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MECHANISMS OF RESISTANCE

Functional Characterization of AbaQ, a Novel Efflux Pump Mediating Quinolone Resistance in *Acinetobacter baumannii*

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ABSTRACT *Acinetobacter baumannii* has emerged as an important multidrug-resistant nosocomial pathogen. In previous work, we identified a putative MFS transporter, AU097_RS17040, involved in the pathogenicity of *A. baumannii* (M. Pérez-Varela, J. Corral, J. A. Vallejo, S. Rumbo-Feal, G. Bou, J. Aranda, and J. Barbé, Infect Immun 85:e00327-17, 2017, https://doi.org/10.1128/IAI.00327-17). In this study, we analyzed the susceptibility to diverse antimicrobial agents of *A. baumannii* cells defective in this transporter, referred to as AbaQ. Our results showed that AbaQ is mainly involved in the extrusion of quinolone-type drugs in *A. baumannii*.

KEYWORDS *Acinetobacter*, efflux pumps, quinolones

Acinetobacter baumannii is a multidrug-resistant (MDR) pathogen that causes hospital-acquired infections (1). In previous work, we identified a new, putative major facilitator superfamily (MFS) transporter in *A. baumannii* strain ATCC 17978, AU097_RS17040. This transporter, referred to as AbaQ (*A. baumannii* quinolone resistance transporter), is involved in surface-associated motility as well as the virulence of *A. baumannii* (2).

Sequence analysis of AbaQ, annotated as an MFS transporter in *A. baumannii* strain ATCC 17978 (accession number WP_000345069), indicated an open reading frame (ORF) of 1,305 nucleotides. According to the deduced amino acid sequence, the protein consists of 434 residues and has a molecular mass of 47.8 kDa and a theoretical isoelectric point (pI) of 9.27. On the basis of predictions of its secondary structure and transmembrane topology, AbaQ is composed of 12 α-helical transmembrane segments, with both the N and C termini located in the cytoplasm (Fig. 1A). Support for this structure came from an independent analysis that revealed the three-dimensional (3D) structure of the protein (Fig. 1B). These data indicated that AbaQ is a drug H^+ antiporter 1 (DHA1), which differs from DHA2-type MFS drug transporters by the presence of 12 rather than 14 transmembrane segments (3). The predicted product of the *abaQ* gene exhibited low amino acid identity and similarity (<24% and <38%, respectively) with other MFS transporters involved in drug efflux in *A. baumannii* (Table 1).

To determine whether a lack of AbaQ alters antimicrobial susceptibilities, the responses of two *A. baumannii abaQ* mutants obtained in previous work (2) to antimicrobials of different classes were tested. Compared to the wild-type (WT) parental strain, *A. baumannii* ATCC 17978, the *abaQ* mutant had 2- to 4-fold higher susceptibilities to trimethoprim and novobiocin (Table 2). The highest susceptibilities (approximately 8- to 32-fold) occurred in response to the quinolone-type antibiotics ciprofloxacin, levofloxacin, and nalidixic acid (Table 2). In contrast, the mutant and its WT parent did not differ in their susceptibilities to β-lactams (meropenem and ampicillin), aminoglycosides (amikacin and gentamicin), macrolides (erythromycin), and polymyxins (colistin) or to other antimicrobials (chloramphenicol, tetracycline, minocycline, and rifampin) (Table 2). To further corroborate the relevance of this MFS in other *A.*

