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المجلة العراقية لبحوث السوق وحماية المستهلك

Subject Review

THE ROLE OF EFFLUX PUMP FOR ANTIBIOTIC RESISTANCE IN BACTERIA

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ABSTRACT

The multi-drug resistant efflux pump is a glycoprotein pump whose function is to push foreign substances. The efflux pump is found in humans, animals. It also has wide-ranging properties in bacteria and fungi. They are found in all species of bacteria, and efflux pump genes can be found in bacterial chromosomes or mobile genetic elements, such as plasmids. The most sensitive function that leads to a global problem is its resistance to antibiotics in bacterial cells, which increases the ability to bacteria from becoming strong virulence factors that most or all antibiotics cannot kill. It also has other important functions. It is related to the defense mechanism of the horse by pushing all harmful substances. This pump is divided into five families. The division depends on the source of energy, symmetry, and the general structure. The major facilitator (MFS) superfamily; the multidrug and toxic compound extrusion (MATE) family and the adenosine-triphosphate (ATP)-binding cassette (ABC); superfamily the resistance-nodulation-cell division (RND) superfamily and the small multidrug resistance (SMR). The ABC family uses ATP as a source of energy for export, while other flow pumps use the driving force of the proton. These hosts participate in many cellular processes such as intercellular communication, formation of cell membranes, virulence factors of bacterial cell, extrusion of toxic substances, disinfectants, toxic metabolic by-products, and antibiotics. It is also one of the important formations in bacteria that give bacteria the ability to expel antibiotics through various and diverse genes that are located either on the chromosome or plasmid of different pump families. Evidence indicates that the efflux pump has physiological functions in bacteria, and its overexpression has a role in inducing multi-drug resistance and biofilm formation. Hence, the regulation of efflux pump action is an orderly, strict action in response to physiological and environmental signals.

Keywords: Efflux pump, pump families, antibiotics resistance, bacteria.

مقال مراجعة الموضوع

دور مضخة الدفق في مقاومة البكتيريا للمضادات الحيوية

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الخلاصة

تكون مضخة الدفق المقاومة للأدوية المتعددة عبارة عن مضخة متكونة من بروتين سكري وظيفتها دفع المواد الغريبة خارج الخلية، وتمتلك خصائص واسعة في البكتريا والفطريات وتقريبا تتوجد في جميع أنواع البكتريا ويمكن العثور على جيناتها في الكروموسومات البكتيرية أو العناصر الوراثية المتنقلة مثل البلازميدات، وتعد مقاومة البكتريا للمضادات الحيوية مشكلة عالمية تمثل تحدي متزايد، اذ ان هذه المقاومة تزيد من قدرة البكتريا في امتلاك عوامل ضراوة قوية لا تستطيع معظم أو كل المضادات الحيوية قتلها او التأثير عليها، كما أن لها وظائف مهمة أخرى. وهي مرتبطة بآلية الدفاع عن طريق دفع جميع المواد الضارة، وتقسم هذه المضخة إلى خمس عوائل هي MFS وMATE وATP-ABC وRND وSMR والذي يعتمد على مصدر الطاقة والتماثل والبنية العامة، وفي هذا السياق فأن عائلة ABC تستعمل ATP كمصدر للطاقة، بينما تستعمل مضخات الدفق الأخرى القوة الدافعة للبروتون، وتتشارك هذه المضافات في العديد من العمليات الخلوية مثل الاتصال بين الخلايا وتشكيل أغشية الخلايا وعوامل ضراوة الخلية البكتيرية وبثق المواد السامة والمطهرات والمنتجات الثانوية الأيضية السامة والمضادات الحيوية، كما أنه أحد التكوينات المهمة في البكتريا التي تمنحها القدرة في طرد المضادات الحيوية من خلال جينات مختلفة ومتنوعة تقع إما على الكروموسوم أو البلازميد لعائلات المضخات المختلفة، وتشير الدلائل إلى أن مضخة الدفق لها وظائف فسلجية اخرى وان الافراط في التعبير لها دور في تحفيز مقاومة الأدوية المتعددة وتكوين الأغشية الحيوية، ومن ثم فإن تنظيم عمل مضخة الدفق يخضع لإجراء منظم وصارم تبعا للإشارات الفسلجية والبيئية.

