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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*, *Falvone*, *Mahonia*, *Dalea versicolor*, *Lycopus 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)

1. Introduction

Acinetobacter spp. was detected around the 20th century (1911) by famous bac-

teriologist Beijerinck [1], but it was not until 1960 that *A. baumannii* was declared in hospital. *A. baumannii* 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 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 reduces largely the valid drug for cure. However using inhibitor components could restore 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.

2. 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] [10] [11] [12] [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 1) modification of drug target, 2) drug inactivation by enzymes, 3) modification of cell wall protein, and 4) activation of drug efflux system.

2.1. 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] [18] [19].

2.2. 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-enyzmes, IMP, and VIM), class C (AmpC-type ADC enzymes) and class D or OXA (OXA-23, OXA-51, OXA-58, and OXA-66) [22] [23] [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] [26] [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].

2.3. 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 demon-

strate very low permeability to cephalosporin's [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].

2.4. 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. baumanii* [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.

3. 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* [43]. However, because of inadequate data, we will concentrate much more on the first 5 families present in **Figure 1** where a totally understanding of structure and regulation is not complete.

3.1. *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 P. aeruginosa* [46]. RND efflux on *A. baumannii* has

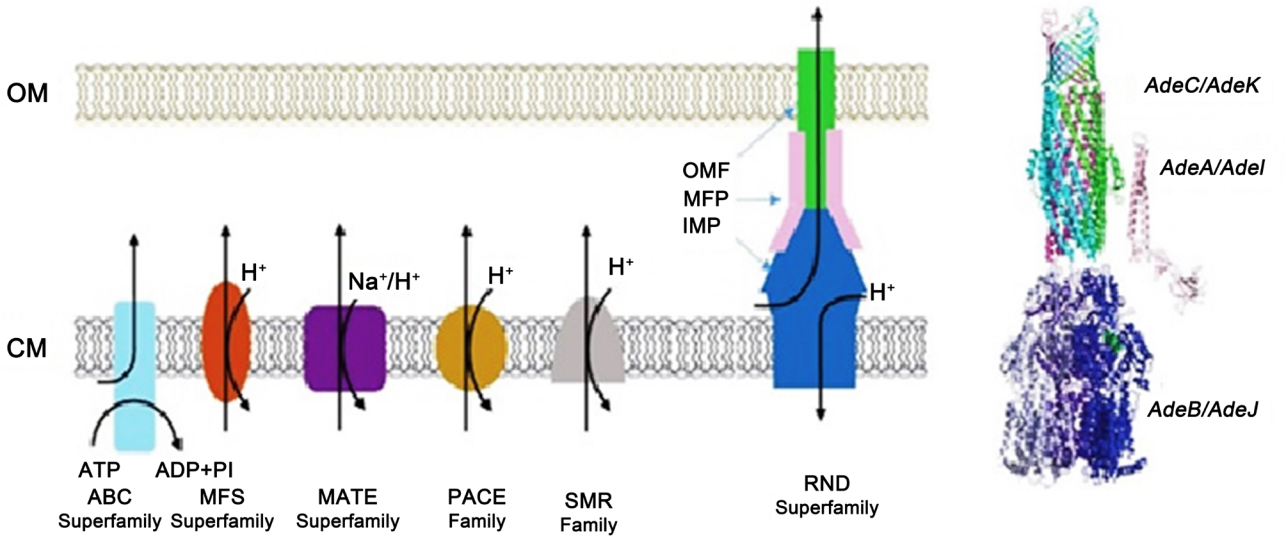

Figure 1. Structure of major families *A. baumannii* efflux pumps + PACE family a newly superfamily identify (adapted from [44] and [45]).

mainly three gene (*adeABC*, *adeIJK*, *adeFGH*) and some special gene (*adeDE*, *adeAA*) [47].

3.2. *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 *P. aeruginosa* 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, flusidic 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 problem [42] [53]. Remarkably, about 20% of *adeC* was found to be involved in tigecycline resistance tests in *A. baumannii* demonstrating that in the *adeABC* gene, *adeAB* 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 determin-

ing role in increasing resistance of *adeABC*. Some result shows that a dysfunction of *adeR* and or *adeS* will increase the resistance of tigecyclin, chloramphenicol, minocyline, 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 ISAba1 insertion produced by the *adeS* mutation confers resistance over expression to tegecycline.

3.3. *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, trimethoprime, 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 *P. aeruginosa mexAB-OprM*. Studies on production and regulation *adeABC* and *adeIJK* resulted in the formation of biofilms [36].

3.4. *adeFGH*

Outstanding variation of *adeABC* and *adeIJK*, has induced the discovery of *adeFGH* operon sometime 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 eryt-

hromycin, 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* (*GDG*3) 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].

3.5. *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, chloramphenicole, fluoroquinolones, tetracycline and rifampicin. *craA* is particularly resistant to chloranphenicol, 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] [71] [72].

3.6. *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 as-

sociated 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, erythrocin, 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]. The recapitulate of Efflux pumps family in A. baumannii and Antimicrobial drug target was listed in Table 1.

