Arrival of *Klebsiella pneumoniae* producing KPC carbapenemase in the United Kingdom

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**Background**: KPC-type carbapenemases are increasingly prevalent in parts of the USA and Israel and are an emerging concern in South America, Europe and China. We investigated the UK’s first two KPC-producing *Klebsiella pneumoniae* isolates.

**Methods**: The isolates were referred to the UK’s national reference laboratory for confirmation of carbapenem resistance. Susceptibilities were determined by agar dilution, and *bla*KPC and Tn4401-like elements were sought by PCR and sequencing. Isolates were compared by PFGE of XbaI- and SpeI-digested genomic DNA.

**Results**: The isolates were from patients in different UK hospitals, with no epidemiological connection. Both were resistant to carbapenems (MICs > 16 mg/L), with imipenem MICs unchanged by EDTA, and also to all other β-lactams (including inhibitor combinations), tobramycin, amikacin and ciprofloxacin. They were susceptible to gentamicin (MICs ≤ 1 mg/L) and colistin (MICs ≤ 0.5 mg/L), with intermediate susceptibility to tigecycline (MICs 1–2 mg/L). The isolates belonged to the same PFGE-defined strain, highly related to a disseminated KPC-producing strain characterized previously in Tel Aviv, Israel. Like this Israeli strain, the UK isolates produced KPC-3 carbapenemase, with the *bla*KPC-3 gene located within a Tn4401-like element.

**Conclusions**: The first KPC-3-producing *K. pneumoniae* isolates detected in the UK were highly genetically related to a KPC-3-producing Israeli *K. pneumoniae* strain. This relatedness was consistent with the history of one UK patient, who had been hospitalized previously in Israel. However, this strain may be circulating more widely since the second UK patient had no identifiable links with Israel or other overseas countries.

Keywords: Enterobacteriaceae, class A β-lactamase, non-metallo-carbapenemase, international clone, mobile genetic element

**Introduction**

The global explosion of CTX-M-type extended-spectrum β-lactamases and, to a lesser extent, acquired AmpC enzymes in clinical isolates of *Escherichia coli* and *Klebsiella* spp. drives therapeutic choice increasingly towards carbapenems. For this reason, carbapenem-hydrolysing β-lactamases (carbapenemases) represent a serious public health threat.

Carbapenemases are diverse, including representatives of β-lactamase molecular classes A, B (metalloenzymes) and D (OXA enzymes). Class A carbapenemases are generally rare, but the KPC (*Klebsiella pneumoniae* carbapenemase) variants are now spreading rapidly. There are currently six recognized variants (note that KPC-1 is synonymous with KPC-2), with KPC-2 and KPC-3 most frequently reported. *K. pneumoniae* is their usual host, but *bla*KPC genes have spread to other members of...
the Enterobacteriaceae and, in Colombia, have been described in 
Pseudomonas spp.\textsuperscript{1} Isolates with KPC enzymes are increasingly 
prevalent in many parts of the USA, particularly in New York.\textsuperscript{2,3} Beyond the USA, there have been hospital outbreaks of infection 
caused by KPC-producing isolates in Israel,\textsuperscript{4,5} and producers are 
an emerging public health concern in Europe,\textsuperscript{6,7} China,\textsuperscript{8,9} and 
Central and South America.\textsuperscript{10}

We report here the characterization of the UK’s first 
*K. pneumoniae* isolates producing KPC enzymes.

**Materials and methods**

**Bacterial isolates**

The first *K. pneumoniae* was isolated, at admission, in 2007 from 
blood cultures of an elderly male in a Scottish hospital (hospital A); he 
had no history of foreign travel in the preceding 6 years, but had 
been hospitalized elsewhere in Scotland. The second isolate was 
from London (hospital B) in 2008, from a urine specimen of an 
elderly female renal transplant patient; her recent history included 
1 month prior to isolation of the bacterium, for the treatment of 
pyelonephritis. Both *K. pneumoniae* isolates were referred to the 
HPA’s Antibiotic Resistance Monitoring and Reference Laboratory 
for confirmation of carbapenem resistance.

Further isolates of *K. pneumoniae*, representing a strain responsible 
for a hospital outbreak in Bnei Brak, Israel, were recovered 
from storage at \(\text{-70}^\circ\text{C}\).

**Antibiotic susceptibility testing and strain typing**

Susceptibilities were determined by BSAC agar dilution methodology 
or Etest (AB Biodisk, Solna, Sweden) and interpreted using 
European Committee for Antimicrobial Susceptibility Testing 
(EUCAST)/BSAC (v.7) breakpoints.

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<tr>
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</tr>
<tr>
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<td>32</td>
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<tr>
<td>Ciprofloxacin</td>
<td>&gt;8</td>
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</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
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</tr>
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Isolates were compared by PFGE, initially using *XbaI*-digested 
genomic DNA, and then with *SpeI*-digested DNA.\textsuperscript{5,11} BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) was used 
to determine relatedness with other referred *K. pneumoniae* isolates.

