adeABC efflux gene in Acinetobacter baumannii

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Abstract

The antimicrobial resistance to Acinetobacter baumannii is significantly high and continues to grow; it has become a global health issue, particularly in regards to carbapenem resistance. The expression of efflux pumps is one of the major mechanisms of antibiotic resistance in A. baumannii by, most prevalently, adeABC of the resistance/nodulation/division family. The detection rate of adeB was the highest in clinical isolates compared to others (adeFGH, adeIJk), although it varied among other strains. In this minireview, we explain the adeABC efflux gene in A. baumannii causing antibiotic resistance and compare adeABC with other efflux genes in order to discern the function of adeABC in A. baumannii resistance, which may help in the discovery of new antibacterial agents.
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Introduction

Acinetobacter baumannii is a common Gram-negative opportunistic pathogen. In recent decades, it has successfully evolved from an ordinary bacterium to an important pathogen of nosocomial infection, causing ventilator-associated pneumonia, bacteraemia, urinary tract infection and secondary meningitis [1]. At present, the infection of A. baumannii is widespread, especially in intensive care units. Statistical data estimated that about 45 000 (41 400−8300) cases of Acinetobacter infections occurred in the United States each year, and about 1 000 000 (600 000−1 400 000) around the world [2]. Moreover, the emergence of multidrug-resistant A. baumannii, extensively drug-resistant A. baumannii and even pandrug-resistant A. baumannii brings about great challenges to global healthcare workers. Therefore, further research is needed to investigate the resistance mechanism and related genes in order to offer more information for the development of new sensitive antibiotics.

The major mechanisms of resistance generally include producing antimicrobial-inactivating enzymes, modifying targets, reducing the membrane permeability and forming biofilm and overexpression of the membrane active efflux system [3]. Antimicrobial inactivating enzymes hydrolyze drugs and confer resistance against drugs. However, the substrates of the inactivated enzyme are often selective. For example, β-lactamases cause the inactivation of β-lactams, and aminoglycosides-modifying enzymes induce the resistance to aminoglycosides [4]. Compared to other resistance mechanisms, active efflux pumps are more widely distributed and have a wider substrate, resulting in more kinds of drug resistance [1]. In addition, recent research has suggested that biofilm formation of A. baumannii is potentially associated with the genes encoding efflux pumps [5]. Finally, minocycline and tigecycline are broad-spectrum antibiotics which show effective activity against multidrug-resistant Acinetobacter. However, with the mutation leading efflux pump overexpression, the susceptibility of A. baumannii to these drugs is limited [4,6].

Efflux Pump in A. baumannii

The first efflux pump of A. baumannii, AdeABC, regulated by AdeRS, was found in multidrug-resistant A. baumannii BM4454

by Magnet et al. in 2001 [7]. The study of the efflux pump system in *Acinetobacter* subsequently developed. *adeDE* [8] and *adeXYZ* [9] were detected in *Acinetobacter* genomic DNA group 3 (GDG3) in 2004 and 2006, respectively. In 2008, the AdeIJk efflux pump was found in BM4454 by Damierpiolle et al. [10]. The *adeFGH* efflux pump was discovered in BM4664 in 2010 [11].

The membrane-active efflux system generally consists of three parts: outer membrane protein (*adeC*), multidrug transporter (*adeB*) and membrane fusion protein (*adeA*). According to the homology of the amino acid sequence, the membrane efflux pump is divided into five superfamilies: ATP-binding cassette (ABC), small multidrug resistance (SMR), multiantimicrobial extrusion (MATE), major facilitator (MFS) and resistance/nodulation/division (RND). The ABC family mainly exists in Gram-positive bacteria, which rely on ATP to provide energy, while for the SMR, MATE, MFS and RND family the proton driving force acts as the energy source [12]. The RND efflux pump superfamily, including *adeABC*, *adeDE*, *adeFGH*, *adeIJK* and *adeXYZ*, is prevalent in *A. baumannii*, and its substrate is the most extensive. At present, *adeABC*, as the pump gene in *A. baumannii* discovered first, is the most studied pump gene; some researchers have even proposed that *adeABC* be used as a sign of resistance of *A. baumannii*.

