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# Genetic Basis of Multidrug Resistance in *Acinetobacter baumannii* Clinical Isolates at a Tertiary Medical Center in Pennsylvania[▽]

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**A total of 49 unique clinical isolates of multidrug-resistant (MDR) *Acinetobacter baumannii* identified at a tertiary medical center in Pittsburgh, Pennsylvania, between August 2006 and September 2007 were studied for the genetic basis of their MDR phenotype. Approximately half of all *A. baumannii* clinical isolates identified during this period qualified as MDR, defined by nonsusceptibility to three or more of the antimicrobials routinely tested in the clinical microbiology laboratory. Among the MDR isolates, 18.4% were resistant to imipenem. The frequencies of resistance to amikacin and ciprofloxacin were high at 36.7% and 95.9%, respectively. None of the isolates was resistant to colistin or tigecycline. The presence of the carbapenemase gene $bla_{OXA-23}$ and the 16S rRNA methylase gene *armA* predicted high-level resistance to imipenem and amikacin, respectively. $bla_{OXA-23}$ was preceded by insertion sequence IS*Aba1*, which likely provided a potent promoter activity for the expression of the carbapenemase gene. The structure of the transposon defined by IS*Aba1* differed from those reported in Europe, suggesting that IS*Aba1*-mediated acquisition of $bla_{OXA-23}$ may occur as an independent event. Typical substitutions in the quinolone resistance-determining regions of the *gyrA* and *parC* genes were observed in the ciprofloxacin-resistant isolates. Plasmid-mediated quinolone resistance genes, including the *qnr* genes, were not identified. Fifty-nine percent of the MDR isolates belonged to a single clonal group over the course of the study period, as demonstrated by pulsed-field gel electrophoresis.**

*Acinetobacter baumannii* is a gram-negative, non-lactose-fermenting organism that is increasingly recognized as a major pathogen causing nosocomial infections including bacteremia and ventilator-associated pneumonia, particularly in patients admitted to intensive care units (23, 25). The organism is characterized by its tendency to acquire resistance to multiple classes of antimicrobials (3). Of note, increasing resistance to carbapenems has been observed worldwide in the past decade, frequently mediated by production of Ambler's class D β-lactamases, which possess carbapenemase activity (26). Several outbreaks caused by multidrug-resistant (MDR) *A. baumannii* have been reported from the United States (21, 22, 28). Additionally, infections due to MDR *A. baumannii* have been observed in military personnel returning from Iraq and Afghanistan (18, 29). The Infectious Diseases Society of America recently identified *A. baumannii* as one of the six particularly problematic pathogens in terms of antimicrobial availability issues arising from resistance (32).

The emergence of *A. baumannii* clinical isolates with resistance to multiple classes of antimicrobials, including carbapenems, aminoglycosides, and fluoroquinolones, was observed at our medical center in the latter half of 2006. For these patients, therapeutic options were limited to salvage agents such as colistin and tigecycline. In the present study, we conducted a detailed investigation of the molecular epidemiology and genetic basis of multidrug resistance among *A. baumannii* clinical isolates identified at our medical center over a 1-year period, with a focus on the mechanisms of carbapenem, aminoglycoside, and fluoroquinolone resistance.

## MATERIALS AND METHODS

**Clinical isolates and definition of MDR.** *A. baumannii* isolates recovered from patient specimens at the University of Pittsburgh Medical Center (UPMC) Presbyterian Campus between August 2006 and September 2007 were included in this study. *A. baumannii* was identified in the clinical microbiology laboratory by using the Gram-Negative Identification Panel (Microscan, Dade Behring Inc., Sacramento, CA). For automated identification in the electronic medical records, MDR was defined as nonsusceptibility to three or more of the antimicrobials that are routinely tested in the clinical laboratory and to which *A. baumannii* would have been expected to be susceptible. These included ampicillin-sulbactam, piperacillin-tazobactam, cefepime, ciprofloxacin, trimethoprim-sulfamethoxazole, a carbapenem (imipenem or meropenem), and an aminoglycoside (amikacin, tobramycin, or gentamicin). As a result, 65 MDR isolates from different patients were identified from this period. Forty-nine of the 65 isolates were available for further analysis in the research laboratory. All the study isolates were aliquoted and stored at −80°C until further use.

