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Tigecycline Efflux as a Mechanism for Nonsusceptibility in *Acinetobacter baumannii*[▽]

Anton Y. Peleg,* Jennifer Adams, and David L. Paterson

Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213

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Tigecycline has an extended spectrum of in vitro antimicrobial activities, including that against multidrug-resistant *Acinetobacter*. After identifying bloodstream isolates of *Acinetobacter* with reduced susceptibilities to tigecycline, we performed a study to assess tigecycline efflux mediated by the resistance-nodulation-division-type transporter AdeABC. After exposure of two tigecycline-nonsusceptible isolates to the efflux pump inhibitor phenyl-arginine-β-naphthylamide (PABN), a fourfold reduction in the tigecycline MIC was observed. Both tigecycline-susceptible and -nonsusceptible isolates were found to carry the gene coding for the transmembrane component of the AdeABC pump, *adeB*, and the two-component regulatory system comprising *adeS* and *adeR*. Previously unreported point mutations were identified in the regulatory system in tigecycline-nonsusceptible isolates. Real-time PCR identified 40-fold and 54-fold increases in *adeB* expression in the two tigecycline-nonsusceptible isolates compared to that in a tigecycline-susceptible isolate. In vitro exposure of a tigecycline-susceptible clinical strain to tigecycline caused a rapid rise in the MIC of tigecycline from 2 μg/ml to 24 μg/ml, which was reversible with PABN. A 25-fold increase in *adeB* expression was observed in a comparison between this tigecycline-susceptible isolate and its isogenic tigecycline-nonsusceptible mutant. These results indicate that an efflux-based mechanism plays a role in reduced tigecycline susceptibility in *Acinetobacter*.

Tigecycline is the first of a new class of modified tetracycline antimicrobials known as glycylcyclines. Its structural modification is the addition of a 9-*t*-butyl-glycylamido side chain to the central skeleton of minocycline (16). This provides the drug with an expanded spectrum of activities, including those against susceptible and multidrug-resistant gram-positive and -negative organisms, anaerobes, and atypical pathogens. As with other tetracycline derivatives, tigecycline inhibits the 30S ribosomal subunit, but its unique feature is its ability to evade the major determinants of tetracycline resistance, the *tet*(A) to *tet*(E) and *tet*(K) efflux pumps and the *tet*(M) and *tet*(O) determinants, that provide ribosomal protection (3). Despite this, tigecycline has poor activities against certain organisms, most importantly *Pseudomonas* spp. and *Proteus* spp. Previous studies have shown that the reduced tigecycline susceptibilities of such organisms are due to chromosomally encoded multidrug efflux pumps from the resistance-nodulation-division (RND) family, specifically the MexXY-OprM pump in *Pseudomonas aeruginosa* and the AcrAB efflux pump in *Proteus mirabilis* (2, 22). Also, reduced tigecycline susceptibility in *Escherichia coli* and *Klebsiella pneumoniae* has recently been attributed to the up-regulation of the multidrug efflux pump AcrAB (6, 19). RND-type efflux pumps are tripartite structures that include an inner membrane transporter, an outer membrane channel, and a membrane fusion protein (17, 18). Such pumps are of particular concern given their wide substrate profiles and their abilities to be selected after exposure to a single antimicrobial agent.

* Corresponding author. Present address: Division of Infectious Diseases, Beth Israel Deaconess Medical Center and Harvard Medical School, 110 Francis St., LMOB Suite GB, Boston, MA 02215. Phone: (617) 632-0233. Fax: (617) 632-7626. E-mail: apeleg@bidmc.harvard.edu.

