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Review article

Multidrug resistant *Acinetobacter baumannii* – the role of AdeABC (RND family) efflux pump in resistance to antibiotics

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Abstract: *Acinetobacter baumannii* is an opportunistic pathogen which play the more and more greater role in the pathogenicity of the human. It is often attached with the hospital environment, in which is able easily to survive for many days even in adverse conditions. *Acinetobacter baumannii* is the species responsible for a serious nosocomial infections, especially in the intensive care units. Option of surviving in natural niches, and in the hospital environment could also be associated with the efflux pump mechanisms. Mechanisms of efflux universally appear in all cells (eukaryotic and prokaryotic) and play the physiological important role. In prokaryote, the main functions are evasion of such naturally produced molecules, removal of metabolic products and toxins. These pumps could also be involved in an early stage of infection, such as adhesion to host cells and the colonization. Importantly, they remove commonly used antibiotics from the cell in therapy of infections caused by these bacteria. Efflux pumps exemplify a unique phenomenon in drug resistance: a single mechanism causing resistance against several different classes of antibiotics. In *Acinetobacter baumannii*, the AdeABC efflux pump, a member of the resistance-nodulation-cell division family (RND), has been well characterized. Aminoglicosides, tetracyclines, erythromycin, chloramphenicol, trimethoprim, fluoroquinolones, some β-lactams, and also recently tigecycline, were found to be substrates for this pump. Drugs, as substrates for the AdeABC pump, can increase the expression of the AdeABC genes, leading to multidrug resistance (MDR). From this reason, treatment failure and death caused by *Acinetobacter baumannii* infections or underlying diseases are common. Because the AdeABC pump is widespread in *Acinetobacter baumannii*, similarly to other pumps in Gram-negative and Gram-positive bacteria, exists a need of searching a new therapeutic solutions. Specific efflux inhibitors of pumps (EPIs), including AdeABC inhibitors, could be suppress the activity of pumps and restore the sensitivity of such important bacteria as *Acinetobacter baumannii* to commonly used antibiotic.

Key words: *Acinetobacter baumannii* - MDR - Efflux pump - AdeABC - RND family

Introduction – *Acinetobacter baumannii*

Bacteria classified to genus *Acinetobacter* play increasingly important role in pathogenesis of human diseases, although included to opportunistic pathogen group. Among members of the genus *Acinetobacter baumannii* species is most frequently isolated from humans [12,113,125]. These organisms are widely distributed in nature and may be present in soil, water and sewage, also are found in variety of foodstuffs [39,113,124].

Very important place of their occurrence is the hospital environment, especially intensive care units [10,43,113,132]. Acinetobacters are the second most commonly isolated nonfermenters in human specimens (*Pseudomonas* being the first). They are inhabitants of healthy human skin as part of normal flora and can be readily isolated from moist areas, especially. Other reservoirs of these organisms may include a range of both moist and dry surfaces and equipment and they can easily survive for many days or weeks, even in dry condition [10,34,113,115,124]. Occurrence in the hospital environment, in the patient and staff favour (favour the spread of diseases in hospital/ amongst other hospitalized patients) hospital-acquired infections.

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Acinetobacter baumannii is the species most often responsible for a wide spectrum of nosocomial infections [68,113,136]. It is infections such as bloodstream infections, ventilator-associated pneumonia, urinary tract infections and wound infections [113,124, 129,136]. Sporadic cases peritonitis, endocarditis, meningitis, osteomyelitis and arthritis have also been reported [10,113]. In addition, community-acquired infections have been reported, such as pneumonias [7], severe wound infections and osteomyelitis caused by multiresistant *Acinetobacter baumannii* [15,23]. There are risk factors such as instrumentation, mechanical ventilation, surgery, treatment with broad-spectrum antibiotics, and admission to an intensive care unit for colonization of patient and environmental cross-infections [5,10,16,21,140].

Treatment failure and death caused by *Acinetobacter baumannii* infections or diseases are common [10]. One of the main reasons is that clinical isolates are frequently resistant to many commonly used antimicrobial agents (multidrug resistant - MDR) [10,33]. Often are susceptible only to carbapenems (imipenem, meropenem), though resistant strains are increasingly reported, and amikacin, polymyxins, but some may be susceptible only to polymyxins [29,35,40,43,56,57,74, 106,129,136]. Colonization of the digestive tract intensive care unit patients is an important epidemiologic reservoir for multi-drug resistant *Acinetobacter baumannii* infections in hospital outbreaks [22].