**Susceptibility testing.** The susceptibilities of the isolates to ampicillin-sulbactam, ceftazidime, cefepime, meropenem, tobramycin, gentamicin, ciprofloxacin, and tetracycline were tested using the standard disk diffusion method on Mueller-Hinton (MH) agar plates (BD Microbiology Systems, Sparks, MD) and using the breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI) (5). MICs of imipenem, amikacin, colistin, and tigecycline were determined by use of Etest strips (AB Biodisk, Solna, Sweden). They were also interpreted according to the CLSI breakpoints, except for tigecycline, for which the breakpoints endorsed by the British Society of Antimicrobial Chemotherapy (BSAC) (MICs, ≤1 μg/ml for susceptibility and >2 μg/ml for resistance) were used. BSAC is the only organization that has defined tigecycline breakpoints for

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TABLE 1. Primers used for amplification of resistance genes

| Primers | Sequences (5′ to 3′)[a] | Target gene(s) | Reference |
|---|---|---|---|
| OXA Set A FOR | ATGAAAAAATTTATACTTCC | $bla_{OXA-24}$, $bla_{OXA-25}$, $bla_{OXA-26}$, $bla_{OXA-33}$, $bla_{OXA-40}$, $bla_{OXA-72}$ | 18 |
| OXA Set A REV | TTAAATGATTCCAAGATTTTC | | |
| OXA Set C FOR | ACAGAARTATTTAAGTGGG | $bla_{OXA-51}$, $bla_{OXA-58}$, $bla_{OXA-64}$, $bla_{OXA-69}$, $bla_{OXA-70}$, $bla_{OXA-71}$, $bla_{OXA-75}$, $bla_{OXA-78}$ | 18 |
| OXA Set C REV | GGTCTACAKCCMWTCCCCA | | |
| OXA 23 FOR | GATGTGTCATAGTATTCGTCG | $bla_{OXA-23}$, $bla_{OXA-27}$, $bla_{OXA-49}$ | 1 |
| OXA 23 REV | TCACAACAACTAAAAGCACTG | | |
| OXA 58 5′ | ATGAAATTATTAAAAATATTGAGTTTAG | $bla_{OXA-58}$, $bla_{OXA-96}$ | 18 |
| OXA58 3′ | TTATAAATAATGAAAAACACCCAAC | | |
| ADC-7 FOR | ATGCGATTTAAAAAAATTTCTTGT | $bla_{ADC-1}$, $bla_{ADC-2}$, $bla_{ADC-3}$, $bla_{ADC4}$, $bla_{ADC-5}$, $bla_{ADC-6}$, $bla_{ADC-7}$ | 18 |
| ADC-7 REV | TTATTTCTTTATTGCATTCAG | | |
| SHV S1 | ATTTGTCGCTTCTTTACTCGC | $bla_{SHV}$ | 37 |
| SHV S2 | TTTATGGCGTTACCTTTGACC | | |
| TEM1 | ATGAGTATTCAACATTTCCGTG | $bla_{TEM}$ | 11 |
| TEM4 | TTACCAATGCTTAATCAGTGAG | | |
| CTX-M/F | TTTGCGATGTGCAGTACCAGTAA | $bla_{CTX-M}$ | 10 |
| CTX-M/R | CGATATCGTTGGTGGTGCCATA | | |
| CTXM 2 FOR | AAATGTGCTGCTCCTTTCGTGAGC | $bla_{CTX-M-2}$ | This study |
| CTXM 2 REV | AGGGTTCGTTGCAAGACAAGACTG | | |
| ArmAf | TGCATCAAATATGGGGGTCT | *armA* | This study |
| ArmAr | GGATTGAAGCCACAACCAAA | | |
| aac(6′)-1b f | TTGCGATGCTCTATGAGTGGCTA | *aac(6′)-Ib* | 24 |
| aac(6′)-1b r | CTCGAATGCCTGGCGTGTTT | | |
| ABA-F | TTTGGCTATGATCCTATG | *aac(6′)-Iad* | 9 |
| ABA-R | CATGTCGAACAAGTACGC | | |
| APH F | ATACAGAGACCACCATACAGT | *aph(3′)-VI* | 36 |
| APH R | GGACAATCAATAATAGCAAT | | |
| gyrA-1 | AAATCTGCCCGTGTCGTTGGT | *gyrA* | 18 |
| gyrA-2 | GCCATACCTACGGCGATACC | | |
| parC-1 | AAACCTGTTCAGCGCCGCATT | *parC* | 18 |
| parC-2 | AAAGTTGTCTTGCCATTCACT | | |
| qnrA f | ATTTCTCACGCCAGGATTTG | *qnrA* | 28a |
| qnrA r | GATCGGCAAAGGTTAGGTCA | | |
| qnrB f | GATCGTGAAAGCCAGAAAGG | *qnrB* | 28a |
| qnrB r | ACGATGCCTGGTAGTTGTCC | | |
| qnrS f | ACGACATTCGTCAACTGCAA | *qnrS* | 28a |
| qnrS r | TAAATTGGCACCCTGTAGGC | | |

