A part of this work was supported by a grant from the Universities of Larissa and Patras.

**Transparency declarations**

None to declare.

### References


### Table 1. MICs of linezolid in correlation with molecular characteristics of linezolid-resistant S. epidermidis

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No. of isolates</th>
<th>MIC of linezolid (mg/L)</th>
<th>C2534T</th>
<th>G2576T</th>
<th>T2504A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>8–10</td>
<td>rrlD, rrlF</td>
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<td>—</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>16–24</td>
<td>rrlD, rrlF</td>
<td>rrlA, rrlD, rrlE, rrlF</td>
<td>—</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>1024</td>
<td>rrlD, rrlF</td>
<td>—</td>
<td>rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>1024</td>
<td>rrlD, rrlF</td>
<td>—</td>
<td>rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>16–24</td>
<td>rrlD, rrlF</td>
<td>rrlA, rrlD, rrlE, rrlF</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>8</td>
<td>rrlD, rrlF</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>64</td>
<td>rrlD, rrlF</td>
<td>rrlA, rrlD, rrlE, rrlF</td>
<td>—</td>
</tr>
</tbody>
</table>

### Acknowledgements

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### Funding

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### Isolation of multidrug-resistant Klebsiella oxytoca carrying bla\textsubscript{IMP-8}, associated with OXY hyperproduction, in the intensive care unit of a community hospital in Spain

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Sir,

The emergence of metallo-β-lactamases in Enterobacteriaceae is a matter of major concern for clinicians worldwide. The VIM type is the most prevalent metallo-β-lactamase in Europe, and Enterobacteriaceae expressing these enzymes have caused outbreaks in some Mediterranean countries, including Spain. From March to August 2009, multidrug-resistant Klebsiella oxytoca isolates were recovered from clinical and surveillance samples of patients admitted to the eight-bed intensive care unit of a community hospital in Spain. Overall, 9 of the 52 patients investigated (17.3%) were found to be colonized and/or infected by this microorganism (11.4 cases/1000 patient-days) [features of the patients are shown in Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Preliminary identification and susceptibility testing were performed by the Wider automated system (Francisco Soria Melguizo, Madrid, Spain). The first isolate of each patient was selected for further study. The MICs of amoxicillin/clavulanate, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ertapenem, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, amikacin, tobramycin and tigecycline were determined by the broth microdilution method, and susceptibility to colistin was determined by the disc diffusion test, following the CLSI guidelines; the results were also interpreted according to the CLSI guidelines. All the isolates were intermediate or resistant to all the β-lactams tested, except for the carbapenems (the imipenem MIC was 2 mg/L; the MIC ranges for meropenem and ertapenem were 0.5–1 and 1–2 mg/L, respectively). The isolates were also resistant to ciprofloxacin and trimethoprim/sulfamethoxazole, intermediate to tobramycin and susceptible to gentamicin, amikacin and colistin. The MIC of tigecycline was 4 mg/L for the nine isolates. This is considered to be intermediate using the FDA breakpoint criteria for Enterobacteriaceae (Tygacil package insert (June 2005), Wyeth Pharmaceuticals Inc., Philadelphia, PA, USA) and resistant using the European Committee on Antibiotic Susceptibility Testing (EUCAST) clinical MIC breakpoints (http://www.eucast.org/). The nine isolates showed a negative confirmatory disc diffusion test for the presence of extended-spectrum β-lactamase (ESBL), carried out in accordance with CLSI guidelines, whereas the modified Hodge test for suspected carbapenemase production was positive for all of them. Analytical isoelectric focusing, using crude supernatants from sonicated cells and the Phast-System (gel pI range, 3.5–9; Pharmacia, Sant Cugat del Vallès, Spain), showed two bands per strain. Their pIs were slightly above 6.5 and 8.2.

Molecular relatedness was determined using PFGE with XbaI, and cluster analysis was performed using the Dice coefficient and unweighted pair group method (UPGMA) with a tolerance of 1.0% and optimization of 1.0%, using the Fingerprinting II software (Bio-Rad Laboratories, Hercules, CA, USA). All the isolates had a genetic relationship of 90.15%, corresponding to five bands of difference (Figure 1).

The first and the last isolates were selected in order to determine the genetic basis of the β-lactam resistance phenotype observed.