In the last few decades *Acinetobacter baumannii* has emerged as important opportunistic pathogen, especially as multiple resistant to the major agents (MDR) used to treat nosocomial infections [33,93,136].

Efflux pumps as mechanism of antibiotics resistance

Bacteria can resist the action of antibiotics through several mechanisms. The transporting systems are one of them (in bacteria efflux pumps). Transporters are present in all organisms (including eukaryotic cells [130,131]) and could be drive various compounds such as physiological substrates, non-antibiotic substrates and antibiotics (different chemical classes) into (influx) or outside cells (efflux) [11,44,63,65,104,116, 117,120,128]. Bidirectional transporters have also been found and these can take various roles [62,77, 127,133]. Bacterial efflux pumps that are involved in clinically relevant resistance to antimicrobial agents, have also role in bacterial pathogenicity (*e.g.* in the colonization and the survival of bacteria in the host) [14,38,45,64,97].

There are five families of efflux-pump proteins that are associated with MDR in bacteria: (1) the multidrug and toxic compound extrusion (MATE) family [13], (2) the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily [134], (3) the small multidrug resistance (SMR) family [90], (4) the major facilitator superfamily (MFS) [76], and (5) the resistance-nodulation-cell division (RND) family [111]. This classification is based on amino acid sequence homology [96], on the energy source that the pump uses, the number of components that the pump has (single or multiple), the number of transmembrane-spanning regions and the types of substrate that the pump exports [97]. Drug efflux pumps are found in Gram-negative and Gram-positive bacteria, but resistance of this type in Gram-negative bacteria is a more complex problem due to the molecular architecture of the cell envelope. Drug resistance in many cases is attributable to synergy between reduced drug intake (mainly due to low outer membrane permeability) [85] and active drug export (via efflux pumps).

The structure of RND-family efflux pumps

Gram-negative bacteria are protected by an outer membrane, therefore efflux transporters of the RND family are organized as three-component systems similar in various species. The best studied members of this group are the AcrAB-TolC system of *Escherichia coli* [70,71] and the MexAB-OprM system of *Pseudomonas aeruginosa* [99]. These efflux pumps comprise the following: a transporter (efflux) protein (*e.g.*, AcrB), which is located in the inner (cytoplasmic) membrane; a periplasmic accessory protein (also known as a membrane fusion protein - MFP) (*e.g.*, AcrA); and an outer membrane protein channel (OMP) (*e.g.*, TolC) [51], which is located in the outer membranes of Gram-negative bacteria [28,51,126,142]. Many RND-type systems were described in Gram-negative bacteria, amongst which multidrug-resistant clinical pathogens constitute particularly a problematic group. Occurrence of the RND antibiotic transporters in a chosen nosocomial pathogens presents table 1. In some species, for example *Pseudomonas aeruginosa*, there are a few systems described which can be active at the same time. Clinical isolates of *Pseudomonas aeruginosa* can express (overexpress) two efflux pumps simultaneously MexAB-OprM and MexXY [66] or MexAB-OprM and MexEF-OprN [102].

In *Acinetobacter baumannii* AdeB is the multidrug transporter protein, AdeA is the MFP and AdeC is the OMP [73]. The efflux transporter (AdeB) captures its substrates either from within the phospholipid bilayer of the inner membrane or the cytoplasm [4] and then transport them into the extracellular medium via OMP (AdeC) [28]. The periplasmatic protein AdeA mediates in the cooperation between AdeB and AdeC components. Drug transport by different pumps families is

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Table 1. RND family efflux pumps caused multidrug resistance in important clinical Gram-negative pathogens