[a] M stands for A or T; R stands for A or G; W stands for A or T; K stands for G or T.

*A. baumannii*. For non-imipenem-susceptible isolates, a phenotypic screening test for metallo-β-lactamase production using sodium mercaptoacetic acid disks was also conducted (2).

**PFGE.** For pulsed-field gel electrophoresis (PFGE), the genomic DNA was digested with ApaI (New England Biolabs, Beverly, MA). The resultant fragments were then separated by PFGE using a temperature-controlled CHEF DR III system (Bio-Rad, Hercules, CA) as described previously (31). For PFGE pattern analysis, Bionumerics software, version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium), with the unweighted-pair group method using average linkages and the Dice setting for clustering analysis was applied. The genetic relatedness of isolates was determined by the criteria of Tenover et al. (33).

**PCR analyses for detection of resistance genes.** PCR analyses were performed for detection of various resistance genes in all isolates. A loopful of bacteria was taken from each fresh overnight culture on MH agar plates (BD Microbiology Systems), suspended in 1 ml of sterile water, and boiled for 10 min. After centrifugation, the supernatant was used as the template. Amplification was performed using a 9700 GeneAmp thermocycler (Applied Biosystems, Foster City, CA). The genes investigated included the carbapenemase genes $bla_{OXA-23}$, $bla_{OXA-40}$, $bla_{OXA-51}$, and $bla_{OXA-58}$; the cephalosporinase gene $bla_{ADC}$; potential extended-spectrum β-lactamase (ESBL) genes $bla_{TEM}$, $bla_{SHV}$, and $bla_{CTX-M}$; the 16S rRNA methylase gene *armA*; the *aac(6′)-Ib*, *aac(6′)-Iad*, and *aph(3′)-VIa* genes, encoding amikacin-modifying enzymes; and the plasmid-mediated quinolone resistance genes *qnrA*, *qnrB*, and *qnrS*. Select PCR products were sequenced by use of an ABI 3100 instrument (Applied Biosystems). For genes with negative results in PCRs in which no positive control was used [$bla_{OXA-40}$, $bla_{OXA-58}$, and *aac(6′)-Iad*], PCR amplifications were repeated at least twice. A negative control was run with every PCR. The quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes of 13 isolates (1 representative isolate for each of the 10 pulsotypes identified in this study and 3 ciprofloxacin-susceptible control isolates from outside the study) were also amplified by PCR and subjected to sequencing. The primers used for the PCR analyses are listed in Table 1.

**Transfer of $bla_{OXA-23}$.** *A. baumannii* HE130 (a clinical strain susceptible to carbapenems) and *Escherichia coli* DH10B were used as the recipients for transformation experiments. AB017 and AB026, two $bla_{OXA-23}$-positive isolates of pulsotype A, were used as the donor strains. The competent cells of the recipient strains were prepared and transformed by electroporation with plasmid DNA extracted from the donor strains by the standard alkaline lysis method. The transformants were selected on Luria-Bertani (LB) agar plates containing 2 μg/ml of meropenem.

