Table 1. Primers pairs used by the KPC Resistance Assay Kit to obtain the specific bla<sub>KPC</sub> amplicons analysed by ESI-MS<sup>3</sup>

<table>
<thead>
<tr>
<th>Primer pair name&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Expected base composition after ESI-MS analysis&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>forward (5′-3′)</td>
<td>reverse (5′-3′)</td>
</tr>
<tr>
<td>bla&lt;sub&gt;KPC-2&lt;/sub&gt; and all other bla&lt;sub&gt;KPC&lt;/sub&gt; subtypes not listed below</td>
<td>A26 G26 C26 T19</td>
</tr>
<tr>
<td>bla&lt;sub&gt;KPC-3&lt;/sub&gt;</td>
<td>A26 G26 C26 T19</td>
</tr>
<tr>
<td>bla&lt;sub&gt;KPC-4&lt;/sub&gt; and bla&lt;sub&gt;KPC-5&lt;/sub&gt;</td>
<td>A26 G26 C26 T19</td>
</tr>
</tbody>
</table>

Expected base composition after ESI-MS analysis:
- bla<sub>KPC-2</sub> and all other bla<sub>KPC</sub> subtypes not listed below: A26 G26 C26 T19
- bla<sub>KPC-3</sub>: A26 G26 C26 T19
- bla<sub>KPC-4</sub> and bla<sub>KPC-5</sub>: A26 G26 C26 T19

<sup>b</sup>Species identification of Enterobacteriaceae was obtained with the same kit using primers specific for the valS housekeeping gene: 358-forward, 5′-TCGTTGGCGCGCTGTTATCGA-3′; and 358-reverse, 5′-TCGGTACGAACTGATCGCGTTGTT-3′.
<sup>c</sup>All primer sequences for KPC detection are based on GeneBank ID EU784136.

<sup>3</sup>Results in bold indicate amplicons with different base compositions that identify different KPC variants.

used to perform epidemiological and infection control studies where large collections of isolates need to be rapidly characterized.

Acknowledgements
We thank Dr Krisztina M. Papp-Wallace for providing the E. coli DH10B control strains carrying bla<sub>KPC</sub> variants.

Funding
This work was supported in part by the Veterans Affairs Merit Review Program (R. A. B.), the NIH (grant RO3-AI081036 to R. A. B.) and the Geriatric Research Education and Clinical Centre VISN 10 (R. A. B.). Ibis Biosciences Inc. provided the KPC Resistance Assay Kit plates free.

Transparency declarations
R. A. B. has received money for research and accepted speaking invitations from various pharmaceutical companies. None of these poses a conflict of interest with the present work. R. S. and D. J. E. are employees of Ibis Biosciences Inc., a subsidiary of Abbott Molecular Inc. Both R. S. and D. J. E. are shareholders of the company. Other authors: none to declare.

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J Antimicrob Chemother 2010
doi:10.1093/jac/dkq196
Advance publication 2 June 2010

Elution and antibacterial activity of meropenem from implanted acrylic bone cement

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Keywords: joint infections, bone infections, antimicrobial delivery, clinical microbiology

Sir,

Meropenem has good tissue penetration and broad-spectrum bactericidal activity. Often employed to treat multiresistant
Gram-negative organisms, meropenem was active against 98.7% of 1657 clinical surveillance Enterobacteriaceae isolates collected in the USA in 2005. Its stability permits combination with polymethylmethacrylate (PMMA) bone cement. We present here the first published account of the use of meropenem-loaded PMMA in human prosthetic joint infection.

The patient, a 66-year-old woman with insulin-requiring type 2 diabetes, polyamalgia rheumatica (treated with 10 mg of prednisolone daily) and nodular prurigo, kindly gave written informed consent to publication. She was 170 cm tall and weighed 102 kg. She had had her left hip replaced for osteoarthritis at another institution in 1996. This prosthesis had functioned excellently for >10 years before becoming unstable; cup revision in September 2006 was complicated by formation of an infected haematoma. The joint was replaced again in October 2006 and the patient put on a long antibiotic course. Recurrent dislocation led to further socket revision in July 2007. The patient was referred to our specialist hip revision service with continuing instability in late 2008. On 17 February 2009, both components were revised. Seven operative tissue specimens were sterile. Antibiotic prophylaxis was with 48 h of vancomycin and gentamicin.

