

Mechanisms Efflux Pumps of *Acinetobacter baumannii* (MDR): Increasing Resistance to Antibiotics

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Abstract

Acinetobacter baumannii has greatly increased its degree of resistance to become multidrug resistant (MDR) over the past 30 years and is on the red line of the most widely replicated bacteria according to World Health Organization (WHO). The efflux pumps are the main cause for the increasing antibiotic resistance of *A.baumannii* originated from nosocomial infection. The progressive resistance of *A.baumannii* even on the recent drugs (tigecycline and fosfomycin) reduces to very effective antibiotic scale. With attention focused on MDR and pan-drug-resistant (PDR) in *A.baumannii* multiple works on efflux pumps chemical inhibitor (NMP, PAβN, omeprazole, verapamil, reserpine, CCCP) are still in progress. Certain inhibitors from plants (Biricodar and timcodar, Flavone, Mahonia, Dalea versicolor, Lycopodium europaeus, and Rosmarinus officinalis) have the capability to have such compounds according to their very significant synergistic effect with antibiotics. In this review we focused on the growth of antibiotic resistance to explain the mechanism of efflux pumps into these different super families and a comprehensive understanding of the extrusion, regulation and physiology role of drug efflux pumps in the essential development of anti-resistivity drugs. We recapitulated the evolution of the work carried out in these fields during the last years and in the course of elaboration, with the aim of increasing the chances of decreasing bacterial resistivity to antibiotics.

Keywords *Acinetobacter baumannii*; RND efflux pumps; Efflux transporters; Multidrug resistant (MDR); Efflux Pumps Inhibitors (EPIs)

Introduction

Acinetobacter spp was detected around the 20th century by famous bacteriologist Beijerinck [1], but it was not until 1960 that *A.baumannii* was declared in hospital. Belongs to the large family of non-fermentable gram-negative bacteria capable of harming patients in surgical intensive care [2]. During the 20 past years it has developed a capital importance and its classification among the nosocomial infections makes it a priority to all the public health organizations considering its increase and recurrence [3]. *A.baumannii* is much more present in humans and is the origins of multiple diseases like septic fever, pneumonitis pachymeningitis and other disease [4]. Over time it has gained its resistance through diverse modifications and is presently resistant to approximately all the various groups of antibiotics even the most widely used drugs (fluoroquinolones, macrolides, trimethoprim, b-lactams, tetracyclines, aminoglycosides, and chloramphenicol) [5]. The bacterial efflux operation causes the formation of toxins and rejects antibiotics from the cells, which confers a specific invulnerability to antibiotics. Multidrug resistant (MDR) efflux pumps are now present in almost all microorganisms, in which bacteria it is one of the main causes of obstruction to action of drugs [6]; several works have concluded that MDR is on origin of the decline progressive of drugs sensitization by bacterial mutation [7] that reduce largely the valid drug for cure. However using inhibitor components could restores bacterial susceptibility to antimicrobial agents. Efflux pumps inhibitors (EPIs) synthetic or natural component is the potential drugs for treatment of MDR or PDR *A. baumannii*. After

describing the general mechanisms of efflux pumps systems in bacterial resistance, we will explain regulation and physiology role of drug efflux pumps in the essential development of anti-resistivity drugs and report the evolution of the work done during the recent years especially in EPIs.

Mechanisms of bacterial resistance to antimicrobial agents

The bacterial resistance to antibiotics has emerged in the face of several pathogenic agents, besides *A.baumannii*, albeit efforts to treat these pathogenic germs still progressing [8]. Bacterial pathogens that have shown resistivity to a single drug or to several agents are considered MDR bacterial. With all the efforts united to resolve the problem via the outgrowth of new line of antibiotics, the bacteria does not cease also to mutate quickly to acquire new mechanism of resistivity or to improve their resistance to antibiotics [1,4,5,9-13]. Many reports showed that bacterial resistance to antibiotics is believed that the pathogenic bacterium transfers certain genetic gene of resistance to drugs from one species to another and automatically acquires resistant phenotypes against the majority of the pre-existing antimicrobial agents [14]. This mechanism of opposition poses a critical problem to bacterial treatments. Another significant cause of this obstruction is the proximity of drugs to environment, agriculture and others, leading to the emergence and development of resistances. Clinically, a low or high or inappropriate use level of antibiotics will also imply in the increase in bacterial resistance [15]. Presently following many research there exist different mechanisms responsible for the bacterial resistance in addition (a) modification of drug target, (b) drug inactivation by enzymes, (c) modification of cell wall protein, and (d) activation of drug efflux system.

Alteration drugs target: Every drug has a specific target for destruction of bacteria, and as such when the target is changed bacteria can easily resist to antimicrobial agents, and these have been observed in quinolones (DNA gyrase variation or Qnr intercede purpose defense), aminoglycosides (16S rRNA methylation), and β -lactams (transformation in penicillin junction proteins). Reduced vulnerability to minocyclin and tigecyclin occurs via transformation in gene encoding S adenosyl-L-methionine-dependent methyltransferase [16]. After undergone several transformation in gene *lpx*, *pmrB* and outer membrane induce the structure of lipopolysaccharide for causing polymyxin resistance [17-19].

