Multicility Outbreak of Carbapenem-Resistant Acinetobacter baumannii Isolates Producing the Carbapenemase OXA-40

Karen Lolan,1 Thomas W. Rice,1 L. Silvia Munoz-Price,2 and John P. Quinn1,3*

John Stroger Hospital, Chicago, Illinois1; Munster Community Hospital, Munster, Indiana,2 and Rush University Medical Center, Chicago, Illinois3

Received 27 January 2006/Returned for modification 17 April 2006/Accepted 18 June 2006

During 2005 we detected a multicility outbreak of infections or colonization due to high-level imipenem-resistant Acinetobacter baumannii (MIC, 64 μg/ml). One hundred isolates from diverse sources were obtained from seven acute-care hospitals and two extended-care facilities; 97% of the isolates belonged to one clone. Susceptibility testing of the first 42 isolates (January to April 2005) revealed broad resistance profiles. Half of the isolates were susceptible to ceftazidime, with many isolates susceptible only to colistin. The level of AmpC β-lactamase expression was stronger in isolates resistant to ceftazidime. PCR and subsequent nucleotide sequencing analysis identified blaOXA-40. The presence of an OXA-40 β-lactamase in these isolates correlated with the carbapenem resistance. By Southern blot analysis, a blaOXA-40-specific probe revealed that the gene was both plasmid and chromosomally located. This is the first time in the United States that such carbapenem resistance in A. baumannii has been attributable to a carbapenemase.

Acinetobacter baumannii is recognized as an increasingly important opportunistic gram-negative pathogen frequently associated with nosocomial outbreaks worldwide (33). Acinetobacter infections may pose treatment difficulties, as nosocomial isolates are typically resistant to a wide variety of antimicrobials. This problem is compounded by the increasing rates of resistance to broad-spectrum antibiotics detected in Acinetobacter (11, 19). The carbapenem-resistant A. baumannii (11, 19). The carbapenem-resistant isolates are typically resistant to a wide variety of antimicrobials. This problem is compounded by the increasing rates of resistance to broad-spectrum antibiotics detected in Acinetobacter (11, 19). The carbapenem-resistant A. baumannii isolates (94 individual patient isolates and 6 environmental isolates) were collected from multiple facilities in Chicago and northwest Indiana. Isolates of an imipenem-susceptible A. baumannii clone, ACB1, were included as control strains in isoelectric focusing (IEF), conjugation, and transformation experiments. This endemic clone had been present for at least 6 years in the primary participating institution (center A).

Bacterial identification and susceptibility testing. Clinical isolates were identified and antimicrobial susceptibilities were determined by the automated system used at each participating institution (Microscan [Dade Behring Inc., Deerfield, IL] and Vitek [bioMerieux Vitek, Inc., Hazelwood, MO]). In vitro susceptibilities were determined for the first 42 IRAB isolates by standard broth microdilution methodology following CLSI (formerly NCCLS) guidelines (6). Testing of susceptibility to tigecycline was performed with medium that was no greater than 12 h old (6). Susceptibilities were interpreted according to CLSI guidelines (6), when available. For colistin, the resistance breakpoint was defined as an MIC ≥4 μg/ml (10). No tigecycline interpretive criteria currently exist for A. baumannii; hence, the Food and Drug Administration-approved breakpoints for members of the family Enterobacteriaceae of ≤2, 4, and ≥8 μg/ml (designating tigecycline susceptible, intermediate, and resistant, respectively) (Michael Dowzicky [Wyeth Research], personal communication) were applied as provisional MIC breakpoints.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared from all isolates by the method of Matushek et al. (23). A contour-clamped homogeneous electric field mapper system (Bio-Rad Laboratories, Richmond, CA) was used to analyze SmaI-digested DNA at a voltage of 6 V/cm at 14°C for 21 h, with pulse times linearly ramped from 1 to 20s. Strain relatedness was determined according to the criteria of Tenover et al. (31).

