

were resistant to aztreonam, which is not hydrolysed by metallo- $\beta$ -lactamases, and because IMP-8 and SHV-12 share the same pI—despite the negative diffusion test for ESBL detection—we carried out a search for *bla<sub>SHV</sub>* 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  $\beta$ -lactamase.<sup>5</sup> The promoter region of the OXY  $\beta$ -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*.<sup>5</sup> The *bla<sub>OXY</sub>* gene was also amplified by PCR (see Table S2), and the sequence analysis indicated that both strains carried a *bla<sub>OXY-2</sub>*-type very similar to *bla<sub>OXY-2-8</sub>* (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,<sup>4</sup> an outbreak caused by this microorganism was reported in the intensive care units of the same hospital between January 1999 and December 2000,<sup>6</sup> as well as the spread of *bla<sub>IMP-8</sub>*-containing multidrug resistance plasmids to *Enterobacter cloacae*.<sup>7</sup> 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/>).

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## Stability of meropenem and doripenem solutions for administration by continuous infusion

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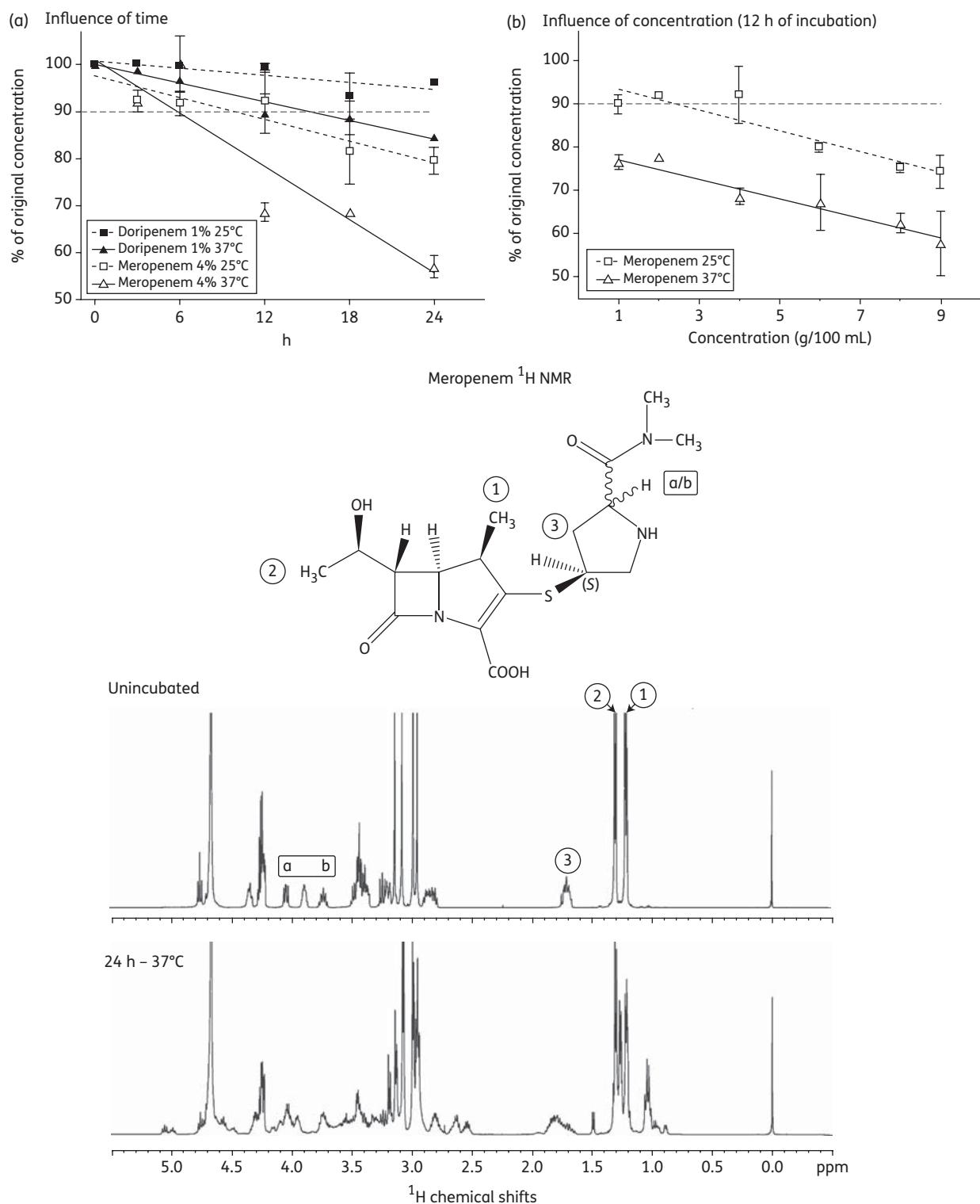
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Sir,

Administration of  $\beta$ -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 ( $fT > MIC$ ) being most closely linked to organism killing] and to facilitate serum concentration monitoring. The issue of instability of  $\beta$ -lactams in concentrated solutions needs, however, to be carefully addressed. To comply with the European Pharmacopoeia,  $\beta$ -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 ( $\geq 10\%$  degradation in  $\leq 5$ –6 h at  $25^\circ\text{C}$ ),<sup>1</sup> in contrast to ceftazidime, piperacillin or temocillin that are stable for 24 h at  $25^\circ\text{C}$ , 20 h at  $37^\circ\text{C}$  and  $> 24$  h at  $37^\circ\text{C}$ ,



