ORIGINAL ARTICLE

Role of efflux pumps in reduced susceptibility to tigecycline in *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is an important human pathogen responsible for a various type of infections. These bacterial strains are generally resistant to numerous antibiotics. Therefore, eradication of such strains is problematic and related to high mortality. We investigated the effect of cyanide 3-chlorophenylhydrazone (CCCP) efflux pump inhibitor in tigecycline-resistant strains of *Acinetobacter baumannii.* In a cross-sectional study, from July until the end of February 2017, eighty isolates of *A. baumannii* were recovered. Antimicrobial susceptibility testing against tigecycline was performed by the disc diffusion method and determination of minimum inhibitory concentration by broth microdilution method, according to Clinical and Laboratory Standards Institute guidelines. Active efflux pumps were detected by CCCP as an efflux pumps inhibitor, and the gene expression of some of the resistance/nodulation/division (RND)-type efflux pumps was measured by semiquantitative RT-PCR (qRT-PCR). Antibiotic susceptibility tests in this study showed that 78 of 80 *A. baumannii* isolates were resistant to tigecycline. The results of phenotypic detection of efflux pumps revealed that 23.07% of tigecycline-resistant *A. baumannii* isolates can contain active efflux pumps. On the basis of conventional PCR, genes coding for *adeF* and *adeJ* were detected in 76 (98%) *A. baumannii* isolates. The results of qRT-PCR showed that the transcript level of the *adeJ* gene increased in 66.6% *A. baumannii* isolates with CCCP-positive tests and was correlated with tigecycline resistance. The results of this study indicate that RND-type efflux pumps appear to play a significant role in the tigecycline resistance of *A. baumannii.*

Keywords: *Acinetobacter baumannii*, antibiotic resistance, efflux pump, tigecycline, gene expression
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Introduction

Globally, Gram-negative bacteria, especially *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* are important and serious hospital-acquired pathogens responsible for a various type of infections, including wound and urinary tract infections, pneumonia, bloodstream infections and secondary meningitis, especially in patients in the intensive care unit [1–5]. *A. baumannii* can persist in different areas of the hospital and can also acquire antibiotic resistance genes or develop resistance mechanisms against antibacterial agents [4,6,7]. However, these bacterial strains are at the same time generally resistant to numerous antibiotics [8]. Previous studies have indicated that a high percentage of *A. baumannii* isolates are multidrug resistant (MDR) to several categories of antibiotics including fluoroquinolones, carbapenems, aminoglycosides and tetracyclines [9,10]. Therefore, eradication of such strains is problematic and is related to high mortality [2,11].

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The US Food and Drug Administration has approved tigecycline to treat skin infections, complex intra-abdominal infections and community-acquired respiratory infections [12]. Furthermore, tigecycline, minocycline and colistin are among the few remaining antibiotics for the treatment of MDR *A. baumannii* infections, and tigecycline has shown significant activity to MDR *A. baumannii* [13]. However, reports indicate that tigecycline resistance is increasingly common [14,15], although minocycline remains effective in several infections [16]. Tigecycline-resistant *A. baumannii* involves overexpression of chromosomally encoded RND efflux pumps [10,17,18]. These efflux pumps are mainly composed of three parts: fusion protein, cytoplasmic membrane-spanning transport protein and outer membrane protein [9,13,19–21]. Previously published studies have found that overexpression of AdeABC, AdeFGH or AdeIJK, as three major RND pumps, contribute to antibiotic resistance in *A. baumannii* clinical isolates [13,22,23]. According to these data [1–22], evaluating the efflux pump effect on tigecycline resistance in *A. baumannii* probably assists with the prevention of this antibiotic resistant.

We therefore investigated the relative gene expression of RND-type efflux pumps' effect on tigecycline resistance in MDR *A. baumannii.*

Materials and Methods

Bacterial isolates and species identification

In a cross-sectional study conducted from July until the end of February 2017, separate clinical isolates of *A. baumannii* were recovered from diverse clinical samples of patients, including both adults and children. Specimens included blood, cerebrospinal fluid, dialysis fluid and shunt. All isolates were identified using conventional biochemical and microbiologic tests, such as growth on MacConkey agar, oxidase, motility, triple sugar iron agar (TSI) and growth at 42°C.