Isoelectric focusing. Bacterial lysates were prepared as described previously (26). Briefly, overnight cultures were grown in 10 ml Trypticase soy broth (containing imipenem [10 μg/ml] for imipenem-resistant isolates), sonicated, and resuspended in 1 ml 0.1 M phosphate buffer, pH 7.0. Identification of β-lactamase bands was facilitated by overlaying the gel with nitrocefin (Becton Dickinson, Sparks, MD). The gels were overlaid with 0.3 mM cloxacillin in 0.1 M phosphate buffer, pH 7.0, prior to nitrocefin development to identify AmpC-type β-lactamas (28). PCR amplification of β-lactamase genes. The isolates were investigated for the presence of blaOXA genes by using primers specific for the OXA-23-, OXA-40-, and OXA-58-type oxacillinases, as described by Heritet et al. (15), and using a genomic DNA template. To verify the presence of blaTEM primers JP2 (5'-T

* Corresponding author. Mailing address: John Stroger Hospital, 1900 W. Polk, Rm. 1258, Chicago, IL 60612. Phone: (312) 864-4874. Fax: (312) 864-9522. E-mail: ESBLman@yahoo.com.
TGAAGACGAGAAGGGCTCTGTG3′) (35) and blaβ (5′-TATGATGAACACT TGGTCGTGACG3′) (30) (designed at nucleotides 1091 to 1068) were used to amplify a 1,085-bp fragment.

Cloning and sequencing of blaOXA gene. The amplification products from the OXA-40 PCR were purified with a QIAquick PCR purification kit (QIAGEN, Valencia, CA), cloned into plasmid pCR-XL-TOPO, and transformed into chemically competent Escherichia coli TOP10 cells by heat shock, as described in detail by the manufacturer of the TOPO XL PCR cloning kit (Invitrogen, Carlsbad, CA). Transformants were screened on kanamycin (50 μg/ml)-containing plates. Recombinant plasmid DNA was isolated with a QIAfilter Midi plasmid preparation kit (QIAGEN); and both strands of the insert were sequenced by ACGT, Inc. (Wheeling, IL), by using primers M13R and M13F (−20) primers.

Hybridization studies. To determine the location of the β-lactamase gene, chromosomal and plasmid DNA were evaluated in two representative isolates of the ACB20 pulsotype. A chromosomal location was investigated by using the endonuclease I-CeuI (New England Biolabs, Beverly, MA), which digests a 26-bp sequence in rnr genes for the 23S large-subunit rRNA (18). Whole-cell DNA was digested with this enzyme and the resulting fragments were separated by PFGE with pulse times of 5 to 60 s. The sizes of the fragments generated with I-CeuI were determined by comparison with those of a bacteriophage lambda DNA molecular weight marker (Cambrex Bio Science, Rockland, ME). Southern transfer was performed with a positively charged nylon membrane (Roche, Indianapolis, IN), as described previously (26), and hybridized to probes specific for blaOXA-40 or 16S rRNA genes, under conditions of high stringency. The probes consisted of the entire blaOXA-40 (generated with primers OXA-40A and OXA-40B [13] and 16S rRNA genes (generated with universal primers A and B [21]) amplified by PCR and labeled with digoxigenin (Roche). Extraction of plasmid DNA was performed by using QIAfilter Midi kit (QIAGEN), and similar hybridization experiments for blaOXA-40 by using uncleaved plasmid DNA were performed. Imipenem-susceptible ACB1-type isolates were included as controls.

Transfer of resistance. Transfer of resistance by liquid and solid mating assays was attempted by using rifampin-resistant E. coli C600 or ACB1-type A. baumannii. Transconjugant screening used plates containing rifampin (50 μg/ml) plus ticarcillin (50 μg/ml) for E. coli C600 or ceftazidime (100 μg/ml) plus imipenem (10 μg/ml) for ACB1-type A. baumannii. A plasmid extract of an ACB20-type A. baumannii isolate was also used to transform chemically competent cells prepared from E. coli DH5α and an ACB1-type A. baumannii (29), with selection with imipenem (1 μg/ml) for E. coli and imipenem (10 μg/ml) for A. baumannii.