Organism	Efflux components			Antibiotic as substrates	Reference
	MFP	Main transporter	OMP		
Acinetobacter baumannii	AdeA	AdeB	AdeC	AG, CM, FQ, NO, TC, TM	[73]
	AdeI	AdeJ	AdeK	?	[75]
Escherichia coli	AcrA	AcrB	TolC	BL, CM, FQ, ML, NO, RF	[32,71]
	AcrA	AcrD	TolC	AG, FU	[27,109]
	AcrE	AcrF	TolC	FQ	[72]
	MdtA	MdtBC	TolC	NO	[8,81]
	YhiU	YhiV	TolC	NO	[86]
Klebsiella pneumoniae and *Klebsiella oxytoca*	AcrA	AcrB	?	FQ	[78]
Proteus mirabilis	AcrA	AcrB	?	NO	[137]
Pseudomonas aeruginosa	MexA	MexB	OprM	AG, BL, CM, ML, NO, TC, TM	[58,99,100]
	MexC	MexD	OprJ	CM, CP, FQ, TC	[98]
	MexE	MexF	OprN	CM, FQ	[48]
	MexH	MexI	OpmD	NO	[2,118]
	MexJ	MexK	OprM/OpmH	EM, TC	[20]
	MexV	MexW	OprM	CM, FQ, TC	[61]
	MexX	MexY	OprM	AG, ML, TC	[3,80,139]
Serratia marcescens	SdeA	SdeB	?	CM, FQ	[53,54]
	SdeC	SdeDE	?	?	[53]
	SdeX	SdeY	?	FQ, TC	[18]
Stenotrophomonas maltophilia	SmeA	SmeB	SmeC	AG, BL, FQ	[60]
	SmeD	SmeE	SmeF	EM, FQ, TC	[6,143]

AG – aminoglycosides, BL – β-lactams, CM – chloramphenicol, CP – cephalosporins, EM – erythromycin, FQ – fluoroquinolones, FU – fusidic acid, ML – macrolides, NO – novobiocin, RF – rifampicin, TC – tetracycline, TM – trimethoprim, ? – unknown

driven by the transmembrane electrochemical gradient of protons. Members of the RND family are proton antiporters, using the proton gradient to power efflux, exchanging one H+ ion for one drug molecule [28,91]. Figure 1 demonstrate the scheme of the structure of the AdeABC. This system was shown to be responsible for decreased susceptibility to a broad spectrum of antimicrobials. Aminoglicosides, tetracyclines, erythromycin, chloramphenicol, trimethoprim, fluoroquinolones, some β-lactams, ethidium bromide [30,37,73,135], and also recently tigecycline [92,110], were found to be substrates for AdeABC, although netilmicin and gentamicin appeared to be the best one for the pump [73].

The secondary structure of RND-type efflux proteins was proposed to consist of 12 transmembrane segments (TMS), with two long loops between TMS 1 and 2 and TMS 7 and 8 [111,126]. The trimeric form of the OMP generates a continous, solvent-accessible channel-like structure that spans both the outer membrane and the periplasmic space [52,73,141]. MFP could be involved in either the bringing of the inner and outer membranes closer or the stabilization of the OMP structure [73,84,142].

The efflux pump system AdeABC, member of RND family, was identified in a multidrug-resistant *Acinetobacter baumannii* strain in 2001 [73]. This transporter is the main efflux system in *Acinetobacter baumannii*. A lot of pumps from this family are widespread amongst Gram-negative bacteria and are associated with multidrug resistance to antibiotics in important pathogens.

Genetic organization – regulation, expression and overexpression

Genes of AdeABC system and function

The genes that encode AdeABC efflux pump are located on the bacterial chromosome [75]. Typically, the genes are organized as an operon – the structural genes *adeA*, *adeB* and *adeC* are contiguous and directly oriented. The gene encoding the periplasmic accessory protein is located adjacent to the gene encoding trans-