The presence of genes coding for class A carbapenemases (blaGES-1, blaGIM, blaIMI, blaOXA) and metallo-β-lactamases (blaGIM, blaIMP, blaIMR and blaVIM) and metallo-β-lactamases (blaGES, blaGIM, blaIMR and blaVIM) was determined by specific PCR [see Table S2, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Only PCR of blaIMP was positive for both isolates, giving an amplicon of 587 bp. Direct sequencing of blaIMP amplicons showed a 100% sequence identity with blaIMP-B. In order to amplify the entire coding region of the blaIMP-B gene, primers IMPF (5′-CAAAGTTAGAAA GGGCAAGTAGG-3′) and IMPR (5′-GAGTTTCACCGCCTGTAGAAA T-3′) were designed, based on the nucleotide sequence of the immediate environment of blaIMP-B, under GenBank accession number AF322577 (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide). The sequence analysis of the amplicons obtained by PCR confirmed that both strains carried blaIMP-B. The metallo-β-lactamase IMP-B was described for the first time in a Taiwanese hospital, in a K. pneumoniae specimen isolated in 1998. The blaIMP-B gene was carried on a multidrug-resistant plasmid also encoding TEM-1 and SHV-12. Because our isolates

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**Table S1.** Isolates and characteristics

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date</th>
<th>Patient</th>
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<tbody>
<tr>
<td>Tracheal aspirate</td>
<td>16/03/2009</td>
<td>2</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>05/04/2009</td>
<td>6</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>20/05/2009</td>
<td>9</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>13/03/2009</td>
<td>1</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>18/03/2009</td>
<td>3</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>24/03/2009</td>
<td>4</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>04/05/2009</td>
<td>7</td>
</tr>
<tr>
<td>Pharyngeal swab</td>
<td>11/05/2009</td>
<td>8</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>04/04/2009</td>
<td>5</td>
</tr>
</tbody>
</table>

---

**Figure 1.** PFGE of XbaI-digested DNA from all nine multidrug-resistant K. oxytoca isolates. The scale above the dendrogram indicates percentage similarity.
were resistant to aztreonam, which is not hydrolysed by metallo-β-lactamases, and because IMP-8 and SHV-12 share the same pl—despite the negative diffusion test for ESBL detection—we carried out a search for bla_{IMP-8} using PCR (see Table S2); this, however, was negative for both strains. Another possible explanation for the aztreonam resistance observed in our strains might be overproduction of their chromosomal class A OXY-β-lactamase. The promoter region of the OXY-β-lactamase was amplified by PCR (see Table S2), and the sequences revealed a mutation in the −10 consensus region of the promoter in both strains; this consisted of the transition (G→A) of the fifth base, described as the most frequent among in vitro mutants and clinical isolates of aztreonam-resistant *K. oxytoca*. The bla_{OXY} gene was also amplified by PCR (see Table S2), and the sequence analysis indicated that both strains carried a bla_{OXY-2-type} very similar to bla_{OXY-2-8} (GenBank accession no. AY055205) with only two substitutions, a serine to glycine at position 23 and an aspartic acid to alanine at position 38, considering position 1 as the starting methionine.

After the first report of IMP-8-producing *K. pneumoniae* in 1998, an outbreak caused by this microorganism was reported in the intensive care units of the same hospital between January 1999 and December 2000, as well as the spread of bla_{IMP-8}−containing multidrug resistance plasmids to *Enterobacter cloacae*. To our knowledge this is the first report in Spain of an outbreak caused by Enterobacteriaceae producing IMP-8.

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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


Stability of meropenem and doripenem solutions for administration by continuous infusion

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Keywords: HPLC, ¹H NMR, concentrated solutions, temperature

Sir, Administration of β-lactams by continuous infusion is gaining popularity as a simple approach to optimize their efficacy (the percentage of the dosing interval during which the free drug concentrations exceed the MIC (T > MIC) being most closely linked to organism killing) and to facilitate serum concentration monitoring. The issue of instability of β-lactams in concentrated solutions needs, however, to be carefully addressed. To comply with the European Pharmacopoeia, β-lactam solutions should always contain at least 90% of intact molecule. We showed that carbapenems are quite unstable in concentrated (6.4 g/100 mL) aqueous solutions (>10% degradation in ≤5–6 h at 25°C), in contrast to ceftazidime, piperacillin or ticarcillin that are stable for 24 h at 25°C, 20 h at 37°C and >24 h at 37°C.