**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.uk).

- 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 bacteremic episodes over a one-month period.

- We sought to determine the mechanism(s) of carbapenem resistance in these three isolates.

**METHODS**

- Protocols for isolation 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 blood-sediment agar. In addition:

  - Isolate E624 was identified by 16S 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 cloxacillin to inhibit AmpC activity.

- Isolates were typed by PFGE of chromosomal DNA. Isolates were screened by PCR for genes encoding known carbapenemases, including for 

  - β-lactamases: (3,4); selected amplimers 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 cloxacillin was used to inhibit AmpC activity before developing gels with nitocefin.

- Outer membrane protein (OMP) profiles were examined by SDS-PAGE analysis (4).

**RESULTS**

**Identification & typing**

- The three isolates were initially identified as Enterobacter sp.; 16S rRNA sequencing confirmed isolate E624 as E. cancerogenus.

- The isolates highly-hydrated by PFGE (Fig. 1) represented a single strain (Fig. 1).

**Antibiotic susceptibility and carbapenemase activity**

- Carbapenem (and some other) susceptibilities of the isolates varied (Table 1).

- Isolate 1 was susceptible to ertapenem (ETP; MIC 2 mg/L) and MEM (MIC 0.5 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).

- Isolate 1 was susceptible to IPM (MIC 0.5 mg/L) and MEM (MIC 0.5 mg/L). Isolate 2 (E624) and 3 lacked a major OMP that was present in susceptible isolate 1 (Fig. 2).

**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 blaKPC 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 this strain from an unidentified source.

- 12 days later (immediately prior to isolation of robust 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 bladP allele, encoding KPC-4.
  - KPC-4 (GenBank AV700571) 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 bladP 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).

**REFERENCES**