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Acinetobacter is a ubiquitous, gram-negative coccobacillus that has emerged as a highly problematic hospital-associated pathogen (13). Its spectrum of illness is wide and includes pneumonia, bloodstream infection, urinary tract infection, skin and wound infection, meningitis, and endocarditis (14), with the distribution of these dependent on the reporting institution. Apart from its resilience in the environment, *Acinetobacter* is characterized by its ability to rapidly acquire resistance determinants, with enzymatic mechanisms predominating (1). More recently, the role of a multidrug efflux pump in *Acinetobacter* drug resistance, namely, AdeABC, has been described (9, 10). Of interest, this efflux pump belongs to the RND family and has a similar three-component structure: AdeB forms the *trans*-membrane component, AdeA forms the inner membrane fusion protein, and AdeC forms the outer membrane protein. AdeABC is chromosomally encoded and is regulated by a two-component system comprising a sensor kinase (AdeS) and its associated response regulator (AdeR) (10). Point mutations in such components have been associated with overexpression of AdeABC leading to multidrug resistance (10). The substrate profile for the AdeABC efflux pump is broad, including tetracyclines, aminoglycosides, fluoroquinolones, chloramphenicol, and some β-lactams (9).

We have recently managed two patients with bloodstream infections caused by tigecycline-nonsusceptible *Acinetobacter baumannii* (15). Given the mechanism of reduced tigecycline susceptibility in *Pseudomonas* spp. and *Enterobacteriaceae* and the similarity between RND-type efflux pumps, we investigated the role of efflux as a cause of tigecycline nonsusceptibility in *Acinetobacter*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains included in this study were as follows: three clinical isolates (A24, B46, and C75) that were obtained from separate

TABLE 1. Primer sequences used for this study

Gene	Primer sequence	Source or reference
adeR	Forward, 5′-ATGTTTGATCATTCTTTTTCTTTTG-3′ Reverse, 5′-TTAATTAACATTTGAAATATG-3′	8
adeS	Forward, 5′-ATGAAAAGTAAGTTAGGAATTAGTAAG-3′ Reverse, 5′-TTAGTTATTCATAGAAATTTTTATG-3′	This study
adeB (qualitative)	Forward, 5′-GTATGAATTGATGCTGC-3′ Reverse, 5′-CACTCGTAGCCAATACC-3′	9
adeB (quantitative)	Forward, 5′-AACGGACGACCATCTTTGAGTATT-3′ Reverse, 5′-CAGTTGTTCCATTTCACGCATT-3′	This study
16S RNA	Forward, 5′-CAGCTCGTGTCGTGAGATGT-3′ Reverse, 5′-CGTAAGGGCCATGATGACTT-3′	5

patients with bloodstream infections due to *A. baumannii* at the University of Pittsburgh Medical Center, PA, two isolates (B46 and C75) cultured from patients who were receiving intravenous tigecycline for other indications at the time of isolation (15), and a tigecycline-susceptible laboratory strain (D54) known to carry the *adeB* gene and the two-component regulatory system comprising *adeR* and *adeS*, which was studied for comparative analysis. The strains were cultivated in Luria-Bertani (LB) broth.

Antimicrobial susceptibility testing. Tigecycline MICs were initially determined by the Etest method (AB Biodisk, Solna, Sweden) and confirmed by agar dilution using Mueller-Hinton II agar (Oxoid, Hampshire, England). Susceptibility testing of other antimicrobials was performed by broth microdilution (9). Bacterial cultures were prepared fresh on the day of testing. Tigecycline (Wyeth, Madison, NJ) powder was obtained from commercial sources and was prepared in a solution by using sterile water, and the solution was then frozen in aliquots at −80°C. Currently, the U.S. FDA and EUCAST have defined susceptibility breakpoints for tigecycline only for *Enterobacteriaceae* (≤2 μg/ml and ≤1 μg/ml, respectively). Neither CLSI nor BSAC have provided breakpoints for tigecycline. For the purposes of this study, we have defined susceptibility as a MIC of ≤2 μg/ml for *Acinetobacter*.

