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Correlation of Antimicrobial Resistance with β-Lactamases, the OmpA-Like Porin, and Efflux Pumps in Clinical Isolates of *Acinetobacter baumannii* Endemic to New York City[▽]

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***Acinetobacter baumannii* strains resistant to all β-lactams, aminoglycosides, and fluoroquinolones have emerged in many medical centers. Potential mechanisms contributing to antimicrobial resistance were investigated in 40 clinical isolates endemic to New York City. The isolates were examined for the presence of various β-lactamases, aminoglycoside-modifying enzymes, and mutations in *gyrA* and *parC*. Expression of the genes encoding the β-lactamase AmpC, the efflux systems AdeABC and AbeM, and the OmpA-like porin was also examined by real-time reverse transcription-PCR. No VIM, IMP, KPC, OXA-23-type, OXA-24-type, or OXA-58 β-lactamases were detected, although several isolates had acquired bla_{SHV-5}. Most cephalosporin-resistant isolates had increased levels of expression of *ampC* and/or had acquired bla_{SHV-5}; however, isolates without these features still had reduced susceptibility to cefepime that was mediated by the AdeABC efflux system. Although most isolates with IS*Aba1* upstream of the bla_{OXA-51}-like carbapenemase gene were resistant to meropenem, several remained susceptible to imipenem. The presence of aminoglycoside-modifying enzymes and gyrase mutations accounted for aminoglycoside and fluoroquinolone resistance, respectively. The increased expression of *adeABC* was not an important contributor to aminoglycoside or fluoroquinolone resistance but did correlate with reduced susceptibility to tigecycline. The expression of *abeM* and *ompA* and phenotypic changes in OmpA did not correlate with antimicrobial resistance. *A. baumannii* has become a well-equipped nosocomial pathogen; defining the relative contribution of these and other mechanisms of antimicrobial resistance will require further investigation.**

The emergence of multidrug-resistant *Acinetobacter baumannii* strains has created severe challenges in the clinical setting, including an increased reliance on polymyxins and tigecycline for therapy. Resistance to penicillins and cephalosporins usually centers on the class C chromosomal β-lactamase AmpC (2, 20). The increased level of expression of *ampC* has been attributed to the acquisition of the promoter IS*Aba1* (17). Resistance to penicillins and cephalosporins may also be mediated by extended-spectrum β-lactamases; SHV-5 has been recovered from some isolates of *A. baumannii* (28), and hyperproduction of this enzyme contributes to β-lactam resistance in *Klebsiella pneumoniae* (12). Isolates of *A. baumannii* have a naturally occurring $bla_{OXA-51-type}$ β-lactamase that has weak carbapenemase activity, but it does not hydrolyze cephalosporins (5, 16, 41). The increased level of expression of bla_{OXA-51}-type enzymes has been also linked to IS*Aba1* and results in reduced susceptibility to carbapenems (19, 40). Additionally, in many isolates from Europe, Asia, and South America, carbapenem resistance in *A. baumannii* is mediated by the acquisition of a class B or a class D carbapenem-hydrolyzing enzyme (30).

Several studies have examined the outer membrane of *A. baumannii*. The major porin (HMP-AB) is a 35.6-kDa protein analogue of OmpA of *Escherichia coli* and OprF of *Pseudomonas aeruginosa* (14). Porins in this family allow entry of β-lactams; the loss of OprF in *P. aeruginosa* may contribute to resistance to penicillins and cephalosporins (34). Conversely, the hyperexpression of *oprF* in selected isolates of *P. aeruginosa* has been correlated with cephalosporin susceptibility, even in the presence of increased levels of *ampC* expression (4). Several other porins in *A. baumannii* have been specifically correlated with carbapenem resistance, including a 43-kDa protein with significant homology with OprD (10), a 33- to 36-kDa porin (1, 8), and a 25- to 29-kDa membrane protein (CarO) (24, 44).