Drug inactivation by *A. baumannii* enzyme: *A. baumannii* synthesizes aminoglycoside-modifying enzymes well as AAD, APH, AAC3, and AAC6 that are frequently encrypted by a quota of *aba* obstruction island- homogeneous gene cassettes including class 1 integrons [20,21]. *A. baumannii* also bring out a high quantity of antimicrobial -inactivating enzymes, which are encoded by plasmids and chromosome for the resistance of antibiotics developed from β -lactamine family [8]. These β -lactamases include, enzymes from 4 class: class A (CTX-M and VEB), class B (NDM, SIM, metallo-enzymes, IMP, and VIM), class C (AmpC-type ADC enzymes) and class D or OXA (OXA-23, OXA-51, OXA-58, and OXA-66) [22-24]. For example, broad-spectrum TEM variants and either narrow-spectrum TEM enzymes (ambler class A); are mutually capable to hydrolyze approximately all β -lactams. In peculiarity, class_B and class_D as well as β -lactamas are implicated to hydrolyze carbapenems, a latest resort of antibiotics opposite several major pathogens [25-27]. *A. baumannii* also raising the presence of some enzyme link to the drug resistance like ADP-ribosyltransferase (rifamycin), chloramphenicol acetyltransferase (chloramphenicol), and alteration enzyme TetX1 (tetracyclin) [28,29].

Modification of cell wall protein: permeableness barrier of OM: Target modification mechanisms, drug-specific inactivation, and efflux drugs crossways the cell membrane barriers play an essential role in influencing the sensitivity of *Acinetobacter spp.* to a wide range of antimicrobials. This trait is due to the non-appearance of conventional high permeability trimmers (porins of enterobacteria spp.) [30] which has poor activity in *Acinetobacter spp* and thus belong to the minor proteins [18]. First, matching who has correctly, study is *A. baumannii* and *Pseudomonas aeruginosa* (*P. aeruginosa*), OM also demonstrate very low permeability to cephalosporins [17,31,32].

The Omp A protein monomer, the major protein of OM *A.baumannii*, has been shown analytically as the main nonspecific slow porin [33,34] which is identical to the slow pores OprF from *P.*

aeruginosa and OmpA from *E. coli* [33]. Overexpressing OmpA gene in *A. baumannii* would resulted in reduced sensitivity to chloranphenicol and aztreonam (8 times decrease MIC), both of which *A. baumannii* is intrinsically resistant [35,36]; Nevertheless, there was only a moderate (≤ 2 times) effect on the colistine MIC values, tigecycline, and imipenem [37], a common antibiotics in *A. baumannii* infection treatment [12,38].

Drug efflux systems: During the recent years because of the poor OM absorbency drug efflux systems has become one of the most complicated mechanisms of bacterial resistance and has played an essential role on drug resistance specially to *A.baumannii* [39]. The indulgence to amikacine and levofloxacin is the result of a negative control of the gene and protein CarO 31-36 kDa [10] and up regulation of 14 genes at OM by varying the amount of physiological NaCl.

MDR Efflux pumps: structure and regulation

The structure of the efflux system is comprised of 3 well-defined parts each playing a function in the drug efflux mechanism, including the outer membrane (OM) [40], the internal membrane (IM) and the fusion protein at the intermediate level (MFP); Each part of the structure EP has a certain factor causing resistance to approximately every groups of antibacterial [41,42]. In reference to further research, it has been proven that there are five different families of efflux pumps present on *A.baumannii*:

- major facilitation super family (MFS);
- multidrug toxic composite extrusion (MATE) transporters;
- resistance nodulation-division (RND) super family
- ATP binding cassette (ABC) transporters;
- Small multidrug resistance (SMR) family;

Recently other studies have reported a sixth efflux family named PACE (proteobacterial antimicrobial composite efflux) present in the *A.baumannii* (Figure 1) [43]. However because of inadequate data, we will concentrate much more on the first 5 families where a totally understanding of structure and regulation is not complete.

RND Efflux pumps: The large family RND efflux is special of the rather complex compared to other family. It is very represented and has a special role in almost all major gram-negative bacteria and developing multiple resistance to antibiotic also call MDR such as *A.baumannii*, *E. coli*, and *Paeruginosa* [46]. RND efflux on *A.baumannii* has mainly three gene (*adeABC*, *adeIJK*, *adeFGH*) and some special gene (*ade DE*, *adeAA*) [47].