3.7. *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

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

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^+)	Acrifl Avine, 6-Diamidine-2-Phenylindole, Daunomycin, Doxorubicin, Fluoroquinolones, Gentamicin, Rhodamine 6G, Tetracycline.
SMR	*abeS*	Proton motive force (H^+)	Acridine Orange, Acrifl 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-tolC* (*baeSR*)	ATP hydrolysis (P-gp)	Erythromycin, Gramicidin
PACE	*aceI*	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

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].

3.8. *ABC* Efflux Pumps

ATP binding 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].

3.9. *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*, *Enterobactre 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 6^{th} group of MDR efflux pumps [44].

3.10. Mechanisms of Transporters in the Efflux Pump Systems

According to structural and bioenergetics characteristics, carriers could be separated into two major groups [80] [86], 1) transporters that hydrolyze ATP as an energy source; they are also summons ABC transporters (ATP binding cassette) [81], and 2) 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 understudy [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 [92] [93].

4. 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 [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 struc-

ture-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, **Figure 2** present the various target of EPI such as 1) Change regulatory steps of efflux pumps expression; 2) Inhibit the practical construction of the multi-component pump; 3) Obstruct the outer membrane ways (*adeC*, *adeK*) with a plug; 4) Disintegrate the energy resource of efflux, direct-specific or indirect-general via a destruction of energy mechanisms of the bacterial transporters; 5) Apply a non-antibiotic molecule to the affinity sites of the efflux pump for competitive or no inhibition; 6) 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.

PAβN 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 μg/ml (PAβN) and 200 ≥ 400 μg/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 μg/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 μg/ml of the two EPIs,

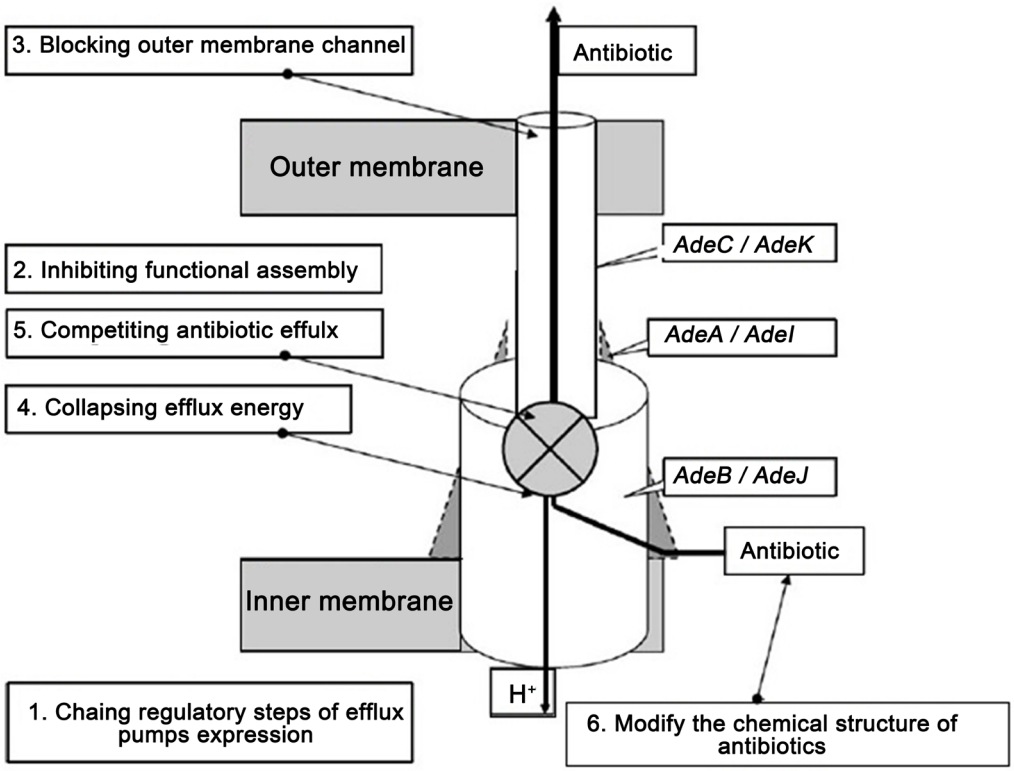

Figure 2. Various targets for inhibition of complex efflux pump (adapted from [6] and [102]).

which means that one or both EPIs have a affirmative effect [55] [103]. It was also noticed that PAβN and NMP, either at 100 μg/ml, restored the susceptibility on tigecycline (double-reduction of MIC) and fluoroquinolone (decrease MIC 2 - 16 times) [104] [105].

In other studies, PAβN at 10 μg/ml decreased predominantly MIC concentrations of trimethoprim, clindamycin and chloramphenicol [97] [102]. Twice a time on clinical isolates, whereas PAβN at 20 μg/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] [108] [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 demontrated 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 kibdelomycin 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] [117] [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 *S. tyhimuriun* and *K. pneumoniae* by restore the sensibility of tetracycline, chloramphenicol and fluoroquinolones [94] [95].

5. 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 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.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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