Tigecycline in vitro exposure. To assess whether tigecycline nonsusceptibility in *Acinetobacter* occurs after tigecycline exposure and whether this resistance is reversible with an efflux pump inhibitor, we performed an in vitro exposure experiment. Fresh cultures underwent serial overnight exposure to tigecycline, starting at a tigecycline concentration of half the MIC and doubling the exposure concentration each 24 h until there was no growth. Exposure was performed with fresh Mueller-Hinton II agar that had been boiled before bacterial and antibiotic inoculation to prevent oxidative degradation of tigecycline (7). A 0.5 McFarland inoculum was used, and MICs were recorded daily. Bacteria from the final day of growth underwent exposure to the efflux pump inhibitor phenyl-arginine-β-naphthylamide (PABN) (11), and tigecycline MIC determinations were repeated. PABN powder was resuspended in pure water to a 25 mg/ml concentration and then added to 250 ml of Mueller-Hinton agar cooled to 50°C. Genomic DNA from the final day's growth was also analyzed by pulsed-field gel electrophoresis (PFGE) and compared to that for the parent strain to confirm isogenicity. An isogenic mutant was selected to undergo comparative assessment of *adeB* gene expression by real-time PCR (RT-PCR).

Accumulation of ethidium bromide. A functional efflux pump assessment was performed by measuring the accumulation of ethidium bromide before and after exposure to carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a protonophore that disrupts membrane proton force. The method was slightly modified from that described previously (4, 9). Briefly, cells were grown overnight, pelleted, and resuspended to an A_{600} of 0.2 in sodium phosphate buffer (pH 7.0). Ethidium bromide was added to the suspension at a final concentration of 2 μg/ml. Ethidium bromide accumulation, which is inversely proportional to the activity of the efflux pump, was recorded by the fluorescence intensity (λ_{excite}, 530 nm; λ_{emit}, 600 nm) on a SpectraMax M2 spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA). The bacterial cells were initially incubated with ethidium bromide for 420 s. Following this, CCCP was added at a final concentration of 100 μM and incubated for a further 180 s. Background fluorescence from bacterial cells alone was subtracted. The results were expressed as percent increases in ethidium accumulation after CCCP exposure.

PCR and nucleotide sequencing. The primers specific for the genes encoding the two-component regulatory system comprising *adeS* and *adeR* and the *trans*-membrane component *adeB* are shown in Table 1. PCR amplification was performed using a 9700 GeneAmp thermocycler (Applied Biosystems, Foster City, CA). PCRs for *adeR* and *adeS* were as described previously (8), except a primer annealing temperature of 50°C was used for *adeS*. For *adeB*, a similar cycling program was used and ran for 40 cycles with an annealing temperature of 60°C. Sequencing of the products was performed by an ABI 3730 DNA analyzer (Applied Biosystems) and then analyzed using Lasergene DNAStar sequencing software (DNAStar, Madison, WI).

RT-PCR. *adeB* gene expression was measured using RT-PCR. Bacterial cells were grown aerobically in LB broth until mid-log phase. DNase-treated RNA templates were prepared using an RNeasy kit (QIAGEN Sciences, MD). The concentrations of the RNA were quantified with a spectrophotometer. Oligonucleotide primers for the *adeB* gene were designed using Primer Express version 2 (Applied Biosystems) and purchased from IDT DNA (Coralville, IA) (Table 1). 16S RNA was used as a housekeeping gene to normalize levels of *adeB* transcripts. Reverse transcription was performed using a high-capacity cDNA archive kit (Applied Biosystems) with 350 ng of RNA in a 50-μl reaction mixture containing 1× (each) reverse transcription buffer, deoxynucleoside triphosphate mix, and random primers as well as 2.5 U/ml multiscribe reverse transcriptase. Negative-control reactions included equal concentrations of RNA and all reagents except reverse transcriptase, which was omitted. Incubation for 10 min at 25°C followed by 2 h at 37°C was carried out with a Tetrad DNA engine (MJ Research). RT-PCR was performed with a 7900HT sequence detection system (Applied Biosystems) with SYBR green PCR master mix (Applied Biosystems) at a 1× concentration containing 125 nM of each primer and a 1:100 final dilution of the cDNA product. The *Taq* activation step of 12 min at 95°C was followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C. Following PCR cycling, melting point data were collected and a dissociation curve was examined for each well. Each sample was run in triplicate. The critical threshold cycle (C_T) numbers were determined by Sequence Detection Systems version 2.2.2 (Applied Biosystems). The ΔC_T for *adeB* was calculated against that for the 16S RNA housekeeping gene, and the $\Delta\Delta C_T$ was calculated against that for the tigecycline-susceptible calibrator sample (D54).

