A nosocomial outbreak of Acinetobacter baumannii isolates expressing the carbapenem-hydrolysing oxacillinase OXA-58

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Objective: The aim of this study was to analyse the spread of the blaOXA-58 gene as a source of carbapenem resistance in Acinetobacter baumannii in the burns unit of a university hospital in Toulouse in 2003–2004.

Methods: Six carbapenem-resistant A. baumannii isolates from six patients, and a carbapenem-resistant environmental A. baumannii isolate were collected in the burns unit of the Rangueil hospital (Toulouse). Susceptibility tests were carried out by disc diffusion and agar dilution methods. The detection of the blaOXA-58 gene was conducted by PCR followed by sequence analysis. Plasmids were extracted and hybridized with a probe specific for blaOXA-58. DNA fingerprints were obtained by pulsed-field gel electrophoresis of Apal- or SmaI-digested chromosomal DNA of the tested strains.

Results: The multidrug-resistant clinical isolates had a similar 30 kb plasmid that encoded the carbapenem-hydrolysing β-lactamase OXA-58. These isolates were clonally related. The unrelated environmental carbapenem-resistant A. baumannii isolate had a similar blaOXA-58-carrying plasmid, suggesting spread of this gene.

Conclusions: A novel oxacillinase was the source of carbapenem resistance in the A. baumannii isolates. Its gene was plasmid-, but not integron-borne.

Keywords: A. baumannii, carbapenem resistance, nosocomial infections

Introduction

Acinetobacter baumannii is an important cause of nosocomial infections such as pneumonia, sepsicaemia, urinary tract infections and wound infections. It may become resistant to a wide range of antibiotics, thus complicating the treatment of nosocomial infections. Carbapenems are drugs of choice for treating Acinetobacter infections, but their effect may be compromised by carbapenem-hydrolysing β-lactamases. Acquisition of IMP-like and VIM-like metallo-β-lactamases (Ambler class B) has been reported worldwide in A. baumannii.

In addition, seven oxacillinases (Ambler class D) with carbapenem-hydrolysing activity have been characterized previously in A. baumannii and were involved in outbreaks in several cases. Oxacillinases OXA-23, OXA-27, and OXA-49 (GenBank AY288523) have 99% amino acid identity, but they share only 60% identity with a second group of oxacillinases consisting of OXA-24, -25, -26 and -40; these latter enzymes differ by a few amino acid substitutions. Outbreaks of OXA-type carbapenemase-producing A. baumannii have been reported: OXA-24 in Madrid, Spain; OXA-23 in Brazil; and endemic carbapenem resistance in A. baumannii in Bilbao, Spain, was associated with OXA-40.

Recently, we characterized a novel carbapenem-hydrolysing oxacillinase, OXA-58 from A. baumannii, from Toulouse, which belongs to a novel group of oxacillinases.

The aim of this study was to analyse the spread of blaOXA-58 as a source of carbapenem-resistance in A. baumannii in the burns unit of a hospital in Toulouse in 2003–2004.

Materials and methods

Bacterial isolates

A. baumannii clinical and environmental isolates (strains 1–8) were isolated from August 2003 to March 2004 and were identified using the API20NE system (bioMérieux, Marcy-l’Etoile, France) and by sequencing of 16S rRNA genes. E. coli NCTC 50192 harbouring...
four plasmids of 154, 66, 38 and 7 kb was used as a size marker for plasmids. Reference strain A. baumannii CIP7010T (Institut Pasteur Strain Collection, Paris, France) was used as the host in conjugation experiments.

Susceptibility testing
The antimicrobial agents and their sources have been referenced elsewhere.5 Antibiotic-containing discs were used for detection of antibiotic susceptibility with Mueller–Hinton agar plates and a disc diffusion assay (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) (www.sfm.fr). MICs of β-lactams were determined by an agar dilution technique as previously reported,5 and results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards.9

PCR amplification
PCR experiments were carried out using primers specific for the bla\textsubscript{OXA-58} gene. Primers preOXA-58prom+ (5′-TTATCAAAATCCAAATTGCC-3′) and preOXA-58B (5′-TAACCTCGAAACTCTATTCC-3′), able to amplify a 934 bp external fragment of the bla\textsubscript{OXA-58}, were used to amplify the entire bla\textsubscript{OXA-58} gene followed by sequencing with an Applied Biosystems sequencer (ABI 300). Primers OXA-58A (5′-CGATCAGAATGTTCGAC-3′) and OXA-58B (5′-ACGATTCTCCCCTGCGC-3′), amplifying a 528 bp internal fragment of the bla\textsubscript{OXA-58} gene, were used to generate a probe for Southern analysis.

Plasmid analysis and hybridization
Plasmid extraction was performed using the Kieser method designed to isolate large-sized plasmids.10 The plasmid extraction of each isolate was submitted to digestion with EcoRI restriction enzyme and restriction patterns of these plasmids were compared. Southern transfer of the plasmid extract was carried out on a Nylon membrane (Hybond N +; Amersham Pharmacia Biotech, Orsay, France), as described previously.5 The membrane was hybridized with a probe specific for bla\textsubscript{OXA-58}. Southern hybridization was carried out as described by the manufacturer using the ECL non-radioactive labelling and detection kit (Amersham Pharmacia Biotech).