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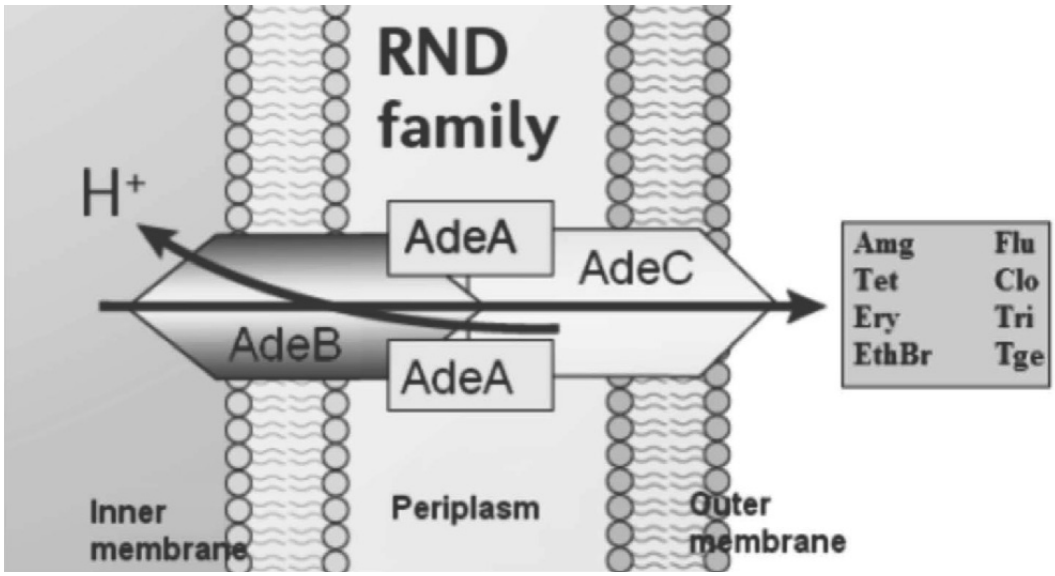

Fig. 1. Multidrug resistance RND efflux pump. Amg – aminoglycosides, Flu – fluoroquinolones, Tet – tetracyclines, Clo – chloramphenicol, Ery – erythromycin, Tri – trimethoprim, EthBr – ethidium bromide, Tge – tigecycline.

Fig. 2. Schematic organization of the *ade* gene cluster (adapted from [73]); 1443, 9208 cluster localization.

porter protein, which is located adjacent to the OMP. There are two regulatory genes, *adeS* and *adeR*, which products are closely related to proteins of two-component regulatory system. These genes, that are transcribed in the opposite direction and are localized upstream from *adeA* (Fig. 2). Two-component system are signal transduction pathways in bacteria that respond to environmental conditions (pump is dependent on substrate) [50,73,75]. The protein AdeR (regulator) consisted of 228 amino acids is typical transcriptional regulator and protein AdeS (sensor kinase) is shorter and demonstrate activity of bacterial histidine kinase, that work together to regulate target gene expression in response to stimuli. The sensor protein monitors the environmental conditions and activates or inactivates the response regulator protein which controls the expression of the efflux pump [73,75].

The sensor kinase autophosphorylates at an internal histidine (the H box) in response to stimulus and that the phosphate group is then transferred to an aspartate residue of the response regulator. Phosphorilation of each domain and the transfer of phosphoryl groups between these domains is reversible by the phosphatase activity of the sensor. The histidine kinases leads to a switch between phosphorylation and dephosphorylation activities and modulates the active state of the regulators, which controls structural genes expression [9,50,138].

Experiments performed by Marchand et al. [75] suggests, that the *adeS* gene appears to be essential for expression of the *adeABC* operon. To asses the role of *adeRS*, the *adeR* and *adeS* genes of *Acinetobacter baumannii* BM4454 were disrupted by insertion of a suicide plasmid following homologous recombination. Inactivation of these genes restored the sensitivity to aminoglycosides and to other substrates for the pump in resulting mutants [75]. In addition, another experiment indicates that expression of the AdeABC pump is under the control of the RS two component system. Spontaneous gentamicin-resistant mutants were obtained in vitro from susceptible *Acinetobacter baumannii* strain. Sequence analysis of their *adeRS* operons showed two mutations. The first one (Thr153→Met) was located in the kinase and the second mutation (Pro116→Leu) was located in the response regulator; these led to constitutive expression of the pump and MDR [75].

Inactivation of structural genes, especially *adeB* encoding the protein of main transporter have also

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Table 2. Levels of identity between proteins from RND-type efflux systems

Proteins		% identity
AdeA	AcrA	38
AdeA	MexA	37
AdeB	AcrB	49
AdeB	MexB	47
AdeB	MexD	53
AdeC	OprM	42
AdeC	TolC	22
AdeX	AdeA	35
AdeX	AdeD	38
AdeY	AdeB	45
AdeY	AdeE	51
AdeZ	AdeC	67

been describe. The derivative recombinant demonstrated 4 to more 32 times lower MICs of antibiotics, which are substrates of the pump than the parental strain [73]. In contrast, examination of the contribution of the *adeC* gene to multidrug resistance, showed that mutants still had been multidrug resistant and the AdeC protein was not essential. That AdeC is not recquired for resistance suggests that AdeAB can utilize another outer membrane constituent. The AdeK OMP associated with the AdeIJK RND efflux pump could be candidate [75]. Some efflux gene clusters RND proteins do not encode an outer membrane protein [3,71,79], but can mobilize other outer membrane channels for creating a functional three-component pumps [31,52,121,142].