Structure and Regulator of *adeABC*

Structure of *adeABC*

At present, the understanding of the molecular mechanisms and functions of the *adeABC* complex are primarily based on the study of the resistant strain *A. baumannii* BM4454 [7]. *adeABC* is located in the chromosome genome of *A. baumannii*. *adeA*, *adeB* and *adeC* are continuous, encoding membrane fusion protein, multidrug transporter and outer membrane channel protein structure, respectively. Their function can be simply explained by the fact that *adeB* captures substrates in the inner membrane of phospholipids bilayer or the cytoplasm, then transports the substrates by *adeC* (membrane channel protein). Therefore, these structural genes can promote drug discharge (Fig. 1).

The expression of *adeABC* is regulated by *adeRS*, and the expression levels of *adeA*, *adeB* and *adeC* are inconsistent. PCR amplification showed that the detection rate of *adeB* was highest in clinical isolates, but the detection rates varied in these studies. In some studies the *adeB* gene was found in all clinically isolated strains, while in other studies the rate just was 70% to 75% [13,14]. By contrast, some studies found that *adeA* has the highest detection rate in clinically isolated strains [15,16]. More researchers incline to the view that *adeB* is the most important gene in *adeABC* and is most associated with the resistance of
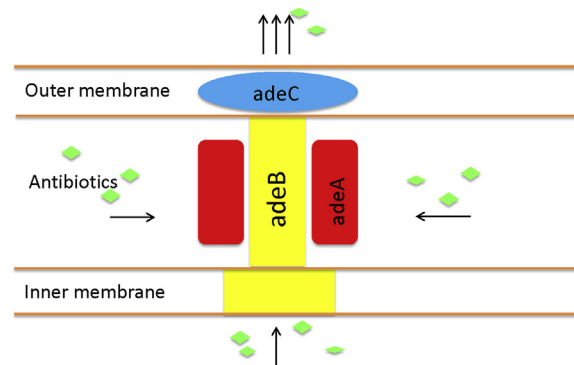

FIG. 1. Function of *adeABC* efflux pump in cell wall of *Acinetobacter baumannii*. *adeA* acts as membrane fusion protein, *adeB* as multidrug transporter and *adeC* as outer membrane protein. *adeB* captures antibiotics in inner membrane of phospholipids bilayer or cytoplasm, then transports substrates out by *adeC* (membrane channel protein).

A. baumannii [17]. In all studies, the detection rate of *adeC* was the lowest [13−16], with the lowest rate of 42% in some studies [13]. However, compared to *adeC*-positive and *adeC*-negative strains, we found that the *adeC*-positive group had more strain resistance to all six antibiotics in the study [13]. It demonstrated that although *adeC* is not necessary in *adeABC* efflux-mediated drug resistance, the presence of *adeC* is more likely to result in multidrug resistance or pan-drug resistance.

adeRS regulates expression of *adeABC*

adeRS is located upstream of *adeABC*, separated by a 133 bp intercistronic spacer between the *adeRS* and *adeABC* operons [18] and the expression direction reverse to *adeABC*. The *adeRS* bicomponent regulation system consists of sensor kinase *adeS* and responsive regulator *adeR*. *adeS* consists of histidine kinase that receives environmental signals and cause autophosphorylation, then transfers the phosphoric acid to the output responder, *adeR*. *adeR* has been recognized as a recognition response factor and acts as a transcriptional activator [19]. In the study of Hassan et al. [20], *adeB* and *adeR* were discovered in mutant cell populations by the fluorescence technique. *adeB* and *adeR* have similar fluorescence intensity higher than that of parents. When Hornsey et al. [6] analysed the nucleotide sequence of the carbendazone-resistant clone strain South-East and OXA-23 clone 1, they found that at position 62 of the AdeS sensor histidine kinase, there was a difference in amino acid, which was methionine in the OXA-23 clone 1 strain and isoleucine in the South-East clone strain. The amino acid sequence of AdeR was not different, however.