Antimicrobial susceptibility tests

The tigecycline susceptibility test was performed using the disc diffusion method (DDM) on Müller-Hinton agar; the minimum inhibitory concentration (MIC) values (mg/L) of tigecycline were determined by the broth microdilution method. Antibiotic disc and powders were purchased from Mast (Bootle, UK) and MilliporeSigma (St Louis, MO, USA; cat. PZ0021). All experiments and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Resistance to tigecycline was defined as a MIC of at least 4 mg/L on the basis of CLSI recommendation. *Pseudomonas aeruginosa* ATCC 27853 was used as a control for DDM and MIC tests.

Identification of RND-type efflux pump genes

The existence of genes encoding the efflux pumps AdeF and AdeJ was screened by PCR. The primers used in this study are listed in Table 1. PCR amplification was performed using a 9700 GeneAmp thermocycler (Applied Biosystems, Foster City, CA, USA); PCR conditions have been described previously [23,24]. PCR products were analysed on agarose gels, stained with DNA-safe stain (SinaClon, Tehran, Iran) and visualized on a UV transilluminator. The sequencing of PCR products was performed by ABI 3730X capillary sequencer (Pishgam; Macrogen, Seoul, Korea). Moreover, *A. baumannii* ATCC 19606 was used as the reference strain.

Treatment of efflux pump inhibitor

The activity of the efflux pump system was screened using efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone (CCCP). To explore the existence of the efflux pump mechanism, CCCP was added to each Müller-Hinton agar plate containing 0.5 to 256 μg/mL tigecycline. The final concentration of CCCP in the Müller-Hinton agar was 25 μg/mL, and MIC for tigecycline was determined again. Finally, a fourfold decrease of the MIC with CCCP compared to tigecycline MIC without CCCP showed presence of active efflux pumps.

RNA extraction and complementary DNA synthesis

A. baumannii strains were cultured in brain–heart infusion medium, and total RNA was extracted from exponentially grown bacteria (OD_{600}, 1.5–2.0) using the RNeasy Mini Kit (SinaClon) according to the supplier's instructions. RNA samples were treated with 20 U of RQ1 RNAse-free DNAse (Promega, Madison, WI, USA) to eliminate any genomic DNA carryover and were suspended in 50 μL of diethylpyrocarbonate-treated water (0.1% v/v). The complementary DNA synthesis was performed using the complementary DNA synthesis kit (Bioneer, Daejeon, Korea; cat. K-2041). Synthesis conditions have been described previously [11].

Semiquantitative RT-PCR

The relative expression levels of *adeF* and *adeJ* genes were assessed by qRT-PCR. qRT-PCR reaction was performed using the Power SYBR Green PCR Master Mix (Bioneer) on a Rotor-

TABLE 1. Primers used for detection of efflux pump genes

Target	5′–3′
adeF	Forward: GGTGTCGACCAAGATAAACG
	Reverse: GTGAATTTGGCATAGGGACG
adeJ	Forward: GCGAATGGACGTATGGTTCT
	Reverse: CATTGCTTTCATGGCATCAC
16S ribosomal RNA	Forward: AACGGACGACCATCTTTGAGTATT
	Reverse: CAGTTGTTCCATTTCACGCATT

Gene RT-PCR machine (Corbett Research, Sydney, Australia; model RG3000, software version 6). The relative expression of the investigated genes was normalized against the 16S ribosomal RNA housekeeping gene and was calculated on the basis of the $2^{-\Delta\Delta Ct}$ method. *A. baumannii* ATCC 19606 was used as the reference strain. The primer sequences used for qRT-PCR are listed in Table 1.