الكلمات المفتاحية: مضخة الدفق، عوائل المضخة، المقاومة للمضادات، بكتريا.

INTRODUCTION

Efflux pump is a protein structure found in all types of bacterial membranes and they have ability to exit antibiotics to the external environment and prevent them from reaching their targets and were first discovered as a mechanism of tetracycline resistance in *E coli* bacteria **(Blanco *et al.*, 2016)** .The different types of efflux pump present in different species of bacteria ,all bacterial genomes studied contain several different efflux pumps; this indicates their ancestral origins. It has been estimated that ~5–10% of all bacterial genes are involved in transport and a large proportion of these encode efflux pumps **(Piddock, 2016)**.The development of bacterial resistance to most antibiotics contributed to an increase in the study of efflux pumps, as well as the role of the genes responsible for this **(Sun *et al.*, 2014)**.The Multi drug resistance efflux systems are classified into five families: the small multidrug resistance (SMR) family; the major facilitator superfamily (MFS); the multidrug and toxic compound extrusion (MATE) family and the adenosine-triphosphate (ATP)-binding cassette (ABC); superfamily the resistance-nodulation-cell division (RND) superfamily as the ABC family transporters use ATP to transport substrates, while the other of the families use proteins with the help hydrogen ion to transport substrates. Efflux pump systems may be specific to one type of antibiotic or one group of antibiotic , or nonspecific including many types of antibiotic group **(Kumar *et al.*, 2005)**. The aim of the this review to comparison of the different types of efflux pump families in terms of their presence or non- presence and the role in multidrug resistance in different bacterial species and their role in antibiotic resistance and biofilm formation .

1. SMR (small multidrug resistance) family

They are small multidrug-resistant proteins ranging from 100-140 amino acids that take the form of four spirals across the membranes of bacteria and are resistant to quaternary ammonium compounds as well as highly hydrophobic so they dissolve in organic compounds, it has the ability to synthesize fat-loving compounds, disinfectants, detergents and antibiotics in the first place, as well as for various drugs, the family of SMR contains a group of diverse subclasses on plasmids and chromosomes with high functional and structural diversity through which it can resist various antibiotics such as cephalosporin and the reason is that there is a great link between its genes and resistance genes in the bacterial cell **(Vrancianu *et al.*, 2020)**. Through many studies, it was found that this family consists of three classes: SMP, SUG, PSMP, and this division came through the genome sequence **(Bay *et al.*, 2008)**. All these gene express in to different type of proteins, When studying a group of SMP proteins based on their functional and structural similarity, it was found that they provide resistance to Gram-negative and Gram-positive bacteria as well as Archea through the expression of a single gene, it consists of proteins encoded by a plasmid for *Escherichia coli* –EmrE, *Staphylococcus aureus* –SMR and intergron encoded for *Klebsiella* QacE Then it included the discovery of many proteins encoded from plasmids and chromosomes **(Vourli *et al.*, 2003)**.

A single radioactive protein of Mycobacterium tuberculosis Mtu-Smr was described, which showed high drug resistance represented by the transport and activity of Ebr-QacC/D **(Evangelopoulos *et al.*, 2019)**. The second class SUG proteins have the ability to inhibit the groEL mutation through the accumulation of proteins, but they lack the recognition of lipophilic dyes, which are shown by the proteins that the genes encoding for SUG proteins are located on the plasmid, as well as a group of proteins encoded from a particular plasmid, with multi-antibiotic resistance **(Chang *et al.*, 2004)**.

SMR a pump encoded by the return chromosome SMR called *Abes* has been described in the multi-resistant strain *Acinetobacter baumannii* to explain its role in antibiotic resistance.

