ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2011, p. 1285–1286 Vol. 55, No. 3
0066-4804/11/$12.00 doi:10.1128/AAC.01142-10

First Identification and Characterization of an AdeABC-Like Efflux Pump in *Acinetobacter* Genomospecies 13TU▽†

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Received 17 August 2010/Returned for modification 24 October 2010/Accepted 25 December 2010

Non-*Acinetobacter baumannii* spp. are emerging among clinical *Acinetobacter* isolates causing nosocomial infections, and some (such as genomospecies 13TU) appear to be multidrug resistant. The prevalence of non-*Acinetobacter baumannii* spp. in the hospital setting is likely understated due to poor identification techniques. We report the first identification of an AdeABC-type efflux pump in an *Acinetobacter* genomospecies 13TU clinical isolate, its contribution to multidrug resistance, and the coexistence of three Ade-type efflux pumps in this strain.

The *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex comprises closely related genomic species belonging to the nonfermentative Gram-negative *Acinetobacter* genus (7). Among them, genomospecies 2 (*Acinetobacter baumannii*), 3, and 13TU are rapidly emerging as opportunistic pathogens and pose an important public health issue in the clinical setting (10, 11). The treatment of infections caused by these strains can be complicated due to the increasing occurrence of multidrug-resistant (MDR) strains. The MDR phenotype in *Acinetobacter* spp. has been associated with transposon-based resistance islands and/or active efflux mechanisms (1, 4, 6, 9, 13, 16, 18).

To date, four main RND efflux pumps have been identified in *Acinetobacter* spp. The AdeABC system, only present in *A. baumannii* clinical isolates, provides resistance to aminoglycosides, tetracycline, erythromycin, chloramphenicol, trimethoprim, and fluoroquinolones (9). The AdeDE pump, which has been identified in genomospecies 3 and in one *Acinetobacter* genomospecies 13TU clinical isolate (4, 5), provides resistance to amikacin, ceftazidime, chloramphenicol, ciprofloxacin, erythromycin, meropenem, rifampin, and tetracycline. The AdeIJK efflux pump has only been found in *A. baumannii* and confers resistance to β-lactams, chloramphenicol, tetracycline, erythromycin, lincosamides, fluoroquinolones, rifampin, and trimethoprim (6). Finally, the AdeXYZ efflux system was identified in 2006 in clinical isolates classified as genomospecies 3, but its role in antimicrobial resistance has not yet been proven (5).

A PCR screen designed to identify *adeB* genes in a collection of *Acinetobacter calcoaceticus-Acinetobacter baumannii* clinical isolates originated a positive amplicon (1.2 kb) from an *Acinetobacter* genomospecies 13TU strain designated strain 158029 (species was identified by amplified rRNA gene restriction analysis [ARDRA]), sequence analysis of the 16S-23S rRNA gene spacer region (internal transcribed spacer [ITS]), and *recA* sequencing (3, 12, 17). The amplified PCR product was sequenced and used to design an initial set of outward primers to fully sequence its flanking regions by stepwise genome walking (see Table S1 in the supplemental material). Several rounds of outward sequencing finally provided a 7,780-bp sequence containing the genes encoding a membrane fusion protein, an inner membrane efflux protein, and an outer membrane protein channel with very good similarities to the *A. baumannii* AYE AdeABC efflux system (96%, 99%, and 95%, respectively). A two-component regulatory system with very good similarity to AdeRS (99% and 94%, respectively) was also found upstream from *adeABC* in an inverted orientation.

To demonstrate the involvement of this system in multidrug resistance, an internal fragment of the *adeB* gene (619 bp) was amplified and cloned into the pGEM-T Easy vector (Promega) by A/T cloning and introduced into strain 158029 by electroporation. Since pGEM-T Easy is suicidal in *Acinetobacter*, transformants selected in LB plates containing 80 μg/ml ticarcillin should result from a single-crossover event leading to the insertional inactivation of the cognate *adeB* gene. *adeB* disruption within the resulting strain, designated JVAB02, was verified by PCR using a combination of primers matching the upstream region of the *adeB* gene and the pGEM-T Easy scaffold.

