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Review Article

Various Molecular Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa*

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❖ ABSTRACT

Antibiotics are those drugs which are commonly used for therapeutic management of variety of bacterial infections. Nowadays, bacteria seem to have developed antibiotic resistance due to inappropriate use of medications. *Pseudomonas aeruginosa* is a nosocomial gram-negative pathogen which causes most fatal infections in humans. This species of pseudomonas is resistant to many antibiotics and is among World Health Organization pathogen list of primary concern for investigation and development of novel antibiotics. Apart from its unique potential to develop intrinsic or innate resistance to several conventional antibiotics, *pseudomonas aeruginosa* can also acquire resistance by mutation in its chromosomes, subsequently altering the membrane permeability, efflux system over-expression, antibiotic inactivating enzyme production and biofilm resistance. *Pseudomonas aeruginosa* seem to be resistant to various antibiotics such as carbapenems, penicillin & other beta-lactams, aminoglycosides and fluroquinolones. Newer antibiotic combinations such as ceftazidime-avibactam, imipenem-cilastatin/relebactam, cefiderocol have shown some promising results in treatment of antibiotic resistant *Pseudomonas aeruginosa*.

❖ **KEYWORDS:** Molecular Mechanisms, Antimicrobial resistance, *Pseudomonas aeruginosa*

ISSN 2515-8260 Volume 09, Issue 07, 2022

❖ INTRODUCTION

Antimicrobials are the drugs which are found especially useful in treating variety of infections caused by microorganisms either by killing them or inhibiting their growth. Antimicrobial includes different classes of antiviral, antibacterial and antifungal agents. *Pseudomonas aeruginosa* was firstly found in the year 1882 by bacteriologist named Carle Gessard and its first case in an infected individual was reported in 1896 (Lister *et al.,* 2009). It is Gram-negative opportunistic bacteria mainly affecting the immuno-compromised patients and causing variety of acute and chronic diseases like malignant otitis, ventilator associated pneumonia, blood infection etc. In the present scenario it has been found that *Pseudomonas aeruginosa* causes only 11 - 13.8 % infections in hospitalized patients whereas the rate of ICUs is found as 13.2 - 22.6 % (Driscoll *et al.,* 2007). It is associated with morbidity and mortality in cystic fibrosis patients and it became apparent after which it has been regarded as a main causative pathogen (Moradali *et al.,* 2017). *Pseudomonas aeruginosa* is among prime nosocomial pathogens which mainly affects hospitalized patients. Antimicrobial agents which are frequently used in the treatment of *Pseudomonas aeruginosa* infections includes aminoglycosides, beta-lactams, fluroquinolones, penicillin with beta-lactamase inhibitors, phosphonic acid & colistin (Botelho *et al.,* 2019). Excessive use of antibiotics has now led to resistance as well as cross resistance between antibiotics which are further leading to multi drug resistance (MDR) in the case of *Pseudomonas aeruginosa*. This bacterium has been found to be highly resistant to fosfomycin and even to cephalosporins, fluroquinolones & beta-lactams (Yayan *et al.,* 2015). A study was done on 80 intensive care unit (ICU) patients to find the prevalence as well resistance pattern of *Pseudomonas aeruginosa.* It has been discovered that prevalence of *Pseudomonas aeruginosa* in isolates was 13.66 % out of which 21 - 40 years and 41 - 60 years were most affected age groups (Patil*et al.,* 2022). The same study also found that the maximum resistance was reported for co-trimoxazole (86.25 %) followed by cefotaxime (83.75 %) whereas most susceptible antibiotics were piperacillin and amikacin (Patil *et al.,* 2022). Hence, it becomes vital for the microbiologists to study the sensitivity pattern of antibiotics so that the physician will be able to select right antibiotic at right time. In this review we provide an update on the resistance pattern of *pseudomonas aeruginosa* as well as the mechanisms behind such resistances.

