# P427 NOVEL CLASS A CARBAPENEMASE, KPC-4, IN AN *ENTEROBACTER* ISOLATE FROM SCOTLAND

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## INTRODUCTION & OBJECTIVES

- Imipenem (IPM) and meropenem (MEM) are agents of last resort for treatment of serious infections caused by many multi-resistant gram-negative bacteria. The emergence and spread of carbapenem-hydrolyzing β-lactamases (carbapenemases) belonging to molecular classes B, D and, more rarely, to class A, has considerable public health importance (1).
  - except in the case of class D enzymes in *Acinetobacter* spp., carbapenemases remain rare.
- A carbapenem-resistant isolate of *Enterobacter* sp. (E624) was identified in the British Society for Antimicrobial Chemotherapy (BSAC) Bacteraemia Resistance Surveillance Programme collection for 2003 (http://www.bsacsurv.org).
  - Two further *Enterobacter* isolates from the same patient, a 65-year-old woman with acute myeloid leukaemia, varied in their degrees of carbapenem susceptibility. The three isolates were from separate bacteraemic episodes over a one-month period.
- We sought to determine the mechanism(s) of carbapenem resistance in these three isolates.

## METHODS

- Protocols for isolate collection within the BSAC Bacteraemia Resistance Surveillance Programme, and methods of identification and susceptibility testing have been described (2). Carbapenems were tested on Mueller-Hinton agar; all other agents on IsoSensitest agar. In addition:
  - isolate E624 was identified by 16*S* rRNA sequencing.
  - IPM MICs were measured with 320 mg/L EDTA to screen for metallo-carbapenemase activity.
  - IPM and MEM MICs were measured with 100 mg/L cloxacilllin to test any contribution of AmpC activity to resistance.
- Isolates were typed by PFGE of *Xba*I-digested genomic DNA.
- Isolates were screened by PCR for genes encoding known carbapenemases, including for $bla_{KPC}$ (3;4); selected amplicons were sequenced using dye-terminator chemistry.
- IPM hydrolysis was investigated by spectrophotometry at 297 nm at 37°C using crude enzyme extracts (4). Isoelectric focusing (IEF) was performed to visualise β-lactamases in these extracts; an overlay of 100 mg/L cloxacilllin was used to inhibit AmpC activity before developing gels with nitrocefin.
- Outer membrane protein (OMP) profiles were examined by SDS-PAGE analysis (4).

**Fig. 1.** PFGE profiles of *E. cancerogenus* isolates. Lanes 1 and 6, concatamer of λ phage DNA; lanes 2 & 4 E624 (different submissions to reference laboratory); lane 3, isolate 1; lane 5, isolate 3.

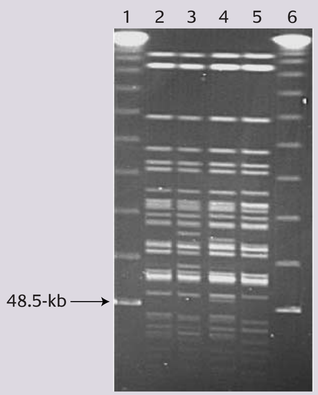


**Table 1.** Antibiotic susceptibilities of *E. cancerogenus* isolates and summary of molecular investigations.

| Antibiotic | MICs (mg/L) | | |
|---|---|---|---|
| | 1 Isolated : 21/07/2003 | 2 (E624) Isolated : 14/08/2003 | 3 Isolated : 19/08/2003 |
| Imipenem (IPM) | 0.5 | >32 | 4 |
| Meropenem (MEM) | 0.25 | >32 | 8 |
| Ertapenem | 2 | >16 | >16 |
| IPM + EDTA | 0.5 | >16 | 2 |
| IPM + cloxacillin | 0.5 | 16 | 0.25 |
| MEM + cloxacillin | 0.06 | 16 | 0.125 |
| Ampicillin | >64 | >64 | >64 |
| Co-amoxiclav | 64 | >64 | 64 |
| Aztreonam | >64 | >64 | >64 |
| Cefotaxime | >64 | >64 | >64 |
| Cefotaxime + clavulanate | >32 | >32 | >32 |
| Ceftazidime | >64 | >64 | >64 |
| Ceftazidime + clavulanate | >32 | >32 | >32 |
| Cefepime | 16 | 64 | 16 |
| Cefepime + clavulanate | 4 | >64 | 16 |
| Cefoxitin | >64 | >64 | >64 |
| Piperacillin | >64 | >64 | >64 |
| Piperacillin + tazobactam | >64 | >64 | >64 |
| Ciprofloxacin | >8 | >8 | >8 |
| Gentamicin | >32 | 1 | 4 |
| Tobramycin | 32 | 1 | 2 |
| Amikacin | 4 | 2 | 4 |
| Minocycline | 32 | 32 | 4 |
| Colistin | 1 | 1 | 1 |
| $bla_{KPC}$/carbapenemase activity | Present | Present | Absent |
| AmpC | Present | Present | Present |
| OMP loss | No | Yes | Yes |

**Fig. 2.** The KPC family of carbapenemases. The predicted cleavable signal peptide is shaded.

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                *       20        *       40        *       60        *       80
KPC-1 : MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYRAEERFPLCSSFKGFLAAAVL
KPC-2 : ................................................................................
KPC-3 : ................................................................................
KPC-4 : -...............................................................................