RESULTS

Beginning in January 2005, a large acute-care hospital (center A) in Chicago, IL, detected as part of routine hospital surveillance an increasing incidence of IRAB compared with that in the previous months. The isolates were characterized by a multiresistance profile that included, in addition to resistance to imipenem, resistance to aminoglycosides (amikacin, tobramycin, and gentamicin), fluoroquinolones, and trimethoprim-sulfamethoxazole. These IRAB isolates also represented the first significant occurrence of high-level imipenem resistance (MIC ≥ 64 μg/ml) identified since surveillance had begun at that facility in 1999. Simultaneously, surrounding institutions were independently observing an increase in the occurrence of IRAB. As part of a collaborative effort, resistant strains were referred from six additional acute-care hospitals and two extended-care facilities in the surrounding Chicago area and from northwest Indiana applying surveillance criteria of any Acinetobacter baumannii or A. baemolyticus isolate resistant to imipenem, according to the clinical laboratory report. These referrals did not represent a comprehensive submission of all IRAB isolates from the collaborating facilities. From among the eight institutions, 100 isolates (94 individual patient isolates and 6 environmental isolates) with verified imipenem resistance were collectively recovered between January 2005 and November 2005.

The first 36 clinical IRAB isolates were obtained from a variety of sources, including sputum (n = 10 isolates), wounds (n = 8 isolates), catheter tips (n = 5 isolates), blood (n = 4 isolates), urine (n = 3 isolates), fluid (n = 2 isolates), bone (n = 1 isolate), and other sources (3 isolates). In addition, two of the eight centers performed environmental sampling. Six isolates were recovered from various sites, including beds, room equipment (intravenous pump, settle plate, electrocardiograph cables), and a hemodialysis catheter; and these all displayed antibiograms identical to those of the associated patient isolates.

These 42 IRAB isolates all displayed unusually high-level carbapenem resistance (imipenem and meropenem MICs, 64 to 128 μg/ml) (Table 1). Half of the isolates were ceftazidime resistant and susceptible (imipenem-resistant0.25 0.5 1 2 4 8 16 32 64 128 256 >256 No (%) resistant

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/ml)</th>
<th>No (%) resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>39</td>
<td>42 (100)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>42</td>
<td>1 13 7 21 (50)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>1 9 12</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Colistin</td>
<td>1 39 2</td>
<td>24 (57)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2 16 19 5</td>
<td>9 (5.9)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>9 29 4</td>
<td>41 (98)</td>
</tr>
<tr>
<td>Amp/sulb</td>
<td>1 8 33</td>
<td>42 (100)</td>
</tr>
</tbody>
</table>

a Boldface type indicates isolates resistant to individual antibiotics according to CLSI criteria, when available. The tigecycline resistance breakpoint was defined as an MIC ≥ 8 μg/ml.

b Amp/sulb, ampicillin-sulbactam, tested at a penicillin/inhibitor ratio of 2:1.

PFGE analysis of all 100 isolates identified only two different A. baumannii clones, designated herein as ACB17 and ACB20. The ACB20 clonal type clearly predominated, accounting for 97% of the isolates. The isolates from the environmental samples were of the same clonal type, ACB20, as the related patient isolate from that corresponding room. These results recognized a widespread geographic distribution of this predominant clonal type. In the course of hospital surveillance at center A, all multidrug-resistant A. baumannii isolates, independent of their imipenem susceptibilities, are subjected to PFGE analysis. As a result, a number of imipenem-susceptible ACB17 isolates have been recovered; however, no imipenem-susceptible ACB20 isolates have been found.

Characterization of β-lactamases by isoelectric focusing was performed with representatives of the ACB17 clone (both imipenem resistant and susceptible) and the ACB20 clone, as well as isolates of the endemic, imipenem-susceptible clone, ACB1. As outlined in Table 2, IEF revealed that all isolates tested produced a pI 5.4 β-lactamase. This pI is suggestive of a TEM-type enzyme, the identity of which was supported by a positive TEM PCR result. Likewise, all isolates produced a pI 9.1 cloxacillin-inhibitable band, consistent with the chromosomal AmpC of A. baumannii (5). The AmpC band intensity was stronger in all isolates displaying ceftazidime resistance than in
TABLE 2. IEF and PCR results for representatives of the two
A. baumannii clones, ACB17 and ACB20, compared to those
for the imipenem-susceptible clone, ACB1