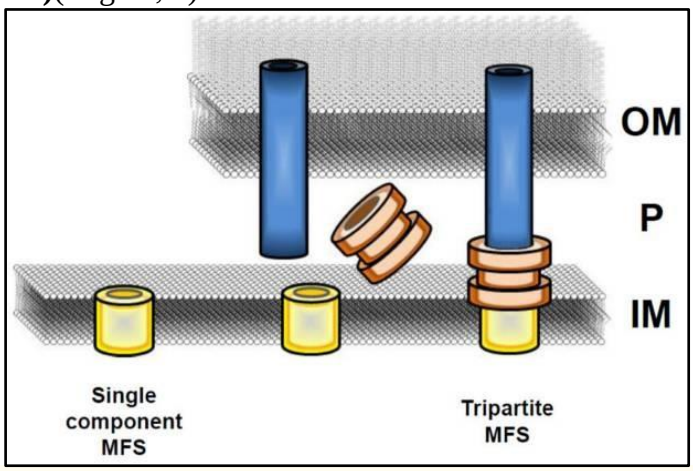
Expression of the *Abes* gene in *Escherichia coli* highly sensitive to KAM32 reduced susceptibility to different classes of agents such as dyes, antibiotics and detergents. When removing the effect of ABC *Abes* gene from *Acinetobacter baumannii* its role in antibiotic resistance was observed **(Wong *et al.*, 2014**).

The pump was determined through the genome data base. It consists of a protein along the length of the SMR protein and its proteins on a separate genome on the chromosome. They exist in pairs or three pairs, depending on the species of the organism. The functional description found homologs of *E.coli* YdgE, referred to as Mdti, Mdtj. The gene expression of Ydgf, YdgE confers the host The ability to resist different antigens in a wide range, and proteins were identified in *Pseudomonas sp.*and *Shigella sp.* through the genome data. In positive bacteria homologs were identified in four distinct loci, which help the host to resist drugs and antigens **(Saier & Paulsen, 2001)**.

The transport in this family is by the electromechanical gradient of the proton, it depends on the proton by transport and through this the flow depends on certain compounds by the proton in the active state of the cell and this indicates **(Robinson *et al.*, 2017)**. The variance in its ability to resist antibiotics according to the activity of the host and therefore its ability to work is different from bacteria to others according to the activity status of the bacteria cells **(Bay *et al.*, 2008**).

2. MFS (major facilitator superfamily) family.

It is a type of efflux pump family that exists in the form of one or three components between the membranes of Gram-negative and Gram-positive bacteria. Its main function is the absorption of sugars, but it was found that it has the ability to flow proteins, and this indicates its contribution to antibiotic resistance in gram-positive bacteria in the monomorphic form and in the negative bacteria in the triple form. MFS efflux pumps contribute to the interaction between bacteria and target host cells, focusing on their role in inducing virulence, in the colonization of plant and animal host cells, and in the formation of cell membranes **(Neuberger *et al.*, 2018)**(Figure, 1).



Figure(1): Schematic overview of the architecture of the efflux pumps of the major facilitator superfamily (MFS) **(Pasqua *et al.*, 2019)**.

It is made by genes organized in a single operon, usually in the triple form. It is encoded by a permanent vector, followed by an encoding from the surrounding protein gene and then the membrane-forming gene, so that there are three consecutive genes that code for the MF family when it is in its triple form **(Alvarez-Ortega *et al.*, 2013)**. This family consists

of two groups, the first containing 12 helixes and the second 14 helixes, both of which originated from a simple prototype, as a result of successive repetition, these two groups are formed, and through this combination, the drug is transferred from the cytoplasm to the outside of the bacterial membrane according to the classical theory, **(Quistgarrd *et al.*, 2016).**

Substrates exit occurs through Tolc protein Tolc is a trimeric outer membrane protein characterized by the presence of an α-barrel projecting across the periplasm and linked to a β-barrel domain embedded in the outer membrane **(Pasqua *et al.*, 2019)**. A protein that not only functions in the RNA family, and therefore shows a distinct diversity of types of substrates **(Barroso *et al.*, 2018)**.