                *       100       *       120       *       140       *       160
KPC-1 : ARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAELSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFR
KPC-2 : ................................................................................
KPC-3 : ................................................................................
KPC-4 : .....................R..........................................................

                *       180       *       200       *       220       *       240
KPC-1 : LDRWELELNSAIPSDARDTSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCGVY
KPC-2 : ............G...................................................................
KPC-3 : ............G...................................................................
KPC-4 : ............G..................................................................G.

                *       260       *       280       *
KPC-1 : GTANDYAVVWPTGRAPIVLAVYTRAPNKDDKHSEAVIAAAARLALEGLGVNGQ*
KPC-2 : ...................................................*
KPC-3 : ..............................Y....................*
KPC-4 : ...................................................*
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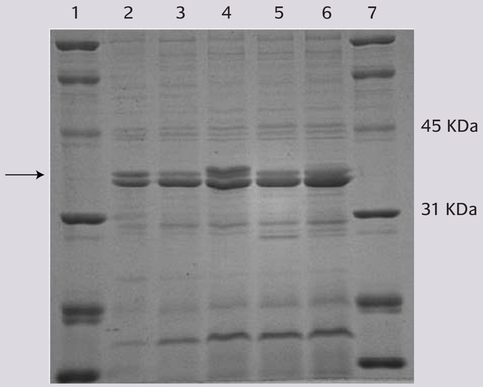
**Fig. 3.** OMP analysis of *E. cancerogenus* isolates. Lane 1 & 7, MW standards; lane 2: 684 (a carbapenem-susceptible *E. cloacae*); lanes 3 & 5, E624 (different submissions to reference laboratory); lane 4, isolate 1; lane 6, isolate 3. The OMP present in only in carbapenem-susceptible isolate 1 is indicated by the arrow.




## RESULTS

### *Identification & typing*

- The three isolates were initially identified as *Enterobacter* sp.; 16*S* rRNA sequencing confirmed isolate E624 as *E. cancerogenus*.
- The isolates were highly-related by PFGE and represented a single strain **(Fig. 1)**.

### *Antibiotic susceptibility and carbapenem therapy*

- Carbapenem (and some other) susceptibilities of the isolates varied **(Table 1)**:
  - isolate 1 was susceptible to ertapenem (ETP; MIC 2 mg/L), IPM (MIC 0.5 mg/L) and MEM (MIC 0.25 mg/L).
  - Isolate 2 (E624) was highly-resistant to all three carbapenems (MICs >16 mg/L).
  - isolate 3 was resistant to ETP (MIC >16 mg/L), but less so to MEM (MIC 8 mg/L) and IPM (MIC 4 mg/L).
- This patient had multiple bacteraemic episodes and antimicrobial therapy was complex;
  - for the episode caused by carbapenem-susceptible isolate 1, the patient received MEM + gentamicin for 10 days.
  - carbapenems had not been administered previously.
  - 12 days later (immediately prior to isolation of resistant variant E624) she received MEM + gentamicin + co-amoxiclav for 4 days.

### *Carbapenem resistance mechanisms*

- Isolates 1 and 2 (E624):
  - hydrolyzed IPM and possessed a novel $bla_{KPC}$ allele, encoding KPC-4.
  - KPC-4 (GenBank AY700571) had 3 amino acid substitutions in comparison with KPC-1, Pro(103)Arg, Ser(174)Gly, and Val(239)Gly **(Fig. 2)**.
  - 2 β-lactamases were apparent by IEF, and were consistent with KPC and an AmpC enzyme.
- Isolate 3:
  - lacked a $bla_{KPC}$ allele; IPM hydrolysis was not detected.
  - regained susceptibility to carbapenems when tested in the presence of 100 mg/L cloxacillin to inhibit AmpC activity **(Table 1)**.
- Isolates 2 (E624) and 3 lacked a major OMP that was present in susceptible isolate 1 **(Fig. 3)**.

## CONCLUSIONS

- *E. cancerogenus* E624 produced a novel carbapenemase, KPC-4. To our knowledge this is the first KPC enzyme detected outside of the United States.
- *E. cancerogenus* is naturally susceptible to carbapenems (5), implying that $bla_{KPC-4}$ was acquired by this strain from an unidentified source.
- Production of KPC-4 alone did not confer high-level carbapenem resistance; rather resistance appeared to require concomitant OMP loss (isolate 1 *vs*. E624).
  - this loss had probably been selected *in vivo* during MEM therapy.
- In the absence of KPC-4, high-level ETP resistance and moderate level IPM and MEM resistance in this *E. cancerogenus* strain was associated with AmpC activity plus OMP loss (isolate 3).

## REFERENCES

(1) Nordmann P & Poirel L. *Clin Microbiol Infect* 2002; **8**:321-331.
(2) Reynolds R, *et al. J Antimicrob Chemother* 2004; **53**:1018-1032.
(3) Yigit H, *et al. Antimicrob Agents Chemother* 2001; **45**:1151-1161.
(4) Woodford N, *et al. Antimicrob Agents Chemother* 2004; **48**:4793-4799.
(5) Stock I & Wiedemann B. *Clin Microbiol Infect* 2002; **8**:564-578.

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