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Identification of 16S rRNA Methylase-Producing *Acinetobacter baumannii* Clinical Strains in North America▽

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Five highly amikacin-resistant *Acinetobacter baumannii* isolates were collected at a medical center in Pennsylvania. The aminoglycoside resistance was due to the production of the 16S rRNA methylase ArmA. Two of the isolates coproduced OXA-23 β-lactamase and were highly resistant to carbapenems as well. The isolates were genetically closely related by pulsed-field gel electrophoresis.

Acinetobacter baumannii is increasingly becoming a major nosocomial pathogen worldwide, particularly in the setting of ventilator-associated pneumonia or bloodstream infections in intensive care units (6). The treatment is complicated by its tendency to acquire resistance to multiple classes of antimicrobials (2). When these strains are encountered, empirical salvage regimens may include such agents as colistin, tigecycline, and amikacin (1, 13). Amikacin is an aminoglycoside that generally continues to retain good activity against *A. baumannii* (3, 11). Resistance to amikacin in *A. baumannii* is primarily mediated by structural modification of the agent through the actions of aminoglycoside-modifying enzymes that are produced by resistant strains (14). In recent years, the production of 16S rRNA methylases has been implicated in aminoglycoside resistance among gram-negative pathogens (4). Five such enzymes have been identified, ArmA and RmtA through -D. They confer high-level resistance to all parenterally formulated aminoglycosides, effectively eliminating the entire class, including amikacin, as a therapeutic option. The presence of these 16S rRNA methylases has already been reported worldwide, but no strains with this resistance mechanism have been reported in North America so far.

Between December 2006 and March 2007, five nonrepetitive *A. baumannii* isolates (isolates A through E) with high-level resistance to amikacin, tobramycin, and gentamicin (defined by MICs of >512 μg/ml) were recovered from inpatients at the University of Pittsburgh Medical Center. Multiplex PCR for the five known 16S rRNA methylase genes (4) yielded amplicons consistent with *armA* for all five isolates, which was verified by sequencing. No amplicons were obtained for the other 16S rRNA methylase genes. In addition, isolates A and B, both of which were highly resistant to carbapenems, were positive for $bla_{\text{OXA-23}}$ by PCR and sequencing (5). OXA-23 is a carbapenem-hydrolyzing β-lactamase that is known to cause clinically relevant carbapenem resistance (10).

The genomic DNA of isolate B was then prepared, digested with HindIII (New England BioLabs, Beverly, MA), and ligated with cloning vector pUC19. Electrocompetent *Escherichia coli* DH10B (Invitrogen Corporation, Carlsbad, CA) was transformed with the resultant recombinant plasmids. As a result, pUCarmA, a pUC19 derivative with a 4-kb insert containing *armA*, was obtained. *E. coli* DH10B(pUCarmA) exhibited high-level resistance (MICs, ≥256 μg/ml) to amikacin, tobramycin, and gentamicin. The genetic environment surrounding *armA* was identical to those reported earlier as Tn*1548* for strains belonging to the family *Enterobacteriaceae* (7, 8). The 3′ end of *orf513*, the gene that encodes a putative transposase that characterizes IS*CR1* and that is commonly associated with class 1 integrons (16), and *tnpU*, another putative transposase gene, were located upstream of *armA*. It was followed downstream by the 5′ end of yet another putative transposase gene, *tnpD*. These findings suggest that Tn*1548* is serving as an efficient vehicle to mobilize *armA* across phylogenetically distant gram-negative species.

A reverse transcription assay was conducted to confirm the expression of *armA* in *A. baumannii*. Total RNAs of isolate B (*armA* positive) and isolate F (*armA* negative) were prepared with the RNeasy Maxi kit (QIAGEN Inc., Valencia, CA). Reverse transcription was performed by the use of a high-capacity cDNA reverse transcription kit (Applied Biosystems). The resultant cDNA was then used as the template for a PCR (4). The presence of mRNA transcripts for *armA* was observed in isolate B (*armA* positive) but not isolate F (*armA* negative). No amplicon was obtained for either isolate when reverse transcriptase was absent. These results confirmed the expression of *armA* in *A. baumannii*.

Curing of *armA* was attempted with *A. baumannii* isolate B by serial passage of the strain in Luria-Bertani broth containing ethidium bromide at a subinhibitory concentration. One of the strains obtained by this procedure, *A. baumannii* 231, was found to be susceptible to amikacin. Loss of *armA* was confirmed by PCR. Conjugation experiments were conducted with the five study isolates as the donors and azide-resistant *E. coli* J53 or colistin-resistant *A. baumannii* 213 as the recipient by using standard broth mating techniques. No amikacin-resistant

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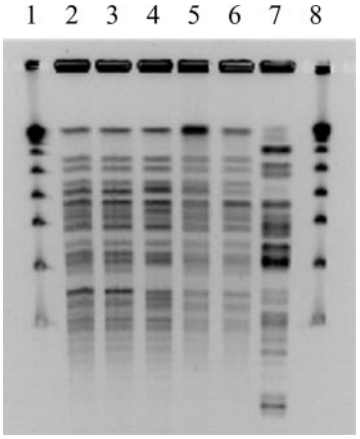

FIG. 1. Result of PFGE. Lanes 1 and 8, molecular weight marker; lanes 2 to 6, isolates A to E; lane 7, *armA*-negative, amikacin-resistant clinical isolate from the study period (control).

transconjugants were obtained for any of the study isolates producing ArmA with either recipient.

To further localize the *armA* gene, both plasmid and genomic DNAs from isolate A were subjected to DNA hybridization. Plasmids from isolate A were extracted and subjected to electrophoresis. Subsequently, they were transferred to a nylon membrane by the method of Southern (15) and hybridized with digoxigenin-labeled *armA* gene fragments by use of the PCR DIG detection system (Roche Diagnostics, Indianapolis, IN). The genomic DNA of isolate A was digested with restriction enzyme CeuI (New England BioLabs) and subjected to pulsed-field gel electrophoresis (PFGE) according to the method of Liu et al. (12). Hybridization was carried out in the same fashion by using probes specific for either *armA* or the 16S and 23S rRNA genes (9). A plasmidic band from isolate A hybridized with the *armA* probe, whereas none of the chromosomal bands obtained by CeuI digestion hybridized with it. These results, along with the curability of *armA*, suggested a plasmidic location of *armA*.

To assess the genetic relatedness of the study isolates, genomic DNA was isolated and digested with ApaI (New England BioLabs). PFGE was performed with the CHEF III system (Bio-Rad, Hercules, CA) with the following run parameters: block I, switch time of 3 to 8 s and run time of 10 h; block II, switch time of 12 to 20 s and run time of 10 h. The five isolates that carry *armA* were either identical or closely related to each other, differing by up to three bands (Fig. 1). Thus, the spread of *armA* in our study population appeared to be due to the dissemination of closely related clones of *A. baumannii* that carry *armA* on a nonconjugative plasmid.

In summary, we report the first identification of 16S rRNA methylase as a mechanism of high-level resistance to aminoglycosides in North America. Some of the *A. baumannii* strains were simultaneously resistant to other classes of antimicrobials, including carbapenems. More research is required to add to the understanding of the increasingly complex nature of the multidrug resistance in this troublesome organism.

Nucleotide sequence accession number. The nucleotide sequence determined in this study appears in the EMBL/GenBank/DDBJ databases under accession number EU014811.

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