On the basis of the above evidence, we suspect that AdeS may play a leading role in the regulation of AdeABC. Some studies have shown that amino acid substitutions at AdeRS of clinical isolates resulted in overexpression of the *adeABC* efflux

pump. Hornsey et al. [6] found Ala-94 → Val substitution in *adeS* in AdeB-overexpressing tigecycline-resistant strains; Coyne et al. [12] found Asp-30 → Gly substitution in the AdeS-sensing domain in the multidrug-resistant strain; Chang et al. [21] found Met-197 → Ile substitution and Gly-200 → Cys substitution in *adeS* DNA combination domain in tigecycline-resistant isolates. In addition, insertion sequence (IS) in *adeRS* also can affect the resistance of *A. baumannii*. Sun et al. [22] found that inserting *ISAbaI* into *adeS* can produce N-terminal truncated free forms of *adeS* and messenger RNA transcripts. The truncated AdeS then enhances *adeABC* gene overexpression. Lopes et al. [23] also demonstrated that the insertion of ISAbaI in AdeS enhanced the expression of *adeABC* efflux pump and reduced the susceptibility of *A. baumannii* to tigecycline. In short, the mutation of *adeRS* or the insertion sequence in AdeRS can cause the overexpression of *adeABC*, resulting in resistance of *A. baumannii*.

How do *adeRS* regulate the overexpression of *adeABC* efflux pumps? Some studies showed that phosphorylated regulators bind to *adeABC* promoter regions and regulate the expression of *adeABC* operon [24,25]. However, Chang et al. [21] explored the interaction between *adeR* and *adeABC* by electrophoresis mobility shift analysis; they found that AdeR and *adeABC* promoters did not interact. Even if *adeS* was present, *adeR* was not found to bind to the promoter region of *adeABC*. Their further studies discovered that *adeR* binds to a direct-repeat motif region between *adeR* and *adeABC*, then regulated *adeABC* expression. They argued that amino acid substitutions of *adeRS* changes the binding ability of AdeR to the direct-repeat motif region, thereby leading to *adeABC* overexpression. However, additional research is required to support this notion. *adeRS* regulating the expression of *adeABC* is defined, but the mode of action is still difficult to specify. In addition, the regulation of the expression of the *adeABC* gene is complex. Under some conditions, the *ISAbaI* insertion does not lead to the overexpression of this pump [26], indicating that other regulators may be involved. One study showed that the other two-component system, *BaeSR*, can regulate *adeA* and *adeB* [27]. More research is needed to explore the regulation mechanism of *adeABC*, and more direct evidence of the association of mutations and regulators involved in antibiotic resistance is required.

adeABC in drug-resistant *A. baumannii*

adeABC has a wide range of substrates, including β-lactams, fluoroquinolones, tetracycline (tigecycline), macrolide (linamides) and chloramphenicol; it confers the clinical resistance of aminoglycosides. Among these, netilmicin and gentamycin appear to be the best substrates for efflux pump *adeABC* [7]. Efflux pumps such as *adeABC* play an important role in the resistance mechanisms of tigecycline by throwing drugs away from the target biding site [28,29]. Studies have also found that the *adeABC* pump has a synergistic effect with carbapenems and aminoglycosidases on drug resistance [30]. A study in China showed a close association between overexpression of AdeABC efflux pump genes and carbapenem (meropenem) resistance in *A. baumannii* without mutation of its regulatory genes [31]. It has been noted that the presence of both Int1 and 16S ribosomal RNA methylases confers resistance to aminoglycosides [32].

