regarded as avirulent, could serve as a reservoir for the spread of resistance genes between different species, leading to dissemination of resistant bacteria between patients and in the environment.

Funding

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Transparency declarations

None to declare.

References


Table 1. Molecular and microbiological features of the four KPC-producing Enterobacteriaceae isolates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Isolate no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1269</td>
</tr>
<tr>
<td>Hospital Source</td>
<td>LMC rectum</td>
</tr>
<tr>
<td>Species</td>
<td>L. adecarboxylata</td>
</tr>
<tr>
<td>blαKPC allele</td>
<td>2</td>
</tr>
<tr>
<td>Size of blαKPC-harbouring plasmid (kb)</td>
<td>65</td>
</tr>
<tr>
<td>Plasmid Inc type</td>
<td>N</td>
</tr>
<tr>
<td>Tn4401 type</td>
<td>c</td>
</tr>
<tr>
<td>Ertapenem MIC (mg/L)</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Meropenem MIC (mg/L)</td>
<td>16</td>
</tr>
</tbody>
</table>

other studies found a higher incidence of A. radioresistens when only looking at Acinetobacter bloodstream isolates, where bacteremia is most often associated with indwelling devices. 3–5 Compelling evidence suggests that the origin of OXA-23, the most commonly acquired oxacillinase in Acinetobacter baumannii, is A. radioresistens, to which this enzyme is intrinsic. 6 In A. baumannii, blaoxa-23 mediates carbapenem resistance when overexpressed and this is caused by the insertion element ISAbaI, which is located upstream where it provides a strong promoter; however, this has not been found in A. radioresistens, so the mechanism of blaoxa-23 mobilization is still not fully understood. Despite possessing OXA-23, carbapenem resistance is rarely described in A. radioresistens and has only been reported when the additional acquired carbapenemases IMP-1 and OXA-58 were present. 7, 8

In this study, we investigated carbapenem resistance and the development of fluoroquinolone resistance in two A. radioresistens isolates that were recovered from a patient 17 days apart. The primer sequences used in this investigation are shown in Table S1 (available as Supplementary data at JAC Online). The ISAcrA-blaOXA-23 nucleotide sequence reported in this paper has been submitted to the EMBL/GenBank nucleotide sequence database under accession no. JQ326202. Isolate F1244 was isolated from a blood culture of a patient with a catheter-related bloodstream infection. Ciprofloxacin was used for treatment. Isolate A1474 was recovered 17 days later from a sputum sample. Species identification was confirmed by rpoB sequencing and the isolates were typed by rep-PCR (DiversiLab) as previously described. 9 The isolates showed 98% similarity in their rep-PCR patterns and confirms their clonality. The only difference we found between the isolates was in their fluoroquinolone susceptibility: isolate F1244 was susceptible to ciprofloxacin, levofloxacin and moxifloxacin, whereas isolate A1474 was resistant (Table 1). Carbapenem MICs were investigated by Etest and showed that both isolates were carbapenem resistant (Table 1). The isolates were negative for other blaOXA genes often associated with Acinetobacter and we investigated the expression of the intrinsic blaoxa-23 by qRT–PCR, qRT–PCR was performed three times in triplicate using freshly prepared RNA and cDNA as previously described, 9 with rpoB as a reference gene. This revealed that F1244 and A1474 expressed similar levels of blaoxa-23 and that it was overexpressed >100-fold when compared with the carbapenem-susceptible control strain A. radioresistens SH164 (data not shown). PCR to detect ISAbaI adjacent to blaoxa-23 proved negative. Analysis of the A. radioresistens genome sequence revealed putative O-sialylglycoprotein- and ATPase-encoding genes flanking blaoxa-23. The primer pair OXA-23-up/OXA-23-down was designed to amplify and sequence the region between these flanking genes and revealed an ~700 bp insertion in the 5’ region of blaoxa-23. Sequencing revealed a novel insertion element in a non-coding region 62 bp upstream of the blaoxa-23 start codon. This insertion element was submitted to the IS Database (http://www-is.biotoul.fr/) and termed ISAcrA1. ISAcrA1 is a 732 bp element that encodes a predicted 220 amino acid transposase that is flanked by 15 bp inverted repeats and a 7 bp target site duplication. The element belongs to the IS1595 family and the transposase showed 47% amino acid identity to ISFtu3 from Franciscella tularensis. The blaoxa-23 gene and putative promoter region was amplified and cloned into shuttle plasmid pWH1266 and transformed into carbapenem-susceptible A. baumannii ATCC 17978 and A. radioresistens SH164, leading to carbapenem resistance (Table 1).