**Cloning and sequencing of $bla_{OXA-23}$.** It has been demonstrated that OXA-23 confers high-level carbapenem resistance on *A. baumannii* when it is expressed under the control of a potent promoter provided by insertion sequence IS*Aba1* (7, 16). To examine if this is the case for our isolates, the genetic environment of $bla_{OXA-23}$ was investigated. Genomic DNA of *A. baumannii* AB017 was digested with XbaI and ligated with pBC-SK(−) (Stratagene, La Jolla, CA). *Escherichia coli* DH10B was then transformed with the ligated products by electroporation. Transformants that possessed recombinant plasmids carrying $bla_{OXA-23}$ were selected on LB agar plates containing chloramphenicol (25 μg/ml) and ampicillin (50 μg/ml). The DNA insert obtained was sequenced on both strands using custom sequencing primers.

**Nucleotide sequence accession numbers.** The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL/DDBJ database under accession no. EU594641.

TABLE 2. PCR results based on pulsotypes

| Pulsotype | Total no. of strains | No. (%) of strains positive for the indicated: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β-Lactamase gene | | | | | | | | Amikacin resistance gene | | | |
| | | $bla_{OXA-23}$ | $bla_{OXA-40}$ | $bla_{OXA-51}$ | $bla_{OXA-58}$ | $bla_{ADC}$ | $bla_{CTX-M-2}$ | $bla_{TEM}$ | $bla_{SHV}$ | *armA* | *aph(3′)-VIa* | *aac(6′)-Ib* | *aac(6′)-Iad* |
| A | 29 | 7 | 0 | 29 | 0 | 29 | 2 | 27 | 0 | 16 | 2 | 9 | 0 |
| B | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| C | 4 | 0 | 0 | 4 | 0 | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| D | 7 | 0 | 0 | 7 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 6 | 0 |
| E | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| F | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| G | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| I | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 49 | 8 (16.3) | 0 (0) | 48 (97.9) | 0 (0) | 40 (81.6) | 5 (10.2) | 36 (73.5) | 0 (0) | 17 (34.7) | 3 (6.1) | 16 (32.6) | 0 (0) |

## RESULTS

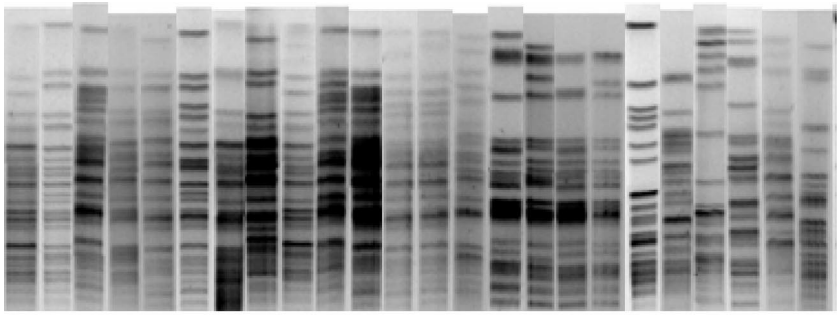
**Antimicrobial susceptibility of MDR *A. baumannii*.** A total of 142 unique *A. baumannii* isolates were identified at the clinical microbiology laboratory at UPMC during the study period. Of those, 65 isolates (45.8%) met the MDR criteria used in the present study. Among the 49 MDR isolates that were available for the study, rates of full resistance were as follows: 95.9% for ciprofloxacin, 87.8% for ceftazidime, 79.6% for cefepime, 40.8% for ampicillin-sulbactam, 18.4% for imipenem, 22.4% for meropenem, 36.7% for amikacin, 61.2% for tobramycin, 77.6% for gentamicin, and 79.6% for tetracycline. Overall, 8 out of the 49 isolates (16.3%) were resistant to six classes of antibiotics tested (ampicillin-sulbactam, ciprofloxacin, a cephalosporin, a carbapenem, tetracycline, and an aminoglycoside). None of the isolates was resistant to colistin or tigecycline. However, 55.1% showed tigecycline MICs between 1.5 and 2 μg/ml, which are interpreted as intermediate according to the BSAC breakpoints.

