Dissemination of imipenem-resistant Acinetobacter baumannii strains carrying the ISAb1–blaOXA-23 genes in a Chinese hospital

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An outbreak of 95 clinical infections with imipenem-resistant Acinetobacter baumannii in a Chinese hospital was investigated and the carbapenemase-encoding genes and their relationship with ISAb1 of these and a further 16 isolates recovered from the intensive care unit (ICU) environment were analysed. Almost all isolates were resistant to a wide range of antimicrobials; the lowest resistance rates were found for polymyxin E (17.1 %), cefoperazone/sulbactam (30.6 %) and ampicillin/sulbactam (67.6 %). Six pattern types defined by DNA macrorestriction patterns were distinguished among the clinical isolates with dissemination of pattern A (50 isolates) to patients in seven hospital units and pattern B (35 isolates) to eight units; the environmental isolates from ICUs were also of pattern A. All isolates were positive for the blaOXA-46 and blaOXA-23 genes. The OXA-23-encoding gene was located 34 bp downstream of ISAb1. No plasmids were detected and conjugal transfer of resistance was not demonstrated. The blaOXA-23 probe hybridized with 200 and 220 kb Apal chromosomal fragments for type patterns A and B, respectively.

INTRODUCTION

Acinetobacter baumannii is an increasingly important nosocomial pathogen (Bergogne-Bérizé & Towner, 1996), causing a wide range of infections, some serious, in patients in intensive care units (ICUs) (Chastre & Trouillet, 2000). The species can survive for long periods in the hospital environment and is a frequent cause of outbreaks of nosocomial infection, which are often difficult to control (Towner, 2000). Effective treatment is compromised by the high level of resistance to antimicrobials exhibited by hospital strains. Indeed, the emergence of carbapenem resistance in A. baumannii has become of global concern, as these β-lactams are often the only active agents against many multiresistant strains (Brown & Amyes, 2006). These agents are being compromised by the emergence of carbapenem-hydrolysing β-lactamasmes (carbapenemases) of molecular classes B and D (Livermore, 2002). Class B carbapenemases (various IMP-type, VIM-type and SIM-1 metallo-β-lactamases) have been found in Acinetobacter spp., but worldwide most A. baumannii strains are resistant as a result of the production of OXA-type carbapenemases (Afzal-Shah et al., 2001). Four of the eight known clusters of OXA-type carbapenemases have been identified in A. baumannii, namely OXA-23, OXA-24, OXA-51 and OXA-58 (Brown et al., 2005; Walther-Rasmussen & Hoiby, 2006). The genes encoding OXA-23-like and OXA-51-like enzymes have been found to be linked to ISAb1, with the OXA-58-like encoding gene adjacent to ISAb2, ISAb3 and IS18 (Turton et al., 2006; Poirel & Nordmann, 2006a).

Over a 17-month period, we observed the dissemination of imipenem-resistant A. baumannii isolates in clinical infections in several units of a university hospital in China. We describe here the molecular epidemiology of the isolates and the identification of the genes encoding resistance to carbapenems.

METHODS

Bacterial isolates. Ninety-five non-repetitive imipenem-resistant isolates of A. baumannii were recovered from clinical infections in hospitalized patients from August 2003 to December 2004 in the First Affiliated Hospital, College of Medicine, Zhejiang University, China. The patients were distributed among ten clinical units, with the majority in the transplant unit (35.6 %) and ICU (18.9 %) (Table 1). An additional 16 A. baumannii isolates were recovered from the environment of the ICU (bed sheets, desks, floors, computer keyboards and mouse, and healthcare workers’ hands) in December 2004. All isolates were assigned to the Acinetobacter calcoaceticus–A. baumannii complex using a Vitek GNI® card (bioMérieux) and species identification was confirmed by sequence analysis of the 16S–23S rRNA gene intergenic spacer region (Chang et al., 2005).
Table 1. Distribution of DNA pattern types of imipenem-resistant A. baumannii in clinical units