Results

In all, 80 *A. baumannii* strains were collected from July until the end of February 2017. On the basis of DDM and MIC results, 78 (98%) of 80 *A. baumannii* isolates were resistant to tigecycline. To confirm the main role of the efflux pump in the tigecycline-resistant phenotypes in 80 *A. baumannii* isolates, we identified the MIC of tigecycline in the presence of the efflux pump inhibitor CCCP, then compared the MICs with and without CCCP. The results of phenotypic detection of efflux pumps revealed that 23.07% of tigecycline-resistant *A. baumannii* isolates can contain active efflux pumps, with a minimum fourfold decline of the MIC with CCCP in contrast with the MIC of tigecycline without CCCP.

Furthermore, after PCR detection, *adeF* and *adeJ* genes were confirmed to be present in 76 *A. baumannii* isolates (98%). Moreover, the relative expression levels of *adeF* and *adeJ* genes were identified in *A. baumannii* isolates by qRT-PCR. Quantitative analysis indicated that *adeF* and *adeJ* gene expression increased from two times to more than 256 times, and from 32 to more than 256 times compared to ATCC strain, respectively. Moreover, the result of qRT-PCR revealed that seven (38.8%) of 18 and 12 (66.6%) of 18 *A. baumannii* isolates with CCCP-positive test results included an increase in *adeF* and *adeJ* gene expression, respectively. In general, four (22.22%) of 18 *A. baumannii* isolates revealed the highest expression in both efflux pump genes (Table 2).

Discussion

The chromosomal RND-type efflux systems play a significant role in the initiation and progress of clinical antibiotic resistance, bacterial pathogenesis, virulence and biofilm maturation in Gram-negative bacteria, especially *A. baumannii* [18,19]. Furthermore, the overexpression of these efflux pumps has been related to MDR in *A. baumannii* [25]. On the basis of World Health Organization reports, the last few years have seen a dramatic surge in the prevalence of MDR and extensively drug-resistant (XDR) bacteria, thus indicating that antibiotic resistance is a major worldwide health problem [26]. Published studies reveal that bacteria can acquire antibiotic resistance in the existence of drug efflux pumps; new findings indicate that these systems, by removing various compounds, contribute to bacteria that has had time to acquire resistance to the various classes of antibiotics, including β-lactams, tetracyclines, aminoglycosides, cephalosporins and fluoroquinolones [21,27]. MDR and XDR strains have been recognized as a major concern in different hospital areas, particularly the intensive care unit [18,19,28]. According to US Food and Drug Administration–published guidelines as well as the results of previous studies, a few antibiotics such as tigecycline, minocycline and colistin can be used for the treatment of MDR *A. baumannii* [12,13], but tigecycline resistance is increasingly being reported [16].

The role of RND-type efflux pumps in tigecycline-resistant *A. baumannii* has been extensively studied [7,11,21]. In the current study, 98% of *A. baumannii* isolates were resistant to tigecycline; the susceptibility of isolates to tigecycline was therefore still low. In addition, our finding in the current study also showed that the MIC of 51.25% was considerably reduced by twofold to fourfold when CCCP was added. CCCP reduced MIC by twofold to fourfold in *A. baumannii* strains [29,30]. The results of present study showed that 19 (24.3%) and 13 (16.6%) of 78 tigecycline-resistant *A. baumannii* isolates showed an increase of gene expression in one and both efflux pumps, respectively. Moreover, results of our previously published study have shown that 50% and 70% of tigecycline-resistant *A. baumannii* isolates with CCCP-positive tests included an increase in *adeB* and *abeM* gene expression, respectively. Moreover, results of a published study showed that the AdeABC efflux pump appeared to play an important role in the tigecycline resistance of *Acinetobacter* species [18]. These findings revealed that drug efflux pumps can be involved in resistance to tigecycline in clinical isolates of *A. baumannii* [31]. This study showed that 76 *A. baumannii* isolates (98%) carried *adeF* and *adeJ* genes; these results were similar to a previous study that reported that 90% of 112 *A. baumannii* isolates carried the *adeJ* gene [32].