3. MATE (multidrug and toxic compound extrusion) family

Multidrug and toxic compound extrusion (MATE) transporters underpin multidrug resistance by using the H^+ or Na^+ electrochemical gradient to extrude different drugs across cell membranes. A broad and large family for the transport of compounds across cell membranes, including antibiotics and drugs, consisting of two sub families Nor-M and Din-F, all of which consist of a helical shape. This family is considered secondary to the transfer process because it depends on ions in the transfer process but it differs from the rest because it uses Na^+ and H^+ ions **(Lu, 2016)**. Nor-M proteins are the first structural and functional proteins of the MATE family consisting two types based on the amino acid sequence NorM-NG (*Neisseria gonorrhoeae*) and NorM-CV (*Vibrio cholerae*) **(Long *et al.*, 2008)**.

The NorM-VC transporter is composed of 12 trans membrane helices and the structure is shown in a V-shape where the transporter is separated into two domains, the first domain N (TM1-6) and the second domain C (TM7-12). It is sometimes possible that the structures of the two domains overlap each other and that the two domains have the same membrane topology. This transporter is credited with the influx of many toxic compounds through the flow of ketones (Na^+) and the ketone binding sites are always within the C domain of the transporter **(He *et al.*, 2010)**.

NorM-NG is a substrate that was discovered in 2013 for Neisseria. The general structure also consists of 12TMS similar to NorM-VC, but the vectors of it diverge from each other towards the extracellular space. There is also a central cavity between the two domains. The lower side of the cavity is tightly closed and protected from the cytoplasm side by a protein structure, and this helps molecules the solvent diffuses freely in the center. It is possible that the transport process of drugs takes place through the rearrangement of the helical membranes attached to the ketone. the ionic transport process is supported by genetic mutations, as the transporter uses a group of amino acids to interact with drugs and ketones . It is also noted that the transfer takes place in the direction of the outer periphery. However, there remains poor information on how MATE turns to the confrontation inside the cell to obtain the substrates **(Lu *et al.*, 2013)**. As for the Dinf transporter, it is a H^+-linked transporter, but it lacks the amino acids that form the ketone bond, and it is a helical structure consisting of 12 structures **(Tanaka *et al.*, 2013)**.

4.RND (resistance-nodulation-division) family

This family is distinct and large, consisting of three components: outer membrane protein (OMP), middle membrane, surrounding membrane fusion protein (MFP), and inner membrane protein Imp **(Toba *et al.*, 2019)**. This family plays a major role in resisting antibiotics and removing toxins from intermediate materials such as metals and solvents and it has a great role in bacterial virulence **(Reygaert *et al.*, 2018)**. The family is related to

resistance in Gram-negative bacteria, and the driving force of the drug across the membrane is H^+ **(Piddock, 2006)**(Figure 2).

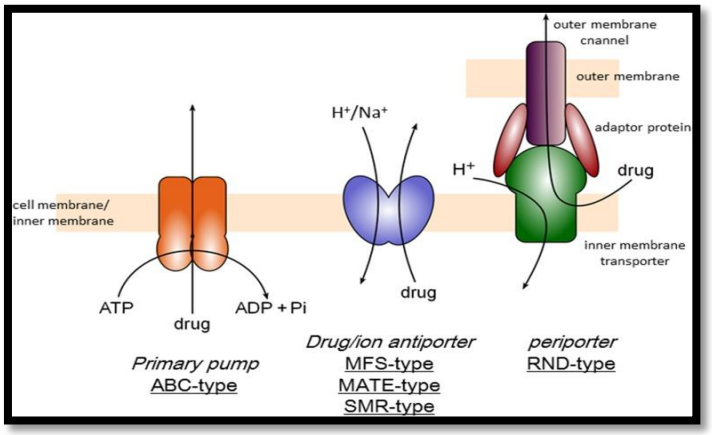


Figure (2): Structure of multidrug efflux transporters **(Yamaguchi *et al.*, 2015)**.

5. ABC (ATP-binding cassette) family

Cast proteins are distributed between two nucleotide-binding domains (NBDs) and two trans membrane domains (TMDs)(**Onelle *et al* .,2019**). *nbd* is characterized by its use of ATP energy after hydrolysis and contains walker A, walker B, and signature motif (**Moussatova *et al*., 2008**).