<table>
<thead>
<tr>
<th>Antibiogram profile</th>
<th>PFGE type</th>
<th>β-Lactamases present by IEF</th>
<th>OXA-40 PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pI 9.1</td>
<td>pI 8.8</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB1</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB1</td>
<td>+++</td>
<td>No</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB17</td>
<td>+++</td>
<td>No</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB17</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB20</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>Caz* Imi*</td>
<td>ACB20</td>
<td>+++</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Caz, ceftazidime; Imi, imipenem.
* pI 9.1, consistent with AmpC by cloxacillin inhibition; +, weaker; ++++, stronger band intensity.
* pI 5.4, consistent with a TEM-type enzyme.
* ND, not determined.

The ceftazidime-susceptible isolates. All IRAB isolates produced an additional β-lactamase with a pI of 8.8, indicating that the production of this β-lactamase might be responsible for the carbapenem resistance.

PCR was performed with primers for the \( \text{bla}_{OXA-23} \), \( \text{bla}_{OXA-40} \), and \( \text{bla}_{OXA-58} \)-related oxacillinases. A ca. 830-bp fragment was amplified from imipenem-resistant isolates of both the ACB17 and ACB20 clones with primers for \( \text{bla}_{OXA-40} \)-like genes (Table 2). No PCR amplification was detected for the imipenem-susceptible isolates of clones ACB1 and ACB17, consistent with the absence of the pI 8.8 β-lactamase on IEF. As the ACB20 clone showed a clear predominance in this outbreak, its amplification product was cloned into pCR-XL-TOPO, and both strands of the insert were sequenced. The sequences obtained were 100% homologous from nucleotides 20 to 802 of the sequence with GenBank accession no. AF509241 (13), consistent with \( \text{bla}_{OXA-40} \).

Both plasmid and chromosomal locations were investigated for the \( \text{bla}_{OXA-40} \) gene in the ACB20 clone. The entire ca. 830-bp OXA-40 PCR amplicon was labeled with digoxigenin and used as the probe in hybridization experiments. Total DNAs of two representative ACB20-type isolates and an ACB1-type A. baumannii isolate were digested with the I-CeuI endonuclease and hybridized successively with 16S rRNA and OXA-40 probes. After digestion with the enzyme, the DNA from these ACB20 A. baumannii isolates resolved nine fragments, six of which subsequently hybridized with the 16S rRNA-specific probe (Fig. 1A and B), identifying these as chromosomal fragments. The \( \text{bla}_{OXA-40} \)-specific probe cohybridized with two of those six bands, an ~450-kb fragment and an ~500-kb fragment (Fig. 1C), indicating two chromosomal locations for \( \text{bla}_{OXA-40} \) in ACB20-type isolates.

Plasmid DNA was extracted from the same ACB1- and

![FIG. 1. Localization of \( \text{bla}_{OXA-40} \) gene in I-CeuI-generated chromosome fragments of ACB20 pulsotype A. baumannii separated by PFGE. (A) Electrophoretic pattern after I-CeuI digestion; (B) hybridization with a probe specific for rRNA genes; (C) hybridization with a probe specific for \( \text{bla}_{OXA-40} \) gene. Lanes: 1, imipenem-susceptible ACB1 type; 2 and 3, imipenem-resistant ACB20 types. Marker sizes are indicated to the left.](http://aac.asm.org/)
ACB20-type isolates used in the I-CeuI experiments. While electrophoretic separation of uncut plasmid DNA detected only a single band in the ACB1 isolate, multiple bands were present in the ACB20 isolates. Under conditions of high stringency, the \( \text{bla}_{\text{OXA-40}} \)-specific probe gave a positive signal with plasmid bands (Fig. 2B). Collectively, the results indicate both chromosomal and plasmid locations for \( \text{bla}_{\text{OXA-40}} \) in these ACB20-type \( A. \ baumannii \) isolates. Attempts to transfer resistance by conjugation or transformation of plasmid DNA into either an \( A. \ baumannii \) or an \( E. \ coli \) recipient were unsuccessful.

