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Contribution of Efflux Pumps, Porins, and β-Lactamases to Multidrug Resistance in Clinical Isolates of *Acinetobacter baumannii*

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We investigated the mechanisms of resistance to carbapenems, aminoglycosides, glycylcyclines, tetracyclines, and quinolones in 90 multiresistant clinical strains of *Acinetobacter baumannii* isolated from two genetically unrelated *A. baumannii* clones: clone PFGE-ROC-1 (53 strains producing the OXA-58 β-lactamase enzyme and 18 strains with the OXA-24 β-lactamase) and clone PFGE-HUI-1 (19 strains susceptible to carbapenems). We used real-time reverse transcriptase PCR to correlate antimicrobial resistance (MICs) with expression of genes encoding chromosomal β-lactamases (AmpC and OXA-51), porins (OmpA, CarO, Omp33, Dcap-like, OprB, Omp25, OprC, OprD, and OmpW), and proteins integral to six efflux systems (AdeABC, AdeIJK, AdeFGH, CraA, AbeM, and AmvA). Overexpression of the AdeABC system (level of expression relative to that by *A. baumannii* ATCC 17978, 30- to 45-fold) was significantly associated with resistance to tigecycline, minocycline, and gentamicin and other biological functions. However, hyperexpression of the AdeIJK efflux pump (level of expression relative to that by *A. baumannii* ATCC 17978, 8- to 10-fold) was significantly associated only with resistance to tigecycline and minocycline (to which the TetB efflux system also contributed). TetB and TetA(39) efflux pumps were detected in clinical strains and were associated with resistance to tetracyclines and doxycycline. The absence of the AdeABC system and the lack of expression of other mechanisms suggest that tigecycline-resistant strains of the PFGE-HUI-1 clone may be associated with a novel resistance-nodulation-cell efflux pump (decreased MICs in the presence of the inhibitor Phe-Arg β-naphthylamide dihydrochloride) and the TetA(39) system.

Acinetobacter baumannii is an important pathogen that causes nosocomial infections associated with high morbidity and mortality (1). Multidrug-resistant (MDR) strains of *A. baumannii* have emerged in the last few decades as a result of the combination of two main factors: (i) a high level of genomic plasticity (2) and (ii) mutation of endogenous genes, alteration of which is associated with antimicrobial resistance, such as overexpression of the chromosomally encoded ADC β-lactamase (AmpC) (3) and the OXA-51-like β-lactamase (4), loss of expression of porins (CarO and Omp33) (5, 6), mutation in the *gyrA* and *parC* genes (7), and overexpression of efflux systems (8).

Overexpression of the OXA-51-like β-lactamase has been associated with resistance to carbapenems and decreased expression of CarO and Omp33 (5, 6, 9).

Efflux pumps have multifactorial roles. These mechanisms are important for detoxification of intracellular metabolites, bacterial virulence (in both animal and plant hosts), intercellular signaling and trafficking, and cell homeostasis (10). Three resistance-nodulation-cell division (RND) systems, AdeABC, AdeIJK, and AdeFGH, have been characterized and reported to cause MDR in *A. baumannii* (8). AdeABC is the RND system most frequently involved in MDR in clinical strains; it has been found in approximately 80% of clinical isolates (the rates reported vary from 53% to 97%) (11) but was not detected in 32 environmental isolates (12). AdeRS is a two-component system that regulates AdeABC expression (13). Mutations in this system and the presence of an IS*Aba1* insertion sequence in this system can lead to overexpression of the AdeABC operon (13–15). However, strains of *A. baumannii* that express AdeABC without mutations have been found in association with AdeRS (16, 17). Recently, the *adeN* gene has been found to be associated with the regulation of the AdeIJK system (18), and mutations in the *adeL* gene have been associated with overexpression of the AdeFGH pump (11). Three other types of efflux systems have been described in *A. baumannii*: CraA (a major facilitator superfamily [MFS] pump), which confers intrinsic chloramphenicol resistance (19); AbeM (a member of the multidrug and toxic compound extrusion [MATE] family of pumps), which extrudes several antimicrobials and biocides (20); and AmvA (an MFS pump), which confers resistance to detergents, disinfectants, dyes, and erythromycin. Overexpression of the AmvA efflux pump has been associated with increased drug resistance in *A. baumannii* clinical isolates (21). Finally, several tetracycline efflux pumps (systems acquired from the MFS superfamily) have been described in *A. baumannii*. The most prevalent of these are TetA, which is associated with resistance to tetracycline, and TetB, which is implicated in resistance to tetracycline, doxy-

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cycline, and minocycline (8). TetA(39) is an important tetracycline resistance mechanism in clinical strains (22).

Because of the complexity of clinical strains of *A. baumannii*, many researchers have used ATCC reference strains to investigate the mechanisms of resistance. However, very few studies have analyzed the combinations of mechanisms and their interrelation in clinical isolates of *Acinetobacter baumannii*. Here, we studied the interplay between the mechanisms of multidrug resistance in clinical *A. baumannii* strains, particularly those involving efflux pumps, the influx of antimicrobials, and chromosomally encoded β-lactamases.

MATERIALS AND METHODS

Bacterial isolates and molecular typing. In 2010, 444 strains of *A. baumannii* were isolated (from 273 patients) in 42 participating hospitals and identified as part of the second multicenter study on this pathogen in Spain (GEIH-REIPI-2010-Ab) (23). The strains were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (24) and amplified rRNA gene restriction analysis (ARDRA) (25). Species identification was confirmed by detection of the OXA-51 gene by PCR (26) and also by detection of the *bsp* gene (a novel target) by real-time quantitative PCR (27, 28).

The clonal relationship between all strains displaying various levels of antibiotic susceptibility ($n = 71$) from a hospital in southern Spain and a hospital in the Canary Islands ($n = 19$) was determined by pulsed-field gel electrophoresis (PFGE) (29, 30) of samples of chromosomal DNA digested with ApaI (Roche, Mannheim, Germany) and embedded in low-melting point agarose. The restriction fragments thus obtained were separated in a CHEF DR-III system (Bio-Rad, Hercules, CA). FPQuest II software, version 4.5 (Bio-Rad), was used to analyze the band patterns in the agarose gel electrophoresis images (cutoff = 85%). Strains of both clones were analyzed by multilocus sequence typing, according to the system developed by Nemec and coworkers (31).

Susceptibility testing. The antibiotic susceptibility profile was determined according to CLSI recommendations (23). In strains of the PFGE-HUI-1 clone, we determined MICs in the presence of Phe-Arg β-naphthylamide dihydrochloride (PAbetaN; a commonly assumed inhibitor of the RND efflux pump) (32).

DNA amplifications studies. We used PCR to detect the genes coding for common aminoglycoside-modifying enzymes (AacA4, AacC1, AacC2, AadB, AadA1, AphA1, AphA6, and AadA2) (33); CHDL enzymes (OXA-23, -24, -51, -58, and -143) (34, 35); MBL enzymes (IMP, VIM, SPM-1, GIM-1, SIM-1, BIC, DIM, and NDM) (36); extended-spectrum β-lactamases (ESBLs), such as GES enzymes; and carbapenemases, such as KPC enzymes. We sequenced the *gyrA* and *parC* genes to study the presence of the mutations. Finally, we analyzed the *tet* genes most commonly detected in isolates of *A. baumannii* [*tetA*, *tetB*, *tetA*(34), and *tetA*(39)] (37, 38).

