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Role of Efflux Pump in Biofilm Formation of Multidrug-Resistant *Pseudomonas aeruginosa*

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Abstract *– Pseudomonas* (*P.*) *aeruginosa* is Gram-negative an opportunistic human pathogen associated with nosocomial infections particularly immuno-compromised patients such as cystic fibrosis, cancer, and diabetes. This organism can also develop high-level intrinsic and acquired antibiotic resistance by different mechanisms when it grows in a biofilm. The formation of biofilm, known as a passive resistance mechanism, inhibits the diffusion of antibiotics due to the polysaccharide structure surrounding the bacteria and makes the bacteria resistant. One of the most important mechanisms responsible for multiple antimicrobial resistance in biofilm structures is the efflux pump. Five families of bacterial drug efflux pumps have been identified that contribute to the efflux pathways including the ATP binding cassette (ABC) family, major facilitator superfamily (MFS), the multidrug and toxin extrusion (MATE) family, the small multidrug resistance (SMR), the resistance nodulation cell division (RND) superfamily. Among these pumps, the RND efflux pumps in *P. aeruginosa* play a major role in MDR. Furthermore, there are 11 types of RND efflux pumps in *P. aeruginosa* for the release of multi-class drugs. Of these, MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY were most important due to their high prevalence in clinical strains. In summary this review focus on the aim to give an overview of the relationship between efflux mediated resistance and biofilm formation in bacteria.

Keywords *– Pseudomonas aeruginosa*, multidrug resistance, biofilm formation, efflux pump

I. INTRODUCTION

Pseudomonas aeruginosa is aerobic Gram-negative bacteria, non-fermentative, nutritionally versatile and ubiquitous in the environment such as biotic and abiotic surfaces. *P. aeruginosa* is considered a potential opportunistic human pathogen especially immuno-compromised patients such as cystic fibrosis (CF), cancer, and diabetes [1]. Moreover it is an important non-fermantative pathogen causing urinary tract infections, gastrointestinal tract infections, soft tissue infections, conjunctival erythema, corneal infections, catheter-related infections, meningitis, abscesses, ventilator-associated pneumonia and nosocamial infections [2]. It is a current problem with its increasing incidence in recent years, showing multiple antibiotic resistance due to its ability to gain resistance even during treatment as well as being naturally resistant to many antibiotics [3], [4]. This organism can also cause serious infections both in hospitals and in the community [5], [6], [1]

Infections caused by *P. aeruginosa* is generally difficult to treat as it exhibits several of mechanisms of antibiotic resistance including AmpC beta-lactamase [7], extended-spectrum beta-lactamase, multi-drug efflux pump [8] and biofilm formation [9] [10]. Because it prefers moist environments, it causes biofilm formation in patients' moist body areas (axilla and ear), as well as in respiratory support systems, moist medical devices (catheters, lenses, implants, artificial heart valves, intrauterine devices).

Infections caused by *P. aeruginosa* are difficult to treat, as biofilm production leads to the formation of highly resistant strains of antibiotics [11], [12]. Therefore, *P. aeruginosa* is a bacterium that causes death and disease most frequently among nosocomial infections [13], [10]. About 30% of 56 million deaths worldwide are infectious diseases. This is a serious public health problem in developing countries [14].

P. aeruginosa is the causative agent in 11% and 13.8% of the cases of nosocomial infection with microbiological causative agents. Its easy adaptability to different physical and chemical conditions and resistance to many antibiotics and disinfectants give this bacterium an effective opportunistic pathogen [15].

A. Biofilm and Antimicrobial Resistance Mechanisms

Biofilms have been defined as highly structured communities of bacterial cells in abiotic or biotic surfaces. Most bacterial cells are often encased in rich extracellular polymeric substances (EPS) in biofilm [16], [17]. EPS are comprised of polysaccharides, proteins, lipids and e DNA that surround the cells, forming a glycocalyx that can prevent the penetration of antibiotics, antimicrobial agents, disinfectants and immune system elements [5]. Therefore, the formation of biofilm provides the protection and growth of microorganisms in hostile environments for their survival.

Biofilm is also a film that causes the development of many chronic and stubborn infections, accelerates the emergence of multi-drug resistant bacteria and is responsible for the formation of nosocomial infections [1], [18]. Recently, some studies have proved this. In the study, the deterioration in the physiological function of the efflux pump has shown that it reduces or even eliminates the formation of biofilm in some bacterial species [18].

P. aeruginosa is one of the most common pathogens known as model organisms for biofilm formation. Biofilms formed by *P. aeruginosa* are thought to be the underlying cause of many chronic and recurrent infectious diseases. Due to its multiple antibiotic resistance, this makes it difficult to treat infections caused by this bacterium [5]. The multi-drug resistant (MDR) phenotypes of *P. aeruginosa* are known to be associated with overexpression of efflux pump systems. The multidrug-resistant (MDR) phenotypes of *P. aeruginosa* in biofilm are known to be associated with overexpression of efflux pump systems [19].

Efflux systems, which allow the removal of antimicrobial agents from *P. aeruginosa* without the need for drug modification, contribute to the MDR phenotype of the bacterium [20], [21]. Firstly, in 1976, it was shown that P-glycoprotein in the mammalian cell membrane causes resistance in cancer drugs, and then it causes tetracycline resistance of Tet pulse pump protein in bacterial cell membrane. Subsequent studies have shown that active pump genes leading to multidrug resistance are common in bacteria and many other pathogenic microorganisms [22].