PFGE. Chromosomal DNA was incubated and digested by ApaI (New England Biolabs, Beverly, MA). The restriction fragments were separated by PFGE using a temperature-controlled CHEF DR III system (Bio-Rad) as described previously (20). The fragments were visualized by using a Bio-Rad Gel Doc 2000 system. For PFGE pattern analysis, Bionumerics software version 4.0 with the unweighted-pair group method using average linkages and the DICE setting for clustering analysis (Applied Maths, Sint-Martens-Latem, Belgium) was applied. The genetic relatedness of isolates was determined by the criteria of Tenover et al. (21).

Nucleotide sequence accession numbers. The GenBank accession numbers for the nucleotide sequences determined and reported in this study are EF520299, EF520297, EF520296, and EF520298 for *adeS* of A24, B46, C75, and D54, respectively, and EF520294, EF520292, EF520291, and EF520293 for *adeR* of A24, B46, C75, and D54, respectively.

TABLE 2. Susceptibility profiles of the bacterial strains included in this study[a]

Strain	MIC (μg/ml) (susceptibility rating) for indicated drug										
	TGC	MIN	GEN	TOB	CIP	CHL	PIP	CAZ	FEP	IPM	MEM
D54	0.125	0.125 (S)	0.25 (S)	0.75 (S)	0.25 (S)	0.125 (S)	16 (S)	4.0 (S)	1.5 (S)	0.125 (S)	0.25 (S)
A24	2.0	32 (R)	64 (R)	4.0 (S)	256 (R)	16 (I)	16 (S)	>256 (R)	16 (I)	0.38 (S)	1.0 (S)
A24 + PABN	2.0	2.0 (S)	48 (R)	3.0 (S)	128 (R)	12 (I)	3.0 (S)	96 (R)	3.0 (S)	0.032 (S)	0.094 (S)
A24A	2.0	12 (I)	256 (R)	3.0 (S)	256 (R)	96 (R)	12 (S)	>256 (R)	12 (I)	0.125 (S)	0.75 (S)
A24B	2.0	16 (R)	256 (R)	4.0 (S)	256 (R)	96 (R)	12 (S)	>256 (R)	12 (I)	0.125 (S)	0.75 (S)
A24C	6.0	16 (R)	512 (R)	6.0 (I)	256 (R)	96 (R)	16 (S)	>256 (R)	16 (I)	0.19 (S)	0.75 (S)
A24D[b]	24	32 (R)	256 (R)	16 (R)	512 (R)	16 (I)	24 (I)	>256 (R)	16 (I)	0.50 (S)	2.0 (S)
A24D + PABN	2.0	8.0 (I)	96 (R)	8.0 (I)	128 (R)	4.0 (S)	1.0 (S)	32 (R)	1.0 (S)	0.064 (S)	0.094 (S)
B46	4.0	0.25 (S)	8.0 (I)	24 (R)	128 (R)	32 (R)	256 (R)	96 (R)	>256 (R)	1.5 (S)	4.0 (I)
B46 + PABN	1.0	0.25 (S)	4.0 (S)	16 (R)	128 (R)	8.0 (S)	256 (R)	8.0 (S)	128 (R)	0.25 (S)	0.25 (S)
C75	16	2.0 (S)	128 (R)	16 (R)	512 (R)	32 (R)	48 (I)	192 (R)	>256 (R)	1.5 (S)	4.0 (I)
C75 + PABN	4.0	1.0 (S)	16 (R)	6.0 (I)	256 (R)	8.0 (S)	8.0 (S)	8.0 (S)	16 (I)	0.125 (S)	0.125 (S)

[a] TGC, tigecycline; MIN, minocycline; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; CHL, chloramphenicol; PIP, piperacillin; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; R, resistant; S, susceptible; I, intermediate.
[b] An isolate that underwent tigecycline in vitro exposure.