A study by Sun et al. [22] found the RNA transcripts of the *adeA*, *adeF* and *adeI* genes in the isolates resistance to ticarcillin were 8.18 (±14.60), 0.03 (±0.07) and 1.65 (±1.64) times, respectively, as those of the reference strain *A. baumannii* ATCC 15151. The expression of the *adeABC* efflux pump gene therefore changes more than *adeFGH* and AdeIJk in ticarcillin-resistant *A. baumannii* isolates. In addition, *adeB* gene expression was not observed in any of the initially sensitive strains. In the study of Rumbo et al. [33], clinical isolates overexpressed the *adeABC* efflux system (expression level 30 to 45 times those of *A. baumannii* ATCC 17978) were resistant to tigecycline, minocycline and gentamycin, and other biological functions were significantly correlated. The high-expression *adeIJk* efflux system (expression level as eight to ten times those of reference *A. baumannii* ATCC 17978) were related to only tigecycline and minocycline resistance.

In an article published in 2010, Coyne et al. [11] pointed out that *adeABC* was detected in 80% (reported rate, 53−97%) in clinically isolated resistant strains and was the most frequently involved in the multidrug-resistant RND system in the clinical setting. Hornsey et al. [6] found a correlation between higher minimum inhibitory concentration (MIC) values and elevated *adeABC* expression. Furthermore, overexpression of AdeABC efflux pump genes is a common mechanism to decrease susceptibility to tigecycline, which is supported by the presence of efflux pump inhibitor (EPI) to reverse the resistance pattern [34]. Further, differences in expression of *adeABC* contributed to both inter- and intraclone variation in tigecycline MICs in *A. baumannii* [34]. One study in China compared tigecycline-susceptible *A. baumannii* and tigecycline-sensitive *A. baumannii* isolates; the study found that overexpression of *adeABC* is the main mechanism for the decrease in resistance to tigecycline [35]. In the study of *adeB*, *adeB* was found to be related to aminoglycoside resistance and mediated tetracycline, chloramphenicol, erythromycin, trimethoprim and ethidium bromide sensitivity levels [36]. The resistance range was similar to all expressions of *adeABC*, which further illustrates the fact that the *adeB* gene plays an important role in *adeABC* pump resistance mechanism.

TABLE 1. Basic properties of *adeABC* and *adeIJk*

Property	adeABC	adeIJk
Distribution strains	Almost always mutant strains, seldom wild	Mutant and wild
Regulator	AdeRS (positive regulation)	adeN (negative regulation)
Kind of antibiotic resistance	Intrinsic antibiotic resistance and acquired antibiotic resistance	Intrinsic antibiotic resistance
Drug resistance	β-Lactams, fluoroquinolons, tetracycline, linamides, chloramphenicol, aminoglycosides	β-Lactams, fluoroquinolons, tetracycline, linamides, chloramphenicol, erythromycin, fusidic acid, neonatal acid, rifampicin, trimethoprim, acridine (dyes), coke (dyes) and sodium dodecyl sulfate

The presence of the *adeABC* gene in sensitive strains is therefore low, and is prevalent in the drug-resistant strains, so some researchers support the notion that *adeABC* can be used as a sign of resistance of *A. baumannii* [14]. To date, however, *A. baumannii* is not particularly sensitive to many drugs, but its sensitivity to colistin remains high [37,38]. One study showed the contribution of *adeABC* in colistin heteroresistance when exposed to colistin by overexpression of *adeB* in clinical isolate [39]. In the study of Gholami et al. [40], the clinical isolates are all sensitive to colistin.