Isolates of *A. baumannii* have an efflux system, AdeABC, that belongs to the resistance-nodulation-division family of transporters. Several antimicrobial agents have been shown to be substrates for this system, including aminoglycosides, tetracycline, fluoroquinolones, trimethoprim, and chloramphenicol (18, 25). The increased level of expression of *adeABC* has also been correlated with a reduced level of susceptibility to tigecycline (33, 37). However, its effect on β-lactams is less clear, and amoxicillin and ceftazidime do not appear to be affected by this efflux pump (25). Efflux pump inhibitors, including 1-(1-naphthylmethyl)-piperazine (NMP), may reduce the MICs of some antimicrobial agents, but their effects may be unrelated to efflux pump inhibition (19, 31). The expression of *adeABC* is governed by the two-component system that includes a response regulator (AdeR) and a sensor kinase (AdeS) (26). The increased level of expression of *adeABC* in isolates grown in vitro in the presence of gentamicin has been correlated with mutations in *adeS* and *adeR* (26). However, these mutations have not been observed in a small number of clinical isolates

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with increased levels of expression of *adeABC* (33, 37). A second efflux pump, AbeM, that belongs to the multidrug and toxic compound extrusion family has also been characterized in isolates of *A. baumannii* (39). Substrates for the AbeM efflux pump include gentamicin, ciprofloxacin, erythromycin, and trimethoprim (39). The contribution of this system to antimicrobial resistance in clinical isolates is unknown.

In this report, we correlated the expression of genes encoding the chromosomal cephalosporinase AmpC, the major outer membrane porin OmpA, and efflux systems AdeABC and AbeM in clinical isolates of *A. baumannii* with antimicrobial resistance. The effects of other β-lactamases and the phenotypic pattern of OmpA were also assessed.

MATERIALS AND METHODS

Bacterial isolates. Forty single patient isolates of *A. baumannii* were selected for analysis. Isolates were gathered from citywide surveillance studies conducted from 2001 to 2006 in Brooklyn, NY (22) and were selected on the basis of their ribotypes and various susceptibilities to antimicrobial agents. Multidrug-resistant isolates belonging to the predominant ribotypes endemic to our region were included. Isolates were confirmed to be *A. baumannii* by ribotyping (13).

Susceptibility testing. The MICs of tobramycin were determined by the agar dilution method (6). Susceptibility testing with the other agents was performed by the Etest methodology (AB Biodisk, Solna, Sweden). Isolates also underwent susceptibility testing by the agar dilution method with Mueller-Hinton agar (6) and selected antimicrobial agents with and without the purported efflux inhibitor NMP (Sigma, St. Louis, MO) (31). The latter agent was added to the agar at a fixed concentration of 100 μg/ml, which was a two- to fourfold concentration below the MICs for all isolates. Also, to assess the potential impact of extended-spectrum β-lactamases, the MICs of meropenem were determined by the agar method with Mueller-Hinton agar (6) with and without 4 μg/ml clavulanic acid. A significant effect of the efflux pump or β-lactamase inhibitor was considered if there was a fourfold or greater decrease in the MIC in the presence of the agent. To assess the level of activity of AmpC in isolates lacking other (SHV or TEM) β-lactamases, the rates of hydrolysis of nitrocefin (Becton Dickinson and Company, Sparks, MD) at 100 μM in 50 mM phosphate buffer, pH 7.0, were measured spectrophotometrically. Crude cellular extracts were prepared by the freeze-thaw method, and the protein concentrations of the extracts were measured by the Bradford method.