RESULTS AND DISCUSSION

Susceptibility profiles of bacterial strains. The susceptibility profiles of the bacterial strains are shown in Table 2. All clinical isolates were resistant to multiple antimicrobial agents, including aminoglycosides, ciprofloxacin, chloramphenicol, and certain β-lactams. The laboratory strain (D54) was susceptible to all tested antimicrobials, including tigecycline (MIC, 0.125 μg/ml). The tigecycline MICs were 2 μg/ml, 4 μg/ml, and 16 μg/ml for isolates A24, B46, and C75, respectively. After exposure of the tigecycline-nonsusceptible clinical isolates (B46 and C75) to the efflux pump inhibitor PABN, the tigecycline MICs were reduced to 1 μg/ml and 4 μg/ml, respectively. Also, reductions in the MICs of gentamicin, tobramycin, chloramphenicol, and β-lactams were observed (Table 2). PABN had no effect on the MIC of tigecycline for the tigecycline-susceptible clinical strain (A24). All four isolates were genetically unrelated (data not shown). Such results support the existence of a multidrug efflux pump but are nonspecific with regard to the type of pump involved and the mechanism of increased pump activity.

Tigecycline in vitro exposure. To provide supporting evidence, we performed an in vitro tigecycline exposure experiment using a tigecycline-susceptible clinical isolate (A24). After exposure of A24 (MIC, 2 μg/ml) to serially increasing concentrations of tigecycline, starting at 1 μg/ml and doubling each day, the MICs were 2 μg/ml (A24A), 2 μg/ml (A24B), 6 μg/ml (A24C), and 24 μg/ml (A24D) on days 1, 2, 3, and 4, respectively. The MICs for other antimicrobials, including minocycline, gentamicin, tobramycin, chloramphenicol, and β-lactams, were also increased. To assess whether the rise in tigecycline MIC was a result of an efflux-based mechanism, A24D was exposed to PABN. The tigecycline MIC was reduced to the level of the parent strain (2 μg/ml). PABN exposure also reduced the MICs for other antimicrobials (Table 2). PFGE of the above-mentioned isolates confirmed isogenicity (data not shown). These results strongly support the theory that tigecycline nonsusceptibility in *A. baumannii* is due to a multidrug efflux pump. Given that tigecycline exposure may increase the activities of many pumps and that PABN is a nonspecific efflux pump inhibitor, this experiment does not clarify the type of pump involved. The increase in the MIC of tigecycline during the in vitro exposure experiment supports a hypothesis for a two-step mutation process; however, this requires further investigation.

Accumulation of ethidium bromide. To further assess the role of an efflux-based mechanism in reduced tigecycline susceptibility in *Acinetobacter*, we performed a functional experiment of efflux pump activity using ethidium bromide accumulation (inversely proportional to pump activity). RND-type pumps mediate the efflux of toxic compounds by using the proton gradient across the membrane, exchanging one H^+ ion for one drug molecule (12). CCCP disrupts this proton gradient and therefore inhibits pump activity, resulting in a rise in ethidium bromide accumulation. The results are expressed as percent increases in ethidium bromide accumulation after CCCP exposure and are presented in Fig. 1. Both the tigecycline-susceptible clinical (A24) and the laboratory (D54) strains showed lower increases in ethidium bromide accumulation after the addition of CCCP (52% and 31%, respectively) than the tigecycline-nonsusceptible clinical strains (B46 and C75) (115% and 206% increases, respectively). Similarly, the