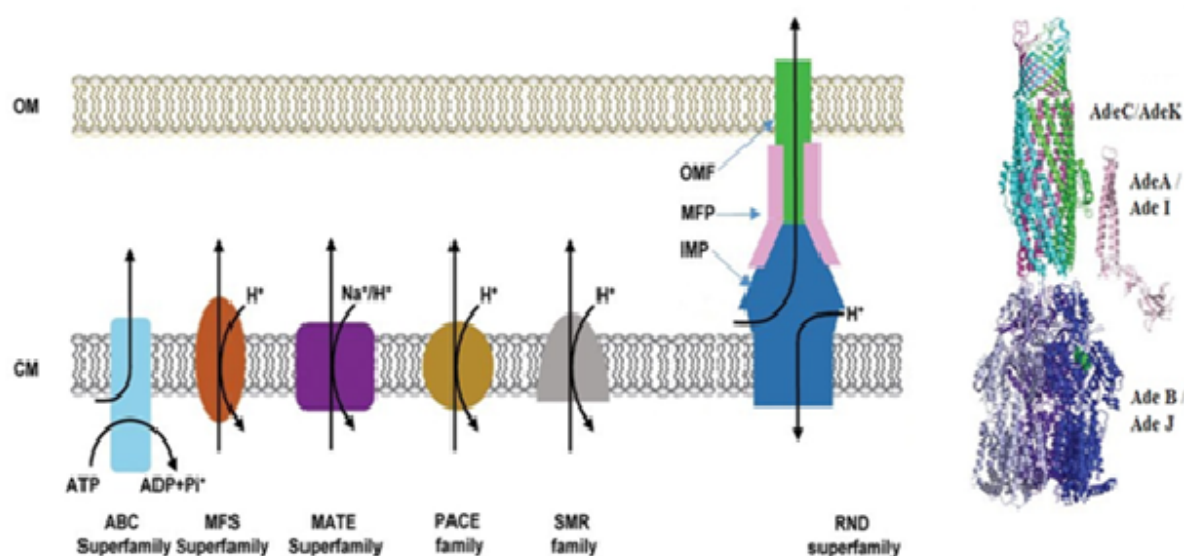


Figure 1: Structure of major families *A. baumannii* efflux pumps + PACE family a newly superfamily identify (Adapted from [44] and [45]).

• AdeABC

The adeABC operon was newly discovered on the antibacterial agents of fluoroquinolones and aminoglycosides in the efflux system RND and divided into 3 part: adeB on inner membrane efflux transporters, adeA on membrane fusion proteins, and adeC on external membrane proteins [48]. AdeB has the largest representation on *A. baumannii* strains (80%), adeA and adeC has 42%, and 40% respectively [49]. The gene adeABC have almost the same structure that genes MexAB-OprM for *Paeruginosa* and genes AcrAB-TolC for *E. coli* [48,50]. Because of this high proportion of adeB gene compare to the others, its inactivation would dramatically cause sensitization to antimicrobial drugs in the hospital for *A.baumannii* [51]. The increasing concentration of MIC would be beneficial to important drug classes like aminoglycosides, tetracyclines-tigecycline, β -lactams, fluoroquinolones, macrolides, trimethoprim, and chloramphenicol [52]. Despite the advancement of research, rifampicin, fusidic acid and sometimes colistin remain resistant to isolate *A.baumannii*. Single last chances of fight against *A. baumannii* isolates are tigecycline but show a hard resistance to adeABC and also it presents a high resistance efflux. The MIC levels of tigecycline remain a clinical problema [42,53]. Remarkably, about 20% of adeC was found to be involved in tigecycline resistance tests in *A.baumannii* demonstrating that in the Ade ABC gene, Ade AB can keep walking without adeC except on [54]. The adeC plays a much more an almost negligible role in RND efflux system.

The two components adeR and adeS are responsible for the regulation of the expression system of adeABC [55]. They are also called protein kinases and are found on both sides of adeABC in different trajectory. AdeRS, plays a determining role in increasing resistance of adeABC. Some result shows that a dysfunction of adeR and or adeS will increase the resistance of tigecyclin, chloramphenicol, minocycline, erythromycine, cefotaxime, tetracycline, fluoroquinolones,

and trimethoprim [19,32]; as well increase the sensitization of amino-glycosides of *A.baumannii* isolate. Recently an intense sight of carbapenem resistance was discovered in *A.baumannii* isolate from the adeABC system such as class D carbapenemases, meropenem, and imipenem, and it remains a serious concern in clinical therapy [13,56]. The ISAbal insertion produced by the adeS mutation confers resistance over expression to tegecycline.