Real-time RT-PCR studies. We used real-time reverse transcriptase PCR (RT-PCR) to examine all isolates for expression of *adeB*, *adeJ*, *adeG*, *abeM*, *craA*, and *amvA* (genes belonging to efflux pumps systems); *oprC*, *oprD*, *ompW*, *ompA*,*carO*, *omp33*, *dcap*-like, *oprB*, and *omp25* (genes harboring porins or outer membrane protein); and finally, the OXA-51 and *ampC* β-lactamase genes. We obtained DNase-treated RNA from late-log-phase cultures (optical density = 0.4 to 0.6 absorbance units) by using a High Pure RNA isolation kit (Roche, Germany) and 50 ng of RNA. Analysis of controls without reverse transcriptase confirmed the absence of contaminating DNA in the samples. We used a LightCycler 480 RNA master hydrolysis probe (Roche, Germany) for the RT-PCR studies. The Universal Probe Library (UPL) TaqMan probes (Roche, Germany) and primers used are listed in Table 1. All were designed from conserved regions of DNA after the alignment of the genomes of the following strains of *A. baumannii*: AB 307-0294, AB 0057, ACICU, SDF, AYE, and ATCC 17978. We adjusted the concentrations of the samples to achieve efficiencies of 90% to 110% and performed all experiments in triplicate from three RNA extractions. For each strain, we normalized the levels of expression of all genes relative to those of the single-copy housekeeping genes *rpoB* and *gyrB*. We then calibrated the normalized expression of each gene of interest relative to its expression by *A. baumannii* ATCC 17978, which was assigned a value of 1.0.

Sequencing of the genes regulating AdeABC and AdeIJK efflux pumps. We sought mutations in the regulatory genes *adeR-adeS* and *adeN*, which have previously been associated with upregulation of the AdeABC and AdeIJK efflux systems, respectively. We amplified the genes by using the primers listed in Table 1. We used the Macrogen program (Macrogen Europe, Amsterdam, Netherlands) for DNA sequencing and the NCBI BLAST (www.ncbi.nlm.gov/BLAST) and CLUSTAL (www.ebi.ac.uk/Tools/msa/clustalw2/) programs for posterior analyses.

Statistical analysis. We categorized the strains into two groups according to antimicrobial susceptibility (not following the CLSI or EUCAST clinical breakpoints). We worked with the Student's *t* test to compare differences in gene expression between groups and thus evaluate any associations with antibiotic resistance. Differences were considered significant at a P value of <0.05.

Nucleotide sequence accession numbers. The nucleotide sequences of the *adeR*, *adeS*, and *adeN* genes from strains of the PFGE-ROC-1 clone were submitted to the GenBank database and have been assigned accession numbers KF147860, KF147861, and KF147862, respectively.

RESULTS

MICs, typing, and PCR detection of genes of the isolates. To study the expression levels of efflux pump systems, porins, and chromosomal β-lactamases, we selected clonally related strains with different antibiotic susceptibilities (39). In Spain, OXA-type enzymes are prevalent in carbapenem-resistant strains of *A. baumannii* (40, 41). Isolates of *A. baumannii* from two hospitals in Spain that showed some clonal relation were designated clone PFGE-ROC-1 (sequence type 2 [ST2]) ($n = 71$; Fig. 1) and clone PFGE-HUI-1 (ST79) ($n = 19$; Fig. 2). Moreover, 53 strains of the PFGE-ROC-1 clone carried the OXA-58 β-lactamase gene (designated PFGE-ROC-$1_{\text{OXA-58;}}$ imipenem MICs, 8 to 64 mg/liter; meropenem MICs, 8 to 16 mg/liter) and 18 isolates carried the OXA-24 β-lactamase gene (designated PFGE-ROC-$1_{\text{OXA-24}}$; imipenem MICs, ≥64 mg/liter; meropenem MICs, 32 to 64 mg/liter). The isolates of clone PFGE-HUI-1 ($n = 19$) were susceptible to carbapenems. We studied the variability in the MICs of glycylcyclines, aminoglycosides, tetracyclines, rifampin, and doripenem for all isolates, with the following results: (i) for PFGE-ROC-$1_{\text{OXA-58}}$ (Table 2) tigecycline MICs were ≤0.25 to 2 mg/liter, gentamicin MICs were 1 to >64 mg/liter, amikacin MICs were <2 to 64 mg/liter, doxycycline MICs were 16 to >64 mg/liter, minocycline MICs were 1 to 8 mg/liter, tetracycline MICs were >64 mg/liter, netilmicin MICs were 1 to >64 mg/liter, rifampin MICs were 1 to 64 mg/liter, tobramycin MICs were <0.5 to 64, and doripenem MICs were 4 to 8 mg/liter. (ii) For PFGE-ROC-$1_{\text{OXA-24}}$ (Table 3), tigecycline MICs were ≤0.25 to 1 mg/liter, gentamicin MICs were 2 to >64 mg/liter, amikacin MICs were <2 to 64 mg/liter, doxycycline MICs were 16 to 32 mg/liter, minocycline MICs were <0.5 to 4 mg/liter, tetracycline MICs were >64 mg/liter, netilmicin MICs were 64 to >64 mg/liter, rifampin MICs were <0.5 to 4 mg/liter, tobramycin MICs were 4 to 64 mg/liter, and doripenem MICs were 64 to >64 mg/liter. (iii) For PFGE-HUI-1 (Table 4), tigecycline MICs were 1 to 2 mg/liter, gentamicin MICs were 16- to >64 mg/liter, amikacin MICs were 4 to 64 mg/liter, doxycycline MICs were <0.5 to 8 mg/liter, minocycline MICs were <0.5 to 1 mg/liter, tetracycline MICs were 4 to >64 mg/liter, netilmicin MICs were 4 to >64 mg/liter, rifampin MICs were 2 to 32 mg/liter, tobramycin MICs were 8 to 64 mg/liter, and doripenem MICs were <0.5 to 2 mg/liter.

TABLE 1 Primers used in this study

Analysis and gene	Orientation	Primer sequence (5′–3′)	UPL probe[a]	Reference or source
RT-PCR analysis				
rpoB	Forward	CGTGTATCTGCGCTTGG	131	This study
	Reverse	CGTACTTCGAAGCCTGCAC		
gyrB	Forward	TGGTGGAACGTGGTCATATTT	76	This study
	Reverse	TGCTCTTGCTTACCCTTTTTG		
adeB	Forward	CGAGTGGCACAACTAGCATC	61	This study
	Reverse	CCTTGTCTTGGCTGCACTCT		
adeJ	Forward	CCTATTGCACAATATCCAACGA	119	This study
	Reverse	AGGATAAGTCGCAGCAATCG		
adeG	Forward	GTCCTGAAATGGTCGTTCGT	43	This study
	Reverse	AGCTTCTGCTTGGCTAGATGA		
craA	Forward	TTCATTGCTTGCGCCTTT	125	This study
	Reverse	CCAGTGCCATGAAACATAATCA		
abeM	Forward	AGGGACGTATTATGGCGAAA	165	This study
	Reverse	CTGCTGTGCTTAGACCAATTTTT		
amvA	Forward	GCAGAGAAATTTTGCACTTGG	10	This study
	Reverse	CGACTAATGGACCAAAAGCTG		
ompA	Forward	GGTATTCAGATAATTTTTCAGCAACTT	129	This study
	Reverse	AACAAATCAAACATCAAAGACCAA		
ompW	Forward	GCCTTATTTGCTCTGCCAAC	60	This study
	Reverse	CGTTTGAAACCATCACCATCT		
dcap-like	Forward	TGATCGACTTCTCGACAAACA	77	This study
	Reverse	GTGTAGTTGGGCCTAGTTTGTAGTT		
oprC	Forward	ACTCGATACAAAGCGGTGGA	9	This study
	Reverse	TTTAATACGTGAACCAAACATACCTC		
oprB	Forward	GCCCCACACTTCTTGAACAG	67	This study
	Reverse	ATGGGCAATCGCTTTCTG		
omp25	Forward	CGAACGTGAAATCGACAACA	128	This study
	Reverse	CGTAACCTTTAACACCTAGAGCAAG		
omp33-omp36	Forward	CAAGTGTTGCTAACCAATTCGCT	FAM-CCAAACTGCTGCTATCCAAAACGACCAA-BBQ	This study
	Reverse	GTTTTCTTGACCGAATGCACC		
carO	Forward	TGTTCATGACAGCTATGCATTCGATA	FAM-CGCTCGTGCTGAAGTAGGTACTACAGGTT-BBQ	This study
	Reverse	CCCAATGCTAAACCTACATATGGGT		
Sequencing analysis				
adeR	Forward	ACTACGATATTGGCGACATT		
	Reverse	GCGTCAGATTAAGCAAGATT		13–15
adeS	Forward	TTGGTTAGCCACTGTTATCT		
	Reverse	AGTGGACGTTAGGTCAAGTT		13–15
adeN	Forward	GCTGTTAGGTTGGGGTCGTA		
	Reverse	CGTGACCAAAAGTACGAATCA		18

[a] FAM, 6-carboxyfluorescein; BBQ, BlackBerry Quencher.