We found a single nucleotide difference between the isolates in gyrA, leading to a Ser-83→Phe amino acid substitution in isolate A1474. In A. baumannii, Ser-83 in GyrA is the most commonly altered amino acid associated with low-level fluoroquinolone resistance (typically ciprofloxacin MICs of 4–32 mg/L). 10 We did not detect any difference in parC between the two isolates. We have previously found efflux to be associated with the development of fluoroquinolone resistance. 3 To test for an efflux phenotype, agar dilution MICs were performed with the following commonly effluxed substrates: tetracycline, chloramphenicol, rifampicin, gentamicin, erythromycin and the dye rhodamine 6-G. Agar dilution MICs did not show any difference in MICs between isolates F1244 and A1474 against these substrates, suggesting efflux was not selected (see Table S2 available as Supplementary data at JAC Online).

In conclusion, this study demonstrates that carbapenem resistance was mediated through overexpression of the intrinsic blaoxa-23 and was associated with the novel ISAcrA1.

Table 1. Carbapenem MICs determined by Etest in A. radioresistens isolates F1244 and A1474, control strains A. radioresistens SH164, A. baumannii ATCC 17978, and their respective blaoxa-23 transformants, and fluoroquinolone agar dilution MICs and the amino acid substitution in GyrA for A. radioresistens isolates F1244 and A1474

<table>
<thead>
<tr>
<th>Strain</th>
<th>IPM (mg/L)</th>
<th>MEM (mg/L)</th>
<th>CIP (mg/L)</th>
<th>LVX (mg/L)</th>
<th>MXF (mg/L)</th>
<th>GyrA Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1244</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>Ser-83</td>
</tr>
<tr>
<td>A1474</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>32</td>
<td>8</td>
<td>2</td>
<td>Ser-83Phe</td>
</tr>
<tr>
<td>A. radioresistens SH164</td>
<td>0.38</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. baumannii ATCC 17978</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. radioresistens SH164 (pOXA-23)</td>
<td>&gt;32</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. baumannii ATCC 17978 (pOXA-23)</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin.
Fluoroquinolone resistance developed during ciprofloxacin therapy and was associated with a gyrA mutation. These data show for the first time the ability of A. radioresistens to develop fluoroquinolone resistance during therapy. Additionally, ISAcar1 has the potential to spread OXA-23-mediated carbapenem resistance in A. radioresistens.

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**Transparency declarations**

None to declare.

**Supplementary data**

Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


**Acquisition of plasmid-borne blaIMP-19 gene by a VIM-1-positive Pseudomonas aeruginosa of the sequence type 235 epidemic lineage**

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**Keywords:** metallo-β-lactamases, integrons, multidrug resistance

Sir,

Acquired metallo-β-lactamases (MBLs) are the most common acquired carbapenemases in Pseudomonas aeruginosa. They confer a broad-spectrum β-lactam resistance profile, including resistance to the antipseudomonal penicillins, cephalosporins and carbapenems, that is not antagonized by the available β-lactamase inhibitors. Most MBL-producing strains exhibit a multidrug resistance profile, also including resistance to non-β-lactams, due to the accumulation of additional resistance determinants.1

Although several types of acquired MBLs have been detected in P. aeruginosa, the IMP- and VIM-type enzymes are currently the most widespread.1 A number of genes encoding these enzymes (e.g. blaIMP-1, blaVIM-2, blaVIM-4 and blaIMP-1) have become associated with high-risk clones, such as clonal complex (CC) 235 and CC111, which has promoted their dissemination in Europe and other continents.2

IMP-19 is an IMP allelic variant that was originally detected in Enterobacter cloacae (GenBank/EMBL accession no. AB201264) and P. aeruginosa (GenBank/EMBL accession no. AB184976) from Japan and, subsequently, in an Aeromonas caviae isolated in France.3 More recently, it has been reported in Achromobacter xylosoxidans and Acinetobacter spp. isolates from Japan.4,5

Here, we report on the first detection of the blaIMP-19 gene in Italy, in a multidrug-resistant (MDR) P. aeruginosa clinical isolate that also produced VIM-1 and belonged to the CC235 epidemic lineage.