❖ BETA-LACTAMS

Beta-lactam antibiotics are used against variety of bacterial infections. They are bactericidal against most of gram-positive as well as gram-negative bacteria. Their bactericidal property is because of their potential to inhibit transpeptidase and carboxypeptidase activity of penicillin binding proteins (PBP). Due to PBP cell wall integrity inhibition, it gets reduced and breakdown of bacteria occurs (Bush *et al.,* 2016). Carbapenems are considered as the first-line treatment in ventilator associated pneumonia and other significant infections caused by *pseudomonas aeruginosa*. Few years back carbapenems were excellent choice of antibiotic for the physicians to treat infections caused by this bacterium due to its potency. However, recently *Pseudomonas aeruginosa* has been observed in developing resistance to carbapenems because of persistent use of this drug in treating infections (Labarca *et al.,* 2014). Due to developed resistance of antimicrobials, morbidity and mortality rates associated with this bacterium have increased tremendously. In a study conducted in ICU patients, 49% patients were isolated having *Pseudomonas aeruginosa* resistant strains. These strains were resistant to various antibiotics including carbapenems (55.02%) (Feretzakis *et al.,* 2019). Another study done by Lake *et al* in pediatric patients concluded that about 10% of isolates of *Pseudomonas aeruginosa* were resistant to carbapenems (Lake *et al.,* 2018). Several case studies and meta analysis reports have concluded that patients who are infected with *Pseudomonas aeruginosa* which show resistance to this antimicrobial may have higher risk of mortality than the susceptible individuals;

ISSN 2515-8260 Volume 09, Issue 07, 2022

though it is not always true (Zhang *et al.,* 2016; Balkhair *et al.,* 2019; Lin *et al.,* 2016). There are three major resistance mechanisms which have been identified for beta-lactam resistance in *Pseudomonas aeruginosa*. All of these mechanisms arise from genetic mutations in the bacteria. It includes reduced uptake of antibiotic through porins, alteration of PBP3 target protein and overexpression of efflux pumps (Reygaert *et al.,* 2018).

1. Alteration of PBP3 target protein

Penicillin binding proteins, also abbreviated as PBP, are the enzymes that are present in the periplasm and are required during peptidoglycan synthesis in a bacterium. This peptidoglycan is utmost cell wall component in bacteria and is important for its survival and for maintaining its cell shape. PBP3 are crucial for the growth of *Pseudomonas aeruginosa*. FtsI gene encodes the PBP3.
Inhibition of this gene causes defect in the cell division (Chen *et al.,* 2017). As PBP3 is found to be essential for *Pseudomonas aeruginosa* growth, it's the main target for beta-lactam antibiotics. These antibiotics bind to PBP3 and causes conformational changes. Resistance developments in these antibiotics are due to mutations or genetic changes in FtsI gene encoding the PBP3 (Glen *et al.,* 2021). Due to this mutation, beta-lactams are not able to bind with PBP3 and show its action.

2. Reduced uptake of antibiotic through porins

Porins are those proteins in the cell wall of bacterial which mediate the entry of essential nutrients and hydrophilic molecules inside the bacterial cell. Several genes play role in the formation of these porins. OprD porin in *Pseudomonas aeruginosa*, encoded by OprD gene, is responsible for uptake of essential amino acids as well as carbapenems & faropenems (Li *et al.,* 2011). Any type of genetic changes or mutation in the OprD can disrupt its function and reduce carbapenems‟ uptake, contributing to its resistance in *Pseudomonas aeruginosa* (Li *et al.,* 2011). Mutations in OprD do not affect susceptibility of other beta-lactams in *Pseudomonas aeruginosa*. Likewise, other porins are involved in uptake of other beta-lactams, like OprF which is involved in piperacillin uptake whereas on other hand, meropenem uptake is done by OpdP (Glen*et al.,* 2021).

3. Overexpression of efflux pump

Strains of *Pseudomonas aeruginosa* that are isolated (MexAB-OprM, MexXY-OprM, and MexCDOprJ) show around 12 efflux system pumps which are responsible for antibiotic resistance (Glen *et al.,* 2021). Efflux pumps are type of channels which export different substrates out of bacterial cell by process called proton motive force (Fernando *et al.,* 2013). MexAB-OprM is the efflux pump that is present in *Pseudomonas aeruginosa*. Their over expression is associated with resistance in many beta-lactam antibiotics (meropenem, carbapenem, ticarcillin & ceftazidime) and their deletion increases susceptibility to antibiotics (Okamoto *et al.,* 2001). Over expression of this gene may occur due to mutation in its repressor proteins such as MexR, NalC etc (Pan *et al.,* 2016). MexR is the main regulator of this gene and plays a vital role in sensing oxidative stress. Moreover, MexR gets detached from the promoter area of MexAB-OprM operon, causing over-expression of pumps (Chen *et al.,* 2010).

