In Vivo Development of Ertapenem Resistance in a Patient with Pneumonia Caused by *Klebsiella pneumoniae* with an Extended-Spectrum β-Lactamase

Eugenne Elliott,1 Adrian J Brink,1 Johan van Greune,2 Zia Els,3 Neil Woodford,4 Jane Turton,4 Marina Warner,4 and David M Livermore4

1Department of Clinical Microbiology, Ampath Laboratories, Milpark Hospital, Johannesburg, and 2Department of Clinical Microbiology, Ampath Laboratories, N1-City Hospital, and 3N1-City Hospital, Cape Town, South Africa; and 4Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency Colindale, London, United Kingdom

Ertapenem is a broad-spectrum carbapenem that is active against most common pathogens, except enterococci, nonfermenters, and methicillin-resistant staphylococci [1]. It remains active against most *Enterobacteriaceae* species with extended-spectrum β-lactamases (ESBLs), although this activity is not quite as universal as it is for other carbapenems. Thus, Paterson et al. [2] reported that 10.9% of ESBL-positive *Klebsiella pneumoniae* isolates collected worldwide from intra-abdominal infections were resistant to ertapenem, compared with 4%–5% that were resistant to imipenem and meropenem, and Jacoby et al. [3] reported that ESBL-producing transconjugants of a porin-deficient *K. pneumoniae* mutant mostly were resistant to ertapenem while remaining moderately susceptible to other carbapenems.

We report the sequential isolation of ertapenem-susceptible and ertapenem-resistant ESBL-producing *K. pneumoniae* from a patient in an intensive care unit (ICU) illustrating in vivo development of resistance in a single strain during ertapenem therapy, and we identify the mechanisms responsible.

**Case report.** An 86-year-old man with chronic obstructive pulmonary disease, for which he was receiving 5 mg of prednisone per day, was admitted to the hospital for investigation of syncopal attacks. Iron-deficiency anemia was detected on admission, and subsequent gastroscopy and biopsy resulted in the diagnosis of gastric carcinoma. Gastrectomy was performed, and the patient was admitted to the ICU postoperatively and was ventilated.

An ertapenem-susceptible, ESBL-producing *K. pneumoniae* isolate was cultured from a sputum specimen, and a diagnosis of nosocomial pneumonia was made. The antibiotic therapy was tailored to 1 g of intravenous ertapenem plus 1 g of intravenous amikacin per day, but an ertapenem-resistant, ESBL-producing *K. pneumoniae* isolate was cultured 5 days later from a tracheal aspirate, and on this basis, the ertapenem-amikacin regimen was replaced with a regimen of 1 g of intravenous imipenem administered every 6 h. A third ESBL-producing *K. pneumoniae* isolate was cultured from an abdominal wound swab specimen and found to be ertapenem resistant, whereas a fourth isolate, cultured from a central venous catheter tip, was found to be ertapenem susceptible. No subsequent blood, urine, and pus samples, central venous catheter tip specimens, and tracheal aspirates grew ESBL-producing *K. pneumoniae*, and treatment with imipenem was stopped after 14 days. The patient recovered and was discharged from the hospital. Informed consent for use of the clinical information in this case report was obtained from the patient.

**Detection, identification, and susceptibility testing.** The ertapenem-resistant organisms were originally detected during routine culture and susceptibility testing in the Ampath service laboratory (Cape Town, South Africa), and they, together with the ertapenem-susceptible isolates, were forwarded to the main Ampath laboratory (Johannesburg, South Africa) for confirmation. Identification was performed using API 20E (BioMérieux). Disc-susceptibility testing was performed and interpreted according to Clinical Laboratory Standards Institute guidelines [4]. ESBL production was identified by the double-disc Jarlier test, using 30 μg of cefpodoxime and 30 μg of ceftaxime in combination with doses containing 20 μg of amoxicillin plus 10 μg of clavulanate.

