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## Molecular and Cellular mechanisms in Drug Resistant Bacteria

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### ABSTRACT

*The presence of antibiotics in the natural systems affects the environment. Their incidence has led to the increased emergence of antibiotic resistant microbes. These multi drug resistant organisms persist and spread globally, thereby causing clinical failures in treating infections and health crises. Treatment of such infections caused by multi drug resistant bacteria has been compromised although it is imperative that such resistance has been attributed to various molecular and cellular changes in the bacteria. This review focuses on the various cellular and molecular modifications that happens in a bacterial cell in general. It is important for us in order to understand these mechanisms for the better management of the antibiotics.*

**Keywords:** Antibiotics, Pathogens, Multi Drug Resistant and Bacterial Resistance.

### INTRODUCTION

Antibiotics have been produced in the nature long time ago, but they are used for first time to treat humans 60 years back, these anti-infection agents have shown to be promising in diminishing human mortality and morbidity rate (D'Costa, V. M. *et al.* 2011; Jose L. Martinez, 2009). However, because of their wide spread and misuse led to the development of resistant strains to most of the regularly utilized antibiotics (Michael *et al.* 2016). In 2000, the World Health Organization warned that "the world might be plunged back to the 'pre-antibiotic era' once individuals usually die from disease that in trendy time are simply treated with antibiotics. It has been suggested that a decline in the antibiotic usage would led the susceptible microbes empower them to outcompete the resistant strains over time (Levin, B. R *et al.*, 2002; Andersson *et al.*, 1999). Multi drug resistant (MDR) bacteria are spread in various environments (Nazret *et al.*, 2015) including rivers, sewage, ocean water and drinking water (Hermanson *et al.*, 1987; Ash *et al.*, 2002; Reinthalr *et al.*, 2003; Schwartz *et al.*, 2003). Agriculture, animal husbandry and as well as the indiscriminate use of antibiotics has resulted in the spread drug-resistance microbes to all parts of the world (Col and O'Conner., 1987). Further, eutrophication of waterways and lakes encouraged the development and survival of enteric pathogens (Olapade *et al.*, 2006) several rivers were shown to become reservoirs of antibiotic resistant microbes (Pathak *et al.*, 1993; Ash *et al.*, 2002; Ram *et al.*, 2003). Increased introduction of antimicrobial agents into the environment via medical therapy, municipal sewage water and wastes (Bruneau *et al.*, 2004; Qadri *et al.*, 2005; Hamwlin *et al.*, 2006) led to the increase of MDR spread in the environments. In 2015, in India nearly 60,000 infants died because they were born with bacterial infections resistant to most known antibiotics (Center for American Progress Action Fund, 2005-2016). It is estimated that 23,000 people die from infections caused by antibiotic resistant bacteria every year, and many people are hospitalized due to drug resistant strains of microorganisms.

"There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness'[resistance]." - Alexander Fleming, 1946

This sentence emphasize that bacteria use circumvent the antibiotics surely before the outcome of therapeutic interventions. The intended modes of action of antibiotics may be counteracted by bacteria via several different means. No single resistance mechanism is responsible for the observed drug resistance in bacteria (Pharmacology Module, Mechanism of Action).

Majorly bacterial resistance strategy involves:

- (1) Reducing the ability of cell penetration by the drug, thereby reducing the chances of reaching its target.
- (2) Employing of general and specific efflux pumps for the removal of antimicrobial agents
- (3) Inactivation by modification or degradation of the drug.
- (4) Modification of the drug target within the bacterial cell

The resistance is also been rendered by different types of mobile DNA segments, such as Plasmids, transposons and Integrons. There are two main mechanisms that play an important role in the development of antibiotic resistance, mutation and acquisition of resistant genes by horizontal gene transfer (HGT) (**Martinez and Baquero**, 2000; **Davies**, 1994).