We detected *tet* genes in both clones: the *tetB* gene in all strains of PFGE-ROC-1 and the *tetA*(39) gene in strains of the PFGE-HUI-1 clone (except for strains 421, 422, 423, 424, and 426).

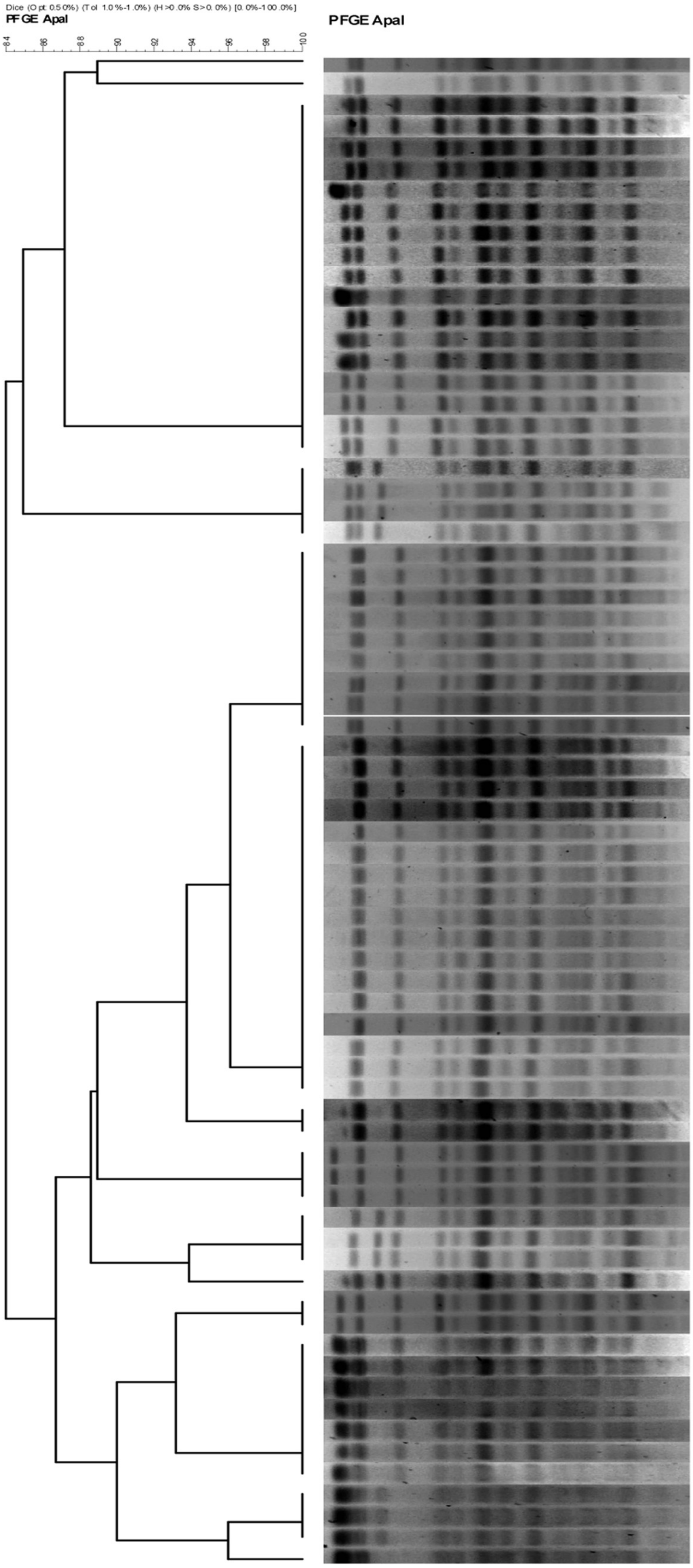
In both *A. baumannii* clones, we detected the AacC1/AphA1/AadB combination of acetylases in strains displaying some resistance to aminoglycosides. We also detected mutations in the *gyrA* ($\text{Ser}_{83} \rightarrow$ Leu) and *parC* ($\text{Ser}_{80} \rightarrow$ Leu) genes in strains showing resistance to quinolones.

Relative gene expression. The levels of expression of the efflux pump genes in the isolates relative to that by *A. baumannii* ATCC 17978 (relative expression ([RE] values) are shown in Tables 2 to 4. For clone PFGE-ROC-1, we applied statistical analysis to genes with RE values higher than 8 (i.e., genes *adeB* and *adeJ*) to determine how gene expression was related to the antibiotic MICs (for strains carrying the OXA-58 β-lactamase gene, see Fig. 3; for strains carrying the OXA-24 β-lactamase gene, see Fig. 4). However, we were not able to analyze the *adeB* gene in strains of clone PFGE-HUI-1, because the internal and external primers used did not amplify the genes in the AdeABC operon of these strains. Moreover, the relative expression of *adeJ* in this clone was not higher than 2.

The RE values of *adeG*, *craA*, *abeM*, and *amvA* in all strains ranged from 0.003 to 1.

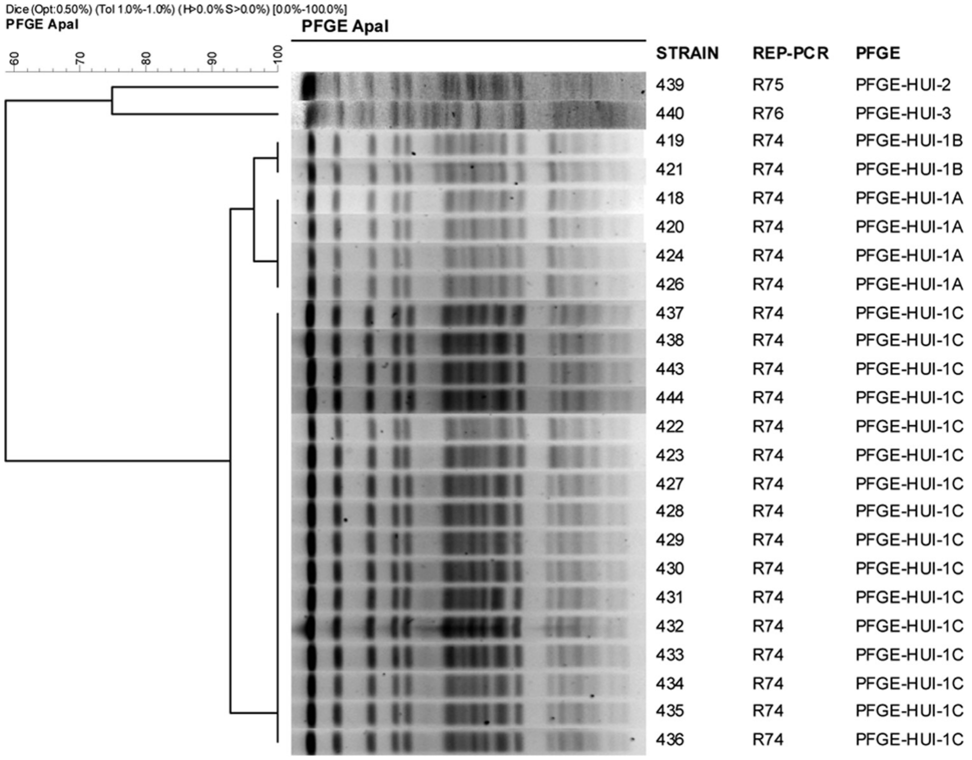
Porin expression was not significantly related to antibiotic resistance in strains of clone PFGE-ROC-1 or PFGE-HUI-1. However, in strains of clone $\text{PFGE-ROC-1}_{\text{OXA-24}}$, the RE values of the *carO* and *omp25* genes were lower than those in strains of clone $\text{PFGE-ROC-1}_{\text{OXA-58}}$ (Fig. 5). However, the RE values of the OXA-51 and *ampC* β-lactamase genes were similar among the isolates.