The genetic organizations of the genes encoding these efflux systems are also similar among different species. However, the genes encoded RND efflux pumps are mainly located on the chromosome, they are detected with the different frequency [19,41,82]. The gene of the main AdeB transporter was detected by Chu et al. [19] with the frequency of the 70% whether the 87% through Nemec et al. [82]. All both structural and two-component regulatory system genes were stated about 40% of examined strains, in the remaining cases all sorts combinations of the presence of these genes were being watched [82].

In the RND family in *Acinetobacter* spp. other pumps are also being enumerated such as AdeDE, AdeIJK and AdeXYZ [19,30,75]. The *adeB* sequence was not detected in any of the other then *Acinetobacter baumannii* genomic DNA groups, suggesting that *adeB* is an active efflux system specific to *Acinetobacter baumannii*. The *adeB* gene is present exclusively in *Acinetobacter baumannii*, whereas *adeE* and *adeY* are present most frequently in *Acinetobacter lwoffii* [19]. Efflux pump AdeIJK has been identified in *Acinetobacter baumannii* and its characterization is under process [75].

The AdeDE has only the membrane fusion protein (MFP) gene *adeD* and the RND transporter gene *adeE* clustered together. The outer membrane protein (OMP) for AdeDE has not been identified. AdeABC differs from AdeDE by protecting the host from cefotaxime, whilst AdeDE increases the host resistance to ceftazidime and rifampicin [17,73].

Structural proteins within the RND family demonstrate the identity in the different degree to AdeA, AdeB and AdeC proteins. The highest identity of main transporters was demonstrated amongst AdeB and MexD proteins (53%); relatively high identity had AdeZ to AdeC (67%) (tab. 2) [19,73,96]. Also a polymorphism of the amino acidic sequence of the AdeB protein is being described (11 sequential types) [42].

Local and global regulation of RND pump gene expression

The regulators involved in efflux gene expression are either local or global regulators. The-best studied example of both local and global cooperation is AcrAB-TolC system of *Escherichia coli*. Most RND MDR efflux pump genes are encoded by operons that are under the control of the local repressor genes (transcriptional repressor); *e.g.* in *Escherichia coli acrR* [87], which regulates negatively the transcription of the **acrAB** operon encoding AcrAB-TolC system [69]. Other regulators demonstrate the activity of positive activators of the transcription process, but their participation in regulation of this type pumps is rare. Higher described *adeR* is an example activator which is a member of two-component system regulating the *adeABC* expression operon in *Acinetobacter baumannii* [47,73,75].

Expression of various efflux pumps is also controlled by different global regulators. The transcription of *acrAB* is primarily mediated by global regulatory pathways such as the *marRAB* operon, *soxRS* operon and *rob*, and that a major function of AcrR is that of a specific secondary modulator [69]. The *acrAB* operon and *tolC* are positively regulated by several transcriptional activators – MarA, SoxS and Rob. The levels of MarA and SoxS are themselves regulated by the repressor MarR and SoxR, respectively. The activity of Rob is modulated by metabolites, such as bile salts and fatty acids [59]. In addition, MarA and SoxS also control *micF*, an antisense RNA, that down-regulate the expression of OmpF channel protein (porin) that is responsible for influx some antimicrobial agents [24,87].

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Level of expression

Two-component regulation systems normally mediate the adaptive responses of bacterial cell to a broad range of environmental stimuli [9,50,75,138]. This system is associated with different pumps of RND family appearing in Gram-negative bacteria [8,25,60, 81,86,94], among others AdeABC of *Acinetobacter baumannii*. Level of the expression of the pump, regulated by such a system, is dependent on the presence of the stimulus (substrate) in environment of the cell, which activating the pump for removing substrate outside the cell. Antibiotics can serve as inducers and regulate the expression of some efflux pumps at the level of gene transcription or mRNA translation, by interacting with regulator systems [108]. This type of expression is named as inductive.