❖ AMINOGLYCOSIDES

Most commonly used antibiotics of this class in the management of community acquired infection by *Pseudomonas aeruginosa* include tobramycin, gentamycin and amikacin. Community acquired infections are generally treated with a combination of antibiotics to avoid resistance. Resistance of aminoglycosides in *Pseudomonas aeruginosa* first time came into notice in 1960s and 1970s. A study done in Cumana, Venezuela found that 65 % patients were resistant to antibiotics out of which 30.7

ISSN 2515-8260 Volume 09, Issue 07, 2022

% were resistant to tobramycin and 29.9 % to amikacin (Teixera *et al.,* 2016). Other studies have also reported more increased frequency of *Pseudomonas aeruginosa* resistance to amikacin and tobramycin (Jones *et al.,* 2013). Mechanisms involved in aminoglycoside resistance to *Pseudomonas aeruginosa* include modification of enzymes, adaptive resistance, impermeability resistance, enhanced efflux. Aminoglycoside modifying enzyme (AME) mediates the same antimicrobial resistance caused by this bacterium. Genetic alteration in enzymes like aminoglycoside phosphoryl transferase (APH), aminoglycoside acetyltransferase (AAC), aminoglycoside adenyl transferase (ANT) takes place which contributes in *Pseudomonas aeruginosa resistance* (Vazari *et al.,* 2011). AME is encoded by four significant genes namely *by* aac (6')-I, aac(6')-II, ant*(2'')-I, and* alpha (3')-VI that gets modified and causes resistance (Vaziri *et al.,* 2011). Different patterns of resistance to aminoglycoside are observed in many countries. In Europe, *aac(6')-II* and ant(2'') were the most significant gene encoding enzymes that were linked with resistance in *Pseudomonas aeruginosa* where as in Korea the most significant were alpha (3')-VI, ant(2')-I, *and* aac (6'')-I (Strateva *et al.,* 2009). Along with the variation in AME, resistance to aminoglycoside also seems to be correlated with methylation in 16S ribosomal RNA (rRNA). This mechanism of resistance initially came to light in 1993 and gene that encodes methylase was named as rmtA (Yokoyama *et al.,* 2003). Till now, rRNA methyl-transferases have been recognized so far are namely rmtA, rmtB, rmtC and rmtD (Driscoll *et al.,* 2007). A study conducted to find out the molecular mechanism of antibiotic resistance in *Pseudomonas aeruginosa* genes deserted from burn infection patients found that 60 % of isolates were having presence of rmt B gene in them, causing resistance (Nadheer *et al.,* 2022). Impermeability is another factor involved in aminoglycoside resistance where accumulation of aminoglycoside is decreased as uptake gets reduced due to reduced permeability (Poole *et al.,* 2005).

❖ FLUROQUINOLONES

Fluroquinolones are the class of antibiotics which are widely used in treatment of variety of infections caused by bacteria since 1900s. Ciprofloxacin is the most commonly used fluroquinolone drug that is among essential medication list created by the World Health Organization (WHO). It is most commonly used effective fluroquinolone antibiotic against *Pseudomonas aeruginosa* (Andriole *et al.,* 2005). Ciprofloxacin is widely used in the management of infections caused by this bacterium such as osteochondritis, ear and eye infections (Raz *et al.,* 1995; Mosges *et al.,* 2011). However it has been observed that there is growing trend in ciprofloxacin resistance cases to *Pseudomonas aeruginosa* worldwide. There are two main mechanisms behind resistance of ciprofloxacin in *Pseudomonas aeruginosa*, which are modification of target site and efflux pumps up-regulation.

1. Target site modification

Ciprofloxacin works by binding non-covalently to DNA gyrase as well as topoisomerase IV, which are essential for the process of replication of DNA. Ciprofloxacin alters DNA replication by interacting with these two enzymes. Mutation takes place in genes that encodes DNA gyrase and topoisomerase IV causing ciprofloxacin resistance in *Pseudomonas aeruginosa* (Robillard *et al.,* 1988). GyrAB and parCE are the genes that encode for DNA gyrase and topoisomerase IVand mutation in affinity of ciprofloxacin to pick out *Pseudomonas aeruginosa* (Breidenstein *et al.,* 2008).