DNA amplification studies. All isolates were screened for the genes encoding the IMP, VIM, KPC, TEM, SHV, OXA-23-type, OXA-24-type, OXA-58, and OXA-51-type β-lactamases by using previously described primers and PCR conditions (3, 30, 32, 42, 44). The isolates were also examined for the presence of the promoter insertion element IS*Aba1* by using previously described primers and PCR conditions (17). To determine the proximity of this element to *ampC*, DNA amplification was carried out with a forward IS*Aba1*-specific primer (17) and a reverse internal *ampC*-specific primer (20). Similarly, the relationship of the $bla_{OXA\text{-}51}$-type β-lactamase with IS*Aba1* was investigated by matching the primers for these two genes (17, 42). The isolates were screened for the presence of the genes encoding aminoglycoside-modifying enzymes common in *A. baumannii* by a PCR multiplex assay (29). The presence of genes encoding modifying enzymes affecting gentamicin, tobramycin, and amikacin was confirmed with additional primers, as described previously (21). For isolates with resistance that could not be explained by the presence of aminoglycoside-modifying enzymes, the isolates were screened for the presence of genes encoding 16S rRNA methylases (*armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD*) by PCR with previously identified primers (9, 45–47). Class 1 integrons were amplified and sequenced with primers derived from the 5′ and 3′ conserved segments (23); additional internal primers were designed to ensure complete identification. Genetic sequencing of the quinolone resistance-determining regions of *gyrA* and *parC* was performed by using previously described PCR conditions (15, 43).

For the construction of the primers and probes used in the real-time reverse transcription-PCR (RT-PCR) studies, conserved regions of genes (ribosomal, *ampC*, *ompA*, *adeB*, and *abeM*) were identified by using the primers found in Table 1. The primers used for amplification of the *adeABC* regulatory genes (*adeR* and *adeS*) are noted in Table 1. DNA sequencing was performed with an automated fluorescent dye terminator sequencing system (Applied Biosystems, Foster City, CA) and were analyzed by using the NCBI BLAST program.

TABLE 1. DNA sequences used in the target gene amplification studies and real-time RT-PCR experiments

Study type and primer or probe	Primer or probe sequence (5′–3′)[a]
Target gene amplification studies	
ribofor	GGACAACATCTCGAAAGGGA
riborev	GCGATTACTAGCGATTCCGA
ompAfor	GGCTTGAGCTTGAACAACAA
ompArev	TGTTCAGCTAAAACAGTACGGC
adeBfor	CGGAAGGCATGGAGTTTAGT
adeBrev	CTGCCATTGCCATAAGTTCA
abeMfor	TGCAACGCAGTTTCATTTTT
abeMrev	CGATGTTTCATCGGCTTTTT
adeRfor	AGCGTATGATGAGTTGAAGCA
adeRrev	AATCCAGCCTTTTTCAATCG
adeSfor	CGTGGCGTGGGATATAGACT
adeSrev	AGGAAAATGCCACAAAATGG
adeS2for	TCAAATGTTAATTAATGTGC GTGG
adeS2rev	TTGTTGTTTGGCATAAAGAG TTGT
Real-time RT-PCR studies	
riboFor	GTAGCGGTGAAATGCGTAGA
riboRev	CTTTCGTACCTCAGCGTCAG
riboProbe	[DFAM]CGAAGGCAGCCATCTG GCCT[DTAM]
ampCFor	TGCTATTTCAAAGGAACCTTCA
ampCRev	TTAATGCGCTCTTCATTTGG
ampCProbe	[DFAM]TGGCTCAACTAACGGTT TCGGAAC[DTAM]
ompAFor	AGCTCTTGCTGGCTTAAACG
ompARev	GAGCAACTGGAGTTGGTTCA
ompAProbe	[DFAM]CAGCAGGCTTCAAGTG ACCACCA[DTAM]
adeBFor	TACGCTTATTCCAGCGATTG
adeBRev	CCGAACATGGTGAGTACGTT
adeBProbe	[DFAM]AGCCGGCAAGCAACAT CACG[DTAM]
adeMFor	GCTATTCCGAAGCATTAGGC
adeMRev	CCAAAGCAGGTATTGGTCCT
adeMProbe	[DFAM]CCCGCCCTGTCACGGTC ATT[DTAM]

[a] DFAM, 6-carboxyfluorescein; DTAM, 6-carboxytetramethylrhodamine.