**DISCUSSION**

Outbreaks of OXA-type carbapenemases have occurred globally but arise only sporadically. Brazil, French Polynesia, Spain, Southern Europe, the Balkans, Turkey, Korea, and Argentina have reported outbreaks of various OXA-type carbapenemases (2, 7, 8, 12, 16, 20, 22, 24). Prior to this study, reports of outbreaks due to OXA-40 enzymes had been limited to the Iberian Peninsula (8, 20), and no carbapenemases have previously been described in \( A. \ baumannii \) isolates from North America.

The atypical phenotype of ceftazidime susceptibility yet imipenem resistance identified in half of the isolates studied hinted to an unusual type of resistance mechanism. The work of Urban and colleagues (32) had previously noted that multidrug-resistant *Acinetobacter* strains exhibited ceftazidime resistance before a progression to becoming carbapenem resistant. In their case, the chromosomal AmpC cephalosporinase, facilitated by the selective pressure of \( \beta \)-lactam use, conferred resistance to cephalosporins. This AmpC, in combination with porin protein mutations, can also confer low-level resistance to the carbapenems (32). In this outbreak, high-level carbapenem resistance was conferred irrespective of the ceftazidime susceptibility, implicating an alternative scenario. Production of these carbapenem-hydrolyzing oxacillinases constitutes an additional mechanistic pathway that reduces the utility of the carbapenems. A surveillance study of *Acinetobacter* strains isolated from hospitalized patients in the United States between 1998 and 2001 found that 92.5% of the isolates were susceptible to imipenem, with the carbapenem class remaining the most active agents overall against *Acinetobacter* (17). With this regional carbapenem resistance due to the clonal spread of OXA carbapenemase-producing \( A. \ baumannii \), the clinical and epidemiological risk factors associated with the emergence of this outbreak are the subject of an active investigation.

Presently, treatment options for infections caused by members of the genus *Acinetobacter* are limited. Colistin has become one of the most commonly used antibiotics for the treatment of imipenem-resistant gram-negative rods. All of our isolates proved to be susceptible to this agent; however, the risk for nephrotoxicity is of clinical concern. The recently approved antibiotic tigecycline has been demonstrated to have activity against \( A. \ baumannii \). A Spanish surveillance study identified tigecycline as the most active agent after polymyxin B (1), while others have reported that it has good in vitro activity against \( A. \ baumannii \), even against isolates resistant to imipenem (27). However, the majority of these isolates were defined as tigecycline nonsusceptible (on the basis of the provisional breakpoint), thereby placing in question the utility of this agent for these cases.

Our evidence for the chromosomal location for \( \text{bla}_{\text{OXA-40}} \) agreed with the single report that had previously investigated its location (13), and data from the molecular typing of all \( A. \ baumannii \) isolates at center A showing a lack of detection of any imipenem-susceptible ACB20-type *Acinetobacter* strain is also consistent with this. Chromosomal mediation has similarly been demonstrated for the other members of the subgroup, OXA-24, -25, and -26 (3). The simultaneous finding of plasmid bands hybridizing with the \( \text{bla}_{\text{OXA-40}} \)-specific probe was unexpected, although genes for OXAs from other subgroups, OXA-23 and OXA-58, are reported to be plasmid mediated (3). Further genetic analysis is needed to definitively identify each individual gene hybridizing with the \( \text{bla}_{\text{OXA-40}} \)-specific probe.

Reports of these carbapenem-hydrolyzing oxacillinases are still relatively rare, as detection may be limited by the overall weak hydrolytic activity of this family of enzymes (13). However, there is a growing global recognition that OXA \( \beta \)-lactamases contribute to carbapenem resistance in \( A. \ baumannii \).

**ACKNOWLEDGMENTS**

We thank Susan Monahan, Robert Hayes, Irene Dusich, Jim Clark, Sunita Mohapatra, David Hines, Vato Bochorishvili, Richard Yu,
Daniel Smith, and Dan Johnson for providing isolates as part of our collaborative effort. This work was funded in part by Merck Research Laboratories, Whitehouse Station, NJ, and by the Chicago Infectious Disease Research Institute, Chicago, IL.

REFERENCES