**Figure 1.** Top panels: change in meropenem and doripenem concentrations upon incubation of aqueous solutions [meropenem, 4 g/100 mL (open symbols); doripenem, 1 g/100 mL (filled symbols)] at 25°C (squares; broken lines) or 37°C (triangles; continuous lines). All values are the means of three independent determinations ( $\pm\text{SD}$ ). The horizontal grey broken line indicates the limit of 90% of the initial drug concentration set by the European Pharmacopoeia. Bottom panels: one-dimensional  $^1\text{H}$  NMR spectra of meropenem 12.5 g/100 mL in deuterated water. The structure of the molecule is shown with the position of three proton groups characteristic of the molecule and identified on the top spectrum; the proton marked a/b is attached to a chiral centre with two epimers present in commercial meropenem and detected as such in the unincubated solution (see the top spectrum). Top spectrum, unincubated solution; bottom spectrum, solution incubated for 24 h at 37°C; the perturbation of the signals of the three characteristic proton groups and the appearance of additional signals indicate a major degradation of the original molecule.

respectively.<sup>1,2</sup> Thus, carbapenems are commonly limited to a 3 h infusion only (extended infusion). Yet, clinical studies have appeared<sup>3–5</sup> in which meropenem, administered by continuous infusion, has been stored for long periods with claims of stability if using dilute solutions and temperatures  $\leq 25^{\circ}\text{C}$ . Here, we re-examine the stability of meropenem in dilute solutions at both 25 and  $37^{\circ}\text{C}$  to better guide its potential clinical use by continuous infusion. We also analyse meropenem degradation using  $^1\text{H}$  NMR spectroscopy, and extend our studies to doripenem, a recently registered carbapenem sharing many of the properties of meropenem and approved for use in a 4 h infusion scheme.

Meropenem and doripenem were obtained as the commercial powder preparations for injection [Meropenem® (AstraZeneca) and Doribax® (Janssen-Cilag)]. For chromatographic studies, solutions were prepared in pyrogen-free water for injection under sterile conditions at concentrations varying from 1 to 9 g/100 mL for meropenem and at 1 g/100 mL for doripenem (limit of solubility; the maximal approved concentration for infusion in clinical use is set at 0.5 g/100 mL) and samples were incubated at 25 or  $37^{\circ}\text{C}$  in sealed vials for up to 24 h. Chromatographic analysis was carried out after diluting all samples to 50  $\mu\text{g}/\text{mL}$ , and using standard HPLC equipment (Waters 2690) connected to a photodiode array detector (Waters Corp., Milford, MA, USA) and using a LiChrospher® 100 RP-18, 5  $\mu\text{m}$  column with isocratic elution [acetonitrile/70 mM ammonium acetate buffer pH 5.0, 28:72 (v/v); 1 mL/min; linear response in 0–150  $\mu\text{g}/\text{mL}$  range ( $R^2 > 0.98$ )]. For NMR studies, meropenem and doripenem solutions [12.5 and 1.04 g/100 mL, respectively (for meropenem, the solution was heated at  $37^{\circ}\text{C}$  for 10 min with stirring to ensure complete dissolution)] were prepared in  $\text{D}_2\text{O}$  (deuterated water; Sigma-Aldrich, St Louis, MO, USA) supplemented with 0.05% trimethylsilyl propionate-d4 (sodium salt) as reference for calibration of NMR spectra. An NMR spectrum was acquired every hour on a sample left at  $37^{\circ}\text{C}$  in a Bruker Avance DRX500 spectrometer operating at 500.20 MHz for  $^1\text{H}$  and equipped with a 5 mm multinuclear inverse z-gradient probe (32 scans per spectrum, with repetition time of 3 s). The signal was Fourier transformed using an exponential multiplication function with a line broadening of 0.3 Hz to a final spectral resolution of 0.13 Hz per point.

Figure 1 (top panels) shows the rates of disappearance of meropenem (4 g/100 mL) and doripenem (1 g/100 mL) upon incubation at 25 and  $37^{\circ}\text{C}$  over 24 h (Figure 1a) and the degradation observed at 12 h for meropenem solutions from 1 to 9 g/100 mL (Figure 1b). As observed previously,<sup>1</sup> meropenem degradation was both time and temperature dependent. We now show that it is also concentration dependent, reaching the threshold of 10% in 12 h at  $>4$  g/100 mL at  $25^{\circ}\text{C}$  and at all concentrations tested at  $37^{\circ}\text{C}$  (the limit of 10% was reached in 6 h for a 6 g/100 mL solution at  $25^{\circ}\text{C}$  and for a 4 g/100 mL solution at  $37^{\circ}\text{C}$ ). Doripenem (1 g/100 mL) was stable for 24 h at  $25^{\circ}\text{C}$  and for  $\sim 12$  h at  $37^{\circ}\text{C}$ . Figure 1 (bottom panels) shows the  $^1\text{H}$  NMR spectra of meropenem (12.5 g/100 mL) in  $\text{D}_2\text{O}$  before (top spectrum) or after a 24 h incubation at  $37^{\circ}\text{C}$  (bottom spectrum), demonstrating major degradation after incubation. No marked

perturbation in the NMR spectrum of doripenem (1.04 g/100 mL) was seen under the same incubation conditions.

Our study shows instability of meropenem when kept at  $37^{\circ}\text{C}$ , but improved stability if: (i) the temperature is kept at  $\leq 25^{\circ}\text{C}$ ; and (ii) solutions of  $\leq 4$  g/100 mL are used. Doripenem seems more stable, but we could only test low concentrations because of solubility limits. Whilst controlling meropenem concentration is easy, controlling the temperature to  $\leq 25^{\circ}\text{C}$  may be more difficult especially in tropical countries<sup>6</sup> or in situations where the containers used for infusion bags are placed close to the patient or other sources of heat. Clinicians need to be aware of these limitations in meropenem stability when using it for continuous infusion.

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