Real-time RT-PCR studies. The 40 clinical isolates were analyzed for the expression of four target genes. DNase-treated bacterial RNA was isolated (RNeasy kit; Qiagen, Inc.) from cultures grown to the late log phase of growth in LB broth. Real-time RT-PCR was performed with an MX3000P system (Stratagene, La Jolla, CA) as described previously (35). The concentrations of primers and probes, given in Table 1, were adjusted to give amplification efficiencies of 90 to 110%. Samples were run in triplicate, and the use of controls without reverse transcriptase confirmed the absence of contaminating DNA in the samples. A total of 25 ng of RNA was used in the target gene studies. The expression of each gene was normalized to that of a ribosomal housekeeping gene. The relative expression of each target gene was then calibrated against the corresponding expression by *A. baumannii* ATCC 19606 (whose expression was set equal to 1.0), which served as the control.

Nucleotide sequence accession numbers. The sequences of the following isolates have been submitted to GenBank and have been given the indicated accession numbers: isolate 1, EU118261 (*ampC*); isolate 2, EU332796 (*ompA*) and EU118260 (*ampC*); isolate 3, EU332795 (*ompA*); isolate 8, EU290755 (*adeR* and *adeS*); isolate 13, EU118262 (*ampC*); isolate 17, EU290754 (*adeR* and *adeS*); isolate 20, EU332797 (*ompA*); isolate 25, EU118263 (*ampC*); isolate 29, EU118265 (*ampC*); isolate 30, EU332798 (*ompA*) and EU290750 (*adeR* and *adeS*); isolate 31, EU118266 (*ampC*); isolate 33, EU332799 (*ompA*); isolate 36, EU290751 (*adeR* and *adeS*); isolate 38, EU290752 (*adeR* and *adeS*); and isolate 39, EU290753 (*adeR* and *adeS*).

RESULTS

Forty clinical isolates underwent evaluation to determine the mechanisms contributing to β-lactam resistance (Table 2). On the basis of ribotype, repetitive PCR, and pulsed-field gel electrophoresis patterns, five major clonal groups (clonal groups α, β, ζ, λ, and *ν*) were identified (data not shown). None were found to harbor β-lactamases belonging to the VIM, IMP, KPC, OXA-23-type, OXA-24-type, or OXA-58 family. All were found to have a $bla_{\text{OXA-51}}$-type β-lactamase, as expected for *A. baumannii* species (41). Three isolates (isolates 6, 8, and 40) and one isolate (isolate 24) possessed $bla_{\text{TEM-1}}$ and $bla_{\text{TEM-116}}$, respectively.

Cephalosporins and aztreonam. Most cephalosporin-resistant isolates had the IS*Aba1*-associated increased level of expression of *ampC*, and several isolates had acquired $bla_{\text{SHV-5}}$, which also contributed to cephalosporin resistance (Table 2). However, even isolates with diminished expression of *ampC* (exemplified by isolates 31 to 37) had reduced susceptibilities to cephalosporins and aztreonam. Compared to the rates of nitrocefin hydrolysis by isolates with increased levels of *ampC* expression (>10 times that of the ATCC control; the isolates also lacked the SHV or the TEM β-lactamase), the rates of nitrocefin hydrolysis by the isolates with reduced levels of *ampC* expression were markedly lower (less than or equal to the level for the ATCC control strain; 0.36 ± 0.53 and 1.8 ± 1.4 nanomoles/microgram protein/minute, respectively). Therefore, factors other than β-lactamases appeared to be contributing to the reduced susceptibilities of these isolates.