Other Efflux Pump Genes

adeDE (containing unidentified outer membrane constituent genes), belonging to the RND family, was first detected in *Acinetobacter* stage GDG3 [8], then was detected separately in GDG13TU and -17 [9]. GDG3 and did not appear with *adeABC* [14], but *adeB* and *adeE* were found in the study of Hou et al. [41] in isolates of resistant strains of *A. baumannii,* indicating that *adeB* and *adeE* can be expressed simultaneously in parts of *A. baumannii.* The expression and the role of *adeDE* in *A. baumannii* remains to be studied. *adeFGH*, RND family; and LysR transcription factor *adeL* are responsible for transcription of *adeFGH*. *adeFGH* overexpression was found in chloramphenicol-resistance−acquired mutant strains, and *adeF* is not associated with resistance to ticarcillin. In all ticarcillin-based extensively drug-resistant *A. baumannii* isolates, the *adeF* gene was the lowest in the three major RND pump genes (*adeABC, adeFGH, adeIJk*) [22].

adeIJK also belongs to the RND family. Its expression is regulated by the TetR family transcriptional regulator AdeN. In sensitive and resistant strains, *adeIJk* has been detected. Studies have shown that *adeIJk* may only cause intrinsic resistance rather than *adeABC*, which will produce intrinsic resistance and acquired resistance. *adeIJK* is resistant to β-lactams, chloramphenicol, tetracycline, erythromycin, linamides, fluoroquinolons, fusidic acid, neonatal acid, rifampicin, trimethoprim, acridine (dyes), coke (dyes) and sodium dodecyl sulfate. Although the average expression level of *adeJ* is relatively low, as long as the expression of the *adeIJk* carried by the

plasmid occurs, it can significantly increase the MIC level of cloxacillin, oxacillin, nitrothromine and ethidium bromide [10]. On this basis, it is speculated that the physiologic effect of *adeIJK* efflux pump may be stronger than that of *adeABC* as well as the properties of *adeABC* and *adeIJk* (Table 1).

Comparison of *adeABC* and *adeIJk* of *A. baumannii* with those of the *AcrAB-TolC* system of *Escherichia coli* showed that under similar conditions, *adeABC* was more effective than the similar level of *AcrAB-TolC* in the resistance to tetracycline but was less effective in lipophilic β-lactams, novobiocin and ethidium bromide. Interestingly, *adeIJK* was more effective than AcrAB-TolC in lipophilic β-lactams, novobiocin and ethidium bromide, although less effective in tetracycline [42]. However, there are no studies directly comparing the effect of *adeABC* and *adeIJk*. Therefore, further study is needed to understand the important effect of *adeIJk* on *A. baumannii*. Genes with unknown mechanisms and drug-resistant efflux pump genes are being found; these genes seem to be related to resistance to certain drugs. For example, in a study of AbeM by the MATE family, it was revealed that it can cause resistance to aminoglycosides and quinolones [43]. TetA is related to tetracycline resistance, while MdfA contributes to ciprofloxacin and chloramphenicol resistance [44]. CraA [45] and CmlA [12] play an important role in chloramphenicol resistance. AmvA [46] has an effect on erythromycin resistance.

Conclusion

Although the efflux gene of *A. baumannii* has been studied for decades, many things remain unclear. Coyne et al. [47] evaluated the expression of the *A. baumannii* efflux pump gene; their microarray chip contained 205 gene probes, including 47 efflux systems, 55 resistance determinants and 35 housekeeping genes. Therefore, the efflux genes of *A. baumannii* are more than those have studied. Except for the above-mentioned genes, many genes remain unclear. *A. baumannii* is also an easy-to-carry drug-resistant gene that moves elements such as plasmids, transposon and insert sequence, leading to its more efficient and complex mechanism. *adeABC* has been widely implicated; other related genes have been less studied. Some

literature has suggested that the expression of *adeIJK* may be a potential gene associated with resistance to *A. baumannii*. What genes play a more extensive and powerful role in drug resistance? Regulation of efflux pump gene expression and whether there are other regulatory genes are also concerns.