In conclusion, unfortunately, with regards to the critical role of drug efflux pumps in the emergence of MDR and XDR strains of bacteria, no inhibitors for drug efflux pumps are available for clinical use [33]. Thus, we suggest that RND-type efflux pumps are interesting targets for inhibition. The relative expression, function and assembly of RND-type efflux pumps can be targeted by numerous strategies, including suppressing efflux pump expression by targeting the regulatory network that controls the expression of efflux pumps, thus altering the molecular design and structures of old antibiotics, disrupting pump assembly by targeting protein–protein interfaces, directly blocking the inner membrane protein and blocking the outer membrane protein [33–36]. Furthermore, the combination therapy of colistin/tigecycline, colistin/

TABLE 2. Synergistic effect of CCCP on tigecycline MIC and expression of RND-type efflux pump in 80 *Acinetobacter baumannii* isolates

					Gene overexpression	
Isolate no.	**MIC-CCCP (μg/mL)**	**MIC range (μg/mL) + CCCP**	**Fold reduction in MIC + CCCP**	**Presence of *adeF* and *adeJ* genes**	***adeF***	***adeJ***
1	>64	<0.25	>16	+	—	+
2	>64	>64	—	+	—	—
3	>64	<0.25	>16	+	—	+
4	>64	<0.25	>16	+	+	—
5	>64	>64	—	+	—	—
6	>64	>64	—	+	—	—
7	32	16	2	—	—	—
8	>64	>64	—	+	—	—
9	>64	>64	—	+	—	—
10	>64	>64	—	+	—	—
11	>64	>64	—	+	—	—
12	>64	>64	—	+	—	—
13	2	—	—	—	—	—
14	>64	>64	—	+	—	—
15	>64	>64	—	+	—	—
16	>64	>64	—	+	—	—
17	>64	>64	—	+	—	—
18	>64	>64	—	+	—	—
19	>64	>64	—	+	—	—
20	64	1	12	+	+	+
21	>64	>64	—	+	—	—
22	>64	>64	—	+	—	—
23	>256	>256	—	+	—	—
24	>256	64	>4	+	+	—
25	>256	>256	—	+	—	—
26	>256	>256	—	+	—	—
27	>256	>256	—	+	—	—
28	>256	>256	—	+	—	—
29	>256	>256	—	+	—	—
30	256	256	—	+	—	—
31	256	128	2	+	—	—
32	256	128	2	+	—	—
33	256	128	2	+	—	—
34	>256	>256	—	+	—	—
35	>256	>256	—	+	—	—
36	>256	>256	—	+	—	—
37	256	128	2	+	—	—
38	128	128	—	+	—	—
39	>256	>256	—	+	—	—
40	>256	>256	—	+	—	—
41	>256	>256	—	+	—	—
42	>256	>256	—	+	—	—
43	>256	>256	—	+	—	—
44	>256	>256	—	+	—	—
45	256	128	2	+	—	—
46	>256	>256	—	+	—	—
47	256	64	4	+	+	—
48	256	128	2	+	—	—
49	256	64	4	+	+	—
50	256	256	—	+	—	—
51	256	128	2	+	+	—
52	256	64	4	+	—	—
53	256	256	—	+	—	—
54	32	16	2	+	—	—
55	2	—	—	—	—	—
56	128	64	2	+	—	—
57	64	16	4	+	+	+
58	64	8	6	+	+	+
59	256	256	—	+	+	—
60	256	128	2	+	—	—
61	128	<0.25	18	+	—	—
62	128	64	2	+	—	—
63	128	64	2	+	—	—
64	128	64	2	+	—	—
65	>256	<0.25	>20	+	—	—
66	>256	>256	—	+	—	—
67	32	<0.25	14	—	—	—
68	>256	>256	—	+	—	—
69	>256	<0.25	>20	+	—	+
70	>256	>256	—	+	—	—
71	>256	>256	—	+	—	—
72	>256	>256	—	+	—	—
73	>256	32	>6	+	—	—
74	>256	>256	—	+	—	—
75	32	1	10	+	—	—
76	64	<0.25	16	+	+	
77	256	125	2	+	+	—
78	256	32	6	+	+	+
79	256	128	2	+	—	—
80	256	256	—	+	—	—

CCCP, cyanide 3-chlorophenylhydrazone; MIC, minimum inhibitory concentration; RND, resistance/nodulation/division.

meropenem and tigecycline/meropenem could be helpful for treatment of tigecycline-resistant *A. baumannii* isolates [28].