• AdeIJK

The second largest pump of the RND family's adeIJK also comprises of adeI, adeJ, adeK genes which occur on the three parts of the pump efflux structure respectively. AdeIJK was described initially in the years 2008 [5,26] with the *A. baumannii* clinical strains fluctuating between 86% and 100% in a presence of the predominant gene adeJ. With various reported a MIC dimness of adeIJK mainly the resistance of *A.baumannii* to β -lactamines, lincosamides, fluoroquinolones, chloramphenicol, trimethoprim, and fusidic acid has been noticed [57]. The selection of the majority gene adeJ, will lead to an amplification in the sensitivity of chloramphenicol, macrolides, lincosamides, tetracyclines and quinolones and β -lactams [58,59]. The regulation of adeIJK is less complex than that of adeABC, but at about 750-850 kbp of adeIJK operon there is a regulator adeN belonging to the class of tetR [35]. The presence of this regulator adeN and mutation in different media led to an increase the resistance to antimicrobial drugs (ertapenem, aztreonam, tigecycline, meropenem, and minocycline) in *A. baumannii* [47]. Several studies have shown that the threshold of expression of adeIJK is lower than that of ABC, which indicated that the level of toxicity of adeIJK in the patient is well regulated [48,52,60]. It has been detected that adeIJK and adeABC have some similarity as the efflux of the same antibacterial drugs (fluoroquinolones, tetracyclines and chloramphenicol) [52] from *A.baumannii* and properties comparable to *Paeruginosa* mexAB-

OprM. Studies on production and regulation adeABC and adeIJK resulted in the formation of biofilms [36].

• AdeFGH

Outstanding variation of adeABC and adeIJK, has induced the discovery of adeFGH operon some time after adeIJK identification. The presence of adeFGH in the genus *A.baumannii* through exposure to certain antibacterial agents (norfloxacin) [22,61] and is also a true source of multidrug. The genes of the adeFGH operon, the adeG is the most representative of more than 80% of the others [37]. AdeFGH has also become popular in the species of *A.baumannii* due to its severe resistance to fluoro-quinolones, tetracyclines, tigecycline, chloramphenicol, trimethoprim, sulfamethoxazole and moderate resistance to erythromycin, rifampicin and aminoglycosides, [61] and also β -lactams. AdeFGH is regulated by LysR (LTTR), also called adeL. The adeL mutation will conduct to the adeFGH level rise. adeXYZ has also been found in *A.baumannii* genospecies 3 and has the same structure and positioning of MFP, OM, IM with propositions 80% (adeX), 89%(adeY) and 87%(adeZ) [39,62,63]. AdeXYZ and adeDE are also regularly present in *A.baumannii* GDG3 as opposed to GDG2 for ade IJK and adeABC. The engagement of adeXYZ in the resistance mechanism is not entirely described. Some research showed that the suppression or perturbation of adeFGH or of another in the RND system does not greatly modify the sensitivity to the antibiotics [2,64].

It has been found that the *A.baumannii* (GDG3) gene and specific resistance to certain antibiotics (ceftazidime, tetracycline, amikacin, ciprofloxacin, erythromycin, rifampin, meropenem, chloramphenicol) [16,26], is due to adeDE gene in *A.baumannii* chromosome, and have resistance to imipenem. Unlike to other efflux system (adeABC, adeIJK), the adeDE gene does not have an outer membrane [27,32]. Previous studies have demonstrated inconsistency between adeDE and adeABC-adeIJK due to the presence of adeABC-adeIJK / inter 1-negative adeS in some isolates for the detection of adeE [65].

MFS Efflux pumps: MFS is the subsequent most studied efflux mechanism in species *A. baumannii* have identified some genes cmlA, tet(A/B), craA, and floR as the most present and appertain to the superfamily MFS [66]. Plural research has explained a particularity of resistance caused by tetA and tetB [61]. These two genes are not involved in resistance to tigecycline, yet tetA leads to a tetracycline resistance while tetB induce the resistance to tetracyclin and minocyclin [67]. In *A. baumannii* isolate resistant to tetracycline, the overexpression rate of tetA is 30-45% whereas tetB is 32-72% [29,67]. The cmlA gene of MFS is resistant to certain β -lactams, chloramphenicol, fluoroquinolones, tetracycline and rifampicin. craA is particularly resistant to chloramphenicol, imipenems, quinolones, aminoglycosides, and tetracyclines [68]. The MFS energy source is proton motive force (H⁺) facilitating H⁺ motive force inhibition to increase the sensitization of antimicrobial drugs [69]. Horizontal transmission was discovered by the association of two tetB-tetR genes in plasmid and the ISCR2 element of the MDR isolate. It has been reported that floR gene and cmlA gene were associate with abaR gene in *A.baumannii* chromosome [70-72].

MATE Efflux pumps: The first and most frequent gene of the MATE family present in *A. baumannii* is the adeM gene. It represents between 63 - 100% in MDR of *A. baumannii* [26, 71]. AdeM protein contains about 447 amino acids and multiform hydrophobic regions. The antimicrobial drugs resistant due to adeM gene are not related to adeABC and totally known. In certain studies it was noted that adeM is not associated with the resistance of β -lactams, or cephalosporin

[73,74]. But it could have an implication of resistance in that of amino glycosides, trimethoprim, fluoroquinolones, erythrocine, and chloramphenicol. The MATE family is powered by double reservoir of energy PMF (motive force of the proton) and sodium ion gradient Na⁺ [75]. This high energy source could be a particular reasons why adeM gene is seen as an important target for elaboration of efflux pumps inhibitory antibiotics that could help restores *A.baumannii* sensitization [76,77].