Reduced susceptibility to cefepime appeared to be mediated in part by the AdeABC efflux system. For the nine isolates with reduced levels of *adeB* expression (less than the level for the control), the addition of NMP had no effect on the MICs for cefepime. However, all eight isolates with negligible β-lactamase activities (the level of *ampC* expression was less than the level for the control, and SHV-5 was absent) but levels of *adeB* expression greater than or equal to the level of expression for the control had significant reductions in MICs with the addition of NMP. For the 24 isolates with background cephalosporinase activity (through either increased levels of *ampC* expression or the presence of SHV-5), the effect of NMP was variable. The effect of NMP on cefepime was more likely to be present in the subgroup with the highest level of expression of *adeB* (>10 times that of the control). However, the level of *adeB* expression did not appear to correlate with aztreonam resistance, and the addition of NMP did not affect susceptibility to this agent. The expression of *abeM* and *ompA* did not correlate with resistance to cephalosporins or aztreonam (Table 2), and there were no phenotypic changes in OmpA that were associated with resistance (data not shown).

Carbapenems. Most isolates resistant to imipenem and/or meropenem had increased levels of expression of *ampC* (Table 2). However, the presence of increased *ampC* activity was certainly not a prerequisite for resistance (as noted for isolate 10). While isolates lacking the association of IS*Aba1* with the $bla_{\text{OXA-51}}$-type β-lactamase remained susceptible to imipenem, a few were still able to achieve resistance to meropenem (isolates 3 and 27). Most meropenem-resistant isolates did have IS*Aba1* linked with the $bla_{\text{OXA-51}}$-type β-lactamase, although several remained susceptible to imipenem (isolates 1, 6, 7, 20, and 25). Although many of the isolates in the β and ζ clonal groups had also acquired $bla_{\text{SHV-5}}$, the addition of clavulanate to meropenem did not change the MICs for the latter agent. The expression of *adeB* did not correlate with carbapenem resistance, and isolates with absent or negligible expression of this system (exemplified by isolates 27 to 29) were still able to achieve high-level resistance. The addition of NMP led to a fourfold reduction in the MIC of meropenem for only one isolate (isolate 8), which also supported the observation that efflux is not an important contributor to carbapenem resistance. The expression of *abeM* and *ompA* and phenotypic changes in OmpA also did not correlate with carbapenem resistance.

Aminoglycosides. Isolates that lacked aminoglycoside-modifying enzymes and that had negligible *adeB* expression (exemplified by isolates 38 and 39) had the lowest MICs of the aminoglycosides (Table 2). Isolates that had detectable *adeB* expression but that lacked modifying enzymes had higher MICs but still generally remained susceptible to the aminoglycosides; one isolate (isolate 9) with markedly increased levels of *adeB* expression was able to achieve intermediate resistance to gentamicin but remained susceptible to tobramycin and amikacin. Isolates resistant to an aminoglycoside generally had a corresponding aminoglycoside-modifying enzyme; the presence of an integron-associated enzyme was the best predictor of resistance to the substrate. Isolates that harbored modifying enzymes and that had markedly increased levels of expression of *adeB* (isolates 8 and 30) did tend to have the highest MICs of the aminoglycosides. However, isolates that had modifying enzymes but that lacked *adeB* expression were still able to achieve frank aminoglycoside resistance (isolates 28 and 29), and the addition of NMP failed to significantly affect the MICs of the three aminoglycosides for any of the isolates. Several isolates (isolates 13 to 18) had resistance to gentamicin without a corresponding modifying enzyme; an increased level of expression of *adeB* or *abeM* or the presence of a 16S ribosomal methylase did not account for this finding.

Fluoroquinolones. All of the isolates resistant to ciprofloxacin possessed a $\text{Ser}_{83}\rightarrow\text{Leu}$ change in GyrA, and many also had a $\text{Ser}_{80}\rightarrow\text{Leu}$ change in ParC (data not shown). Although all of the isolates with these changes were also resistant to levofloxacin, the MICs of this agent were more varied (Table 2). The presence of NMP resulted in significant reductions in the fluoroquinolone MICs for isolates belonging to the ζ and λ clonal groups; this effect was independent of *adeB* and *abeM* expression. It is noteworthy that the OmpA phenotype of the isolates in these two groups was markedly different from that of isolates unaffected by NMP (data not shown). It appears that NMP either had an effect unrelated to efflux pump inhibition (e.g., altered membrane permeability) or, less likely, affected an unidentified efflux system expressed only in the ζ and λ clonal groups.