Polymorphisms of the regulatory genes of the AdeABC and AdeIJK efflux pumps. Strains of the PFGE-ROC-1 clone that overexpressed the AdeABC efflux pump had three mutations in the *adeS* gene ($\text{Ala}_{94} \rightarrow$ Val, $\text{Gly}_{186} \rightarrow$ Val, and $\text{Phe}_{214} \rightarrow$ Leu) and



STRAIN	REP-PCR	PFGE	OXA
118	R24	PFGE-ROC-1M	OXA-58
126	R24	PFGE-ROC-1N	OXA-58
40	R24	PFGE-ROC-1D	OXA-24
44	R24	PFGE-ROC-1D	OXA-24
46	R24	PFGE-ROC-1D	OXA-24
48	R24	PFGE-ROC-1D	OXA-24
60	R24	PFGE-ROC-1D	OXA-24
61	R24	PFGE-ROC-1D	OXA-24
62	R24	PFGE-ROC-1D	OXA-24
63	R24	PFGE-ROC-1D	OXA-24
67	R24	PFGE-ROC-1D	OXA-24
72	R24	PFGE-ROC-1D	OXA-24
73	R24	PFGE-ROC-1D	OXA-24
74	R24	PFGE-ROC-1D	OXA-24
75	R24	PFGE-ROC-1D	OXA-24
95	R24	PFGE-ROC-1D	OXA-24
96	R24	PFGE-ROC-1D	OXA-24
45	R24	PFGE-ROC-1D	OXA-24
47	R24	PFGE-ROC-1D	OXA-24
64	R24	PFGE-ROC-1H	OXA-58
86	R24	PFGE-ROC-1H	OXA-58
87	R24	PFGE-ROC-1H	OXA-58
65	R24	PFGE-ROC-1H	OXA-58
88	R24	PFGE-ROC-1E	OXA-58
89	R24	PFGE-ROC-1E	OXA-58
90	R24	PFGE-ROC-1E	OXA-58
91	R24	PFGE-ROC-1E	OXA-58
93	R24	PFGE-ROC-1E	OXA-58
94	R24	PFGE-ROC-1E	OXA-58
111	R24	PFGE-ROC-1E	OXA-58
119	R24	PFGE-ROC-1E	OXA-58
120	R24	PFGE-ROC-1E	OXA-58
36	R24	PFGE-ROC-1C	OXA-58
38	R24	PFGE-ROC-1C	OXA-58
51	R24	PFGE-ROC-1C	OXA-58
56	R24	PFGE-ROC-1C	OXA-58
92	R24	PFGE-ROC-1C	OXA-58
99	R24	PFGE-ROC-1C	OXA-58
100	R24	PFGE-ROC-1C	OXA-58
101	R24	PFGE-ROC-1C	OXA-58
106	R24	PFGE-ROC-1C	OXA-58
107	R24	PFGE-ROC-1C	OXA-58
108	R24	PFGE-ROC-1C	OXA-58
109	R24	PFGE-ROC-1C	OXA-58
110	R24	PFGE-ROC-1C	OXA-58
112	R24	PFGE-ROC-1C	OXA-58
125	R24	PFGE-ROC-1C	OXA-58
55	R24	PFGE-ROC-1C	OXA-58
57	R24	PFGE-ROC-1C	OXA-58
35	R24	PFGE-ROC-1B	OXA-58
37	R24	PFGE-ROC-1B	OXA-58
113	R24	PFGE-ROC-1K	OXA-58
114	R24	PFGE-ROC-1K	OXA-58
115	R24	PFGE-ROC-1K	OXA-58
102	R24	PFGE-ROC-1G	OXA-58
124	R24	PFGE-ROC-1G	OXA-58
58	R24	PFGE-ROC-1G	OXA-58
41	R24	PFGE-ROC-1F	OXA-58
116	R24	PFGE-ROC-1L	OXA-24
117	R24	PFGE-ROC-1L	OXA-24
32	R24	PFGE-ROC-1A	OXA-24
34	R24	PFGE-ROC-1A	OXA-58
50	R24	PFGE-ROC-1A	OXA-24
53	R24	PFGE-ROC-1A	OXA-58
78	R24	PFGE-ROC-1A	OXA-58
83	R24	PFGE-ROC-1A	OXA-58
84	R24	PFGE-ROC-1A	OXA-58
79	R24	PFGE-ROC-1I	OXA-58
81	R24	PFGE-ROC-1I	OXA-58
82	R24	PFGE-ROC-1I	OXA-58
80	R24	PFGE-ROC-1J	OXA-58

FIG 1 Pulsed-field electrophoresis of strains of the PFGE-ROC-1 clone. REP-PCR, repetitive element palindromic PCR.



STRAIN	REP-PCR	PFGE
439	R75	PFGE-HUI-2
440	R76	PFGE-HUI-3
419	R74	PFGE-HUI-1B
421	R74	PFGE-HUI-1B
418	R74	PFGE-HUI-1A
420	R74	PFGE-HUI-1A
424	R74	PFGE-HUI-1A
426	R74	PFGE-HUI-1A
437	R74	PFGE-HUI-1C
438	R74	PFGE-HUI-1C
443	R74	PFGE-HUI-1C
444	R74	PFGE-HUI-1C
422	R74	PFGE-HUI-1C
423	R74	PFGE-HUI-1C
427	R74	PFGE-HUI-1C
428	R74	PFGE-HUI-1C
429	R74	PFGE-HUI-1C
430	R74	PFGE-HUI-1C
431	R74	PFGE-HUI-1C
432	R74	PFGE-HUI-1C
433	R74	PFGE-HUI-1C
434	R74	PFGE-HUI-1C
435	R74	PFGE-HUI-1C
436	R74	PFGE-HUI-1C

FIG 2 Pulsed-field electrophoresis of strains of the PFGE-HUI-1 clone.

one mutation in the *adeR* gene ($Ala_{136} \rightarrow Val$). Only two strains of this clone had mutations in the *adeN* regulatory gene in the AdeIJK efflux pump ($His_{111} \rightarrow Pro$, $Ile_{112} \rightarrow Phe$). The *adeS* and *adeR* genes in strains of clone PFGE-HUI-1 were not successfully amplified. In all strains of this clone, the *adeN* gene had one mutation ($Pro_{16} \rightarrow Lys$).

Relation between relative gene expression and MICs. Possible interactions between the mechanisms of resistance of the clinical strains of the two clones are summarized in Table 5.

(i) Carbapenems. For the carbapenems (imipenem, meropenem, and doripenem), resistance was associated with the presence of the OXA-type enzymes (OXA-24 and OXA-58 β-lactamases) in strains of clone PFGE-ROC-1.

(ii) Aminoglycosides. In gentamicin-resistant strains (MICs $>$ 8 mg/liter) of clone PFGE-ROC-1, the AdeABC system was overexpressed and/or acetylases (AacC1/AphA1/AadB) were present. Moreover, in strains of both clones (PFGE-ROC-1 and PFGE-HUI-1), the presence of acetylases (AacC1/AphA1/AadB) was associated with resistance to netilmicin (MICs $>$ 16 mg/liter), tobramycin (MICs $>$ 8 mg/liter), and amikacin (MICs $>$ 16 mg/liter).