None of the non-imipenem-susceptible isolates gave a positive result with the phenotypic screening test for metallo-β-lactamase production.

**Molecular typing and clonal detection through PFGE.** A total of 10 pulsotypes that comprised genetically indistinguishable or closely related isolates were observed by PFGE. Pulsotype A was predominant, comprising 29 isolates identified at different time points during the study period. Pulsotypes B, C, and D were possibly related to pulsotype A. The other six pulsotypes (E through J) were all different from each other as well as from pulsotype A (Table 2; Fig. 1).

**β-Lactamase genes.** The carbapenemase gene $bla_{OXA-23}$ was identified in all eight isolates for which imipenem MICs were >32 μg/ml. In contrast, none of the isolates without $bla_{OXA-23}$ had imipenem MICs of >32 μg/ml (Tables 2 and 3). $bla_{OXA-40}$ and $bla_{OXA-58}$, the other frequently reported carbapenemase genes in *A. baumannii*, were not detected in any of the isolates. A $bla_{OXA-51}$-like gene was identified in most isolates. OXA-51-like oxacillinases constitute a group of β-lactamases with low-level catalytic efficiency for carbapenems (15). $bla_{ADC}$ genes, a group of cephalosporinase genes commonly found in *A. baumannii* and related species (17), were detected in 81.6% of the isolates. Only one isolate was susceptible to both ceftazidime and cefepime. Therefore, it was not possible to correlate cephalosporin resistance with the presence of $bla_{ADC}$.

In the investigation of ESBL genes, five isolates were found to encode $bla_{CTX-M}$, which was identified as $bla_{CTX-M-2}$, upon the sequencing of the entire structural gene (Table 2). These isolates were all resistant to cefepime but not necessarily to ceftazidime or ampicillin-sulbactam. $bla_{TEM}$ was detected in 36 isolates. Sequencing of select amplicons revealed that they encoded TEM-1. $bla_{SHV}$ was not identified in any of the isolates.



**A1 A2 A3 A4 A5 A6 A7 A8 A9 B1 B2 C1 C2 C3 D1 D2 D3 D4 E F G H I J**

FIG. 1. PFGE patterns of all pulsotypes identified in the study.

TABLE 3. MICs of imipenem in the presence or absence of $bla_{OXA-23}$

| $bla_{OXA-23}$ status (no. of isolates) | No. of isolates with a MIC (μg/ml) of: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | >32 | 32 | 24 | 16 | 8 | 6 | 4 | 3 | 2 | 1.5 | 1 | 0.75 | 0.5 | <0.38 |
| Positive (8) | 8 | | | | | | | | | | | | | |
| Negative (41) | | | 1 | | 3 | 1 | | 1 | 10 | 9 | 4 | 5 | 5 | 2 |

TABLE 4. MICs of amikacin in the presence or absence of *armA*

| *armA* status (no. of isolates) | No. of isolates with MICs (μg/ml) of: | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | >256 | 256 | 128 | 64 | 48 | 32 | 24 | 16 | 12 | 8 | 6 | 4 | 2 | <1 |
| Positive (17) | 17 | | | | | | | | | | | | | |
| Negative (32) | | | 1 | | 1 | | 2 | 3 | 5 | 9 | 4 | 4 | 3 | |

**Aminoglycoside resistance genes.** Eighteen isolates were resistant to amikacin. Among these, 17 were positive for *armA*, a 16S rRNA methylase gene (Table 2). All of the *armA*-positive isolates had amikacin MICs of >256 μg/ml (Table 4). The only amikacin-resistant, *armA*-negative isolate had an amikacin MIC of 128 μg/ml. This isolate did not yield positive PCR results for any of the three amikacin-modifying enzyme genes investigated. *aac(6′)-Ib*, an aminoglycoside acetyltransferase gene, was present in 16 isolates. The deduced amino acid sequences of select amplicons were consistent with AAC(6′)-Ib, but not with AAC(6′)-Ib-cr, which has been implicated in low-level resistance to fluoroquinolones. MICs of amikacin for *aac(6′)-Ib*-positive isolates ranged from 2 μg/ml to 12 μg/ml, except for eight isolates that were *armA* positive as well. Three isolates were positive for *aph(3′)-VIa*, an aminoglycoside phosphotransferase gene. Two of them were also *armA* positive. The only *aph(3′)-VIa*-positive, *armA*-negative isolate had an amikacin MIC of 12 μg/ml. None of the study isolates was positive for *aac(6′)-Iad*, the other aminoglycoside acetyltransferase gene implicated in amikacin resistance in *A. baumannii*.