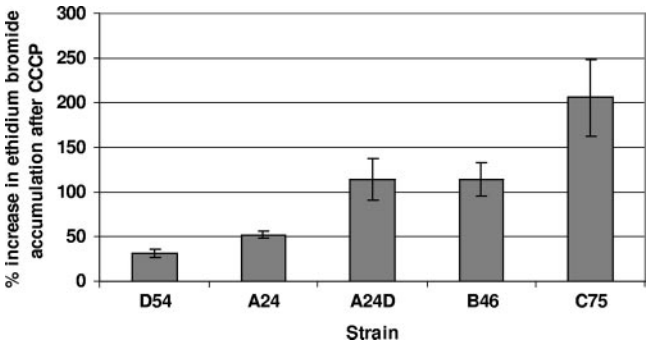

FIG. 1. Percent increases in ethidium bromide accumulation after exposure to CCCP, a protonophore that disrupts membrane active transport, in two tigecycline-nonsusceptible clinical isolates (B46 and C75), a tigecycline-susceptible clinical isolate (A24), its tigecycline-nonsusceptible isogenic mutant (A24D), and a tigecycline-susceptible laboratory strain (D54). Error bars represent standard errors.

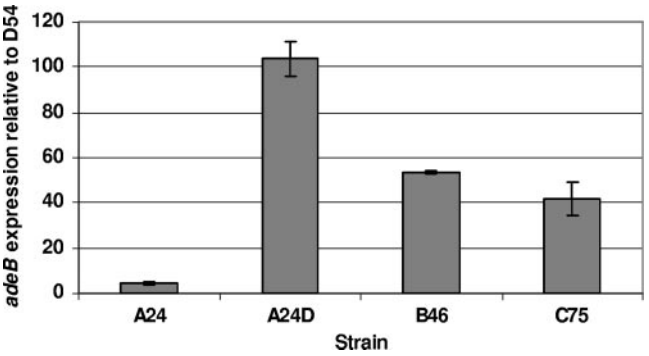

FIG. 2. Expression of *adeB* mRNA transcripts as assessed by RT-PCR. The relative quantification of *adeB* expression in the tigecycline-nonsusceptible clinical isolates (B46 and C75), the tigecycline in vitro-exposed isolate (A24D), and its isogenic parent strain (A24) compared to that in the reference tigecycline-susceptible laboratory strain (D54) is shown. Error bars represent standard errors.

strain that developed tigecycline resistance after in vitro tigecycline exposure (A24D) showed a 114% increase in ethidium bromide accumulation after CCCP addition.

Analysis of *adeB* expression. The gene *adeB* codes for the transmembrane protein of the AdeABC multidrug efflux pump. All isolates in the present study were found to carry the *adeB* gene. As described by Magnet et al., disruption of this gene leads to the loss of multidrug resistance (9). Because of its necessity for AdeABC function, we investigated its expression in a range of isolates with various tigecycline MICs using RT-PCR. We first compared *adeB* expression in tigecycline-nonsusceptible clinical isolates (C75 and B46) with that in our tigecycline-susceptible laboratory strain (D54). Approximate 40-fold and 54-fold increases in *adeB* expression were observed, respectively (Fig. 2). In contrast, only a fourfold increase in *adeB* expression was seen with the tigecycline-susceptible clinical strain (A24). We then compared *adeB* expression in our tigecycline-susceptible clinical strain (A24) and its isogenic tigecycline-nonsusceptible mutant (A24D). A 25-fold increase in *adeB* expression was observed, which equated to a 100-fold increase compared to the level in the tigecycline-susceptible laboratory strain (D54). These data support the hypothesis that increased expression of *adeB* is associated with increased MICs of tigecycline. However, in the absence of an *adeB* gene knockout experiment, the overall contribution of the AdeABC efflux pump to tigecycline nonsusceptibility cannot be ascertained. This is an important limitation of the current study, as the phenotype may well be explained by increased expression in another efflux system. Further research activities are focused on this area.