(iii) Glycylcyclines. Resistance to tigecycline (MICs $>$ 0.5 mg/liter) was associated with overexpression of the RND systems (AdeIJK efflux pump in PFGE-ROC-1_{OXA58} [Fig. 3] and AdeABC efflux pump in PFGE-ROC-1_{OXA24} [Fig. 4]) and the TetA(39) effux pump. The presence of PAbetaN (an inhibitor of the RND system) in strains of clone PFGE-HUI-1 was associated with decreased resistance to tigecycline. In the strains of this clone with no TetA efflux pump (strains 421, 422, 423, 424, and 426), the tigecycline MIC decreased from 1 to ≤0.25 mg/liter (with PAbetaN). In those strains of clone PFGE-HUI-1 with a TetA system, the tigecycline MIC decreased from 2 to 1 mg/liter in the presence of PAbetaN. The AdeIJK and AdeABC efflux pumps (in strains of clone PFGE-ROC-1) were associated with resistance to minocycline (MICs $>$ 2 mg/liter). However, PFGE-$ROC_{OXA\text{-}58}$ displayed resistance to this antibiotic, possibly because of overexpression of the AdeIJK and TetB efflux pumps.

(iv) Tetracyclines. Resistance to tetracyclines was associated with TetB and TetA(39) efflux pumps. In doxycycline-resistant isolates (strains of PFGE-ROC-$1_{OXA\text{-}58}$ with MICs of $>$16 mg/liter), AdeIJK was overexpressed together with Tet systems.

(v) Quinolones. Finally, mutations of the *gyrA* and *parC* genes conferred resistance to ciprofloxacin without any variations in MICs.

DISCUSSION

The impact of the interplay between different mechanisms of antimicrobial resistance in the susceptibility or resistance to antibiotics has been addressed in previous studies. Here, we focused on two of these studies in relation to the present study. One of these studies involved clinical strains of *P. aeruginosa* isolated from cystic fibrosis patients (representative of the Liverpool epidemic strain) (39), and the other involved strains isolated from bloodstream infections (40). In the present study, we attempted to determine if similar conclusions can be applied to clinical strains of *A. baumannii* in which resistance is associated with a multifactorial mechanism. We analyzed strains of two different clones, PFGE-ROC-1 and PFGE-HUI-1. The PFGE-ROC-1 clone included 53 strains carrying the OXA-58 β-lactamase gene (PFGE-ROC-$1_{OXA\text{-}58}$) and 18 strains carrying the OXA-24 carbapenemase gene (PFGE-ROC-$1_{OXA\text{-}24}$). The enzymes encoded by both of these genes are highly prevalent in isolates of *A. baumannii* in

TABLE 2 MICs and RE of genes harboring efflux pumps[c]

	MIC[d] (mg/liter)										RE[b]					
Strain[a]	TIG	GEN	AMK	DOX	MIN	NET	TET	RIF	TOB	DOR	*adeB*	*adeJ*	*adeG*	*craA*	*abeM*	*amvA*
65	≤0.25	8	<2	16	2	1	>64	2	1	4	1.13	**3.10**	1.00	0.43	0.21	0.002
34	0.5	2	32	32	2	>64	>64	64	32	8	**27.73**	0.55	0.70	1.31	0.60	0.003
35	0.5	4	16	32	2	>64	>64	64	64	8	**31.97**	0.40	0.82	1.12	0.66	0.003
37	0.5	4	16	32	2	>64	>64	64	32	8	**28.10**	0.42	0.70	1.25	0.78	0.003
41	0.5	4	32	16	2	>64	>64	64	64	4	**26.89**	0.57	0.66	1.34	0.50	0.003
51	0.5	4	16	32	4	>64	>64	64	64	8	**16.21**	1.62	0.41	0.65	0.70	0.004
53	0.5	4	16	32	4	>64	>64	64	32	8	**17.03**	1.06	0.49	0.70	0.48	0.003
55	0.5	4	16	32	4	>64	>64	64	64	8	**18.25**	1.38	0.85	0.64	0.37	0.004
57	0.5	4	16	32	4	>64	>64	64	64	8	**10.43**	**4.69**	0.37	0.70	0.78	0.003
78	0.5	32	<2	16	2	4	>64	64	<0.5	4	**23.88**	**5.44**	0.96	1.14	0.40	0.007
88	0.5	4	32	16	2	>64	>64	32	32	4	**11.77**	**6.60**	0.92	0.73	0.24	0.003
118	0.5	32	16	32	2	>64	>64	32	64	4	**35.08**	0.61	1.31	0.64	0.27	0.003
126	0.5	16	<2	2	4	4	>64	32	<0.5	4	1.77	1.06	1.27	0.64	0.32	0.004
36	1	4	16	32	2	>64	>64	64	64	4	**32.25**	0.61	0.80	1.03	0.69	0.004
38	1	4	32	32	2	>64	>64	64	64	8	**30.51**	1.34	0.78	1.14	0.51	0.002
58	1	4	32	32	4	>64	>64	64	64	8	**26.93**	**2.20**	0.44	0.85	0.45	0.005
83	1	2	32	32	4	>64	>64	64	32	4	**33.76**	**7.98**	1.37	0.80	0.19	0.002
84	1	2	32	32	4	>64	>64	64	64	4	**13.45**	**10.02**	1.08	0.85	0.24	0.003
86	1	64	8	32	4	32	>64	2	2	4	**22.39**	**6.85**	0.97	0.69	0.19	0.004
87	1	64	4	64	4	16	>64	2	2	4	**43.48**	**6.82**	0.92	0.67	0.08	0.003
89	1	>64	4	64	4	32	>64	64	64	4	**7.25**	**5.61**	1.17	0.89	0.21	0.003
90	1	2	16	64	4	>64	>64	64	32	8	**20.86**	**10.33**	1.00	0.72	0.23	0.004
110	1	2	16	16	4	>64	>64	32	64	4	**30.22**	**5.18**	1.25	0.31	0.30	0.003
111	1	2	16	16	2	>64	>64	32	32	4	**9.86**	**5.39**	1.55	0.31	0.21	0.003
112	1	2	8	16	2	>64	>64	32	32	8	**11.45**	**3.06**	1.23	0.21	0.18	0.36
113	1	2	<2	32	2	4	>64	32	<0.5	4	**24.40**	**4.27**	0.89	0.31	0.25	0.006
114	1	1	<2	32	2	4	>64	32	1	4	**7.01**	**3.87**	0.77	0.27	0.26	0.004
115	1	1	<2	16	2	4	>64	16	<0.5	4	**30.32**	**3.13**	0.82	0.75	0.19	0.003
119	1	32	16	32	2	>64	>64	32	32	4	**43.01**	0.60	1.23	0.54	0.33	0.003
120	1	32	16	16	2	>64	>64	32	32	4	**35.43**	0.66	0.75	0.65	0.19	0.003
124	1	4	64	64	4	>64	>64	32	64	4	**46.58**	0.72	0.79	0.63	0.30	0.004
125	1	4	16	32	8	>64	>64	64	32	4	**32.77**	**8.97**	0.45	0.70	0.30	0.005
56	2	2	<2	32	4	8	>64	64	1	8	**21.05**	**5.82**	0.48	0.87	0.38	0.003
64	2	64	4	32	2	16	>64	2	4	4	**68.98**	**2.94**	0.36	0.62	0.21	0.004
79	2	>64	4	16	1	16	>64	2	2	8	**37.66**	**12.58**	1.57	0.59	0.22	0.004
80	2	>64	8	32	4	32	>64	1	2	8	**32.70**	**10.77**	0.97	0.63	0.19	0.002
81	2	64	4	32	4	16	>64	2	2	4	**46.54**	**11.16**	0.92	0.86	0.18	0.002
82	2	64	<2	32	2	16	>64	1	1	4	**27.63**	**10.04**	1.00	0.97	0.15	0.002
101	2	2	16	32	4	>64	>64	32	32	4	**12.44**	**4.13**	1.25	0.24	0.13	0.003
102	2	2	16	32	4	>64	>64	32	32	4	**8.91**	**7.26**	1.17	0.36	0.28	0.005
106	2	2	8	64	8	>64	>64	64	32	4	**12.06**	**10.55**	1.38	0.76	0.31	0.006
107	2	2	32	64	8	>64	>64	64	32	4	**16.38**	**10.80**	0.91	0.25	0.32	0.004
108	2	4	32	64	8	>64	>64	64	32	8	**23.92**	**10.94**	1.07	0.21	0.29	0.002
109	2	4	32	64	8	>64	>64	64	32	8	**23.37**	**6.32**	0.68	0.32	0.22	0.003

[a] Strains are ranked according to the MIC of tigecycline.