The antibiotic susceptibilities of 158029 and JVAB02 were studied by Etest. Strain JVAB02 proved more susceptible than 158029 to a wide range of antibiotics (Table 1), including β-lactams, chloramphenicol, tetracyclines, quinolones, trimethoprim, aminoglycosides, and the novel glycylcycline, tigecycline. The substrate specificity was similar to that of the AdeABC pump from *A. baumannii* (9, 15), although we found an 8-fold decrease in the MIC of ceftazidime (which is not a substrate for the AdeABC pump in *A. baumannii* but is for the AdeDE pump of *Acinetobacter* genomospecies 3 [4]), as well as a profound effect on the MIC of chloramphenicol. Although RND-type efflux pumps have been shown to extrude chloramphenicol in *Acinetobacter baumannii*, a novel MFS-type pump (CraA) has recently been associated with the intrinsic chloramphenicol resistance displayed by this organism (14). In this work, the use of *craA*-specific primers failed to detect the

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† Supplemental material for this article may be found at http://aac.asm.org/.

▽ Published ahead of print on 3 January 2011.

TABLE 1. MICs of antimicrobial agents for the investigated strains

Antimicrobial agent	MIC (μg/ml)		Difference in susceptibility (fold)
	158029	JVAB02 (*adeB*::*Tic*)	
Piperacillin	6	6	1
Aztreonam	32	3	11
Ceftriaxone	16	3	5
Cefoxitin	96	4	24
Ceftazidime	4	0.5	8
Cefotaxime	12	0.5	24
Imipenem	0.5	0.25	2
Meropenem	1	0.064	16
Nalidixic Acid	12	1.5	8
Ciprofloxacin	1	0.016	63
Norfloxacin	16	0.38	42
Gentamicin	12	0.094	128
Amikacin	12	1	12
Kanamycin	6	0.75	8
Tobramycin	3	0.38	8
Tetracycline	16	0.75	21
Trimethoprim	>32	0.75	>42
Tigecycline	3	0.064	47
Chloramphenicol	>256	8	>32

presence of this pump in strain 158029 (data not shown). The lack of CraA might result in a more predominant role of AdeABC in chloramphenicol resistance in *Acinetobacter* genomospecies 13TU.

We also looked for the presence of additional Ade-type efflux pumps in this strain using specific primers matching internal regions of the *A. baumannii adeJ* and *Acinetobacter* genomospecies 3 *adeE* and *adeY* genes. The use of *adeE*-specific primers originated an amplification product of approximately 750 bp with 90% identity to the *adeE* gene from *Acinetobacter* genomospecies 3 strain 4365 (4). Amplification with the *adeJ*- and *adeY*-specific primers also yielded amplification products of approximately 750 bp for both, with 95% and 89% identity to *adeJ* and *adeY*, respectively. It is worth mentioning that, although described as independent pumps, AdeIJK and AdeXYZ share 93% identity at the nucleotide level and 99% similarity at the protein level. Similar percentages can be found when comparing *adeJ* or AdeJ sequences from different *A. baumannii* strains.

The protein identified in this work seems to be more similar to AdeJ than to AdeY, and therefore, this would be the first time that this pump has been described in an *Acinetobacter* genomospecies other than *A. baumannii*. We believe, however, that AdeIJK and AdeXYZ represent the same pump, which is most likely present in *Acinetobacter* genomic DNA groups 2, 3, and 13TU, among others (2).

Overall, these results were highly unexpected, since previous studies seemed to indicate that the presence of *adeABC* and *adeDE* was species specific, with *adeABC* being restricted to *Acinetobacter baumannii* and *adeDE* to *Acinetobacter* genomospecies 3 (8). This is the first time that the AdeABC pump has been described and characterized in a non-*Acinetobacter baumannii* strain and that all three pumps have been shown to coexist. A larger number of strains belonging to this group should be analyzed in order to confirm whether this carriage of efflux pumps is widespread. Nonetheless, the presence of three major efflux systems in an emerging multidrug-resistant pathogen is alarming.

Nucleotide sequence accession number. The full length of the *adeSRABC* sequence was submitted to GenBank and assigned accession number GU319112.

This study was supported by the Spanish Ministry of Health (FIS 08/00195), by grant 2009SGR1256 from Generalitat de Catalunya, and by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III-FEDER, Spanish Network for Research in Infectious Diseases (grant REIPI RD06/0008). P.E was also supported by a fellowship from Programme Alβan E07D401559CO. This work has also been supported by funding from the European Community (TROCAR contract HEALTH-F3-2008-223031).

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