Nucleotide sequencing of the AdeABC regulatory system. To further assess the mechanism of increased *adeB* expression, mutations in the two-component regulatory system of the AdeABC efflux pump comprising *adeR* and *adeS* were investigated. All isolates in the present study were found to carry *adeR* and *adeS*. Point mutations in *adeR* (Pro116→Leu) and *adeS* (Thr153→Met) known to cause AdeABC overexpression (10) were not identified in any of our included isolates. The *adeR* and *adeS* sequences of the isolates included in this study, as well as a previously sequenced *Acinetobacter baumannii* clinical isolate found to have overexpression of the AdeABC pump (BM4454) (9, 10), were compared (Table 3). There were no point mutations found only in the tigecycline-nonsusceptible isolates (B46 and C75) and BM4454 compared with the tigecycline-susceptible isolates (D54 and A24). The tigecycline-susceptible clinical isolate (A24) had six point mutations compared with the tigecycline-susceptible laboratory isolate (D54), whereas the two tigecycline-nonsusceptible clinical isolates (B46 and C75) had eight point mutations. When the sequences of the tigecycline-susceptible clinical isolate (A24) were compared to those of its tigecycline in vitro-exposed intermediates (A24A, B, and C) and a final mutant (A24D), no change in sequences was observed (data not shown). It is possible that multiple point mutations in the two-component regulatory system of AdeABC may lead to pump overexpression. However, the lack of mutations in A24D compared to its isogenic parent strain (A24), despite increases in MICs of tigecycline and in *adeB* gene expression, indicates that other mechanisms for increased pump activity are also involved. It has previously been suggested that amino acid substitutions in an efflux pump protein are able to make it more efficient at export (17); however, this would not explain an increase in *adeB* gene expression.

Acinetobacter is proving to be a highly resilient and adaptable microorganism that has the capacity to cause problematic outbreaks in health care institutions (13, 14). The organism has an ever-increasing list of resistance determinants that can rapidly nullify most of our therapeutic armamentarium. Thus far, enzymatic mechanisms of resistance, including a wide array of β-lactamases and aminoglycoside-modifying enzymes, have predominated (1). More recently, the roles of multidrug ef-

TABLE 3. Differences between the amino acid sequences of the *adeR* and *adeS* gene products for the indicated strains[a]

Strain	Amino acid at indicated position of protein encoded by indicated gene										
	adeR			*adeS*							
	120	142	158	59	60	186	227	245	279	293	327
D54	V	I	H	V	D	G	D	I	A	S	V
A24	I	L	H	V	D	V	D	V	V	G	V
B46	I	L	L	I	D	G	H	V	A	G	E
C75	I	L	L	I	Y	G	H	V	A	G	V
BM4454	I	L	H	V	D	V	D	V	V	G	V

[a] The strains used were a tigecycline-susceptible laboratory strain (D54), a clinical strain (A24), two tigecycline-nonsusceptible clinical strains (B46 and C75), and a previously sequenced *Acinetobacter baumannii* isolate found to have overexpression of the AdeABC pump (BM4454) (9, 10). V, valine; A, alanine; I, isoleucine; D, aspartic acid; Y, tyrosine; G, glycine; H, histidine; L, leucine; S, serine; and E, glutamic acid.

flux pumps have been described, particularly that of the RND-type pump AdeABC (5, 9, 10). Our results indicate that an efflux-based mechanism plays a role in reduced tigecycline susceptibility in *Acinetobacter*. The contribution of the AdeABC pump compared to those of other efflux systems is yet to be confirmed; however, given the increased *adeB* gene expression in tigecycline-nonsusceptible strains, this pump is at least partly involved. This is the first description, to our knowledge, of the proposed mechanism of tigecycline nonsusceptibility in *Acinetobacter*. The ability of *Acinetobacter* to rapidly acquire resistance to this new glycylcycline antimicrobial is cause for concern and adds further stimulus for the discovery of newer antimicrobials with activities against this problematic organism.

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