[b] Increased gene RE values of ≥2 are indicated in bold.

[c] The reference strain used was *A. baumannii* ATCC 17978. RNA (50 μg/ml) was from strains of the PFGE-ROC-1_{OXA58} clone.

[d] TIG, tigecycline; GEN, gentamicin; AMK, amikacin; DOX, doxycycline; MIN, minocycline; NET, netilmicin; TET, tetracycline; RIF, rifampin; TOB, tobramycin; DOR, doripenem.

the Iberian Peninsula (41, 42). Only strains of the PFGE-HUI-1 clone ($n = 19$ strains) have previously shown susceptibility to carbapenems (23).

Overexpression of the AdeABC system (RE, 30- to 45-fold) was significantly associated with resistance to gentamicin (MICs > 8 mg/liter) in strains of PFGE-ROC-1 (which produce the OXA-58 and OXA-24 β-lactamases) (8). Moreover, in strains of PFGE-ROC-1_{OXA-24}, resistance to tigecycline (MICs > 0.5 mg/liter) and minocycline (MICs > 2 mg/liter) was also significantly associated with expression of this efflux pump, as previously reported (8). All strains of the PFGE-ROC-1 clone had mutations in the *adeR* (Ala$_{136}$ → Val) and *adeS* (Ala$_{94}$ → Val, Gly$_{186}$ → Val, and Phe$_{214}$ → Leu) genes. Hornsey et al. associated the Ala$_{94}$ → Val substitution with overexpression of the AdeABC efflux pump in *A. baumannii* strains representative of the prevalent United Kingdom lineage, OXA-23 clone 1 (16, 43). However, the other mutations have not previously been described. Peleg et al. (17) reported that increased (40- to 54-fold) expression of the *adeB* gene was

TABLE 3 MICs and RE of genes harboring efflux pumps[c]

	MIC[d] (mg/liter)										RE[b]					
Strain[a]	TIG	GEN	AMK	DOX	MIN	TET	NET	RIF	TOB	DOR	*adeB*[b]	*adeJ*	*adeG*	*craA*	*abeM*	*amvA*
44	≤0.25	2	32	16	1	>64	>64	2	32	64	**26.60**	1.69	0.68	1.47	0.39	0.002
45	≤0.25	2	32	16	1	>64	>64	1	64	>64	**15.00**	1.21	0.67	0.71	0.43	0.001
47	≤0.25	4	16	16	1	>64	>64	1	64	64	**19.50**	1.29	0.80	0.53	0.45	0.002
61	≤0.25	4	16	16	4	>64	>64	2	64	64	**23.25**	1.24	0.34	0.99	0.15	0.005
62	≤0.25	2	32	16	1	>64	>64	1	64	>64	**20.19**	1.67	0.49	0.49	0.17	0.002
95	≤0.25	4	32	16	1	>64	>64	<0.5	32	>64	**33.95**	1.33	1.06	0.32	0.22	0.002
40	0.5	4	32	16	1	>64	>64	1	64	64	**12.88**	0.25	0.59	0.91	0.36	0.001
46	0.5	2	32	16	1	>64	>64	1	64	>64	**10.82**	1.70	0.84	1.18	0.38	0.002
48	0.5	8	32	16	1	>64	>64	1	64	64	**10.28**	1.13	1.04	0.81	0.33	0.002
50	0.5	2	64	16	<0.5	>64	>64	2	64	64	**11.91**	1.25	0.44	0.70	0.41	0.002
60	0.5	4	64	16	1	>64	>64	1	64	64	**29.20**	1.52	0.36	1.18	0.41	0.003
72	0.5	2	32	16	<0.5	>64	>64	1	32	>64	**24.94**	1.19	0.87	0.73	0.23	0.004
73	0.5	2	32	16	<0.5	>64	>64	2	64	>64	**7.53**	1.21	0.60	0.72	0.17	0.004
74	0.5	>64	<2	32	4	>64	64	4	4	>64	**22.19**	1.08	0.51	0.68	0.17	0.003
75	0.5	>64	4	16	4	>64	64	4	4	>64	**13.95**	0.84	0.49	0.52	0.17	0.002
96	0.5	4	32	16	1	>64	>64	1	64	64	**8.55**	1.46	0.97	0.31	0.22	0.002
63	1	4	16	16	1	>64	>64	1	32	64	**43.11**	1.87	0.41	0.59	0.23	0.005
67	1	64	32	16	<0.5	>64	>64	1	64	>64	**44.95**	0.81	0.63	0.45	0.19	0.002

[a] Strains are ranked according to the MIC of tigecycline.

[b] Increased gene RE values of ≥2 are indicated in bold.

[c] The reference strain used was *A. baumannii* ATCC 17978. RNA (50 μg/ml) was from strains of the $\text{PFGE-ROC-1}_{\text{OXA24}}$ clone.

[d] TIG, tigecycline; GEN, gentamicin; AMK, amikacin; DOX, doxycycline; MIN, minocycline; TET, tetracycline; NET, netilmicin; RIF, rifampin; TOB, tobramycin; DOR, doripenem.

associated with tigecycline MICs of 4 to 16 mg/liter in *A. baumannii*. We found that tigecycline-susceptible strains (MIC = 0.5 mg/liter) were associated with increased expression of the *adeB* gene (about 20- to 30-fold), which could indicate the role of the AdeABC efflux pump in others functions necessary for the pathogenesis of clinical strains of *A. baumannii*, such as colonization, infection, and the persistence of microorganisms in the host (10).

We did not detect the AdeABC efflux pump or regulator genes in clinical strains of *A. baumannii* clone PFGE-HUI-1 (susceptible to carbapenems). As mentioned above, this efflux pump is present in 80% (range, 53% to 97%) of clinical isolates studied so far (8).

Increased expression of the AdeIJK efflux pump (RE, 8- to 10-fold) was significantly associated with tigecycline resistance (MICs > 0.5 mg/liter) and minocycline resistance (MICs > 2

TABLE 4 MICs and RE of efflux pumps[c]

	MIC[d] (mg/liter)										RE[b]					
Strain[a]	TIG	GEN	AMK	DOX	MIN	TET	NET	RIF	TOB	DOR	*adeB*	*adeJ*	*adeG*	*craA*	*abeM*	*amvA*
421	1	>64	64	<0.5	<0.5	4	64	2	64	1	NA	1.44	1.23	1.20	0.04	0.01
422	1	16	16	<0.5	<0.5	8	8	2	8	<0.5	NA	1.37	2.11	0.82	0.05	0.02
423	1	16	16	<0.5	<0.5	8	8	2	16	1	NA	1.21	1.16	0.63	0.07	0.02
424	1	16	64	1	1	4	8	16	32	<0.5	NA	1.41	0.89	0.68	0.04	0.02
426	1	64	32	1	1	8	64	16	32	<0.5	NA	1.69	1.00	0.45	0.04	0.02
427	2	16	16	8	<0.5	>64	8	4	8	1	NA	1.46	0.83	0.42	0.04	0.02
428	2	16	16	4	<0.5	>64	4	2	8	1	NA	1.64	0.89	0.49	0.05	0.02
429	2	64	4	4	<0.5	>64	8	4	8	1	NA	1.75	0.91	0.48	0.04	0.01
430	2	32	16	8	<0.5	>64	16	4	16	1	NA	1.22	0.76	0.48	0.04	0.02
431	2	64	4	8	1	>64	32	32	32	1	NA	1.28	1.00	0.44	0.04	0.02
432	2	16	16	4	<0.5	>64	4	4	16	2	NA	1.61	0.90	0.53	0.04	0.02
433	2	16	16	8	<0.5	>64	8	4	16	2	NA	1.51	0.89	0.54	0.04	0.02
434	2	16	16	4	<0.5	>64	8	4	8	2	NA	1.82	1.09	0.50	0.04	0.02
435	2	32	32	4	<0.5	>64	8	2	32	1	NA	1.27	0.90	0.34	0.05	0.01
436	2	16	16	8	1	>64	16	4	8	1	NA	1.32	0.79	0.43	0.04	0.01
437	2	16	16	4	1	>64	4	4	16	<0.5	NA	0.72	1.18	0.78	0.03	0.03
438	2	32	16	8	1	>64	8	4	16	<0.5	NA	1.25	1.51	0.60	0.03	0.02
443	2	16	16	4	<0.5	>64	8	4	8	1	NA	0.91	0.80	0.52	0.04	0.01

[a] Strains are ranked according to the MIC of tigecycline.