<table>
<thead>
<tr>
<th>DNA type</th>
<th>Transplant unit</th>
<th>ICU</th>
<th>EICU</th>
<th>Kidney unit</th>
<th>Medical dept</th>
<th>Respiratory unit</th>
<th>VIP unit</th>
<th>Neurology unit</th>
<th>GI unit</th>
<th>Vascular unit</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>23</td>
<td>16</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>2</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>2</td>
<td>35</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td>2</td>
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<td>6</td>
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<tr>
<td>E</td>
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<td>12</td>
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<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>95</td>
</tr>
</tbody>
</table>

Susceptibility testing. The MICs of nine antimicrobial agents for all isolates were determined by the agar dilution technique on Mueller–Hinton agar plates containing serially twofold-diluted agents following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). The agents tested were imipenem (MerckKGAa), meropenem (Sumitomo Pharmaceuticals), ampicillin/sulbactam, cefoperazone/sulbactam, piperacillin/tazobactam, polymyxin E, minocycline and trimethoprim/sulfamethoxazole, was determined by Etest (AB Biodisk). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls. Results were interpreted according to published recommendations (CLSI, 2006).

PFGE. DNA was prepared from isolates and cleaved with 40 U Apal (Sangon) as described previously (Gouby et al., 1992). Electrophoresis was performed on a 1 % agarose gel (Sangon) in 0.5 M Tris/borate/EDTA buffer on a CHEF-Mapper XA PFGE system (Bio-Rad) for 22 h at 14 °C, with run conditions of 6 V cm⁻¹, a pulse angle of 120° and pulse times from 5 to 20 s. A λ DNA ladder (Amersham Biosciences) was used as molecular mass marker and bands were stained with ethidium bromide (0.5 mg l⁻¹) and photographed under UV light. Band profiles were interpreted by the criteria of Tenover et al. (1995).

Isolation and characterization of β-lactamases. Crude β-lactamase extracts from bacterial suspensions were prepared by sonication (Yu et al., 2004) and plts were determined by electrophoresis in a PhastSystem (Amersham Pharmacia Biotech) following staining with nitrocefin (Oxoid). Reference standard proteins were stained with Coomassie brilliant blue R-250. An OXA-23-producing A. baumannii isolate was used as a positive control (Yu et al., 2004). Patterns were analysed according to published recommendations (CLSI, 2006).

Detection of metallo-β-lactamases. An imipenem/EDTA double disc synergy test was performed according to Lee et al. (2003). IMP-producing A. baumannii (Chu et al., 2001) and VIM-2-producing P. aeruginosa (Yu et al., 2006) were used as positive controls.

PCR amplification of the carbapenemase-encoding gene. The PCR system and parameters used have been described previously (Jeon et al., 2005; Lee et al., 2005; Woodford et al., 2006). The primers used to amplify the IMP-like, VIM-like, SIM-1, blaOXA-23-like, blaOXA-24-like, blaOXA-58-like and blaOXA-51-like genes are listed in Table 2. PCR mapping experiments using combinations of the ISAbat1 primers and the blaOXA-23-like and blaOXA-51-like reverse primers were performed to detect the ISAbat1/blaOXA-23-like, ISAbat1/blaOXA-58-like and ISAbat1/blaOXA-51-like/ISAbat1 sequences.

Sequencing of PCR products. Purified PCR products were cloned into the pGEM-T Easy vector (Promega) according to the manufacturer’s instructions. The resultant product was transformed into competent E. coli DH5α cells and selected on MacConkey agar supplemented with 50 μg ampicillin ml⁻¹. Plasmid DNA was prepared with the aid of a plasmid extraction kit (Sangon), digested with EcoRI (Sangon) and sequenced with an ABI 3730 automatic sequencer using the Sanger chain-termination method. Sequences were analysed using the BLAST network service of the National Center for Biotechnology Information.

Conjugation and plasmid extraction. Conjugation experiments were performed as described previously (Yan et al., 2000), with rifampicin-resistant E. coli 600 as the recipient. Mueller–Hinton plates supplemented with rifampicin (512 μg ml⁻¹) and imipenem (2 μg ml⁻¹) were used to select for transconjugants. A Qiagen Plasmid Midi kit was used to extract plasmids of imipenem-resistant A. baumannii strains.

Southern blot hybridization analysis of the OXA-23 gene. For Southern blot hybridization, plasmids and Apal-digested PFGE genomic DNA were transferred from electrophoreses gels to nylon membranes (Bio-Rad) and hybridized with [32P]dCTP (DuPont)-labelled OXA-23 gene fragments. After washing, the membrane was compressed with a storage phosphor screen (Kodak, Japan) for 48 h and then scanned for hybridization reactions.