SMR Efflux pumps: AdeS gene was characterized in 2009 initially in the efflux pump system by increasing the MIC (5-6 times) on an *E.coli* strain for the resistance of novobiocin and erythromycin. AdeS gene is the main efflux pump of SMR family present in *A. baumannii*. This gene adeS belongs to a particular resistance for fluoroquinolones, novobiocin, erythromycin, detergents (benzalkonium chloride), chloramphenicol, and dyes [8,21,49]. AdeS is identical at 52% to emrE (*E. coli*) found in the genome *A.baumannii* genome. AdeS is composed of about 108 acid amines. Because of its constant need for energy (H⁺), the suppression of this energetic source would restore susceptibility to drugs on MDR *A.baumannii* [70,78,79].

ABC Efflux pumps: ATPbinding cassette (ABC) of super family efflux pumps are recognized to be censurable for multidrug-resistance due of P-glycoprotein (ABCB1) [80]. ABC proteins are including in the cytoplasm (inner) membrane of germ, and membranes in eukaryotes. In the human body, ABC proteins encodes for 49 proteins, a particular fraction has been distinguished in function and biochemistry terms to others [39,81]. They have been organized into 7 sub-families established on phylo-genetic examination. P-glycoprotein (ABCB1) contains 170 kDa trans-membrane glycoprotein and practically the most at largely studied transporters that promote cancer cells to develop drug resistance. Unlike the other family of efflux pumps, ABC family is powered by hydrolysis energy sources of ATP (ADP + Pi) which gives cellular resistance to large number of drug molecules [82,83]. The ABC proteins functionally contain two areas for substrate transport and 2 areas of NBD (nucleotide binding) with ATP hydrolyse in the process. ABC family is recognized in *A.baumannii* to have resistance to erythromycin and gramicidin, but it is very present on cancer cells [30].

PACE Efflux pumps: The proteo-bacterial antimicrobial compound efflux family (PACE) is uncommon of the newest families of efflux pumps identified in the latest 15 years [84]. PACE family described on plural gram-negative bacteria like *E.coli*, *K. pneumoniae*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *P. aeruginosa*, *Enterobacter cloacae*, and serovar *Typhi* [36,43,85]. Its homologous aceI gene found to be resistant to chlorhexidine and its overexpression also lead to resistance to dequalinium, benzalkonium chloride, and acriflavine. In *A.baumannii*, aceI also induces the resistance of chlorhexidine and oxidants [85]. The aceI gene of PACE family could be the 6th group of MDR efflux pumps [44].

Mechanisms of transporters in the efflux pump systems

According to structural and bioenergetics characteristics, carriers could be separated into two major groups [80,86], (i) transporters that hydrolyze ATP as an energy source; they are also summons ABC transporters (ATP binding cassette) [81], and (ii) transporters that use the proton H⁺ (and/or Na⁺ sodium MATE family) for energy source [81]. Transporter of proton is the main common conveyance present in gram-negative bacteria especially in multidrug resistant. The mechanisms of transporter expression and regulation in bacterial

present a complex structure with different variable which are still under study (Table 1) [53,87].

The First Transporters: ATP binding cassette Transporters. The mammalian P-glycoprotein (P-gp, MDR1) is one of the particularly studied ABC transporters, and their utilization in chemotherapy has shown that their expression confers resistance to cytotoxic compounds [88]. Ubiquitous ABC transporters have many different functions in transport including drugs, metabolites and the flow of toxins [89]. ABC transporters particularly constitutes, of two hydrophobic transmembrane domains and two cytoplasmic domains which binding ATP [90].

The Second transporters: in MDR bacterial efflux pumps system are represented in four families: RND, MFS, MATE, and SMR family [81,89]. The MFS protein have 12 or 14 transmembrane segments

(TMS) coming from two separate congregate and they are also responsible for transport of drugs, sugars, and intermediate metabolites [66]. Membrane proteins of the SMR family are engaged in the activity of lipophilic cationic drugs in *A.baumannii* [91]. These are the slightest known drug efflux proteins, with just 4 TMS predicted. They can function as either hetero- or homo-oligomeric complexes. Unlike pumps MFS families, RND and SMR, which act as anti-proton / anti-drug, the 12-TMS collapse pumps MATE family afresh recognize (108) are mainly anti-drugs Na⁺ [90]. RND efflux systems presented a 3D structure of proteins of tripartite which is not totally understood by the configuration of these systems [26,60]. It was also noted that RND pumps of *A. baumannii* and gram- negative a tripartite system. RND efflux protein are combined of 12 TMS including 2 large periplasmic which provide specify substrate [91-93].