**QRDRs and resistance genes.** The sequencing results for the QRDRs of *gyrA* and *parC*, encoding DNA gyrase and DNA topoisomerase IV, respectively, revealed the presence of S83L and S80L substitutions in the respective enzymes for all eight ciprofloxacin-resistant isolates sequenced. The QRDRs of the two ciprofloxacin-intermediate isolates in the study and of the three susceptible control isolates did not possess substitutions implicated in fluoroquinolone resistance in the amino acid sequence of either gene. In addition, the V101I substitution in *gyrA* was observed in all of the susceptible, intermediate, and resistant isolates; this likely represented a polymorphism that did not affect susceptibility to fluoroquinolones.

None of the isolates gave positive PCR results for the plasmid-mediated quinolone resistance gene *qnrA*, *qnrB*, or *qnrS*.

**Transfer of $bla_{OXA-23}$.** Both AB017 and AB026 yielded $bla_{OXA-23}$-positive transformants with *A. baumannii* HE130 as the recipient. Imipenem and meropenem MICs were 4 to 8 μg/ml and 6 to 12 μg/ml for the transformants compared with 0.5 μg/ml and 0.125 μg/ml for the recipient, respectively. No transformants could be obtained with *E. coli* DH10B as the recipient.

**Genetic environment of $bla_{OXA-23}$.** A cloning experiment yielded a recombinant plasmid with a 4.0-kb insert carrying $bla_{OXA-23}$. A schematic representation of its genetic environment is given in Fig. 2. As has been reported earlier for strains from Europe and East Asia, $bla_{OXA-23}$ was preceded by insertion sequence IS*Aba1*, encoding a transposase in the opposite orientation. Promoter sequences consisting of the −35 sequence (TTAGAA) and the −10 sequence (TTATTT), known to be responsible for the overexpression of β-lactamase genes located downstream of them (7, 16), were identified between 87 and 60 bp upstream of $bla_{OXA-23}$. However, the left inverted repeat of IS*Aba1* (i.e., the 5′ end of IS*Aba1*) was located closer to the 5′ end of $bla_{OXA-23}$ due to the presence of a 7-bp deletion compared with the corresponding sequence that was initially characterized in Tn*2006* from France (7). This particular deletion is also observed in sequences that have been submitted from several other countries (nucleotide accession no. AJ132105, EF120622, and EF016357). Furthermore, unlike Tn*2006*, the transposon identified in this study, tentatively designated Tn*2008*, was not part of a composite transposon. The sequences flanking Tn*2008* were distinct from those flanking Tn*2006*. An open reading frame with moderate identity to a putative DNA binding protein described in several *A. baumannii* genome sequences was identified downstream of Tn*2008* (Fig. 2).

## DISCUSSION

MDR *A. baumannii* has emerged as a substantial clinical problem worldwide (23, 25, 26). This trend has paralleled the overall increase in the prevalence of *Acinetobacter* spp., including *A. baumannii*, as nosocomial pathogens. For example, data from the National Nosocomial Infections Surveillance System indicate that the proportion of *Acinetobacter* spp. associated with pneumonia in intensive-care units increased from 4% in 1986 to 7% in 2003 in the United States (13). We recently reported the emergence of two MDR *A. baumannii* isolates that were highly resistant to both carbapenems and aminoglycosides due to production of both the OXA-23 carbapenemase and the ArmA 16S rRNA methylase, respectively (8). The present study was conducted to define the genetic basis of multidrug resistance in *A. baumannii* by using a larger set of isolates.