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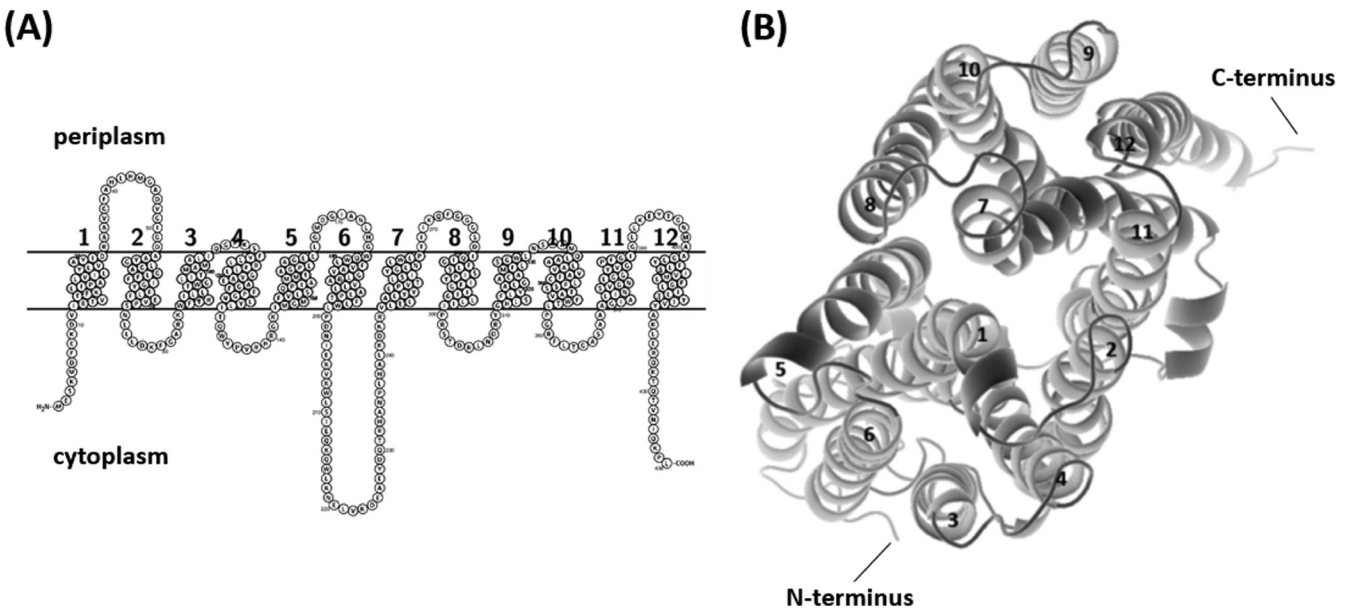

FIG 1 (A) Prediction of the structure of AbaQ using Protter (10). The putative protein is shown parallel to the cytoplasmic membrane. (B) 3D representation of AbaQ viewed along the plane of the membrane from the periplasmic side using RaptorX Structure Prediction (26) and visualized using PyMOL software (27). The 12 transmembrane α-helices are numbered (1 to 12); both the N and C termini are located in the cytoplasm.

baumannii strains, we analyzed the *abaQ* knockout mutant derived from *A. baumannii* strain MAR002, a biofilm-hyperproducing strain recently isolated from a wound sample collected from a patient at the Hospital del Mar in Barcelona (2, 4, 5). Assays of the mutant indicated that inactivation of the AbaQ homologue in *A. baumannii* MAR002 (99% amino acid identity) also caused the highest reduction (approximately 8- to 32-fold) in the MICs of quinolone-type antibiotics (data not shown).

To complement the mutants, the *abaQ* gene, including its own promoter, was amplified from the genome of *A. baumannii* strain ATCC 17978 using the AbaQFXbaI and AbaQRXbaI oligonucleotides (5′-ACTGTCTAGAGGAATATCACAGCTTGCAGCG and 5′-ACTGTCTAGATTACAAAGGCTTTTGAATATTC, respectively), cloning them into the XbaI restriction site of the pET-RA vector (6). Complementation of the *abaQ*-mutant derived from *A. baumannii* ATCC 17978 completely restored antimicrobial susceptibility to the same levels determined in the WT parental strain (Table 2). The recovery of the WT phenotype was also observed in the complemented *abaQ* MAR002 mutant derivative (data not shown). These results provided clear evidence of the critical role of AbaQ in the efflux of quinolone-type antibiotics in *A. baumannii*.

To assess whether AbaQ confers resistance to quinolones through an active efflux mechanism, the MIC of ciprofloxacin in the presence of the efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone ([CCCP] Sigma) was determined in *A. bau-*

TABLE 1 MFS transporters described in *A. baumannii*

MFS[a]	Accession no.	Main antimicrobial exported	Identity (%)[b]	Similarity (%)[b]	Reference
AmvA	ACQ82816	Erythromycin	17.8	31.0	7
TetA	AAO38186	Tetracycline	17.6	28.5	18
TetB	AFV67369	Minocycline	19	33.8	19
CraA	ABO13543	Chloramphenicol	19	36.9	20
FloR	AQT19056	Chloramphenicol	16.9	28.2	21
CmlA	AMD83513	Chloramphenicol	17.5	28.3	22
AbaF	ABO11759	Fosfomycin	23.7	37.6	23
EmrB	ABO12199	Colistin	17.2	30.7	24
AedC	ABO11341	Tetracycline-chloramphenicol	18.5	32.7	25

[a]MFS, Major facilitator superfamily.
[b]With respect to AbaQ of *A. baumannii* strain ATCC 17978, using the Basic Local Alignment Search Tool ([BLAST] http://www.ncbi.nlm.nih.gov) and Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