Efflux pumps families	Efflux pumps genes and (regulators)	Energy resource	Substrates
RND	adeABC (adeSR, baeSR)	Proton motive force (H ⁺)	Aminoglycosides, Benzalkonium Chloride, B-Lactams, Tetracycline, Chloramphenicol, Deoxycholate, Ethidium Bromide, Erythromycin, Tigecycline, Fluoroquinolones, Nalidixic Acid, Methyl Viologen, Sodium Dodecyl Sulfate.
	adeAA2B (baeSR)		Tigecycline
	adeFGH (adeL)		Sodium Dodecyl Sulfate, Tetracycline, Tigecycline, Nalidixic Acid, Sulfonamides, Ethidium Bromide, Fluoroquinolones, Erythromycin,
	adeIJK (adeN, baeSR)		Azithromycin, Benzalkonium Chloride, B-Lactams, Farnesol, Chloramphenicol, Clindamycin, Crystal Violet, Deoxycholate, Fusidic Acid, Erythromycin, Fluoroquinolones, Minocycline, Nalidixic Acid, Rifampicin, Sodium Dodecyl Sulfate, Triclosan, Tetraphenylphosphonium, Trimethoprim, Tetracycline.
MFS	craA	Proton motive force (H ⁺)	Chloramphenicol
	cmlA		Chloramphenicol
	floR		Chloramphenicol, Florfenicol
	tetA(B) (tetR)		Tetracycline
MATE	abe M	Proton motive force (Na ⁺ /H ⁺)	Acridil Avine, 6-Diamidine-2-Phenylindole, Daunomycin, Doxorubicin, Fluoroquinolones, Gentamicin, Rhodamine 6G, Tetracycline.
SMR	abeS	Proton motive force (H ⁺)	Acridine Orange, Acridil Avine, Benzalkonium Chloride, B-Lactams, Chloramphenicol, Ciprofl Oxacin, Deoxycholate, Ethidium Bromide, Tetraphenylphosphonium, Erythromycin, Novobiocin, Sodium Dodecyl Sulfate,
	Smr (A1S_0710)		Deoxycholate, Sodium Dodecyl Sulfate
ABC	macAB-toiC (baeSR)	ATP hydrolysis	Erythromycin, Gramicidin
		(P-gp)	
PACE	acel	Proton motive force (H ⁺)	Chlorhexidine
Acinetobacter Genospecies 3			
RND	adeDE	Proton motive force (H ⁺)	Ceftazidime, Amikacin, Ciprofloxacin, Chloramphenicol, Erythromycin, Ethidium Bromide, Meropenem, Rifomycin, Tetracycline
RND	adeXYZ		B-Lactams, Ciprofloxacin, Tetracycline, Rifampin, Chloramphenicol

Table 1: Efflux pumps families in *A. baumannii* and Antimicrobial drug.

Mechanisms of efflux pumps inhibitors (EPI)

In fighting bacterial resistance which has increased, it would be useful to employ inhibitors of resistance efflux pumps to restore the fundamental action of antibiotic. Efflux pumps are the newest bacterial

resistance mechanism allowing resistance to almost all antibiotics (Figure 2) [94]. Some molecules (chemical or natural) have the capacity to act specifically on the efflux system to restore the action of antibiotics and commonly called efflux pumps inhibitors (EPIs) [95].

In the *A.baumannii* species several chemical inhibitors have already been tested [96]. Only certain inhibitor has shown conclusive results but remains difficult to apply in clinical due to high levels of toxicity for the human organism [97,98]. To discover adequate EPI, different strategies can be considered depending on the cause gene or the level of cellular resistance [99]. Given the enormous variety of drugs, it would be cost-effective and economical to focus more on the classes of antibiotics that could have a serious impact on *A.baumannii* with respect to pharmacokinetics and toxicity. Finally, the screening of banks or chemical compounds emitted by biodiversity may allow the identification of performing compounds, which could be further enhanced by experiments with structure-activity relationships [100]. The mechanisms implicated in inhibition of efflux pump systems are

not clearly understood [6,101] but it has been suggested that inhibition of efflux pump performance in *A.baumannii* may be completed by different channel as we expect EPI to (i) Change regulatory steps of efflux pumps expression; (ii) Inhibit the practical construction of the multi-component pump; (iii) Obstruct the outer membrane ways (adeC, adeK) with a plug; (iv) Disintegrate the energy resource of efflux, direct-specific or indirect-general via a destruction of energy mechanisms of the bacterial transporters; (v) Apply a non antibiotic molecule to the affinity sites of the efflux pump for competitive or no inhibition; (vi) Modify the chemical structure of useful antibiotics in order to reduce its relationship for efflux identification and limiting sites or to obstruct the efflux transport.