**Characterization of bla\textsubscript{KPC} and Tn\textsubscript{4401}-like elements**

Isolates were screened for *bla\textsubscript{KPC}*, using primers \(5^\prime\)-TGT CAC TGT ATC GCC GTC-3\textsuperscript{\prime} and \(5^\prime\)-CTC AGT GCT CTA CAG AAA ACC-3\textsuperscript{\prime},\textsuperscript{2,3} and the amplicons were sequenced using dye-terminator 
chemistry on a CEQ8000 Genetic Analyser (Beckman–Coulter, 
High Wycombe, UK). Sequences were compared and aligned with 
Blast.cgi) and CLUSTAL W (http://www.ebi.ac.uk/clustalw).

Location of the *bla\textsubscript{KPC}* genes within Tn\textsubscript{4401}-like elements was 
investigated using the six primer pairs described by Naas et al.\textsuperscript{12} 
*K. pneumoniae* isolate CL-5761 (KPC-3)\textsuperscript{1} and *Enterobacter* sp. 
 isolate E624 (KPC-4, GenBank no. AY700571) were used as positive controls for PCR assays.

In addition, a *bla\textsubscript{OXA-9}-like* allele was sought with primers 
\(5^\prime\)-GCA TAT GTT GTT CG-3\textsuperscript{\prime} and \(5^\prime\)-TTG CTC CTT ACC GGC TGC-3\textsuperscript{\prime}; \textsuperscript{2,3} and the amplicons were sequenced using dye-terminator 
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Results and discussion

The two *K. pneumoniae* isolates were referred, 5 months apart, 
from different UK hospitals (one in Scotland and the other in 
London), with no discernible connections between the cases. 
Carbapenem resistance was recognized by the sending laboratories 
and was subsequently confirmed for all analogues by MIC 
determination (MICs > 16 mg/L; Table 1). Imipenem MICs

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**Table 1.** Antibiotic susceptibilities (MICs, mg/L) of two KPC-3-producing *K. pneumoniae* 
isolates from the UK

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Woodford *et al.*
were unchanged by EDTA, which ruled out production of a class B metallo-carbapenemase. The isolates were resistant to all other β-lactams tested, including combinations with clavulanic acid and cloxacillin, which are inhibitors of class A and C β-lactamas, respectively. They were resistant to tobramycin, amikacin and ciprofloxacin, showed susceptibility or intermediate susceptibility to tigecycline (MICs 1–2 mg/L) and retained susceptibility only to gentamicin (MICs 0.5–1 mg/L) and colistin (MICs ≤ 0.5 mg/L).

A blaKPC allele was detected by PCR in both of the isolates, and PFGE of XbaI-digested DNA revealed that they belonged to the same strain. Comparison with other K. pneumoniae in our database revealed high relatedness (>85% banding pattern similarity) to a cluster of KPC-producing isolates received in 2007 from a hospital outbreak in Bnei Brak, Israel; no other closely related isolates were identified.

Since KPC-producing K. pneumoniae strains with similar antibiograms (susceptibility only to gentamicin and colistin) have been reported previously in Tel Aviv, Israel, we sought also to compare the UK and Bnei Brak isolates with the most widely disseminated Tel Aviv strain, designated clone Q. We compared the banding patterns of SpeI digests with an electronic gel image of SpeI-digested DNA from clone Q (an isolate of the Tel Aviv clone was not available for testing in parallel). The profiles were indistinguishable (Figure 1): both the UK and the Bnei Brak isolates belonged to Tel Aviv clone Q.

Consistent with the reported characteristics of clone Q, both the UK and the representative Bnei Brak isolates were shown by sequencing to produce KPC-3 carbapenemase, a variant with a His-272→Tyr substitution first reported in New York. Further PCR analyses confirmed that their blaKPC genes were flanked by upstream and downstream copies of ISKpm7 and ISKpm6, respectively, located within larger Tn4401-like elements (approx. 10 kb). All of the isolates also harboured a blaoxa-9-like allele linked to blaTEM-1, as found on Tn1331, and associated previously with blaKPC.

In summary, the first two K. pneumoniae isolates with KPC carbapenemase detected in the UK represented a single strain, although there were no discernible epidemiological connections between the cases. Furthermore, this strain was indistinguishable from a KPC-3-producing strain currently circulating in Israel, both in Tel Aviv, and elsewhere (as demonstrated in this study). An ‘Israel connection’ was consistent with the history of the London (hospital B) patient, suggesting that particular isolate was probably imported into the UK. However, we cannot exclude the possibility that this KPC-positive strain may be circulating more widely outside Israel, since the provenance of the Scottish (hospital A) isolate remains unclear, with no links established to other countries. K. pneumoniae with KPC carbapenemases were originally isolated in the USA and, besides Israel, have also been reported in China, France and Greece. Given the limited therapeutic options available for infections caused by these multiresistant K. pneumoniae strains, there is an urgent need to compare producer isolates from different countries to monitor the potential international dissemination of resistant clones.

Acknowledgements
We are grateful to Yehuda Carmeli (Tel Aviv) for helpful discussions and for providing the digitized image of the PFGE profile (SpeI digest) of clone Q.

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N. W. and D. M. L. have received research grants and speaking invites from various pharmaceutical companies. D. M. L. has a diversified share portfolio, including holdings in pharmaceutical
companies. None of these poses a conflict of interest with this work. Other authors: none to declare.

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