TABLE 2 MICs of various antimicrobials in wild-type *A. baumannii* ATCC 17978 and the *abaQ* mutant derivative

Antimicrobial	MIC (mg/liter)		Fold change[a]	MIC (mg/liter)		Fold change[b]
	WT	AbaQ$^-$		AbaQ$^-$ plus pET-RAØ	AbaQ$^-$ plus pET-RA+AbaQ	
Ciprofloxacin	0.25	0.0078	32	0.0078	0.25	32
Levofloxacin	0.125	0.0078	16	0.0078	0.125	16
Nalidixic acid	8	1	8	1	8	8
Trimethoprim	16	4	4	4	16	4
Novobiocin	8	4	2	4	8	2
Meropenem	2	2	1	2	2	1
Ampicillin	16	16	1	16	16	1
Amikacin	1	1	1	1	1	1
Gentamicin	0.5	0.5	1	0.5	0.5	1
Erythromycin	4	4	1	4	4	1
Colistin	0.5	0.5	1	0.5	0.5	1
Chloramphenicol	16	16	1	16	16	1
Tetracycline	4	4	1	4	4	1
Minocycline	0.062	0.062	1	0.062	0.062	1
Rifampin	4	4	1	NA[c]	NA	NA

[a]Ratio of the MICs of the wild type (WT) versus *abaQ* mutant derivative (AbaQ$^-$).
[b]Ratio of the MICs for the *abaQ* mutant carrying the pET-RA+AbaQ plasmid (complemented mutant) versus the *abaQ* mutant carrying the empty pET-RA plasmid (pET-RAØ).
[c]NA, not applicable (the pET-RA plasmid carries rifampin resistance).

mannii strains carrying (WT and AbaQ$^-$ plus pET-RA+AbaQ) or lacking (AbaQ$^-$ and AbaQ$^-$ plus pET-RAØ) the *abaQ* gene. The addition of CCCP (20 mg/liter) decreased the ciprofloxacin MIC 16-fold in AbaQ-carrying cells (from 0.25 to 0.015 mg/liter) but only 4-fold in cells lacking the *abaQ* gene (from 0.0078 to 0.0019 mg/liter). The addition of CCCP alone did not inhibit bacterial cell growth in any of the *A. baumannii* strains, indicating that the above results were not due to the toxicity of CCCP itself. Accordingly, when ampicillin, which is not a substrate of the AbaQ transporter (Table 2), was used instead of ciprofloxacin, there was no difference in the antimicrobial susceptibilities in the presence or absence of CCCP in any of the cultured *A. baumannii* strains (data not shown). These results unequivocally demonstrated that the *abaQ* gene product confers decreased susceptibility to quinolones by encoding an active efflux transporter.

The majority of the MFS transporters involved in drug-efflux described thus far in *A. baumannii* mediate chloramphenicol efflux, with a smaller number mediating the efflux of other antimicrobials, such as erythromycin, tetracycline, minocycline, fosfomycin, and colistin (Table 1). Among the latter group of transporters is AmvA, which participates in the efflux of erythromycin and different classes of disinfectants, detergents, and dyes but also confers modest resistance to quinolone-type antimicrobials (7). In *amvA* mutant strains, susceptibilities to both ciprofloxacin and norfloxacin were 2-fold higher than in the WT strain, whereas the susceptibility to nalidixic acid was unchanged (7). Other transporters belonging to different families and involved in the extrusion of a wide range of antimicrobials, including quinolones, have been described in *A. baumannii*: AdeABC (8), AdeDE (9), AdeFGH (10), AdeIJK (11), AdeM (12), and AdeT (13), all belonging to the resistance/nodulation/division (RND) superfamily; AbeM, AbeM2, and AbeM4 transporters, belonging to the multiple antimicrobial toxin extrusion (MATE) family (12, 14); and AbeS, belonging to the small multidrug resistance (SMR) family (15). No role in quinolone efflux has been detected in the only ATP-binding cassette (ABC) transporter described so far in *A. baumannii* (A1S_1535) (16) or in any of the transporters that make up the most recently discovered family of efflux pumps: the proteobacterial antimicrobial compound efflux (PACE) transporters (17).

To our knowledge, AbaQ, which is widely present in *A. baumannii* clinical isolates and involved in both surface-associated motility and virulence (2), is the first MFS efflux pump shown to play an important role in the extrusion of quinolone-type antimicrobials in this MDR nosocomial pathogen.

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