Post-operatively she developed a wound haematoma. The wound started to discharge and she returned to theatre on 10 March for a washout; the components were retained and the wound closed. Five of five tissue specimens grew *Klebsiella pneumoniae* susceptible to co-amoxiclav, cefotaxime, piperacillin/tazobactam, carbapenems, ciprofloxacin, amikacin and trimethoprim, but resistant to amoxicillin and gentamicin. Intravenous co-amoxiclav 1.2 g thrice daily was administered from 10 to 27 March, followed by oral co-amoxiclav 625 mg thrice daily until 20 April.

Infection persisted, and extensive osteomyelitis developed in the proximal femur. A decision was made to proceed to one-stage revision. Both joint components and the proximal femur were replaced on 21 April. One of three acetabular specimens grew *K. pneumoniae* (susceptibilities as above), while two of three grew *Morganella morganii* susceptible to the cephalosporins, piperacillin/tazobactam, carbapenems, ciprofloxacin, amikacin and gentamicin, and resistant to co-amoxiclav, colistin and trimethoprim. From 24 April to 12 May the patient received 1.2 g of co-amoxiclav intravenously thrice daily.

On 12 May, a large abscess connected superficial and deep tissues. This was washed out. The acetabular component was removed. Meropenem (10 g) was crushed in a sterile vacuum mixing bowl (Optivac® FusionTM; Biomet, Bridgend, UK); two 40 g mixes of sterile orthopaedic bone cement (Palacos; Biomet; each mix containing 1.8 g of gentamicin and 1.8 g of clindamycin preloaded by the manufacturer) were added. The resulting cement was used to fix the replacement acetabular prosthesis. A third cement mix combined with 5 g of meropenem was used to coat the stem. Intravenous meropenem and amikacin and serial vac dressings were initiated. Samples of pus, fluid and hip tissue each grew scant *K. pneumoniae* susceptible to ciprofloxacin, cephalosporins, ertapenem and meropenem, but resistant to co-amoxiclav, piperacillin/tazobactam, gentamicin and amikacin.

On 13 May, drain fluid was collected. An ISO susceptibility test agar plate was seeded with the patient’s *K. pneumoniae* isolate; a second plate was seeded with fully susceptible *Escherichia coli* strain ATCC 25922. Drain fluid (20 μL) was placed at the centre of each plate. Plates were incubated aerobically at 36°C for 18 h. Inhibition zones suggested that antibacterial activity in the vicinity of the prosthesis was sufficient to inhibit growth of the patient’s *K. pneumoniae* (and therefore also her more susceptible *M. morganii*).

An aliquot of 13 May drain fluid was sent to the UK Antimicrobial Reference Laboratory, Bristol, where its meropenem concentration was measured (by HPLC) at 73.5 mg/L. Drug concentrations in pre- and post-meropenem-dose serum samples (also collected on 13 May) were considerably lower (9.3 and 12.5 mg/L, respectively), suggesting that meropenem was eluting from the cement. The accepted meropenem susceptibility breakpoint is 4 mg/L.

The patient received intravenous meropenem 1 g thrice daily until 30 July, with intravenous amikacin 1 g daily for the first two post-operative weeks. By 30 July, she was well and mobilizing, and was discharged home off antibiotics.

Antibiotic loading of PMMA is routine practice in joints with suspected or proven infection. The aim is to achieve high antibiotic levels at the site of infection while minimizing systemic toxicity. The antibiotic used must be heat stable (since cement polymerization is strongly exothermic) and water soluble (to allow diffusion from cement to tissues). The most common antibiotics used are gentamicin, vancomycin and cefazolin, either alone or in combination. Unfortunately, this patient’s *Klebsiella* isolate was gentamicin resistant, cefazolin is not available in the UK and other cephalosporins are not heat stable. Previous studies have suggested that meropenem elutes from small PMMA discs in vitro, but the present report is the first to provide useful in vivo data. Meropenem should be considered for inclusion in bone cement in patients with difficult-to-treat prosthetic joint infections.

**Funding**

This study was carried out as part of our routine clinical duties: no specific funding was obtained.

**Transparency declarations**

None to declare.

**References**