial resistance shows how the war against pathogens will always persist and its solution is not so simple. The presence of genes resistant to antimicrobial agents are present throughout different types of bacterial chromosomes and DNA plasmids. Because these genes are present in plasmids, they are prone to being spread through the bacterial population through conjugation. Bacteria have conjured a variety of mechanisms to counter the fast development of antimicrobials each suited best for themselves. The large variety of mechanisms and even ways the bacteria has mutated has made the issue of AMR all a more difficult problem to deal with. Has there been global mobilization on addressing issues of AMR focused on monitoring its growth.

4. Discussion

Of course, as bacteria are fighting back against human efforts to defeat them, mainly achived by four mechanisms, including inactivation of the drug, alteration of targeted areas, alteration of cell permeability, and efflux pumps. Meanwhile, society itself has increased its fighting effort against this resistance. There are currently some global efforts being taken to address growing antimicrobial resistance. Firstly, the one health approach is the idea of unifying the resolve of the issue of antimicrobial resistance. This idea puts the welfare of humans, animals, and plants into consideration. It aims to control antimicrobial resistance by bringing together stakeholders to establish communication and collaboration, implement policies and monitoring programs. Some countries adopted the Global Action Plan on antimicrobial resistance (GAP) during the 2015 World Health Assembly to discuss the implementation of action plans and the One Health approach. There has also

been an establishment of increasing awareness of antimicrobial resistance. World AMR resistance week is a global campaign to raise awareness communicating practices with the public [9].

The AMR national plans actions have been developed by 178 countries since November 2023. They've aligned with the GAP to establish multisectoral governance to mobilize resources and effectively implement plans of monitoring mechanisms [9].

Like mentioned earlier, misuse of antibiotics has been a major contributor to the growing prevalence of antimicrobial resistance. In developing countries less than 40% of patients in the public sector have irrational treatments. Addressing the issue of misusing antibiotics will be essential in slowing down the development of AMR. Incorporating empirical antibiotic therapy should be a good way to combat this issue. Establishing guidelines for antibiotic use that includes the type of antibiotics will be helpful [10].

References

[1] *OLCreate: Fleming Fund C Introducing antimicrobial resistance: Figure 15 An overview of the mechanisms of antibiotic resistance. | OLCreate*. (n.d.). https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=159673&extra=thumbnailfigure_idm450

[2] Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, *4*(2). https://doi.org/10.1128/microbiolspec.vmbf-0016-2015

[3] *Fig. 5. Schematic model for TetM-mediated tetracycline resistance. (A. . .* (n.d.). ResearchGate. https://www.researchgate.net/figure/Schematic-model-for-TetM-mediated-tetracycline-resistance-A-and-B-Upon-TetM-binding-to_fig5_274965372

[4] Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, *4*(3), 482–501. https://doi.org/10.3934/microbiol.2018.3.482

[5] Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochimica Et Biophysica Acta (BBA) - Proteins and Proteomics*, *1794*(5), 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005

[6] Fernández, L., & Hancock, R. E. W. (2012). Adaptive and mutational resistance: Role of porins and Efflux pumps in drug resistance. *Clinical Microbiology Reviews*, *25*(4), 661–681. https://doi.org/10.1128/cmr.00043-12

[7] Roy, S., Naha, S., Rao, A., & Basu, S. (2021). CRISPR-Cas system, antibiotic resistance and virulence in bacteria: Through a common lens. *Progress in Molecular Biology and Translational Science*, 123–174. https://doi.org/10.1016/bs.pmbts.2020.12.005

[8] Sharma, A., Gupta, V. K., & Pathania, R. (2019). Efflux pump inhibitors for bacterial pathogens: From bench to bedside. *The Indian Journal of Medical Research*, *149*(2), 129. https://doi.org/10.4103/ijmr.ijmr_2079_17

[9] World Health Organization: WHO. (2023, November 21). *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

[10] Uchil, R. R., Kohli, G. S., Katekhaye, V. M., & Swami, O. C. (2014). Strategies to combat antimicrobial resistance. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/jcdr/2014/8925.4529