2. Overexpression / up regulation of efflux pumps

This is the major mechanism that is responsible in *Pseudomonas aeruginosa* resistance to antibiotics. In this, increased efflux of antibiotic within the cells occurs, due to which its intracellular concentration decreases. There are four main efflux pumps that seem to be associated with fluroquinolones efflux namely MexCD-OprJ, MexEF-OprN, MexAB-OprM and MexXYOprM (Goli

ISSN 2515-8260 Volume 09, Issue 07, 2022

et al., 2016; Llanes *et al.,* 2011). These efflux pumps are modulated by some specific regulatory proteins in which mutation might take place resulting in over expression of pumps (Sun *et al.,* 2014). Two pumps are mainly involved in ciprofloxacin resistance to *Pseudomonas aeruginosa* namely MexCD-OprJ and MexEF-OprN (Goli *et al.,* 2016; Llanes *et al.,* 2011). The genes that encode for MexCD-OprJ and MexEF-OprN are NfxB and MexS respectively. When mutations occur in these genes, production of these pumps increases, leading to MDR (Rehman *et al.,* 2019).

❖ MACROLIDE

Azithromycin and erythromycin are the most commonly used macrolides against infections caused by *Pseudomonas aeruginosa*. They work by interfering with the bacterium protein synthesis as they bind to 50S ribosomal sub-unit and blocking translation process. Cells of *Pseudomonas aeruginosa* are found to be intrinsically resistant to these macrolides (Rozgonyi *et al.,* 1989). Minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa* was discovered as > 256 $mg.L^{-1}$ in standard growth medium (Buyck *et al.,* 2012). Two mechanisms are involved behind macrolide resistance in *Pseudomonas aeruginosa* namely multi drug efflux system and mutation in 23S rRNA. It has been seen that *Pseudomonas aeruginosa* uses multi drug efflux system like many other antibiotics to flush out macrolide drugs from within the cells (Chalmers *et al.,* 2017). Another mechanism that is responsible for resistance of macrolides is mutation or changes in the V domain of 23S rRNA (Mustafa *et al.,* 2017). This mutation turned out to be correlated with changes in three main positions i.e., 2045, 2046 and 2598 (Mustafa *et al.,* 2017; Vester *et al.,* 2001). It has also been observed that mutation in position 2045 and 2046 are associated with higher level of resistance as compared to mutation in 2598 position (Douthwaite *et al.,* 2000). This is mainly because 2045 and 2046 position are a part of binding site of macrolides whereas 2598 position alters conformation of binding site (Strome *et al.,* 1977).

❖ POLYMYXINS

Polymyxins were first isolated from B*acillus polymyxa* (gram positive soil bacteria) in 1947 which was later renamed in 1993 as *Paenibacillus polymyxa* (Ash *et al.,* 1994; Strom *et al.,* 1977). 15 different variants of polymyxins have been identified so far such as polymyxin E and polymyxin M that are therapeutically named as Colistin and Mattacin respectively (Martin *et al.,* 2003; Choi *et al.,* 2009; Tambadou *et al.,* 2015). There are two majorly used polymyxins in clinical practice namely polymyxin B and Colistin (Orwa *et al.,* 2001). The only difference between these two polymyxins is D-leucine in place of D-phenylalanine in the peptide ring structure (Yu *et al.,* 2015). Polymyxins are commonly used for the treatment of cystic fibrosis patients as well as ophthalmic infections (Falagas *et al.,* 2010). Polymyxin B is given directly whereas colistin is given in its pro-drug form (colistin methane sulfonate) which then later gets hydrolyzed in the body into colistin (Landman *et al.,* 2008; Nation *et al.,* 2014). Colistin exerts its anti-bacterial effect by displacement of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions which act as membrane stabilizers from the lipopolysaccridal (LPS) membrane and thereby disrupting the membrane stability and loss of cellular contents, leading to bacteria's death (Mandes *et al.,* 2009). Nowadays, these drugs are used as last line treatment for multi-drug resistant bacterial infections such as *Pseudomonas aeruginosa* (Nation *et al.,* 2014), though there has been an increased reporting of colistin resistant strains in *pseudomonas aeruginosa*. According to the 2016 reports of European Centre for Disease Prevention and Control (ECDC) only 51.3 % of *Pseudomonas aeruginosa* isolates were sensitive to colistin (European Centre 2017). There are two main mechanisms behind colistin resistances in *Pseudomonas aeruginosa* that have been recognized so far. Those are either through mutation or via adaptive resistance.