RESULTS

A total of 1018 A. baumannii isolates were recovered from various specimens in 2003, and 17.6 % were resistant to imipenem. Specimens from which A. baumannii was isolated comprised respiratory secretions (53.7 %), blood (20 %), urine (10.5 %), sterile fluids and cather tips (8.4 %), and wounds (7.4 %).

Antibiotic susceptibility

All of the imipenem-resistant isolates of A. baumannii exhibited high resistance rates (>95 %) to the penicillins, cephalosporins, extended-spectrum cephalosporins, carbapenems, monobactams, quinolones, minocycline and aminoglycosides. The resistance rates for cefoperazone/sulbactam and ampicillin/sulbactam were 30.6 and 67.6 %, respectively, and the most active agent was polymyxin E with 17 % resistance.
Molecular typing

The 95 clinical isolates were grouped into six clonal patterns by PFGE. The two predominant patterns, A represented by 50 isolates and B by 35 isolates, exhibited slight variation (between one and three band differences) within each profile, but this was insufficient to distinguish subtypes confidently within the profile. Pattern C was represented by six isolates, pattern D by two isolates and patterns E and F by single strains (Fig. 1 and Table 1).

The first isolates of pattern A were from the sputum of a patient in the ICU in August 2003 and this type first appeared in the transplant unit in October 2003; the ICU environmental isolates were also of pattern A. Clonal pattern B first appeared in an ICU patient in October 2003 and pattern C in the ‘emerging’ ICU ward in December 2003. The remaining types were isolated in the following 6 months.

Carbapenemase of A. baumannii

All isolates were negative by the imipenem/EDTA double disc synergy test. Isoelectric focusing analysis revealed the presence of a single band of pI 6.64, which is identical to the reported value for OXA-23 (Donald et al., 2000). All isolates were positive by PCR for blaOXA-23-like and blaOXA-31-like genes, and cloning and sequencing confirmed that the PCR products were 100% identical to the OXA-23 and OXA-66 genes (GenBank accession nos AY795964 and AY949204). All isolates were positive for ISAba1/blaOXA-23-like but negative for ISAba1/blaOXA-31-like and blaOXA-51-like/ISAba1 genes (Fig. 2). No IMP-like, VIM-like, SIM-1, OXA-58-like or OXA-24-like genes were detected.

Repeated attempts to transfer the OXA-23-encoding gene by conjugation failed and no plasmids were detected or hybridized with the blaOXA-23 probe. However, chromosomal

Table 2. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Target</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>OXA-23-likeF</td>
<td>GATGTGTCACTAGTATTCGTCGT</td>
<td>blaOXA-23-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>OXA-23-likeR</td>
<td>TCAACAACCTAAAAAGCACTG</td>
<td>blaOXA-23-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>OXA-24-likeF</td>
<td>ATGAAAAATATTATCCTATATCAG</td>
<td>blaOXA-24-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>OXA-24-likeR</td>
<td>TATTTGTTGATCGGCTT</td>
<td>blaOXA-24-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>OXA-51-likeF</td>
<td>TAATGCTGTAGGCGGCTT</td>
<td>blaOXA-51-like</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>OXA-51-likeR</td>
<td>TGAGTTGACATTTCTATG</td>
<td>blaOXA-51-like</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>OXA-58-likeF</td>
<td>AAGTTATGCGGGCTGTCG</td>
<td>ISAba1/blaOXA-23-like</td>
<td>This study</td>
</tr>
<tr>
<td>OXA-58-likeR</td>
<td>CCCCTGTGGCGCTTCATAC</td>
<td>ISAba1/blaOXA-31-like</td>
<td>This study</td>
</tr>
<tr>
<td>ISAba1F1</td>
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<td>ISAba1/blaOXA-51-like</td>
<td>This study</td>
</tr>
<tr>
<td>ISAba1F2</td>
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<td>This study</td>
</tr>
<tr>
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<td>blaOXA-51-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>ISAbaiR3</td>
<td>CACATTCAAATAACGTCAA</td>
<td>blaOXA-51-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>IMP-likeF</td>
<td>CATGGTTTGTGGCTTGT</td>
<td>blaIMP-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>IMP-likeR</td>
<td>ATAATTTGCGCFFACTTGT</td>
<td>blaIMP-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>VIM-likeF</td>
<td>ATGGGCTCATTTGGACGGCTG</td>
<td>blaVIM-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>VIM-likeR</td>
<td>TGCTACTAACAGCGACGTG</td>
<td>blaVIM-like</td>
<td>Jeon et al. (2005)</td>
</tr>
<tr>
<td>SIM-1F</td>
<td>TACAAGGGATCGCGCATG</td>
<td>blaSIM-1</td>
<td>Lee et al. (2003)</td>
</tr>
<tr>
<td>SIM-1R</td>
<td>TAATGGCCGCTTGGCATG</td>
<td>blaSIM-1</td>
<td>Lee et al. (2003)</td>
</tr>
</tbody>
</table>