Genetic mechanisms that are known to mediate HGT are

- (1) Conjugation (**Lederberg and Tatum** 1946, **article 4**): It occurs by normal cell contact, wherein particular plasmids or transposons are exchanged from donor to recipient cells. An example of this mechanism, *floR* gene encoding florofenicol resistance in *E.coli* that is generally found in cattle (**Cloekaert et al.**, 2000). These conjugative plasmids have been identified in various Gram-negative and Gram-positive bacteria. Some of these conjugative plasmids are known to even mobilize chromosomally located genes to recipient cells (**Haas and Holloway**, 1976)
- (2) Transformation (**Avery et al.**, 1994): It involves the uptake of cell free DNA and its incorporation into the bacterial cell and as well as stable inheritance of that DNA. There are several factors that play a role in acquiring the DNA from the environments such as cell wall structure and bacterial species. Bacteria should be competent enough to accept foreign DNA.  
*Campylobacter* Spp are thought to be naturally competent. Specific sequences are required for the new DNA to be taken up by the bacteria.
- (3) Transduction (**Zinder and Lederberg**, 1952): Genetic material is introduced into the bacterium by bacteriophage. This concept is being employed in molecular biology, especially in cloning for presenting vector DNA; antimicrobial resistance, through bacteriophage 'λ' (**Sambrook et al.**, 1989). The transduction mechanism is mediated by two mechanisms. In specialized transduction, specific genes (bacterial in origin) from the phage genome are coordinated into the bacterial chromosome. But whereas in generalized transduction, any gene that is already packed in the phage from the host bacteria can be transferred into another bacteria. Phages are known to exhibit narrow host range of infection, for which transduction may not be able to contribute to the gene exchange among distantly related bacteria.

Certain sections of bacterial DNA chromosome such as Transposons and Integrons followed by the extra chromosomal plasmids are also involved in the drug resistance mechanism (**Davies**, 1978; **Herwig**, 1997). The plasmid transfer in between various bacteria is known to happen in various terrestrial environments of soil and aquatic in origin (**Mach, P.A. et al.**, 1982; **Bale, M.J et al.**, 1988; **Smit, E et al.**, 1995). Number of genes that encode for multiple drug resistance are known to be present on a single plasmid (**Kobori, H. et al.**, 1984). Plasmids are known to be transferred in between different bacteria by conjugation and transformation process. Mostly resistance genes are often located on transposons (**Tenover**, 1991), that has the ability to move from genetic locus to another, irrespective of same or different group of bacteria (**Ochman et al.**, 2000). Transposons are also transferred by conjugation, transformation and transduction process that play a pivotal role in antimicrobial resistance. Integrons, mobile DNA elements, are also known to transfer the antimicrobial resistance with specific conserved regions flanking the central region (**Ochman et al.**, 2000). The genes encoding the resistance will be either located on the 5' or 3' end of the gene cassette (**Hall and Collis**, 1995; **Rowe-Magnus and Mazel**, 1999; **Ochman et al.**, 2000). Integrase enzyme linearizes the circular gene sequence that has been exchanged and inserts at the integration site of the integron, thereby spreading antimicrobial resistance. The spread of antibiotic resistance by integrons has been observed in enteric bacteria such as *Campylobacter* spp., *E.coli*, and *Salmonella enterica* serotype *Typhimurium* (**Ochman, H. et al.**, 2000; **Lucey, B. et al.**, 2000; **Briggs, C.E. et al.**, 1999).

Table 1. Mobile genetic elements and their antibiotic resistance

Genetic Element	General characteristics	Resistance Determinant(s) Specified/Examples
Plasmid	Variable size (1- >100 kb), conjugative and mobilizable	R factor: multiple resistances
Insertion sequence	Small (<2.5 kb), contains terminal inverted repeats, and specifies a transposase	IS1, IS3, IS4, etc.
Composite (compound) Transposon	Flanked by insertion sequences and/or inverted repeats	Tn5: Kan, Bleo, and Str
Complex transposon	Large (>5 kb), flanked by short terminal inverted repeats, and specifies a transposase and recombinase	Tn1 and Tn3: $\beta$ -lactamase Tn7: Tmp, Str, Spc Tn1546: glycopeptides
Conjugative transposon	Promotes self-transfer	Tn916: Tet and Mino Tn1545: Tet, Mino, Ery, and Kan
Transposable bacteriophage	A bacterial virus that can insert into the chromosome	Mu
Other transposable elements	Other than composite, complex, and conjugative transposons	Tn4: Amp, Str, Sul, and Hg Tn1691: Gen, Str, Sul, Cm, and Hg
Integron	Facilitates acquisition and dissemination of gene cassettes; specifies an integrase, attachments sites, and transcriptional elements to drive expression of multiple resistance genes	Class 1: Multiple single determinants and MDR efflux pump (Qac)b Class 2: Tmp, Strp, Str, and Spc (Tn7) Class 3: carbapenems Class 4: <i>Vibrio</i> spp. super-integron

Abbreviations: Amp: ampicillin; Bleo: bleomycin; Cm: chloramphenicol; Ery: erythromycin; Fus: fusidic acid; Gen: gentamicin; Hg: mercury; Kan: kanamycin; Mino: minocycline; Spc: spectinomycin; Sul: sulfonamide; Tet: trimethoprim; Van: vancomycin (adapted from **Michael N. Alekshun** and **Stuart B. Levy.**, 2007).