It is noteworthy that the current studies on the resistance mechanism of efflux pumps are focused on *in vitro* studies. The detection of expression and resistance of efflux pump genes are also carried out *in vitro*. Therefore, some genes such as *adeC* are thought to be unnecessary in mediating drug resistance, but *in, vivo,* its specific role has not been studied; whether it plays a role in the interaction of the strain and the host is not understood. The efflux pump inhibitor is a drug class that does not itself have a bactericidal effect but that can inhibit the efflux pump in combination with antibiotics to reduce the MIC, just as sulbactam in combination with cefoperazone. However, the existing efflux pump inhibitors have a wide range of substrates, and the toxicity is high. If these characters can lead to further resistance is not known. Using more rational drug according to the efflux pump, and developing less toxic and more selective drugs are extremely urgent.

Conflict of Interest

None declared.

Acknowledgements

References

[1] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii:* emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.
[2] Spellberg B, Rex JH. The value of single-pathogen antibacterial agents. Nat Rev Drug Discov 2013;12:963.
[3] Gordon NC, Wareham DW. Multidrug-resistant *Acinetobacter baumannii:* mechanisms of virulence and resistance. Int J Antimicrob Agents 2010;35:219–26.
[4] Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of *Acinetobacter baumannii:* pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol 2017;7:55.
[5] He X, Lu F, Yuan F, Jiang D, Zhao P, Zhu J, et al. Biofilm formation caused by clinical *acinetobacter baumannii* isolates is associated with overexpression of the *AdeFGH* efflux pump. Antimicrob Agents Chemother 2015;59:4817.
[6] Hornsey M, Ellington MJ, Doumith M, Thomas CP, Gordon NC, Wareham DW, et al. *AdeABC*-mediated efflux and tigecycline MICs for epidemic clones of *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:1589–93.
[7] Magnet S, Courvalin P, Lambert T. Resistance−nodulation−cell division−type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 2001;45:3375.
[8] Chau SL, Chu YW, Houang ET. Novel resistance−nodulation−cell division efflux system AdeDE in *Acinetobacter* genomic DNA group 3. Antimicrob Agents Chemother 2004;48:4054–5.
[9] Chu YW, Chau SL, Houang ET. Presence of active efflux systems *AdeABC, AdeDE* and *AdeXYZ* in different *Acinetobacter* genomic DNA groups. J Med Microbiol 2006;55(pt 4):477–8.
[10] Damier-Piolle L, Magnet S, Bremont S, Lambert T, Courvalin P. *AdeIJK,* a resistance−nodulation−cell division pump effluxing multiple antibiotics in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2008;52(2):557–62.
[11] Coyne S, Rosenfeld N, Lambert T, Courvalin P, Périchon B. Overexpression of resistance−nodulation−cell division pump *AdeFGH* confers multidrug resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:4389–93.
[12] Coyne S, Courvalin P, Perichon B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. Antimicrob Agents Chemother 2011;55:947–53.
[13] Wieczorek P, Sacha P, Czaban S, Hauschild T, Ojdana D, Kowalczuk O, et al. Distribution of AdeABC efflux system genes in genotypically diverse strains of clinical *Acinetobacter baumannii*. Diagn Microbiol Infect Dis 2013;77:106.
[14] Lin L, Ling BD, Li XZ. Distribution of the multidrug efflux pump genes, adeABC, adeDE and adeIJK, and class 1 integron genes in multiple-antimicrobial-resistant clinical isolates of Acinetobacter baumannii-Acinetobacter calcoaceticus complex. Int J Antimicrob Agents 2009;33(1):27–32.
[15] Modarresi F, Azizi O, Shakibaie MR, Motamedifar M, Valibeigi B, Mansouri S. Effect of iron on expression of efflux pump (*adeABC*) and quorum sensing (*luxI, luxR*) genes in clinical isolates of *Acinetobacter baumannii*. APMIS 2015;123:959–68.
[16] Wei J, Li C, Zhang H, Li G, Liu X, Wei J. Prevalence of genes of OXA-23 carbapenemase and AdeABC efflux pump associated with multidrug resistance of *Acinetobacter baumannii* isolates in the ICU of a comprehensive hospital of Northwestern China. Int J Environ Res Public Health 2015;12:10079–92.
[17] Yoon EJ, Balloy V, Fiette L, Chignard M, Courvalin P, Grillotcourvalin C. Contribution of the *Ade* resistance−nodulation−cell division−type efflux pumps to fitness and pathogenesis of *Acinetobacter baumannii*. MBio 2016;7. e00697-16.
[18] Marchand I, Damierpiolle L, Courvalin P, Lambert T. Expression of the RND-type efflux pump *AdeABC* in *Acinetobacter baumannii* is regulated by the *AdeRS* two-component system. Antimicrob Agents Chemother 2004;48:3298.
[19] West AH, Stock AM. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biol Sci 2001;26:369–76.
[20] Hassan KA, Cain AK, Huang TT, Liu Q, Elbourne LDH, Boinett CJ, et al. Fluorescence-based flow sorting in parallel with transposon insertion site sequencing identifies multidrug efflux systems in *Acinetobacter baumannii*. MBio 2016;7:e01200–16.
[21] Chang TY, Huang BJ, Sun JR, Perng CL, Chan MC, Yu CP, et al. AdeR protein regulates *adeABC* expression by binding to a direct-repeat motif in the intercistronic spacer. Microbiol Res 2016;183:60.
[22] Sun JR, Perng CL, Lin JC, Yang YS, Chan MC, Chang TY, et al. *AdeRS* combination codes differentiate the response to efflux pump inhibitors in tigecycline-resistant isolates of extensively drug-resistant *Acinetobacter baumannii*. Eur J Clin Microbiol Infect Dis 2014;33:2141.