Approximately 20% of the MDR isolates were resistant to carbapenems. We observed a clear correlation between the presence of the OXA-23 gene and high-level carbapenem resistance (Table 3). Indeed, transfer of $bla_{OXA-23}$ to a susceptible strain led to an 8- to 96-fold increase in carbapenem MICs. This is in contrast to the other reports of carbapenem-resistant *A. baumannii* outbreak investigations in the United States, where carbapenem resistance was attributed to the production of the OXA-40 carbapenemase or reduced expression of outer membrane proteins in the absence of carbapenemase activities (21, 28). Production of OXA-23 is the most frequently encountered mechanism of carbapenem resistance in *A. baumannii* worldwide (26). The degree of resistance in our isolates was likely accentuated by the presence of strong promoter sequences provided by IS*Aba1*, leading to overproduction of the enzyme, as has been demonstrated with European strains (7). Of note, the origin of $bla_{OXA-23}$ was recently iden-

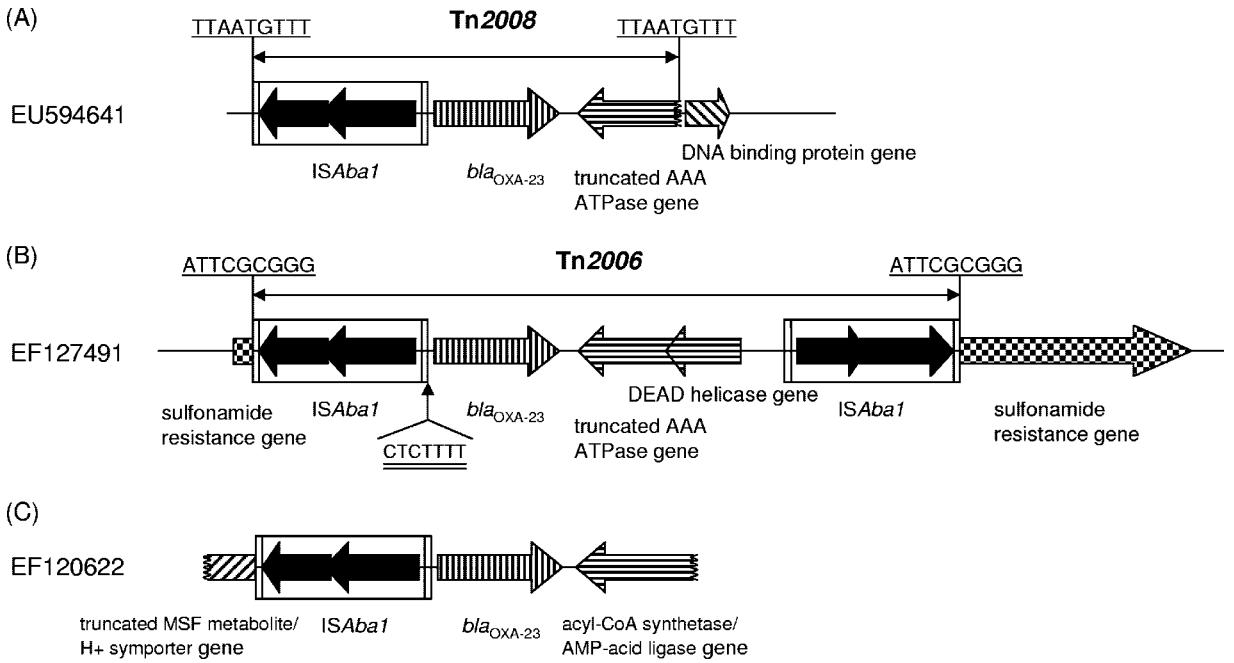



FIG. 2. Schematic representation of the genetic environments of $bla_{OXA-23}$ from the United States, France, and Turkey. (A) Strain AB017 (investigated in the present study); (B) strain AB13 (7); (C) strain AcKOU1 (GenBank accession no. EF120622). The boundaries of Tn*2006* and Tn*2008* are indicated, along with the target site duplications (underlined). The 7-bp difference in the site of insertion of IS*Aba1* for strain AB13 is double underlined. Target site duplication was not identified within the available sequence for strain AcKOU1.