[b] Primers for *adeA* and *adeC* genes were also used. NA, not applicable.

[c] The reference strain used was *A. baumannii* ATCC 17978. RNA (50 μg/ml) was from strains of the PFGE-HUI-1 clone (susceptible to carbapenems).

[d] TIG, tigecycline; GEN, gentamicin; AMK, amikacin; DOX, doxycycline; MIN, minocycline; TET, tetracycline; NET, netilmicin; RIF, rifampin; TOB, tobramycin; DOR, doripenem.

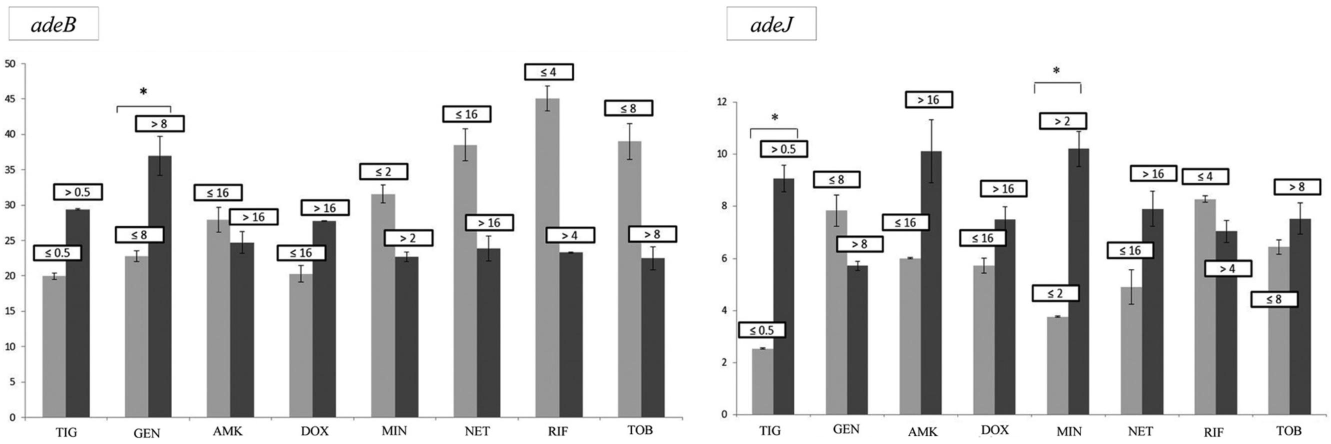


FIG 3 Relative expression of the *adeB* and *adeJ* genes from strains of the PFGE-ROC-1_{OXA-58} clone in relation to the MICs of different antibiotics.*, $P < 0.05$ (Student's *t* test). Light gray bars, strains susceptible to several antibiotics (grouped by MIC [in mg/liter]); dark gray bars, strains resistant to several antibiotics (grouped by MIC [in mg/liter]); TIG, tigecycline; GEN, gentamicin; AMK, amikacin; DOX, doxycycline; MIN, minocycline; NET, netilmicin; RIF, rifampin; TOB, tobramycin.

mg/liter) in strains of PFGE-ROC-1_{OXA-58}. However, this system was not significantly associated with resistance to netilmicin or tobramycin (aminoglycosides). These results are consistent with those obtained by Coyne et al. (44). These authors also noted that overexpression of this pump is always lower than that of the AdeABC system. These results could confirm the theory that high-level expression of the AdeIJK efflux pump is toxic to the host cell (45). The *adeJ* gene was not overexpressed in strains of the PFGE-ROC-1_{OXA-24} and PFGE-HUI-1 clones. Only two strains of the PFGE-ROC-1_{OXA-58} clone had two new mutations in a gene regulating the AdeIJK pump (*adeN*; $His_{111} \rightarrow Pro$, $Ile_{112} \rightarrow Phe$), and all strains of PFGE-HUI-1 had a $Pro_{16} \rightarrow Lys$ substitution in the *adeN* gene. None of these mutations have been associated with overexpression of AdeIJK, although other possible mechanisms of regulation cannot be ruled out (18).

Expression of *adeG* (AdeFGH), *craA*, *abeM*, and *amvA* was not increased (RE, 0.003 to 1) in strains of the PFGE-ROC-1 or PFGE-HUI-1 clone.

Gram-positive bacteria are the origin of *tet* genes detected in Gram-negative bacteria, such as *A. baumannii* (22, 38). Here, we detected the *tetB* gene in strains of the PFGE-ROC-1 clone, all of which were resistant to tetracycline (MICs = 16 to 64 mg/liter) and doxycycline (MICs = 16 to 64 mg/liter). Moreover, in some strains (PFGE-ROC-1_{OXA-58}), overexpression of AdeIJK together with the presence of this acquired efflux pump was possibly associated with resistance to minocycline (MICs = 2 to 4 mg/liter). In

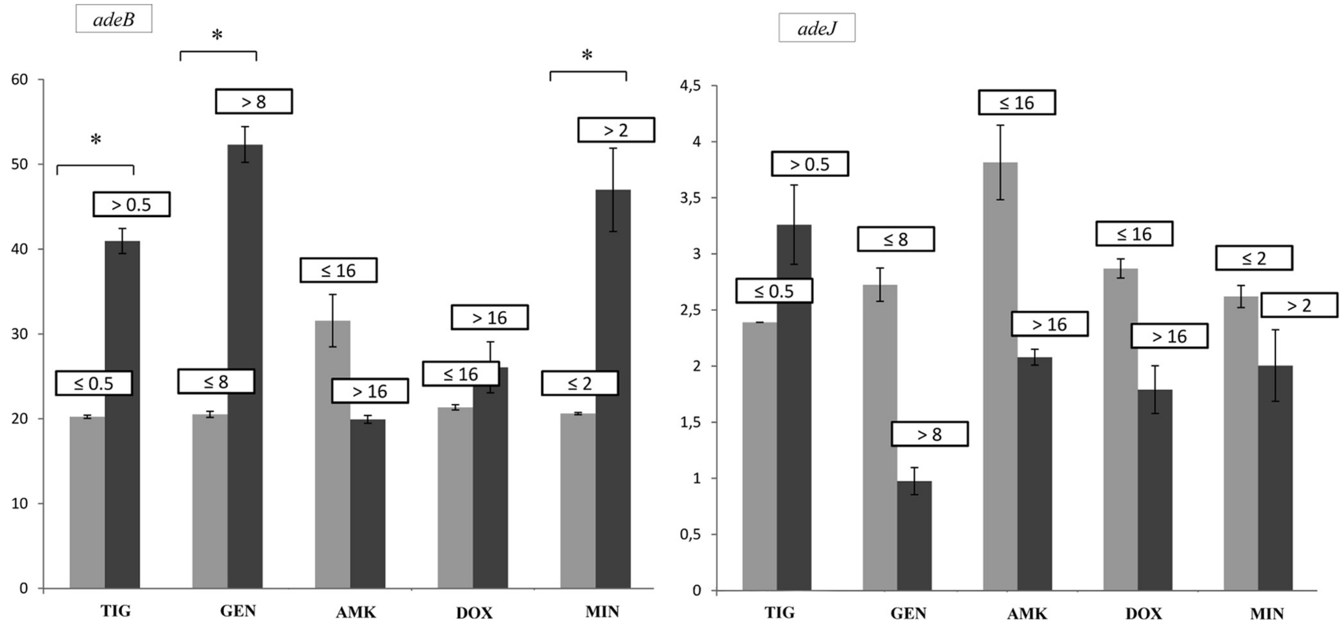


FIG 4 Relative expression of the *adeB* and *adeJ* genes from strains of the PFGE-ROC-1_{OXA-24} clone in relation to the MICs of different antibiotics.*, $P < 0.05$ (Student's *t* test). Light gray bars, strains susceptible to several antibiotics (grouped by MIC [in mg/liter]); dark gray bars, strains resistant to several antibiotics (grouped by MIC [in mg/liter]); TIG, tigecycline; GEN, gentamicin; AMK, amikacin; DOX, doxycycline; MIN, minocycline.

