Characterization of the Carbapenem-Hydrolyzing Oxacillinase Oxa-58 in an Acinetobacter Genospecies 3 Clinical Isolate

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Based on imipenem resistance in an Acinetobacter genospecies 3 clinical isolate, we were able to identify, for the first time in this genomic species, a plasmid-encoded blaOXA-58 gene that was 100% homologous to the same gene in Acinetobacter baumannii.

Since 1986 members of the genus Acinetobacter are determined by DNA-DNA hybridization. Genospecies 1 (Acinetobacter calcoaceticus), 2 (A. baumannii), 3, and 13TU are genetically closely related and are commonly known as the A. calcoaceticus-A. baumannii complex. With the exception of genospecies 1, the other members of this complex have been involved in nosocomial infections and have the ability to spread in hospitals (3, 9, 19, 23, 25, 26). Treatment of these nosocomial infections is becoming a problem because increasing resistance to antibiotics, especially in the case of nosocomial infections is becoming a problem because increasing resistance to antibiotics, especially in the case of nosocomial infections.

In the last decade, carbapenem resistance in Acinetobacter spp. has been reported worldwide (3, 16, 23), mostly genospecies 1, the other members of this complex have been involved in nosocomial infections and have the ability to spread in hospitals (3, 9, 19, 23, 25, 26). Treatment of these nosocomial infections is becoming a problem because increasing resistance to antibiotics, especially in the case of nosocomial infections.

Acinetobacter calcoaceticus-A. baumannii (10). The S1 nuclease transforms supercoiled plasmids into linear molecules (1). Digested genomic DNA and plasmids were separated by gel electrophoresis (PFGE) with ApaI (Promega, Madrid, Spain) and determined that both strains had an imipenem MIC of >32 µg/ml (Table 1). The breakpoints for imipenem were those proposed by the Clinical and Laboratory Standards Institute (5).

PCR analysis with specific primers for all class D β-lactamases (Table 2) determined the presence of the blaOXA-58 gene in both strains; A. baumannii strain Ac057 was also positive for the blaOXA-51 gene. Additional primers were designed at the beginning and end of the blaOXA-58 gene (Table 2) to amplify the whole fragment. This gene presented 100% homology with the blaOXA-58 gene from A. baumannii listed in GenBank.

Plasmid DNA identification was attempted by using genomic mapping with I-CeuI (10) and by digestion with the S1 nuclease (1). I-CeuI cuts a 26-bp site in the rrl gene (23S rRNA), shearing the bacterial genome into an analyzable number of fragments (10). The S1 nuclease transforms supercoiled plasmids into linear molecules (1). Digested genomic DNA and plasmids were sepa-

<table>
<thead>
<tr>
<th>Strain</th>
<th>AMP</th>
<th>PIP</th>
<th>CEF</th>
<th>FOX</th>
<th>CAZ</th>
<th>FEP</th>
<th>SAM</th>
<th>IMP</th>
<th>MEM</th>
<th>CIP</th>
<th>GEN</th>
<th>TOB</th>
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<th>DOX</th>
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<td>256</td>
<td>512</td>
<td>256</td>
<td>256</td>
<td>8</td>
<td>8</td>
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<td>&gt;32</td>
<td>8</td>
<td>32</td>
<td>&lt;1</td>
<td>0.25</td>
<td>0.5</td>
<td>&lt;0.5</td>
<td>4</td>
<td>2</td>
<td>2</td>
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<td>Ac058</td>
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<td>512</td>
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<td>256</td>
<td>256</td>
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<td>64</td>
<td>&gt;32</td>
<td>8</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>256</td>
<td>16</td>
<td>128</td>
<td>2</td>
<td>1</td>
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Abbreviations: AMP, ampicillin; PIP, piperacillin; CEF, cephalothin; FOX, cefoxitin; CAZ, ceftazidime; FEP, cefepime; SAM, ampicillin-sulbactam; IMP, imipenem; MEM, Meropenem; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; DOX, doxycline; AZM, azithromycin; RIF, rifampin; PMB, polymyxin B.