TMDs are known as translocation substrates across the cell membrane and contain 6 hydrophobic membrane domains, one NBD domain fuses with one TMD domain to form a half-size protein or more than that, the family has the ability to push sugars, proteins, amino acids, antibiotics, heavy metals, and inorganic ions, and it represents the largest family in effluent pumps that perform the efflux function in the largest way for antibiotics(**Schultz *et al* .,2011**).Most of this family is found in gram-positive bacteria, and rarely in gram-negative bacteria, as it is found in *E.coli* bacteria**(Lin *et al* .,2009**)(Figure3).

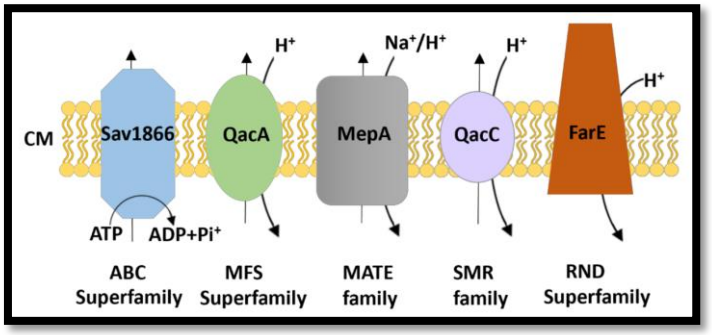


Figure (3): The five efflux pumps families **(Abolfazl & Brown, 2021)**.

Gene expression

RNA family genetic regulation is carried out by three genes, namely AcrB of the inner compartment, AcrA of the peripheral accessory protein, and Tolc of the outer membrane protein(44). AcarA mediates the cooperative environment between AcrB and Tac. Studies in *E. coli* also revealed that the genome is highly homologous between the pump genes and the amino acid sequences of different species, and the genes are regulated in such a way that the regulator is located next to the gene that codes for the extra protein surrounding the protein,

which is located next to the gene that codes for a protein efflux pump **(Parker & Gottesman, 2016)**.

There are other genes *oqxA* and *oqxB*, which belong to the RND family, as they provide resistance against many antibiotics (chloramphenicol, trimethoprim, quinolones) and many disinfectants and detergents where they are located on the chromosome or plasmid, as there can be a lateral transfer between the genes of the chromosome and the plasmid. Note this in *Klebsiella* as it is worth Note that this does not happen with all bacteria **(Li *et al.*, 2019)**. The RND family contains genes located on the plasmid and it is considered one of the best genes for resistance to beta-lactamase and cephalosporin called blaTEM **(Hussain *et al.*, 2021)**. The MF family contains genes located on the plasmid, TetA and TetB, which are highly resistant to tetracycline and are found only in Gram-negative bacteria and are rarely found on the chromosome due to the genetic transfer between the plasmid and the chromosome **(Alcalde-Rico *et al.*, 2016)**.

The *TetB* gene is rarely found in *Klebsiella*. There is also another resistance gene in this family .the qacEΔ1 gene which is often derived from the *qacE* gene which has the ability to make the pump flow of ammonium compounds dyes and xanthine a gene that is often encoded by a plasmid **(De-Oliveira *et al.*, 2020)**. There are many families of genes belonging to the MF family that are located either on the chromosome or plasmid, including (MarR, qacA-B, bltR, salR, salA, emrR, emrK, emrY, mrR, mrA, mrB), the MAET family also found genes responsible for increasing resistance, including the MAET-Nor gene as well as VcmA and VcmM within *Cholera* bacteria, which are genes located on the chromosome **(Pasqua *et al.*, 2019)**.

CONCLUSIONS

There is not enough information about each family and its existence and function in all types of bacteria, and there are large numbers of discovered and undiscovered genes that regulate the function of efflux pump families. Lateral transfer of genes makes the gene encode on the chromosome in one type of bacteria and in another type on the plasmid is considered a family RND is one of the large family of positive and negative bacteria with multiple functions as it removes toxins, resistance to drugs and other toxins.

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