Tigecycline. There was a clear association between *adeB* expression and susceptibility to tigecycline (Table 2). The isolates with the highest level of expression of *adeB* (isolates 8, 9, 18, and 30) possessed the highest tigecycline MICs (2 to 3 μg/ml). Conversely, isolates with negligible or absent *adeB* expression (isolates 27 to 29, 38, and 39) had the lowest tigecycline MICs (0.09 to 0.25 μg/ml).

TABLE 2. Susceptibility results, mRNA expression studies, and identification of other mechanisms contributing to antimicrobial resistance

Clonal group and isolate no.	MIC (μg/ml)[a]										Relative expression[c]				Aminoglycoside-modifying enzyme gene(s)[b]	Presence of bla_{SHV-5}	IS*Aba1* linked to bla_{oxa-51}-type β-lactamase
	FEP	CAZ	ATM	IMP	MEM	TGC	LVX	GEN	TOB	AMK	*ampC*	*ompA*	*adeB*	*adeM*			
α clonal group																	
1	24	>256	48	2	4	1	16	1	<0.25	1.5	5.2	0.5	0.67	1.1		−	+
2–5	16–32	>256	48–>256	0.38–6	1.5–12	1.0–1.5	12–>32	12–24	0.5–1	2–3	10–83	0.59–2.5	0.94–4.4	1.3–8.5	*aacC1*, *aadA1*	−	−
6	16	>256	64	3	12	0.75	6	8	<0.25	1.5	9.2	1.5	2.3	3.6	*aacC1*, *aadA1*	−	+
7	32	>256	>256	4	>32	0.75	6	2	0.5	2	114	5.9	6.3	7.2		+	+
8	>256	96	64	6	24	2	32	192	1	4	76	1.8	27	4.2	*aacC1*, *aadA1*	−	+
β clonal group																	
9	>256	48	>256	12	>32	3	>32	6	2	4	108	5.6	42	1.8		−	+
10	24	48	>256	16	>32	1	>32	4	16	6	NA[d]	3.4	4.2	1.6	*aacA4*	+	+
11	64	128	>256	>32	>32	1	>32	2	0.5	3	4.9	6.4	1.4	1		+	+
12	48	>256	>256	32	>32	1	>32	>256	>32	24	17	1.2	2.5	1.6	*aacA4*, *aacC2*	+	+
13–17	32–>256	48–>256	96–>256	16–>32	>32	0.5–1.5	>32	8–48	32–>32	8–64	4.1–401	0.8–11	0.75–14	0.92–2.1	*aacA4*	+	+
18	>256	96	>256	12	>32	3	>32	64	>32	24	9.2	2.3	29	2	*aacA4*	+	+
ζ clonal group																	
19 and 20	32–48	24–32	24–32	1–24	1.5–>32	0.75–1	12–32	>256	4	3	12–87	1.1–3	1.8–3.3	1.7–6.2	*aacC2*	−	+
21	48	32	32	6	16	1.5	>32	>256	4	3	186	12	7	0.68	*aacC2*	+	+
22	128	>256	>256	16	>32	2	16	>256	>32	48	271	11	9.5	13.6	*aadA1*, *aacA4*, *aacC2*	+	+
23	16	16	16	>32	>32	1.5	12	>256	>32	24	35	1.4	1.5	2.7	*aadA1*, *aacA4*, *aacC2*, *aphA6*	−	+
24–26	96–>256	>256	>256	1.5–>32	2–>32	1–1.5	16–32	>256	32–>32	32–64	16–53	1.1–4.4	0.64–2.6	2.0–3.6	*aadA1*, *aacA4*, *aacC2*	+	+
λ clonal group																	
27	24	>256	24	4	32	0.19	3	1.5	0.5	<.5	270	0.59	0.03	1.5	*aadB*, *aadA1*	−	−
28 and 29	24	>256	>256	16–32	>32	0.19–0.25	8	16–24	16	3	7–18	1.3–1.8	NA	0.78–1.0	*aadB*, *aadA1*	−	+
30	48	>256	>256	8	>32	2	16	>256	>32	48	17	4.2	29	3.2	*aadB*, *aadA1*	−	+
ν clonal group																	
31 and 32	12–16	4–8	12–16	0.25–0.5	0.38–0.5	0.5–1	12–>32	>256	32–>32	4–6	0.19–0.25	1.5–2.2	3.2–4.9	2.3–2.4	*aadB*, *aadA2*	−	−
33 and 34	12–16	6–8	12–24	0.25–0.38	0.38–0.75	0.5–1	4–32	2–4	1	3–6	0.18–0.49	0.71–1.6	1.3–5.4	1.6–1.8		−	−
η clonal group																	
35 and 36	16–24	12–16	48–64	0.25	0.75	0.25–1	6–>32	3	1	3–4	0.28–0.95	1.4–2.1	1–2	0.01–0.04		−	−
Unique clonal groups																	
37	48	12	12	0.5	1.5	0.25	6	6	2	6	0.12	0.53	1.2	1.5		−	−
38	4	16	96	0.19	0.38	0.09	0.13	0.25	<0.25	1.5	0.12	1.1	0.08	1.2		−	−
39	4	4	16	0.25	0.25	0.25	0.09	0.38	<.25	1.5	0.03	1.6	NA	1.8		−	−
40	>256	>256	>256	>32	>32	0.75	>32	>256	>32	32	12	0.14	1.3	2.7	*aadA1*, *aacA4*, *aacC2*	+	+