tified as the chromosome of *Acinetobacter radioresistens*, a commensal species of the human skin (27). Taking this together with the fact that IS*Aba1* is commonly found in various *Acinetobacter* spp. (30), we may speculate that mobilization of $bla_{OXA-23}$ from *A. radioresistens* to *A. baumannii* occurs concurrently under selective pressure from carbapenems in different geographic areas. The diversity observed in the structures of transposons carrying IS*Aba1* and $bla_{OXA-23}$ in the United States and Europe supports this hypothesis. The likelihood that high-level carbapenem resistance may be acquired by *A. baumannii* through transposon-mediated gene transfer from a commensal organism underscores the importance of continued efforts to limit carbapenem use in order to retain susceptibility to these agents.

Resistance to amikacin was seen in nearly 40% of the study isolates. Most of the resistant isolates were highly resistant (MIC, >256 μg/ml), and this resistance coincided with the presence of the 16S rRNA methylase gene *armA* (Table 4). Among the series of acquired 16S rRNA methylases, ArmA appears to be the most common enzyme worldwide to date. Although *armA* was initially identified in 2002 (12), it has been shown that it was present in clinical isolates as early as 1997 (19). *armA* has been found mostly in *Enterobacteriaceae*, but its presence has been documented in *A. baumannii* as well, mostly in East Asia (20, 38). It is somewhat puzzling that we are observing an increasing number of *A. baumannii* isolates that produce ArmA, while the overall systemic use of aminoglycosides has decreased at our facility in the past several years (data not shown). One possibility is the presence of collateral selective pressure from other resistance genes that are located near *armA*. *armA* has been shown to be borne on an IS*26*-based composite transposon in *E. coli*, which likely plays a role in its mobilization (14). However, the only resistance genes contained in this transposon were *ant(3[dprime])-9*, *sul1*, and *dfrXII*, which confer resistance to streptomycin, sulfonamides, and trimethoprim, respectively. Preliminary sequencing of the genetic environment of our isolates revealed an identical structure at least in proximity to *armA* (8). We are currently conducting further investigations of the genetic environment of *armA* in order to clarify the mode of its mobilization in *A. baumannii*.

In contrast to the strong association observed between the presence of *armA* and high-level aminoglycoside resistance, including amikacin resistance, the roles of the amikacin-modifying enzyme genes *aac(6′)-Ib* and *aph(3′)-VIa* in amikacin resistance were less clear. One possible explanation, at least for *aac(6′)-Ib*, is that the gene is typically carried on integrons as a gene cassette lacking its own promoters, and thus its expression may be suboptimal depending on its distance from the common promoter sequences located at the 5′ conserved segment of the integrons (6).

All of the isolates were resistant or intermediate to ciprofloxacin. Resistance was associated with the typical substitutions in the QRDRs of DNA gyrase and DNA topoisomerase IV in all ciprofloxacin-resistant pulsotypes (34, 35). On the other hand, no plasmid-mediated quinolone resistance genes were detected in any of the isolates. These findings suggest that resistance to fluoroquinolone in MDR *A. baumannii* depends on target modification conferred by substitutions in the QRDRs. Given the very high rate of resistance, however, this class is unlikely to have any clinical role in the treatment of MDR *A. baumannii* at our medical center.

More than half of the MDR isolates in the study belonged to the same clonal type, i.e., pulsotype A, as evidenced by PFGE (Table 2). Indeed, seven of eight isolates that were resistant to six classes of antimicrobials belonged to this pulsotype and carried both $bla_{OXA-23}$ and *armA*. However, $bla_{OXA-23}$ and *armA* were also detected in different pulsotypes, indicating that these genes are likely disseminating among *A. baumannii* strains by means of horizontal transfer as well as clonal spread.

In conclusion, we have described the genetic basis of resistance in MDR *A. baumannii* at a tertiary medical center in Pennsylvania. Multidrug resistance was conferred predominantly by the production of OXA-23 carbapenemase, ArmA 16S rRNA methylase, and resistance substitutions in the QRDRs of DNA gyrase and DNA topoisomerase IV. As the use of salvage agents such as colistin and tigecycline to treat infections caused by these MDR organisms increases, close monitoring of susceptibility to these agents is also warranted.

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