[23] Lopes BS, Amyes SG. Insertion sequence disruption of *adeR* and cipro-floxacin resistance caused by efflux pumps and *gyrA* and *parC* mutations in *Acinetobacter baumannii*. Int J Antimicrob Agents 2013;41:117–21.

[24] Cheung J, Hendrickson WA. Sensor domains of two-component regulatory systems. Curr Opin Microbiol 2010;13:116.

[25] Hornsey M, Loman N, Wareham DW, Ellington MJ, Pallen MJ, Turton JF, et al. Whole-genome comparison of two *Acinetobacter baumannii* isolates from a single patient, where resistance developed during tigecycline therapy. J Antimicrob Chemoth 2011;66:1499.

[26] Sun JR, Perng CL, Chan MC, Morita Y, Lin JC, Su CM, et al. A truncated AdeS kinase protein generated by *ISAba1* insertion correlates with tigecycline resistance in *Acinetobacter baumannii*. PLoS One 2012;7: e49534.

[27] Lin MF, Lin YY, Lan CY. The role of the two-component system BaeSR in disposing chemicals through regulating transporter systems in *Acinetobacter baumannii*. PLoS One 2014;10:e0132843.

[28] Singh H, Thangaraj P, Chakrabarti A. *Acinetobacter baumannii:* a brief account of mechanisms of multidrug resistance and current and future therapeutic management. J Clin Diagn Res 2013;7:2602–5.

[29] Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis 2010;2:291–304.

[30] Yoon EJ, Chabane YN, Goussard S, Snesrud E, Courvalin P, Dé E, et al. Contribution of resistance–nodulation–cell division efflux systems to antibiotic resistance and biofilm formation in *Acinetobacter baumannii*. MBio 2015;6:e00309–15.

[31] Dou Q, Zou M, Li J, Wang H, Hu Y, Liu W. *AdeABC* efflux pump and resistance of *Acinetobacter baumannii* against carbapenem. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2017;42:426–33.

[32] Chen F, Wang L, Wang M, Xie Y, Xia X, Li X, et al. Genetic characterization and in vitro activity of antimicrobial combinations of multidrug-resistant *Acinetobacter baumannii* from a general hospital in China. Oncol Lett 2018;15:2305–15.