Drug resistance may also arise due to the modification or lack of certain components of bacterial cell wall. Based upon the peptidoglycan and internal cell membrane bacteria are classified as Gram positive and negative. The Gram positive bacteria are known to contain both of them but whereas peptidoglycan is absent in Gram negative organisms, for which the gram negative have an advantage against the antibiotics that act on cell wall (**Young**, 2011). Peptidoglycan is absent in humans for which it has become an ideal target for antibiotics.  $\beta$ -lactam antibiotics inhibit bacterial cell wall biosynthesis and are one of the most widely available antibiotics in treating number bacterial infections. Their mode of action involves by disrupting the linkages in the peptidoglycan. Disrupting the formation or linkages of peptidoglycan disrupts the structural integrity of the cell thereby leading to the collapse or burst of the cell wall, resulting in the death of the microorganism. For this reason  $\beta$ -lactams are classified as bactericidal drugs (MIC test strip technical sheet, 2013). Resistance to  $\beta$ -lactam antibiotics is caused by  $\beta$ -lactamases. Production of these  $\beta$ -lactamases is a major threat to  $\beta$ -lactam antibiotics (**Jun Lin et al.**, 2015). Antibiotics such as Penicillin and Cephalosporin binds to the enzymes that assemble the precursor molecules of the peptidoglycan. Carbapenem prevents the linkage of different peptidoglycan molecules. Vancomycin inhibits by binding to the precursor molecule of peptidoglycan, thereby disrupting the microorganism's ability to form new peptidoglycan molecules. As Vancomycin has a large size and it cannot cross through the porins of the outer cell membrane of gram negative bacteria, for which it is only used to treat infections caused by gram positive organisms (**Mahon et al.**, 2011). Polymyxins are also another class of antibiotics that disrupt the structural integrity of the cell, they specifically target cell membrane of Gram negative bacteria (MIC test strip technical sheet, 2013). Lipopolysaccharides are the structural components of the gram negative cell membrane and are negatively charged. Polymyxins have a stronger positive charge than Calcium and Magnesium, by which it can strongly bind to lipopolysaccharide with respect to the others, due to this there will be a loss of integrity of the outer cell membrane there by causing cell death (**Falagas and Vardakas**, 2010). Aminoglycosides are another class of antibiotics that involve aminoglycoside kinases and aminoglycoside phosphotransferases, which modify the activity of the aminoglycosides. It is necessary for the cell to create proteins

to survive, cells first copy their DNA into RNA molecule that can be further translated into protein. Antibiotics also function by disrupting the process of DNA replication. Initially, a DNA strands are unwound by helicase enzyme and topoisomerase II (DNA gyrase) that will helps DNA stabilize and relieve the strain from being unwound by helicase. There is another class of antibiotics called Quinolones that will target the complex of DNA and topoisomerase II and prevents DNA replication from occurring. Generally, after a DNA strand is unwound, a RNA copy is made from the DNA by RNA polymerase. Bacterial RNA polymerase differs slightly from that found in eukaryotic cells. Rifampicin works by binding to the RNA polymerase thereby preventing the RNA elongation. Rifampicin is the only drug currently available that is able to bind RNA polymerase and stop the transcription of RNA. It can be bactericidal and bacteriostatic based on the drug concentration (Rifadin drug information, 2013). Bacterial cells contain ribosomes which are composed of 30s and the 50s ribosomal subunit, while eukaryotic cells contain ribosomes which are 40s and 60s ribosomal subunit. Antibiotics against bacteria target the 30s and 50s ribosomal subunit. Aminoglycosides and Tetracycline targets the 30s subunit. The binding of an aminoglycoside to the 30s subunit prevents the transfer of RNA, which can leads to incorrect proteins. Tetracycline block how the RNA molecule rotates into the ribosome, which cause the RNA molecule to be released prematurely, leaving an incomplete peptide. It is important to note that these effects are reversible and if the drug concentration reduces in the patient, then the microorganism will be able to function normally. Thus, these drugs are bacteriostatic (Mahon *et al.*, 2011). Sulfonamide also functions in the similar manner by disrupting the production of DNA and RNA, by acting on folic acid synthesis which used in the synthesis of nucleotides. Many bacteria convert para-amino benzoic acid to folic acid. Sulfonamide has similar structure to para-amino benzoic acid by which it decreases the production of the cell's folic acid and thus effecting the production of DNA and RNA (Henry, 1943). Trimethoprim functions similar to sulfonamide, but the difference is that it binds with different enzyme that is involved in the production of folic acid. Both the antibiotics disrupt the production of a metabolic product the microorganism needs and are both bacteriostatic (May, 2015). There are also many different ways in which antibiotics can kill or inhibit microorganisms, wherein many mechanisms of resistance that microorganism possess or have developed over time. It is possible that by one mechanism, an organism can become resistant to many different classes of antibiotics, especially if the antibiotics function in a similar manner. Sometimes resistance can be shared between individual bacteria by the production of resistance plasmids (Clewell, 2014). Another common way of interfering with antibiotics is by preventing the entry of the drug into the cell. Gram negative bacteria have an external cell membrane, and drugs must pass through porins. A gene mutation can result in an altered porin makes the drug difficult to enter into the cell, the antibiotic is still functionally active but can't reach to its target site. A microorganism can develop resistance to multiple drug classes at once in this manner. Gram negative bacteria are generally resistant to large drugs like vancomycin, which is too large to pass through the porins even before a mutation occurs (Galdiero *et al.*, 2012). Microorganism becomes resistant to antibiotics by efflux pump. An efflux pump is a biological pump that can force antibiotics out of the cell, so that it can't reach to its target site. This method of antimicrobial resistance may often also create resistance to more than one class of antibiotics. Sometimes antibiotic functions by competing for an enzyme binding site in order to disrupt the production of a product, a microorganism will develop resistance by producing more of that enzyme. This will increase the concentration of the enzyme, so that even if all antibiotic molecules bind to an enzyme, there will still be enough to create the metabolic product. Additionally, a microorganism could obtain the compound that it needs to survive from another source. For example, sulfonamides bind to an enzyme in order to disrupt the production of folic acid. Microorganism can render sulfonamides ineffective by taking folic acid from the external environment.