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rated by PFGE (Fig. 1). Probes were marked with the PCR DIG probe synthesis kit (Roche, Barcelona, Spain), and detection was performed with anti-digoxigenin antibody conjugated to alkaline phosphatase and the color substrates NBT/BCIP (Roche) according to the manufacturer’s instructions. In Fig. 1a, the most intense bands would represent fragments of genomic DNA, and the faded bands represent plasmid DNA. Hybridization with probes for the \(\text{bla}_{\text{OXA-58}}\) gene (Fig. 1c) and the 23S rRNA gene (Fig. 1b) suggest that in both isolates the \(\text{bla}_{\text{OXA-58}}\) gene is present in a plasmid. With the S1 nuclease (Fig. 2a), the highest band would be the genomic DNA and the remaining bands would be linear plasmids. Hybridization with the probe for the OXA-58 gene (Fig. 2c) gives the same pattern as obtained with I-CeuI. The hybridization signal with the probe for the 23S rRNA gene was only observed in the undigested genomic DNA (Fig. 2b). Although conjugation experiments did not show any plasmid transfer between strains, Southern blot analysis suggests that the \(\text{bla}_{\text{OXA-58}}\) gene could be present in a plasmid in both strains, and the plasmid from \(A.\, baumannii\) is possibly different from the plasmid in the \(Acinetobacter\) genospecies 3 isolate.

In order to determine the genetic structure surrounding the \(\text{bla}_{\text{OXA-58}}\) gene, DNA from both isolates was digested with MspI “CCG” (Promega). The fragments obtained were autoligated overnight at 16°C using a T4 DNA ligase (Promega).

**TABLE 2. Oligonucleotide sequences used in this study**

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Sequence (5’-3’)</th>
<th>Size (bp)</th>
<th>Use</th>
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<tbody>
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<td>OXA51-1</td>
<td>AACAAGCGCTATTTTTATTCGAG</td>
<td>641</td>
<td>Detection (\text{bla}_{\text{OXA-51}}) variants</td>
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<tr>
<td>OXA51-2</td>
<td>CCCATCCCCAACCACCTTTTT</td>
<td>641</td>
<td>Detection (\text{bla}_{\text{OXA-21}}) variants</td>
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<tr>
<td>OXA23-1</td>
<td>GATGTTGCTATAGTATCCTGCTG</td>
<td>825</td>
<td>Detection (\text{bla}_{\text{OXA-23}}) variants</td>
</tr>
<tr>
<td>OXA23-2</td>
<td>TCAACAACAATCAAGCACCTGT</td>
<td>453</td>
<td>Detection (\text{bla}_{\text{OXA-58}}) variants</td>
</tr>
<tr>
<td>OXA58-1</td>
<td>AGTATTGCGGCTCTTGTCT</td>
<td>843</td>
<td>Total gene amplification</td>
</tr>
<tr>
<td>OXA58-2</td>
<td>AACTTTCCCTGCTAATTTTG</td>
<td>843</td>
<td>Total gene amplification</td>
</tr>
<tr>
<td>OXA58-inv1</td>
<td>GAGGCAGAGGGAGGAGATCGTC</td>
<td>323</td>
<td>Genetic surrounding</td>
</tr>
<tr>
<td>OXA58-inv2</td>
<td>CTCACGACAAAGGCCCATACT</td>
<td>825</td>
<td>Genetic surrounding</td>
</tr>
<tr>
<td>OXA58-inv3</td>
<td>AAGCCATGCAAGCAGCTACA</td>
<td>825</td>
<td>Genetic surrounding</td>
</tr>
<tr>
<td>OXA58-inv4</td>
<td>CATCTCTTCACCTGTGCTGAA</td>
<td>825</td>
<td>Genetic surrounding</td>
</tr>
</tbody>
</table>

\(\text{OXA58-1TOT}\) and \(\text{OXA58-2TOT}\) were used for detecting the \(\text{bla}_{\text{OXA-58}}\) gene and also to generate the probe for Southern blot analysis.

**FIG. 1.** Plasmid identification by genomic mapping with I-CeuI. (a) PFGE gel. (b) Hybridization with probe for the 23S rRNA gene. (c) Hybridization with probe for the OXA-58. Lane 1, \(A.\, baumannii\) strain Ac058; lane 2, \(Acinetobacter\) genospecies 3 strain Ac057.

**FIG. 2.** Plasmid identification by digestion with S1 nuclease. (a) PFGE gel. (b) Hybridization with probe for the 23S rRNA gene. (c) Hybridization with probe for the OXA-58. Lane 1, \(A.\, baumannii\) strain Ac058; lane 2, \(Acinetobacter\) genospecies 3 strain Ac057.
The fragment of DNA containing the blaOXA-58 gene was used as a template for a PCR with inverse primers designed from the blaOXA-58/H11022 sequence number for the resistant strains can be erroneously classified as A. baumannii. 

**Nucleotide sequence accession number.** The GenBank accession number for the blaOXA-58 in Acinetobacter genospecies 3 is EU642594.

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**REFERENCES**