[a] AMK, amikacin, ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; GEN, gentamicin, IMP, imipenem; LVX, levofloxacin, MEM, meropenem, TGC, tigecycline, TOB, tobramycin.
[b] Underlined enzymes were recovered on a class 1 integron.
[c] Relative expression compared to that in *A. baumannii* ATCC 19606 (whose expression was set equal to 1.0).
[d] NA, not amplifiable.

Analysis of regulatory genes *adeR* and *adeS*. Analysis of the genomic sequences of *adeR* revealed several point mutations that were common within clonal groups (data not shown). None of the resulting amino acid changes appeared to correlate with the altered expression of *adeB*. None possessed the Pro_{116}→Leu change previously recognized in an isolate with induced resistance (26). Two isolates belonging to the same clonal group (isolates 28 and 29) had *adeR* that could not be amplified; both of these isolates lacked an amplification product for *adeB* in the real-time RT-PCR studies, suggesting a major disruption in this operon. Mutations effecting AdeS were common in the clonal groups and did not correlate with *adeB* expression; none possessed the Thr_{153}→Met change noted previously (26) in a laboratory isolate.

DISCUSSION

It is apparent that multiple factors are at play in determining antimicrobial resistance in clinical isolates of *A. baumannii*. In isolates endemic to our region, resistance to cephalosporins and aztreonam centered on the presence of the SHV-5 β-lactamase and/or an increased level of expression of *ampC*. However, several isolates without these features had increased cefepime MICs. In another report, the presence of an AmpC inhibitor had no effect on the cefepime MICs for most isolates of *A. baumannii*, suggesting that alternative pathways contribute to resistance to this agent (7). Our results indicate that in the absence of cephalosporinase activity, efflux (due to AdeABC in some isolates) provides the primary mechanism for reduced susceptibility to cefepime. However, in the presence of an effective cephalosporinase, efflux pumps assume a secondary role.