So now that the mechanism of antibiotic action and resistance are discussed, it may be further helpful to consider how microorganism acquire and develop resistance over time. Sometimes genetic mutations occur when the DNA of an organism gets copied. Some sort of random genetic mutation can occur in a large population of the bacteria as bacteria can quickly copy the DNA when compared with eukaryotic cells (Taylor, 2011). However such mutations may not have a phenotypical effect or have a detrimental effects. But, it is possible that the mutation might have produced a slightly altered porin, or is able to produce a new enzyme or product. Sometimes, this mutation can also be shared in between individual bacteria through R- plasmid. Sometimes microorganisms need to compete for space and resources and such mutations may not benefit the organism in the absence of antibiotic, but when antibiotics are present then there is a greater advantage for such mutated organism to survive. The normal strain of the organism will be inhibited and the resistant strain of the organism is able to grow with little competition, if it is able to survive the immune response of the immune response of the host. Eventually, most or all of that organism's species in the host will be resistant to the antibiotic that was consumed. The antibiotic that was initially effective at the infection will not be very effective against the new resistant strain of organisms. The host is also now carrying the resistant strain, which could potentially spread to other people. By this way, an infectious and antibiotic resistant strain can emerge. The discovery of new antimicrobials as well as finding the new strategies to increase the life of existing antibiotics delays

antimicrobial resistance. Bacteria however, possess diversity of genes that allow them sooner or later to counteract the action of newly invented antibiotics.

## CONCLUSION

Even since antibiotics began to be widely used to treat infections in the 1940's, the incidence of antibiotic resistant strains has increased. These MDR organism cause a serious issues including longer illness, more fatality rate and increased the rate cost of the treatment. Surprisingly, certain organisms are becoming difficult to treat by antibiotics rendering antibiotic become less effective. The issues of drug resistant bacteria has gained lots of attention from the government, public, researchers, and also hospital personnel. It is important for everyone working in health-care to have attempt to control the spread of antibiotic resistant infections. It is important for everyone who working in health care to know the basic understanding of the issue of the antibiotic resistance. By knowing the mechanism of antibiotic and drug resistant bacteria, how and why resistance develops, and the problems associated with antibiotic resistant infections, health care workers will be better equipped to work with the problem of antibiotic resistance. Ultimately, Personal hygiene has no alternative, washing thoroughly with soap after contact with contamination can prove to be effectively safe. The Misuse of antibiotic for application and dosage is an additional contributing factor for the development of antibiotic resistance. On the other hand, it is also reflected that the development of new antibiotics, it is imperative to study molecular basis of resistance developments so that we can prevent and overcome antibiotic resistance by targeting resistance mechanism, which will make the existing and novel antibiotics more effective and sustainable.

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