ISSN 2515-8260 Volume 09, Issue 07, 2022

1. Mutation

Any type of alteration or genetic change that takes place in bacteria can result in resistance to known antibiotics. Example of such mutation can be elaborated by over expression of efflux pumps. This type of mutation is inherited by bacteria and is independent of presence of antibiotics (Falagas *et al.*, 2005). The MexAB-OprM and the MexXY-OprM are efflux pumps that are scientifically important in *Pseudomonas aeruginosa* and their over expression or up regulation results in development of resistance to antibiotics (Fernando *et al.*, 2013; Guenard *et al.*, 2014).

2. Adaptive resistance

As we know that polymyxins basically act by altering the calcium and magnesium ions which are integral part of lipopolysaccharide (LPS) membrane. So, any type of change in them can result in resistance to polymyxins. Some studies demonstrated that *Pseudomonas aeruginosa* has potential to develop adaptive resistance to polymyxins by modifying its lipopolysaccharide membrane with LAra4N that gets stimulated by regulatory systems such as pmrA/pmrB and phoP/phoQ TCSs (McPhee *et al.*, 2003; Moskowitz *et al.*, 2012). Several strains of *Pseudomonas aeruginosa* have found to have mutation in pmrB. This genetic variation has eventually led to modification of lipid A with L-Ara4N (Olaitan *et al.*, 2014). Some strains having double mutation in pmrB have also been reported and these multi-mutated strains show higher resistance level as compared to single mutation strains (Moskowitz *et al.*, 2012). So far, a single study has reported colistin resistance owing to mutation in pmrA (Lee *et al.*, 2014). Another regulatory system whose activation can cause resistance of colistin in *Pseudomonas aeruginosa* is phoP/phoQ TCS. Several mutations of the phoQ gene can results in deletion, frame shifts or truncations eventually leading to resistance in *Pseudomonas aeruginosa* (Miller *et al.*, 2011). Colistin MICs is found to be 8 to > 512 mg/L in most cases of *Pseudomonas aeruginosa* that have mutation in their phoP/phoQ genes (Olaitam *et al.*, 2014).

❖ DISCUSSION

Pseudomonas aeruginosa is a prominent nosocomial pathogen which is major reason for infections in hospitalized patients and is a considerable cause of morbidity and mortality in them. The infection management caused by *Pseudomonas aeruginosa* has become very challenging due to significantly arising cases of multi drug resistance in them. *Pseudomonas aeruginosa* has very intricate resistance mechanisms as it can develop intrinsic resistance as well as adaptive resistance. Due to variety of resistance mechanisms *Pseudomonas aeruginosa* has become resistant to standard antibiotic treatment. Here, we have summarized variable resistance mechanisms of *Pseudomonas aeruginosa* to various classes of antibiotics which are used as conventional therapies in other infections. Thus, the result indicates increasing need for appropriate approaches to deal with growing resistance in *Pseudomonas aeruginosa*. Multiple strategies need to be employed to curb the rising trend of AMR like continuous monitoring of resistance from antimicrobials, developing new antimicrobials, revert resistance back to susceptibility with the aid of antibiotic adjuvants (Hernando *et al.*, 2020). Polymyxins were used as a last resort of treatment in multi-drug resistant *Pseudomonas aeruginosa* but there has been an increase in researches reporting isolates which have developed resistance to even polymyxins. In our study we also encountered a problem regarding the molecular mechanism behind the resistance of macrolides in *Pseudomonas aeruginosa*. We did not find much evidence and other researches stating the detailed mechanism behind macrolide resistance, so it is still unclear. More researches need to be done in the nearby future so as to pinpoint the exact mechanism behind *Pseudomonas aeruginosa* resistance to macrolide antibiotics.

ISSN 2515-8260 Volume 09, Issue 07, 2022

❖ CONCLUSION

There has been a steady decline in susceptibility of antibiotics to *Pseudomonas aeruginosa*. This retrieved and identified molecular mechanisms behind the multi-drug resistance of *Pseudomonas aeruginosa* to different antibiotics such as fosfomycin, cephalosporins, beta-lactams, aminoglycosides, macrolides. WHO has declared carbapenem resistant *Pseudomonas aeruginosa* as critical priority as per pathogen priority list. It is required to have further studies to identify various molecular mechanism of drug resistance development for *Pseudomonas aeruginosa.*

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