Identification of 16S rRNA Methylase-Producing Acinetobacter baumannii Clinical Strains in North America
doi:10.1128/AAC.00560-07

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Received 28 April 2007/Returned for modification 14 May 2007/Accepted 20 August 2007

Five highly amikacin-resistant Acinetobacter baumannii isolates were collected at a medical center in Pennsylva-
vania. 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 re-
ported 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 ver-
ified 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 blaoxa-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 Esche-
richia 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 con-
taining armA, was obtained. E. coli DH10B(pUCarmA) exhibited high-level resistance (MICs, ≥256 μg/ml) to amikacin, tobramycin, and gentamicin. The genetic environment sur-
rounding armA was identical to those reported earlier as Tn1548 for strains belonging to the family Enterobacteriaceae (7, 8). The 3′ end of orf513, the gene that encodes a putative transposase that characterizes ISCR1 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 Tn1548 is serving as an efficient vehicle to mobilize armA across phylo-
genetically 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 RNasy 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 expres-
sion 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 con-

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Published ahead of print on 4 September 2007.

{http://aac.asm.org/ on October 22, 2015 by Queensland Library}
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 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) 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.

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.

We thank the clinical microbiology laboratory staff at University of Pittsburgh Medical Center for the identification and provision of the study isolates.

Y.D. is supported by NIH training grant T32 AI007333. D.L.P. is supported in part by NIH research grant R01AI070896-01A1.

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