Some pumps are independent of environmental stimuli and permanently active on the defined level. Such an activity is being described as constitutive. A recent study has demonstrated that the assembly of the AcrA, AcrB and TolC proteins into a functional AcrAB-TolC pump (member of RND family pumps) is constitutive [123], occurring in the presence or absence of substrate molecules. The AdeABC pump of *Acinetobacter baumannii* can change the expression from inductive to the high level of the constitutive type, including the activity of the pump on the very high level called as overexpression. It is possible in the result of various mutations or inactivation by insertion sequences in the local regulatory genes, including the activator *adeR* in *Acinetobacter baumannii* [73,75]. In other bacteria, which genes encoding pumps are regulated by repressors also could be derepressed by mutations in this genes [1,112]. These changes lead to multiple antibiotic resistance of bacteria.

Spontaneous gentamicin-resistance, which is associated with single point mutations in *adeR* (Pro116→Leu) and *adeS* (Thr153→Met) are known to cause AdeABC constitutive overexpression of the efflux pump and MDR in *Acinetobacter baumannii* [73,75]. Up-regulation the transporter AdeB can accompany mutations on different genes conditioning resistance to the given antibiotic. Higgins et al. [37] found a 20-fold increase in mRNA transcript of the *adeB* gene from two outbreak strains of *Acinetobacter baumannii*, which were clones of the pretherapy strain obtained earlier in the outbreak. It was parallel with mutations in *gyrA* and *parC* gene encoding DNA gyrase and topoisomerase IV, respectively. *Acinetobacter baumannii* has been shown to mutate rapidly in *gyrA* and *parC* under the selective pressure of fluoroquinolones in vivo and at the same time to up-regulate the efflux pump AdeB. Ciprofloxacin pressure was responsible for the selection of the efflux phenotype in addition to the topoisomerase mutations. Importantly, these data illustrate the propensity for *Acinetobacter baumannii* to develop multi-drug resistance rapidly [37].

The newest examinations show the participation of the AdeABC efflux pump in the resistance to newer antibiotics in *Acinetobacter*. Experiment performed by Ruzin et al. [110] suggests, that AdeABC MDR efflux pump decreased susceptibility to tigecycline – the first member of a new class of modified tetracycline antimicrobials known as glycylcyclines. In this study the *adeB* gene was disrupted by insertional inactivation using a suicide plasmid in two derivative mutants constructed from two clinical isolates. Insertional inactivation of the *adeB* gene resulted in a decrease in the MICs of tigecycline and other antimicrobials. In addition, analyses of the *adeRS* locus were performed to determine the reason for constitutive overexpression of *adeABC* in two parental clinical strains. The *adeS* gene was disrupted by an insertion sequence IS_{ABA-I}, whereas it remained intact in tigecycline-susceptible strains [110]. IS_{ABA-I} element was previously identified in *Acinetobacter spp.* and is though to affect the expression of antibiotic resistance genes that are adjacent to the site of insertion [114].

The results received by Peleg *et al.* [92] can also indicate that efflux mechanism play a role in reduced tigecycline susceptibility in *Acinetobacter*. In two strains they found multiple point mutations in *adeR* and *adeS* genes, which may lead to pump overexpression. They also put forward a hypothesis, that increased expression of *adeB* is associated with increased MICs of tigecycline. They compared parent strain with isogenic derivative strain, which was *in vitro* exposed to tigecycline. A 12-fold increase in MIC tigecycline and a 25-fold increase in *adeB* expression was observed, however, the lack of mutations in this strain compared to its isogenic parent strain, indicates that other mechanisms for increased pump activity are also involved. The MICs for other antimicrobials were also increased. The tigecycline MIC was reduced to the level of the parent strain using PAβN as inhibitor various pumps. The MICs for other antimicrobials were also reduced. These results strongly support, that participation of the MDR pumps in the resistance to tigecycline is evident, as well as tigecycline exposure may increase the activities of many pumps.