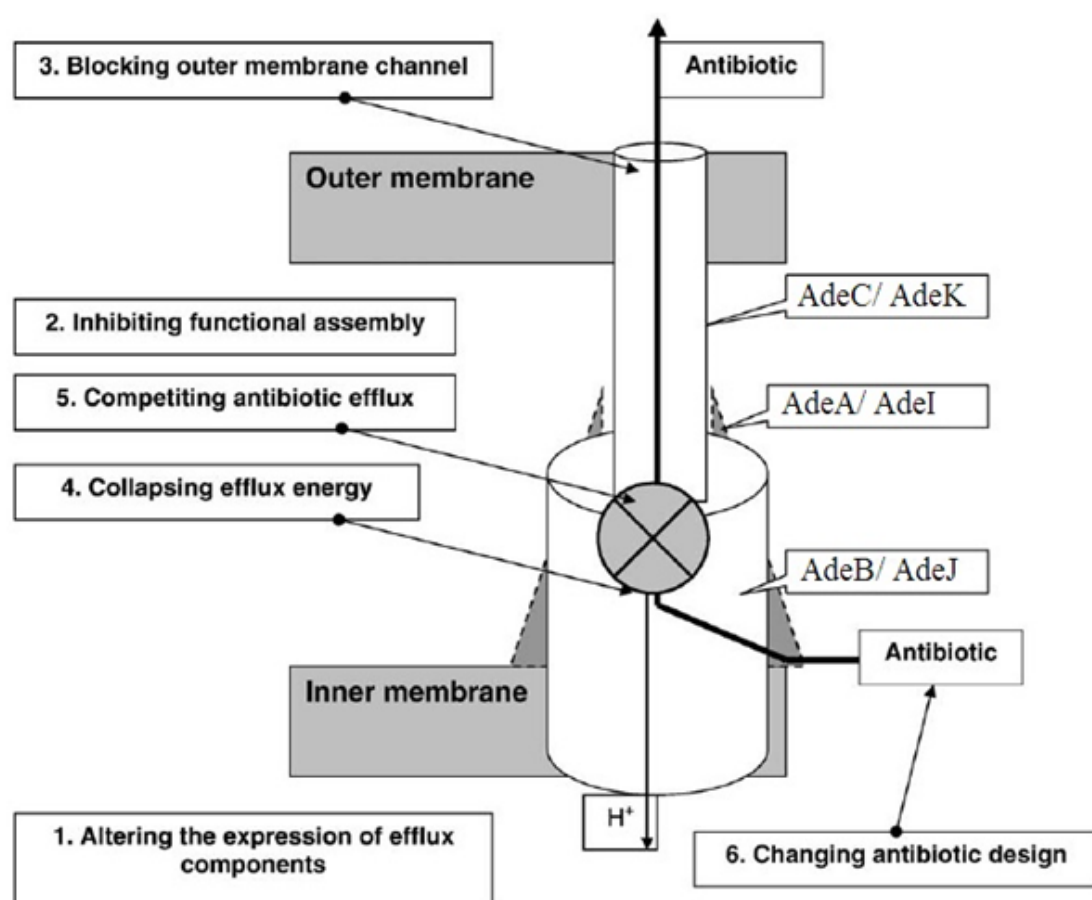


Figure 2: Various targets for inhibition of complex efflux pump (Adapted from [6] and [102])

PA β N and 1- (1-naphthylmethyl) -piperazine (NMP) are commonly used for the mechanism of inhibition efflux pump, they were tested in combination with different antimicrobial drugs facing *A. baumannii* [96,102]. At MIC values ≥ 400 $\mu\text{g} / \text{ml}$ (PA β N) and $200 \geq 400$ $\mu\text{g} / \text{ml}$ (NMP) we observed intense antibacterial activity in the behavior of these two agents. The work done by Pannek.S et al on these two EPI reveals a reversal of the resistance phenotype or a limitation in the sensitization of bacterial cells with a low concentration at 25 $\mu\text{g}/\text{ml}$ [101]. When MIC decrease eight-fold some antimicrobial drugs like levofloxacin, chloramphenicol, linezolid, ciprofloxacin, clarithromycin,

tetracycline, and rifampicin, restores sensitivity to drugs with a density 100 $\mu\text{g} / \text{ml}$ of the two EPIs, which means that one or both EPIs have a affirmative effect [55,103]. It was also noticed that PA β N and NMP, either at 100 $\mu\text{g} / \text{ml}$, restored the susceptibility on tigecycline (double-reduction of MIC) and fluoroquinolone (decrease MIC 2- 16 times) [104,105].