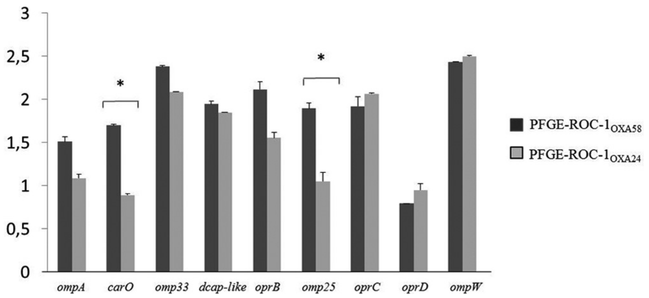


FIG 5 Relative expression of porin genes by strains of the PFGE-ROC-1$_{\text{OXA-58}}$ and PFGE-ROC-1$_{\text{OXA-24}}$ clones. *, $P < 0.05$ (Student's *t* test).

other pathogens, such as *Escherichia coli*, the combination of AcrAB-TolC and TetA has been associated with a high degree of resistance to tetracycline (46). Our results showed that detection of the *tetA*(39) gene in all strains of this clone was associated with resistance to tetracycline and doxycycline. Agersø and Guardabassi (47) analyzed the presence of this gene in *A. baumannii* strains. These authors located the gene in both environmental and clinical strains, and they found the *tetA*(39) gene in 33 tigecycline-resistant strains (MICs ≥ 16 mg/liter). We noted that in strains of PFGE-HUI-1 harboring the *tetA*(39) gene, the tigecycline MIC was lower (2 to 1 mg/liter) in the presence of PAbetaN (an RND efflux pump inhibitor), and the MIC decreased from 1 to 0.25 mg/liter in the *A. baumannii* strains without this gene. This suggests the involvement of a new RND efflux pump, together with the TetA(39) system, in the resistance to tigecycline.

In relation to porins and unlike in other pathogens, such as *Pseudomonas aeruginosa*, in which OprD expression plays an important role in resistance to carbapenem antibiotics (39), we found that decreased expression of a porin was significantly associated with antimicrobial resistance. We observed decreased RE only of the *carO* and *omp25* genes, on comparing strains of PFGE clone-ROC-1$_{\text{OXA-58}}$ and PFGE clone-ROC-1$_{\text{OXA-24}}$. This decrease was not associated with resistance to carbapenems, which is known to be associated with the presence of the β-lactamases (9). Moreover, the carbapenem resistance was not associated with expression of the OXA-51 and AmpC chromosomal β-lactamases. Overall, our data revealed that the presence of OXA-type enzymes (OXA-24 and/or OXA-58) is sufficient to confer resistance to carbapenem in the *A. baumannii* strains under study, as previously found (41, 42). Moreover, resistance to doripenem was also associated with the presence of the β-lactamases OXA-58 (MICs = 4 to 8 mg/liter) and OXA-24 (MICs = 64 to >64 mg/liter) (compared with MICs for strains of the PFGE-HUI-1 clone of 0.5 to 1 mg/liter). Marti and colleagues (48) analyzed the activity of doripenem against clinical isolates of *A. baumannii* and concluded that doripenem was more active than imipenem and meropenem in strains carrying the OXA-58 β-lactamase gene. However, in the present study, doripenem, imipenem, and meropenem MICs were high for the clinical strains producing the OXA-24 enzyme.

Quinolone resistance did not vary between the strains under study and was associated with previously reported mutations in *gyrA* and *parC* (7). Aminoglycoside-resistant isolates of clones PFGE-ROC-1 and PFGE-HUI-1 showed acetylases known to be common in *A. baumannii* strains (AacC1, AphA1, and AadB) (49).

In conclusion, (i) the clinical strains of *Acinetobacter baumannii* under study possess efflux systems and other mechanisms (possibly connected) that enable them to develop resistance to various antimicrobials and that also have other functions necessary in bacterial pathogenesis. (ii) Overexpression of the AdeABC system was found to be associated with resistance to glycylcycline (tigecycline and minocycline) and aminoglycosides (gentamicin), and possibly other biological functions, in the clinical strains under study. (iii) Hyperexpression of the AdeIJK efflux pump was significantly associated with resistance to tigecycline and minocycline but did not appear to be involved in other functions related to the pathogenesis of the bacterium. This efflux pump may be related to the TetB system and, thus, to minocycline resistance. (iv) Porins, AmpC β-lactamases, and OXA-51 were not involved in the antimicrobial resistance observed in the present study in the presence of OXA-type enzymes (OXA-24 and OXA-58). (v) The OXA-24 and OXA-58 β-lactamases were associated with resistance to meropenem, doripenem, and imipenem (especially the OXA-24 β-lactamase). (vi) The presence of the Tet efflux pumps in *A. baumannii* isolates was associated with resistance to tetracyclines and doxycycline. (vii) Finally, a new RND efflux pump may

TABLE 5 Interplay of mechanisms of resistance to several antibiotics of the strains of clones PFGE-ROC-1 and PFGE-HUI-1 under study

	Mechanism of resistance		
Antibiotic(s)	PFGE-ROC-1$_{\text{OXA-58}}$	PFGE-ROC-1$_{\text{OXA-24}}$	PFGE-HUI-1
Tigecycline	Overexpression of AdeIJK	Overexpression of AdeABC	New RND efflux system/TetA(39) efflux pump[c]
Gentamicin	Overexpression of AdeABC/acetylases (*aacC1*, *aphA1*, *aadB*)	Overexpression of AdeABC/acetylases (*aacC1*, *aphA1*, *aadB*)	Acetylases (*aacC1*, *aphA1*, *aadB*)
Minocycline	Overexpression of AdeIJK/TetB efflux pumps	Overexpression of AdeABC[b]	
Netilmicin, tobramycin, and amikacin	Acetylases (*aacC1*, *aphA1*, *aadB*)	Acetylases (*aacC1*, *aphA1*, *aadB*)	Acetylases (*aacC1*, *aphA1*, *aadB*)
Imipenem, meropenem, and doripenem	OXA-58 β-lactamase	OXA-24 β-lactamase	
Ciprofloxacin	Mutations in *gyrA* and *parC*	Mutations in *gyrA* and *parC*	Mutations in *gyrA* and *parC*
Doxycycline	Overexpression of AdeIJK[a]/TetB efflux pumps	TetB efflux pump	TetA(39) efflux pump[c]
Tetracyclines	TetB efflux pump	TetB efflux pump	TetA(39) efflux pump[c]

[a] Nonsignificantly increased expression relative to strains with doxycycline resistance.
[b] Only three isolates, 61, 74, and 75.
[c] Except for strains 421, 422, 423, 424, and 426.

act in combination with the TetA(39) system to confer resistance to tigecycline in the absence of the AdeABC efflux pump and overexpression of the other systems in *A. baumannii* clinical strains susceptible to carbapenems.

The main limitation of the study was that we were not able to study the complex mechanisms of resistance to carbapenems in strains that did not produce OXA-type enzymes.

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