Carbapenem resistance has been attributed to the association of the promoter sequence IS*Aba1* with the bla_{OXA-51}-like carbapenemase in *A. baumannii* (19, 38). This association was also evident in most of our meropenem-resistant isolates; however, several of these isolates remained susceptible to imipenem. The OXA-51 β-lactamase possesses only slow hydrolytic activity against imipenem and is not active against cephalosporins and meropenem (5). A closely related enzyme, OXA-69, causes the low-level hydrolysis of imipenem and meropenem but not the cephalosporins (16). When a high-copy-number plasmid containing the bla_{OXA-51}-like carbapenemase was inserted into *E. coli*, there was no change in the MICs for cephalosporins and meropenem and either no or only a modest effect on the MICs for imipenem (16, 19). The precise contribution of this enzyme to β-lactam resistance remains to be determined. Assessment of the expression of the bla_{OXA-51}-type β-lactamase, along with other potential mediators, such as porins and penicillin-binding proteins (11, 36), will be necessary to further define the mechanisms contributing to β-lactam resistance.

Compared to the outer membranes of members of the family *Enterobacteriaceae*, the outer membrane of *A. baumannii* is relatively impermeable and is a contributor to intrinsic antimicrobial resistance. The major porin in *A. baumannii* is a 35.6-kDa OmpA-like protein (14), which is part of a family of porins that serve as a channel for β-lactams. However, we could not demonstrate an association between antimicrobial resistance and either the expression of *ompA* or phenotypic changes in OmpA. Other porins, such as the 33- to 36-kDa protein (1, 8), a 43-kDa OprD-like protein (10, 24, 27), and an ~25- to 29-kDa porin (CarO) (24, 44) may contribute to carbapenem resistance. Additional genetic expression studies, along with phenotypic characterization, of these membrane proteins will help clarify the role of membrane permeability in β-lactam resistance.

Our results suggest that *adeB* expression is not an important contributor to overt aminoglycoside resistance in isolates endemic to our region. While increased levels of *adeB* expression may augment aminoglycoside MICs, frank resistance typically required the presence of an aminoglycoside-modifying enzyme. In other reports, knockout mutants involving *adeB* generally had 8- to 32-fold decreases in aminoglycoside MICs compared to the MIC of the isogenic parent that had increased levels of expression of the gene (25, 37). However, the parent isolates were already susceptible to most aminoglycosides, and elimination of the efflux pump further increased the susceptibility. The addition of an efflux inhibitor has also been reported to have only a minimal effect on aminoglycoside MICs, regardless of *adeB* expression (31, 33), a finding substantiated with our isolates. Therefore, efflux pump inhibitors are unlikely to be successful in restoring aminoglycoside susceptibility in many clinical isolates. Similarly, an increased level of expression of *adeB* by itself is not an important contributor to fluoroquinolone resistance. All of the resistant isolates in this study possessed changes in *gyrA* and/or *parC* that accounted for fluoroquinolone resistance. Although the MICs of levofloxacin were affected by the AdeABC system in one study (37), the expression of *adeB* did not explain the variabilities in the levofloxacin MICs in our isolates. In our isolates, the efflux inhibitor NMP also reduced the fluoroquinolone MICs only in clonally related groups, and its presence did not correlate with the expression of efflux systems.

The MICs of tigecycline correlated well with increased *adeB* activity. Increased levels of expression of this efflux system have clearly been linked to a reduction in tigecycline susceptibility (33, 37). Conversely, several of our isolates with clearly diminished expression of *adeB* had unusually low tigecycline MICs. Understanding the pathogenesis of altered *adeB* expression in these isolates may hold important therapeutic implications for preserving the utility of this antimicrobial agent. We did not find any changes in the *adeR* and *adeS* regulatory genes that correlated with increased levels of expression of this efflux system.

Because of the effects of confounding variables, assessment of the contribution of several resistance mechanisms in clinical bacterial isolates is admittedly a difficult task. Ultimately, the performance of gene knockout studies (particularly knockout of the genes for β-lactamases and efflux systems) and restoration of the genetic support for deficient mechanisms (e.g., porins) will further define their roles in these clinical isolates.

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