Natural functions of RND efflux pumps

The natural functions of the different RND pumps are still a topic of debate. The physiological role of these systems is evasion of such naturally produced molecules, thereby allowing the bacterium to survive in its ecological niche. Natural functions suggested for efflux pumps include: removal of metabolic products (*e.g.*, fermentation end products and toxins), removal of toxins, buffering the organisms against surges in

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pools of potentially toxic metabolites [36]. It has been suggested that the natural function of RND pumps might also include efflux of signal molecules required for cell-to-cell signaling (quorum sensing) [103]. *Pseudomonas aeruginosa* secretes a quinolone, designated PQS (*Pseudomonas* quinolone signal), which acts as quorum-sensing signal [95] and overexpression of MexEF-OprN was shown to affect PQS signalling [49]. This is a very interesting finding as quinolones constitute a widely used group of antimicrobials and are substrates for RND pumps.

It has also been shown in tissue culture studies that components of RND efflux pumps are important in the invasion, adherence, and/or colonization of the host cell. Decreased cellular invasion was observed with mutant *Pseudomonas aeruginosa* PAO1 with inactivated *mexB* [38].

The AcrAB pump of *Escherichia coli* was found to have the highest affinity for bile salts. The natural habitat of *Escherichia coli* is the enteric tract and efflux is a protective mechanism [14,55,83,101,122]. Other functions of this pump are participation in adherence, colonization and invasion of host cells [14]. AcrAB-TolC also could play a role in the transport of the calcium-channel components in the *Escherichia coli* membrane [46], might export hormones [26].

Efflux pump inhibitors

The importance of efflux as a mechanism of resistance to a variety of antimicrobial agents makes developing efflux inhibitors an attractive strategy, especially in the case of a broad spectrum pumps of multidrug resistance bacteria such as *Pseudomonas* or *Acinetobacter*. Inhibition of these pumps may be achieved at different levels: by inhibiting drug binding to the inner membrane pumps, by inhibiting the interactions of different components of a multi-component pump, by targeting the energy source of pumps, or by targeting the regulatory networks that control the expression of efflux pumps. A few putative bacterial efflux pump inhibitors have been described [88,119].

The first broad-spectrum RND pump inhibitor, MC-207,110 (phenylalanyl-arginyl-β-naphthylamide) was reported to be capable of reversing the MDR phenotype of *Pseudomonas aeruginosa* and several other Gram-negative bacteria [67,105]. It has also been tested in *Acinetobacter baumannii* clinical isolates [89,92,107]. Reduction in the MIC of nalidixic acid after PAβN addition was observed, though there was no effect in the case of ciprofloxacin [107]. Recently study, demonstrated decrease of tigecycline MIC and other antimicrobials, including gentamicin, tobramycin, chloramphenicol, and several β-lactams after exposure to PAβN [92]. Activity of another putative efflux pump inhibitor, 1-(1-naphthylmethyl)-piperazine (NMP), was also analysed and compared to PAβN activity in MDR isolates of *Acinetobacter baumannii*. PAβN at low concentration (25 mg/L) had highly selective action on the reduction in the MIC of rifampicin and clarithromycin. At a higher concentration (100 mg/L), NMP was more active than PAβN. NMP can partially reverse MDR in *Acinetobacter baumannii* and differs in its activity from that of PAβN in this species [89].

Because human cells use efflux pumps as well, the development of a truly broad-spectrum efflux pump inhibitor would theoretically increase the risk of toxicity. Despite these challenges, continued research into development of inhibitors of resistance mechanisms is clearly needed.

Conclusions

During the last 20 years *Acinetobacter baumannii* has become an important opportunistic pathogen cause of nosocomial infections with multiple outbreaks. *Acinetobacter baumannii* cause serious morbidity and mortality in the immunocompromised patient, particularly in intensive care units.

Efflux mechanisms are widespread in bacteria and have a role in establishing the level of both susceptibility and resistance to various antimicrobial agents. Multidrug resistant *Acinetobacter baumannii* strains and other bacteria determine the very considerable therapeutic problem as a result of the activity of efflux pumps with a broad spectrum even if AdeABC.

Recent advances in genome sequence analyses, availability of molecular structures and a more profound understanding of the function and regulation of efflux systems will facilitate exploitation of pumps as new drug targets. Efflux pump inhibitors would be invaluable tools to help clear bacterial infection because of their dual function: restoration of the activity of agents to which efflux pumps confer resistance and reduction in the ability of bacteria to colonize their host or even to cause infection. Identification and clinical development of an effective and safe efflux-pump inhibitors to be used in combination with existing or new antimicrobials is particularly appealing.

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