Conjugation and transformation experiments
Direct transfer of β-lactam resistance markers into rifampicin-resistant A. baumannii CIP7010T was attempted by liquid and solid mating-out assays. Transconjugants were selected on trypticase soy (TS) agar plates containing rifampicin (50 mg/L) plus ticarcillin (50 mg/L).

The plasmid extract of A. baumannii isolate 2 was also used for electrottransformation experiments in A. baumannii CIP7010T. Electroporation products were selected on ticarcillin (50 mg/L)-containing plates.

Pulsed-field gel electrophoresis
PFGE analysis was performed according to the manufacturer’s instructions (Bio-Rad). In brief, whole-cell DNA of the A. baumannii isolates was digested with Apal overnight at 25°C (New England Biolabs, St Quentin en Yvelines, France), or with SmaI overnight at 37°C (Amersham Pharmacia Biotech). Electrophoresis was carried out with a CHEF DRII apparatus (Bio-Rad) through a 0.8% agarose gel in 0.5× Tris/borate/EDTA buffer. Migration conditions were as follows: temperature, 14°C; voltage, 6 V/cm; and switch angle, 120°, with one linear switch ramp of 3 to 20 s for 10 h, then 12 to 20 s for 10 h.

Results and discussion

Bacterial identification and susceptibility tests
From August 2003 to March 2004, seven carbapenem-resistant A. baumannii isolates were collected (isolates 2 to 8). Six strains

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Source (patient name or environment)</th>
<th>Imipenem susceptibility</th>
<th>Date of first isolation (month-day-year)</th>
<th>Date of hospitalization (month-day-year)</th>
<th>Source of isolation</th>
<th>Infected/colonized</th>
<th>Treatment for A. baumannii infection</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A (patient name or environment)</td>
<td>susceptible</td>
<td>09-23-03</td>
<td>08-14-03</td>
<td>skin</td>
<td>colonized</td>
<td>imipenem and amikacin</td>
<td>improved</td>
</tr>
<tr>
<td>2</td>
<td>A (patient name or environment)</td>
<td>resistant</td>
<td>08-21-03</td>
<td>08-14-03</td>
<td>skin</td>
<td>colonized</td>
<td>imipenem and amikacin</td>
<td>improved</td>
</tr>
<tr>
<td>3</td>
<td>B (patient name or environment)</td>
<td>resistant</td>
<td>08-28-03</td>
<td>08-04-03</td>
<td>skin</td>
<td>infected</td>
<td>imipenem and amikacin</td>
<td>died</td>
</tr>
<tr>
<td>4</td>
<td>C (patient name or environment)</td>
<td>resistant</td>
<td>11-21-03</td>
<td>10-28-03</td>
<td>tracheal aspirate</td>
<td>infected</td>
<td>imipenem and amikacin</td>
<td>improved</td>
</tr>
<tr>
<td>5</td>
<td>D (patient name or environment)</td>
<td>resistant</td>
<td>11-24-03</td>
<td>10-21-03</td>
<td>urine</td>
<td>infected</td>
<td>imipenem and amikacin</td>
<td>died</td>
</tr>
<tr>
<td>6</td>
<td>E (patient name or environment)</td>
<td>resistant</td>
<td>01-27-04</td>
<td>01-16-04</td>
<td>tracheal aspirate</td>
<td>colonized</td>
<td>imipenem and amikacin</td>
<td>died</td>
</tr>
<tr>
<td>7</td>
<td>F (patient name or environment)</td>
<td>resistant</td>
<td>03-16-04</td>
<td>03-01-04</td>
<td>tracheal aspirate</td>
<td>infected</td>
<td>piperacillin + tazobactam, amikacin and vancomycin</td>
<td>died</td>
</tr>
<tr>
<td>8</td>
<td>air of the surgery room</td>
<td>resistant</td>
<td>08-25-03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
(2 to 7) were from six patients hospitalized in the burns unit and one was an environmental isolate (isolate 8), collected during the same period of time in the surgical room of the burns unit during systematic air sampling (Table 1). A carbapenem-susceptible *A. baumannii* isolate (isolate 1) collected from one of the six patients was also retained in our study, in order to compare this isolate with carbapenem-resistant isolates.

The age of the patients ranged from 20 to 91 years and all of them except patient F had been treated with imipenem previously (Table 1). Infection with multidrug-resistant *A. baumannii* was considered as being colonized whereas the other was infected (Table 1).