Fig. 1. ApaI-digested PFGE patterns of selected imipenem-resistant A. baumannii strains. Strains 58, 97, 19, and 109 are pattern A; strains 135 and 134 are pattern B; strain 108 is pattern C; strain 131 is pattern D; and strain 129 is imipenem susceptible. M, λ DNA ladder marker.
PFGE patterns A and B, respectively (Fig. 3), which is indicative of a chromosomal location for the \( \text{bla}_{\text{OXA-23}} \) gene of \( A. \text{baumannii} \) in our hospital.

**DISCUSSION**

Carbapenems have the most extended antimicrobial spectrum of antibacterial activity among all of the \( \beta \)-lactams. However, carbapenem resistance is emerging and increasing in clinical isolates, especially in \( P. \text{aeruginosa} \) and \( A. \text{baumannii} \). A limited number of antimicrobial agents maintain high activity against OXA-23-producing \( A. \text{baumannii} \), including polymyxin, sulbactam and minocycline (Dalla Costa et al., 2003). Sulbactam has a direct antimicrobial effect against \( A. \text{baumannii} \) and also acts synergistically with ampicillin against this organism (Levin et al., 2003). Nebulized polymyxin E is increasingly being used for the treatment of respiratory infections caused by multiresistant \( A. \text{baumannii} \) due to the high concentrations that can be achieved in the lung, and is sometimes combined with intravenous administration, despite concerns of nephrotoxicity previously reported in the literature (Li et al., 2005; Lolans et al., 2006).

For all of the patients studied, imipenem-resistant strains of \( A. \text{baumannii} \) were all recovered at least 72 h after initial hospitalization. This strongly suggested nosocomial acquisition of the organism and was supported by the widespread environmental contamination found for the predominant strain type in the ICU. This is a very common feature of hospital outbreaks due to multiresistant opportunist Gram-negative organisms such as acinetobacters and augmented, no doubt, by the increased resistance to desiccation observed with epidemic strains of \( A. \text{baumannii} \) (Jawad et al., 1998), which promotes their persistence in the hospital ward environment. It is therefore important to monitor and control the spread of such strains by the use of molecular epidemiological tools to inform infection control measures.

In recent years, OXA-23-producing \( A. \text{baumannii} \) strains have been reported around the world (Poiré & Nordmann, 2006b), whilst \( \text{bla}_{\text{OXA-66}} \) belonging to the OXA-51/69 cluster was recently found to be the intrinsic \( \beta \)-lactamase gene in \( A. \text{baumannii} \) isolates from Greece (Ikonomidou et al., 2006). In this study, \( \text{bla}_{\text{OXA-23}} \) and \( \text{bla}_{\text{OXA-66}} \) genes were detected in all isolates and \( \text{bla}_{\text{OXA-23}} \) was located chromosomally. \( \text{ISAb}1 \) occurred upstream of the \( \text{bla}_{\text{OXA-23}} \) gene. This is consistent with the finding of Turton et al. (2006), who concluded that \( \text{ISAb}1 \) may have an important role in the expression of OXA-23. It is noteworthy that these characteristics were shared by a number of PFGE-defined genotypes in this study and this might suggest that the \( \text{ISAb}1/\text{bla}_{\text{OXA-23}} \) unit is mobile.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