[33] Rumbo C, Gato E, López M, Ruiz dAC, Fernández-Cuenca F, Martínez-Martínez L, et al. Contribution of efflux pumps, porins, and β-lactamases to multidrug resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2013;57:5247.

[34] Lin MF, Lan CY. Antimicrobial resistance in *Acinetobacter baumannii:* from bench to bedside. World J Clin Cases 2014;2:787.

[35] Deng M, Zhu MH, Li JJ, Bi S, Sheng ZK, Hu FS, et al. Molecular epidemiology and mechanisms of tigecycline resistance in clinical isolates of *Acinetobacter baumannii* from a Chinese university hospital. Antimicrob Agents Chemother 2014;58:297–303.

[36] Zhang T, Wang M, Xie Y, Li X, Dong Z, Liu Y, et al. Active efflux pump *adeB* is involved in multidrug resistance of *Acinetobacter baumannii* induced by antibacterial agents. Exp Ther Med 2017:1538–46.

[37] Savari M, Ekrami A, Shoja S, Bahador A. Plasmid borne carbapenem-hydrolyzing class D beta-lactamases (CHDLs) and *AdeABC* efflux pump conferring carbapenem–tigecycline resistance among *Acinetobacter baumannii* isolates harboring TnAbaRs. Microb Pathog 2017;104: 310–7.

[38] Hua X, Liu L, Fang Y, Shi Q, Li X, Chen Q, et al. Colistin resistance in *Acinetobacter baumannii* MDR-ZJ06 revealed by a multiomics approach. Front Cell Infect Microbiol 2017;7:45.

[39] Machado D, Antunes J, Simões A, Perdigão J, Couto I, Mccusker M, et al. Contribution of efflux to colistin heteroresistance in a multidrug resistant *Acinetobacter baumannii* clinical isolate. J Med Microbiol 2018;67:740–9.

[40] Gholami M, Hashemi A, Hakemi-Vala M, Goudarzi H, Hallajzadeh M. Efflux pump inhibitor phenylalanine-arginine beta-naphthylamide effect on the minimum inhibitory concentration of imipenem in *Acinetobacter baumannii* strains isolated from hospitalized patients in Shahid Motahari Burn Hospital, Tehran, Iran. Jundishapur J Microbiol 2015;8:e19048.

[41] Hou PF, Chen XY, Yan GF, Wang YP, Ying CM. Study of the correlation of imipenem resistance with efflux pumps *AdeABC, AdeIJK, AdeDE* and *AbeM* in clinical isolates of *Acinetobacter baumannii*. Chemotherapy 2012;58:152–8.

[42] Sugawara E, Nikaido H. Properties of *AdeABC* and *AdeIJK* efflux systems of *Acinetobacter baumannii* compared with those of the *AcrAB-TolC* system of *Escherichia coli*. Antimicrob Agents Chemother 2014;58:7250–7.

[43] Su XZ, Chen J, Mizushima T, Kuroda T, Tsuchiya T. *AbeM*, an H$^+$-coupled *Acinetobacter baumannii* multidrug efflux pump belonging to the MATE family of transporters. Antimicrob Agents Chemother 2005;49:4362.

[44] Vila J, Martí S, Sánchez-Céspedes J. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2007;59:1210–5.

[45] Roca I, Marti S, Espinal P, Martínez P, Gibert I, Vila J. *CraA*, a major facilitator superfamily efflux pump associated with chloramphenicol resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2009;53:4013–4.

[46] Rajamohan G, Srinivasan VB, Gebreyes WA. Molecular and functional characterization of a novel efflux pump, *AmvA*, mediating antimicrobial and disinfectant resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:1919–25.

[47] Coyne S, Guigon G, Courvalin P, Perichon B. Screening and quantification of the expression of antibiotic resistance genes in *Acinetobacter baumannii* with a microarray. Antimicrob Agents Chemother 2010;54: 333–40.