*A. baumannii* isolates 2 to 8 were resistant to carbapenems, MICs of imipenem and meropenem being ≥32 mg/L (Table 2). Isolate 1 was susceptible to carbapenems with MICs of imipenem and meropenem of 0.5 mg/L (Table 2). The carbapenem-resistant isolates were resistant to all aminoglycosides, fluoroquinolones and most β-lactams, but remained susceptible to rifampicin and colistin (Table 2). Sulbactam retained activity against carbapenem-resistant *A. baumannii* isolates 2 to 8, but these isolates remained resistant to the combination of ampicillin and sulbactam (MICs, 32 mg/L; Table 2).

**PCR amplification and genetic location of the blaOXA-58 gene**

PCR experiments using primers specific for the *blaOXA-58* gene gave positive results for the seven carbapenem-resistant *A. baumannii* isolates. The carbapenem-susceptible *A. baumannii* isolate was *blaOXA-58*-negative.

The carbapenem-resistant *A. baumannii* isolates had an ~30 kb plasmid whereas no plasmid was detected in the carbapenem-susceptible *A. baumannii* isolate (data not shown). An additional ~17 kb plasmid was found in *A. baumannii* isolate 8.

A Southern membrane was hybridized with a 528 bp PCR-generated probe specific for the *blaOXA-58* gene giving a positive signal with the 30 kb plasmid for the seven carbapenem-resistant *A. baumannii* isolates.

The plasmid extraction of each isolate was submitted to digestion with EcoRI restriction enzyme and restriction patterns of these plasmids were compared. Gel electrophoresis analysis identified an identical plasmid with a positive hybridization signal, a fragment of DNA of ~11 kb, with the *blaOXA-58* probe (Figure 1).

Plasmid location of the *blaOXA-58* gene was confirmed by electrophoretion of the 30 kb plasmid (pMAD) into *A. baumannii* CIP7010T giving rise to a *A. baumannii* CIP7010T transformant that had a β-lactam resistance pattern consistent with the expression of β-lactamase OXA-58 (Table 2). No other antibiotic resistance marker was co-transferred in *A. baumannii* CIP7010T. However, this plasmid was non-conjugative since all conjugation experiments failed using *A. baumannii* CIP7010T as recipient strain.

**DNA fingerprinting**

Genotypic comparison was carried out for evaluating clonality of the isolates. PFGE analysis after digestion of whole-cell DNAs with *Apa* I and *Sma* I restriction enzymes showed that six (isolates 2 to 7) out of the seven carbapenem-resistant isolates probably corresponded to a single strain according to the Tenover criteria (data not shown). The environmental isolate (isolate 8) was of a different genotype, but it contained the same *blaOXA-58*-carrying plasmid (Figure 1).

Identification of the same *blaOXA-58*-carrying plasmid in two different isolates of *A. baumannii* indicated the spread of

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**Table 2. MICs (mg/L) of β-lactams for *A. baumannii* strains**

<table>
<thead>
<tr>
<th>β-Lactam(s)</th>
<th><em>A. baumannii</em> isolate 1</th>
<th><em>A. baumannii</em> isolates 2 to 8</th>
<th><em>A. baumannii</em> CIP7010T (pMAD)#</th>
<th><em>A. baumannii</em> CIP7010T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>&gt;512</td>
</tr>
<tr>
<td>Amoxicillin + CLA#</td>
<td>512</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>512</td>
</tr>
<tr>
<td>Ampicillin + SUL*</td>
<td>4</td>
<td>32</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Sulbactam</td>
<td>2</td>
<td>16</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>4</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>4</td>
</tr>
<tr>
<td>Ticarcillin + CLA</td>
<td>4</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>4</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>4</td>
<td>256</td>
<td>256</td>
<td>4</td>
</tr>
<tr>
<td>Piperacillin + TZB#</td>
<td>4</td>
<td>256</td>
<td>128</td>
<td>4</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>4</td>
<td>128</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>8</td>
<td>32</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1</td>
<td>256</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>2</td>
<td>256</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>64</td>
<td>32</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
<td>32</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.5</td>
<td>&gt;64</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Colistin</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

#Plasmid pMAD is the natural OXA-58-positive plasmid from *A. baumannii* isolate 2.
#CLA, clavulanic acid at a fixed concentration of 2 mg/L.
*SUL, a ratio of 2:1 was used for ampicillin with sulbactam.
#TZB, tazobactam at a fixed concentration of 4 mg/L.

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this plasmid in clinical and environmental isolates. This study constitutes the second description of an outbreak of carbapenem-resistant A. baumannii producing a plasmid-encoded carbapenem-hydrolysing oxacillinase, after that of OXA-23-producing strains in Brazil.6

As observed for other genes that encode carbapenem-hydrolysing oxacillinase, blaOXA-58 was not present as a gene cassette in a class 1 integron.8 Thus, the spread of this β-lactamase gene was not associated with class 1 integrons. This contrasts with the situation found with most other oxacillinase genes.12

The identification of blaOXA-40 in Bilbao,7 and now of blaOXA-58 in Toulouse indicates that distantly-related oxacillinases may be present in outbreak strains of carbapenem-resistant A. baumannii in hospitals located in southern Europe.

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