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Conflict of Interest

None declared.

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References

[1] Beheshti M, Talebi M, Ardebili A, Bahador A, Lari AR. Detection of AdeABC efflux pump genes in tetracycline-resistant *Acinetobacter baumannii* isolates from burn and ventilator-associated pneumonia patients. J Pharm Bioallied Sci 2014;6:229–32.

[2] Azimi L, Namvar AE, Lari AR, Jamali S, Lari AR. Comparison of efflux pump involvement in antibiotic resistance among *Pseudomonas aeruginosa* isolates of burn and non-burn patients. Arch Pediatr Infect Dis 2016;4:e36160.

[3] Azimi L, Talebi M, Pourshafie MR, Owlia P, Lari AR. Characterization of carbapenemases in extensively drug resistance *Acinetobacter baumannii* in a burn care center in Iran. Int J Mol Cell Med 2015;4:46–53.

[4] Blanco N, Harris AD, Rock C, Johnson JK, Pineles L, Bonomo RA, et al. Risk factors and outcomes associated with multidrug-resistant *Acinetobacter baumannii* upon intensive care unit admission. Antimicrob Agents Chemother 2018;62. e01631-17.

[5] Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of *Acinetobacter baumannii:* clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 2012;67:1607–15.

[6] Coyne S, Courvalin P, Périchon B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. Antimicrob Agents Chemother 2011;55:947–53.

[7] Gerson S, Nowak J, Zander E, Ertel J, Wen Y, Krut O, et al. Diversity of mutations in regulatory genes of resistance–nodulation–cell division efflux pumps in association with tigecycline resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2018;73:1501–8.

[8] Leus IV, Weeks JW, Bonifay V, Smith L, Richardson S, Zgurskaya HI. Substrate specificities and efflux efficiencies of RND efflux pumps of *Acinetobacter baumannii*. J Bacteriol 2018;200. e00049-18.

[9] Li H, Wang X, Zhang Y, Zhao C, Chen H, Jiang S, et al. The role of RND efflux pump and global regulators in tigecycline resistance in clinical *Acinetobacter baumannii* isolates. Future Microbiol 2015;10:337–46.

[10] Nowak J, Seifert H, Higgins PG. Prevalence of eight resistance-nodulation-division efflux pump genes in epidemiologically characterized *Acinetobacter baumannii* of worldwide origin. J Med Microbiol 2015;64:630–5.

[11] Owrang M, Karimi A, Azimi L, Motaghi Nezhad R, Fallah F. Relative gene expression of RND-type efflux pumps in tigecycline resistant *Acinetobacter baumannii* isolated from training hospitals in Tehran, Iran. Int J Pediatr 2018;6:8669–74.

[12] Pagdepanichkit S, Tribuddharat C, Chuanchuen R. Distribution and expression of the Ade multidrug efflux systems in *Acinetobacter baumannii* clinical isolates. Can J Microbiol 2016;62:794–801.

[13] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii:* emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.

[14] Pournaras S, Koumaki V, Gennimata V, Kouskouni E, Tsakris A. *In vitro* activity of tigecycline against *Acinetobacter baumannii:* global epidemiology and resistance mechanisms. Adv Exp Med Biol 2016;897:1–14.

[15] Shariati A, Azimi T, Ardebili A, Chirani A, Bahramian A, Pormohammad A, et al. Insertional inactivation of *oprD* in carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from burn patients in Tehran, Iran. New Microbe. New Infect 2018;21:75–80.

[16] Stein GE, Babinchak T. Tigecycline: an update. Diagn Microbiol Infect Dis 2013;75:331–6.

[17] Sun Y, Cai Y, Liu X, Bai N, Liang B, Wang R. The emergence of clinical resistance to tigecycline. Int J Antimicrob Agents 2013;41:110–6.

