Insertion sequence IS18 mediates overexpression of bla\textsubscript{OXA-257} in a carbapenem-resistant Acinetobacter bereziniae isolate

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Sir, Acinetobacter bereziniae, previously known as Acinetobacter genomic species 10, has been isolated primarily from clinical specimens and the hospital environment and more rarely from various other sources, including vegetables, soil and animals.\textsuperscript{1,2} Antibiotic resistance is rarely reported in this species. Over the last decade, carbapenem resistance in Acinetobacter spp., mainly Acinetobacter baumannii, has emerged as a threat in hospitals around the world.\textsuperscript{2} The most widespread mechanism resulting in carbapenem resistance in Acinetobacter spp. is mediated through carbapenem-hydrolysing class D β-lactamases, also known as oxacillinas. The overexpression of bla\textsubscript{OXA} genes is often associated with insertion sequences (IS) located upstream and providing strong promoters. Carbapenem resistance in A. bereziniae has previously been associated with the metallo-β-lactamases IMP, SIM and VIM or overexpression of OXA-229, a variant of the intrinsic OXA-228-like, which was mediated by a mutated promoter.\textsuperscript{3} To date, OXA-228-like has not been associated with an IS.

In the present study, we investigated a carbapenem-resistant Acinetobacter strain isolated from the bronchial secretions of a patient in Germany in 2012. Isolate KH243 was initially identified as Acinetobacter guillouiae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. However, rpoB sequencing revealed 100% similarity to the A. bereziniae type strain CIP 70.12 (accession no. DQ207475).\textsuperscript{1,4} By Etest, carbapenem MICs were 12 and >32 mg/L for imipenem and meropenem, respectively. Multiplex PCR for OXA subclasses that are associated with carbapenem resistance in Acinetobacter spp. (OXA-51, OXA-23, OXA-40, OXA-58, OXA-143 and OXA-235) was negative.\textsuperscript{5,6} Based on published A. bereziniae sequences,\textsuperscript{3} primers were designed to amplify and sequence the intrinsic bla\textsubscript{OXA} and its surrounding region from isolate KH243 (Table 1). PCR revealed an unexpected large amplicon of ≏2.1 kb. Sequencing of the purified PCR product by primer walking identified IS\textsubscript{bza}OXA\textsubscript{257} by the Lahey β-lactamase database (http://www.lahey.org/Studies/) and was submitted to GenBank. OXA-257 possessed six amino acid differences compared with OXA-228. The IS18:bla\textsubscript{OXA-257} nucleotide sequence reported in this paper has been submitted to EMBL/GenBank under accession number KC567681.

The IS18 insertion element encoded a transposase that harboured eight amino acid changes compared with the IS18 sequence available in the IS database (http://www-is.biotoul.fr/). IS18 was flanked by a 3 bp target site duplication (TTT) and 26 bp imperfect inverted repeats. In Acinetobacter spp., IS are often located upstream of bla\textsubscript{OXA} genes and, by providing strong promoters, lead to overexpression of the OXA, resulting in carbapenem resistance.\textsuperscript{7} For example, the intrinsic bla\textsubscript{OXA-51-like} and the acquired bla\textsubscript{OXA-58-like} in A. baumannii are often associated with IS\textsubscript{Aba1} and IS\textsubscript{Aba3}, respectively.\textsuperscript{7} IS18 has also been associated with bla\textsubscript{OXA-58-like}.\textsuperscript{8} Other IS elements include IS\textsubscript{AcrA}, which was recently identified and overexpressed by bla\textsubscript{OXA-23} in a carbapenem-resistant Acinetobacter radioresistens isolate.\textsuperscript{9}

Two predicted promoters were found upstream of bla\textsubscript{OXA-257} with both −35 boxes located within the right inverted repeat of IS18. One was a hybrid promoter based on those previously described in A. bereziniae isolates Nec (bla\textsubscript{OXA-229}) and Baz (bla\textsubscript{OXA-228}).\textsuperscript{3} The −35 and −10 boxes were identical to the −35 box in Nec and the −10 box in Baz, respectively, and were

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target/purpose</th>
<th>5'–3' sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla\textsubscript{OXA-228-like}_F1</td>
<td>sequencing and bla\textsubscript{OXA-228-like} standard curve</td>
<td>GCTAAAGTTTCTGCTGAGGA</td>
<td>this study</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-228-like}_R1</td>
<td></td>
<td>CAGTTACCCCAACAACT</td>
<td>this study</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-228-like}_F2</td>
<td></td>
<td>TAGTTGCGATTTCAGTT</td>
<td>this study</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-228-like}_R2</td>
<td></td>
<td>ACCGTGACTGTCGTTGAT</td>
<td>this study</td>
</tr>
<tr>
<td>IS_F</td>
<td>cloning primer (used in combination with bla\textsubscript{OXA-228-like}_R1) A. bereziniae rpoB standard curve</td>
<td>GAGTTACGACATCACATTAC</td>
<td>this study</td>
</tr>
<tr>
<td>rpoB_beriR1</td>
<td></td>
<td>CGTATTGCGATTTCATTAGG</td>
<td>this study</td>
</tr>
<tr>
<td>rpoB_beriR2</td>
<td></td>
<td>A. bereziniae rpoB qRT–PCR</td>
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</tr>
<tr>
<td>rpoB_beriF2</td>
<td></td>
<td>AGTGGTTTACCTTACGAC</td>
<td>this study</td>
</tr>
<tr>
<td>rpoB_beriR2</td>
<td></td>
<td>CGAGACGATGAGAAGTTGG</td>
<td>this study</td>
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</tbody>
</table>

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separated by 22 bp instead of 17 bp (see Figure S1, available as Supplementary data at JAC Online). An additional promoter was predicted by Softberry BPRM promoter prediction and consisted of the −35 box of IS182 and a −10 box separated by 13 bp (Figure S1, available as Supplementary data at JAC Online). Interestingly, the TTCAT −35 box identical to that from Baz (blaOXA-228) was adjacent to the left inverted repeat.

OXA-228-like expression in isolate KH243 was compared with that in carbapenem-susceptible A. bereziniae isolate G3-59 by semi-quantitative RT–PCR (qRT–PCR) using ropB as the reference gene. The primers used for qRT–PCR are shown in Table 1. Three independent experiments were performed using freshly prepared RNA and cDNA and revealed a 56-fold (±3.84) overexpression of blaOXA-228-like in isolate KH243 compared with that in G3-59. To investigate the potential to mediate carbapenem resistance, IS18:blaOXA-257 was cloned into the shuttle vector pWH1266, but we were unable to transfer this into A. bereziniae G3-59. However, the construct was successfully transferred into A. baumannii ATCC 17978 by electroporation, as previously described in this species so far. IS18:blaOXA-257 in A. baumannii ATCC 17978 raised both imipenem and meropenem MICs from 0.25 to >32 mg/L, demonstrating that IS18:blaOXA-257 is able to confer carbapenem resistance.

In conclusion, this study has detected an IS upstream of the intrinsic blaOXA in A. bereziniae, a phenomenon that has not been described in this species so far. IS18 conferred overexpression of OXA-257, which mediated carbapenem resistance in A. bereziniae and A. baumannii. Moreover, because IS18 has previously been described adjacent to acquired blaOXA in A. baumannii, these data suggest a potential for dissemination of OXA-257 in the genus Acinetobacter.

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Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

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