In *P. aeruginosa*, the uptake of nutrients and ions into the cell, the excretion of metabolic end products, and their relationship with the environment of bacteria are regulated by active pump system proteins, members of a large family of proteins. These proteins, five families of bacterial drug efflux pumps have been identified that contribute to the efflux pathways including the ATP binding cassette (ABC) family, major facilitator superfamily (MFS), the multidrug and toxin extrusion (MATE) family, the small multidrug resistance (SMR), the resistance nodulation cell division (RND) superfamily. Among these pumps, the RND efflux pumps in *P. aeruginosa* play a major role in MDR [23] (Figure 1). The RND superfamily is found only in Gram-negative bacteria, while the efflux pump systems of the other four families: MFS, ABC, SMR and MATE, are widely distributed in both Gram-positive and negative bacteria [18], [24].

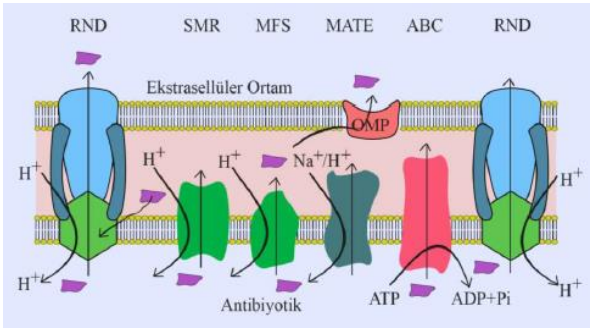

Figure 1. Efflux pump system in *P. aeruginosa*

B. Efflux Pumps in P. aeruginosa

- ATP binding cassette (ABC) family

ABC transport proteins are the largest family of proteins. It is known to be present in prokaryotes and eukaryotes and has a very wide substrate width. Unlike other efflux pump systems, these primary carrier systems obtain the energy required for ATP hydrolysis [25].

- Major facilitator superfamily (MFS)

The MFS family belongs to the secondary carriers category. They make up about 25% of known prokaryotic membrane proteins. Membrane transporter proteins of this family are found in many organisms from bacteria to high eukaryotes [26].

- Multidrug and toxin extrusion (MATE) family

MATE pumps are secondary carriers such as RND and MFS pumps and have 12 or 14 transmembrane segments. They use proton movement force as energy source. But some MATE pumps can use sodium ion gradient [27].

- Small multidrug resistance (SMR)

SMR pulse pumps consist of approximately 110 amino acids and 4 alpha helix transmembrane segments. In Gram negative bacteria, pumps belonging to this family are encoded on the bacterial chromosome [28].

- Resistance nodulation cell division (RND) superfamily

The pumps of the RND family are secondary carriers and have a triple structure. Inner membrane pump protein, periplasmic protein, and outer membrane porin protein are examined in 3 parts (Figure 2).

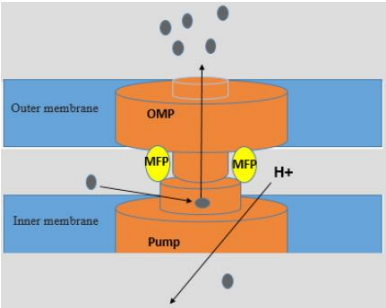

Figure 2. Schematic Representation of Drug Efflux Pumps and Pathway of Drug Efflux Across the inner membrane and outer membrane in *Pseudomonas aeruginosa* [29].

The RND system acts as an antiport with proton movement and transports by the displacement of hydrogen and a molecule. It has very broad substrate specificities. Among these pumps, the RND efflux pumps in *P. aeruginosa* play a major role in MDR. Furthermore, there are 11 types of RND efflux pumps in *P. aeruginosa* for the release of multi-class drugs. *P. aeruginosa* from the RND superfamily; Four different active efflux pump systems have been identified as MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM. Of these, MexAB-OprM plays a role in both natural and high levels of drug resistance [22], [22], [23].

N-3-oxododecanoyl-homoserine lactone (3-oxo-C12-HSL), known as the QS signaling molecule, responsible for the production of virulence factors in *P. aeruginosa*, is pumped out of the cell by the MexAB-OprM excretory system [30]. *P. aeruginosa* is important for resistance to all β-lactam group antibiotics and quinolones except for imipenem with MexAB-OprM active pump system. The MexCD-OprJ active pumping system is responsible for resistance to generation cefolosporins and the MexEF-OprN active pumping system is responsible for resistance to carbapenem group antibiotics [31], [32]. The other pumps (MexEF-OprN, MexJK, MexCD-OprJ, MexGHI-OpmD, MexPQ-OpmE, MexVW-OprM, and MexMN-OprM) are not in the wild type strains, but are expressed in resistant isolates and may participate in biocide or antibiotic resistance [29], [33], [34].

II. CONCLUSION

Most chronic and persistent bacterial infections are associated with biofilm. Because biofilm is a film that accelerates the emergence and rapid spread of multidrug-resistant bacteria. It is known that bacteria that cause biofilm formation for years are very difficult to destroy compared to planktonic species. The multi-drug resistant (MDR) phenotypes of *P. aeruginosa* are known to be associated with overexpression of efflux pump systems. Efflux pumps have an important role in developing resistance to antimicrobial agents, especially in *P. aeruginosa*.

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