[18] Yang YS, Chen HY, Hsu WJ, Chou YC, Perng CL, Shang HS, et al. Overexpression of *AdeABC* efflux pump associated with tigecycline resistance in clinical *Acinetobacter nosocomialis* isolates. Clin Microbiol Infect 2019;25:512. e1–6.

[19] Yoon EJ, Balloy V, Fiette L, Chignard M, Courvalin P, Grillot-Courvalin C. Contribution of the Ade resistance–nodulation–cell division-type efflux pumps to fitness and pathogenesis of *Acinetobacter baumannii*. MBio 2016;7. e00697-16.

[20] 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.

[21] Yoon EJ, Courvalin P, Grillot-Courvalin C. RND-type efflux pumps in multidrug resistant clinical isolates of *Acinetobacter baumannii:* major role of *AdeABC* overexpression and *AdeRS* mutations. Antimicrob Agents Chemother 2013;57:2989–95.

[22] Zgurskaya HI, Nikaido H. Multidrug resistance mechanisms: drug efflux across two membranes. Mol Microbiol 2000;37:219–25.

[23] Peleg AY, Adams J, Paterson DL. Tigecycline efflux as a mechanism for nonsusceptibility in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51:2065–9.

[24] Fernando DM, Xu W, Loewen PC, Zhanel GG, Kumar A. Triclosan can select for an *AdeIJK*-overexpressing mutant of *Acinetobacter baumannii* ATCC 17978 that displays reduced susceptibility to multiple antibiotics. Antimicrob Agents Chemother 2014;58:6424–31.

[25] Mobasseri P, Azimi L, Salehi M, Hosseini F, Fallah F. Distribution and expression of efflux pump gene and antibiotic resistance in *Acinetobacter baumannii*. Arch Clin Infect Dis 2018. https://doi.org/10.5812/archcid.67143.

[26] World Health Organization. Antimicrobial resistance: global report on surveillance. 2014. Available at: https://www.who.int/drugresistance/documents/surveillancereport/en/.

[27] Ranjbar R, Tolon SS, Zayeri S, Sami M. The frequency of antibiotic resistance and ESBLs among clinically *Acinetobacter baumannii* strains isolated from patients in a major hospital in Tehran, Iran. Open Microbiol J 2018;12:254–60.

[28] Ramadan RA, Gebriel MG, Kadry HM, Mosallem A. Carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa:* characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. Infect Drug Resist 2018;11:1261–9.

[29] Abdi-Ali A, Nikasa P, Rahmani-Badi A, Al-Hamad A. *In vitro* evaluation of proton motive force–dependent efflux pumps among multidrug resistant *Acinetobacter baumannii* isolated from patients at Tehran hospitals. Jundishapur J Microbiol 2013;6:e6792.

[30] Szejbach A, Mikucka A, Bogiel T, Gospodarek E. Usefulness of phenotypic and genotypic methods for metallo-beta-lactamases detection in carbapenem-resistant *Acinetobacter baumannii* strains. Med Sci Monit Basic Res 2013;19:32–6.

[31] Ardebili A, Talebi M, Azimi L, Lari AR. Effect of efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone on the minimum inhibitory concentration of ciprofloxacin in *Acinetobacter baumannii* clinical isolates. Jundishapur J Microbiol 2014;7:e8691.

[32] 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;33:27–32.

[33] Venter H, Mowla R, Ohene-Agyei T, Ma S. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. Front Microbiol 2015;6:377.

[34] Blair JM, Smith HE, Ricci V, Lawler AJ, Thompson LJ, Piddock LJ. Expression of homologous RND efflux pump genes is dependent upon *AcrB* expression: implications for efflux and virulence inhibitor design. J Antimicrob Chemother 2014;70:424–31.

[35] Du D, Wang Z, James NR, Voss JE, Klimont E, Ohene-Agyei T, et al. Structure of the *AcrAB–TolC* multidrug efflux pump. Nature 2014;509(7501):512.

[36] Nakashima R, Sakurai K, Yamasaki S, Hayashi K, Nagata C, Hoshino K, et al. Structural basis for the inhibition of bacterial multidrug exporters. Nature 2013;500(7460):102.