In other studies, PA β N at 10 $\mu\text{g}/\text{ml}$ decreased predominantly MIC concentrations of trimethoprim, clindamycin and chloramphenicol [97,102]. Twice a time on clinical isolates, whereas PA β N at 20 $\mu\text{g}/\text{ml}$ reduced nicidixic acid MIC to 16-fold but showed little effect on

sensitiveness to ciprofloxacin [106]. At 100 µg/ml PAβN are also sensible minocycline activity by decreasing ≥ 04-fold MIC values [107]. Delightful, one study has propose a contradictory effect of NMP at 64 µg/ml on susceptibility to tetracyclines (i.e., increased susceptibility to minocycline, tetracycline, doxycycline) and tigecycline (reduced susceptibility) [107-109]. Presumably, the EPIs have powerful effect on resistivity reversal with molecules that have relatively acute MIC values such as clindamycin, chloramphenicol, linezolid rifampicin, trimethoprim clarithromycin [107,108-110]. Moreover, another study also examine the effect of phenothiazines, omeprazole (prochlorperazine, chlorpromazine, and promazine), verapamil and reserpine, on susceptibility cells with phenothiazines being the only emissary capable to re-establish sensibility to some antibiotics (≥ 8 time MIC decrease) [97,109].

Recently some research demonstrated the collision on colistin susceptibility of colistin-susceptible and colistin-resistant bacteria gram (-) including *A.baumannii*

By using the effect of CCCP (carbonyl cyanide m -chlorophenyl hydrazone), NMP, PAβN, omeprazole, verapamil, reserpine [108,111]. The expression status of any drug efflux pump was not evaluate, and only carbonyl-cyanide m -chlorophenyl hydrazone (CCCP) was reveal to particularly offers influence on reversing colistine resistant for *A. baumannii*. Nevertheless, proton channel suchlike CCCP act on dislocation of proton motive energy crossways the cytoplasm membrane and do not active on pump perse [111]. Efficacy of EPI carbonyl-cyanide m -chlorophenyl hydrazone (CCCP) on colistin resistance is exotics [98,108]. Serum agents, N-tert-butyl-2- (1-tert-butyltetrazol-5-yl) sulfanylacetamide and (E) -4- (4-chlorobenzylidene) amino) benzenesulfonamide were combined to find accumulation and potentiating the improvement of the minocycline activity of several antimicrobials opposite *A. baumannii* [73,74].

The perfect results of EPIs could stimulate the action of new antimicrobial drugs. The compound, 3-(phenylsulfonyl)-2-pyrazinecarbonitrile, is an agent developed fronting resistant nosocomial pathogens [112,113]. The combine of PAβN can decrease this MIC value by four time of *A. baumannii* at MIC is 64 µg/ml [97,102]. Another lately kibelomycin natural antibiotic was found, exhibits a broad-spectrum effect with the MIC 90 value of 0.125 facing *A. baumannii* [112], this agent appears to be a distressed substratum of efflux pumps. Finally, any agents that can traverse the OM of *A. baumannii* are expected to counter the activity of the efflux pumps in augmentation the drug ingress to their targets [114,115].

In this regard, many plant extracts of EPI (in addition steroidal alkaloids conessine) are clever to break down the OM barrier to exert a synergistic efficacy on the amelioration of the activity of divers antimicrobials facing *A. baumannii* [116-118]. Natural efflux pump inhibitors (plant extracts): Biricodar and timcodar, Falvone, Berberis, Mahonia, Dalea versicolor, Lycopus europaeus, Rosmarinus officinalis, are the most common use against bacteria [116]. The analytical results of the natural inhibitor Rosmarinus officinalis and Lycopus europaeus have shown great efficacy on efflux pumps to restore the sensitivity of antibiotics against MDR strains of *A.baumannii* and *P. aeruginosa* [119]. The natural extract geranium coespitosum, punica granatum and euphorbiaceae can inhibit the potentiating activity of strains MDR staphylococcus aureus to restore the sensibility of erythromycin, fluoroquinolone, gentamicin, ampicillin, tetracycline, chloramphenicol [120,121]. Extracts of Berberis aetnensis coming from volcano region can reduce the resistance of ciprofloxacin for *P. aeruginosa*, *S. aureus*,

and *E. coli* [122, 123]. The natural inhibitors Mellisa officinalis Daucus carota Levisticum officinale, Glycyrrhiza glabra, has demonstrated a great activity facing Salmonella tyhimuriun and Klebsiella pneumoniae by restore the sensibility of tetracycline, chloramphenicol and fluoroquinolones [94, 95,124].

Conclusion

Drug efflux mechanisms are serious global problems for the fight of nosocomial infections including *A.baumannii* in clinic. RND families are the greatly complex and resist numerous types of antimicrobial drugs. Despite of the development and use of chemical molecules (NMP, PAβN, omeprazole, verapamil, reserpine, CCCP) as an EP inhibitor, many research having present results that are approximately conclusive in vitro always face elevated degree of toxicity to the physical body if it is applied in clinic. Hence the importance for future research that focuses more on natural inhibitor extract from plants (Berberis, Mahonia, Dalea versicolor, Lycopus europaeus, Rosmarinus officinalis). The development of these new type inhibitors could constitute a better and effective voice to resolve definitively the bacterial MDR problem (including *A.baumannii*). Therefore, control pharmacokinetic, pharmaco-dynamic complete and combined will give high efficacy and acceptable degree of toxicity.

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