The isolates were shipped to the Health Protection Agency’s Centre for Infections (London, United Kingdom), where MICs...
were determined by the British Society for Antimicrobial Chemotherapy’s agar dilution method [5]. Drug susceptibility testing included tests for ertapenem and imipenem (Merck) and meropenem (AstraZeneca).

**Molecular and biochemical characterization.** Isolates were screened for bla\textsubscript{CTX-M} alleles by PCR, initially with universal primers MA1 and MA2 (amplicon size, 554 base pairs), and then with primers specific for various bla\textsubscript{CTX-M} Groups [6]. Cycling conditions were as described elsewhere [7]. DNA fingerprinting was performed by PFGE of XbaI-digested genomic DNA, as described elsewhere [7].

Outer-membrane proteins were extracted with sodium lauryl sarcosinate from logarithmic-phase cultures, variously grown in nutrient, Luria-Bertani and IsoSensitest broths, and were profiled by sodium dodecyl sulfate polyacrylamide gel electrophoresis, using the method of Chart [8].

**Susceptibility testing.** Initial disc test results for the isolates were confirmed by MIC results, as shown in table 1. The first and final isolates were susceptible to ertapenem and to the other carbapenems tested, whereas the second and third isolates were resistant to ertapenem, with MICs >16 \(\mu\)g/mL. The second and third isolates also had intermediate resistance to meropenem (MICs, 4–8 \(\mu\)g/mL) and reduced susceptibility to imipenem (MICs, 0.5–1 \(\mu\)g/mL). All isolates were broadly resistant to cephalosporins, as was expected for ESBL producers; the ertapenem-resistant isolates, but not the susceptible isolates, also were resistant to cephalosporin-clavulanate combinations.

**Molecular characterization.** PFGE of XbaI-digested DNA indicated that all 4 isolates belonged to the same strain, because of the consistency between their profiles (figure 1). PCR for bla\textsubscript{CTX-M} gave products with universal and CTX-M group 1–specific primers for all 4 isolates, whereas no products were obtained with primers specific for other CTX-M groups.

Examination of outer-membrane protein profiles revealed that the 2 resistant isolates lacked a prominent protein band of a molecular weight of 35–37 kD, whereas this component was present in both of the susceptible isolates (figure 2). We believe this band to be OmpK36, which is the sole porin expressed by most ESBL-producing *K. pneumoniae* [9]; OmpK35, which runs above OmpK36 in this gel system [10], was not apparent, even when cultures of ertapenem-susceptible or ertapenem-resistant representatives were grown in nutrient broth as a low osmolality medium. The retained major outer-membrane protein, present even in the resistant isolates, is the OmpA homologue, which is not a porin.

**Discussion.** Previous studies have shown that, although the MICs of ertapenem for *K. pneumoniae* with ESBLs and AmpC \(\beta\)-lactamases are mostly \(\leq 1\ \mu\)g/mL, they nevertheless are slightly higher than MICs for strains without these mechanisms, whereas this is not the case for imipenem, doripenem, and meropenem. Thus, Livermore et al. [11] found modal MICs of 0.031 and 0.0078 mg/L for ESBL-positive and ESBL-negative *K. pneumoniae*, compared with 0.12 and 0.12 mg/L, respectively, for imipenem. These data imply that ertapenem may not evade ESBLs as completely as other carbapenems, and this inference is supported by the observations that ESBLs conferred resis-

### Table 1. MICs for extended-spectrum \(\beta\)-lactamase–producing *Klebsiella pneumoniae* isolates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Isolate</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tr>
<td>Date of culture</td>
<td>20 January 2005</td>
<td>25 January 2005</td>
<td>1 February 2005</td>
<td>4 February 2005</td>
<td></td>
</tr>
<tr>
<td>Source of isolate</td>
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<td>Tracheal aspirate</td>
<td>Abdominal swab</td>
<td>Central venous catheter tip</td>
<td></td>
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<tr>
<td>MIC, (\mu)g/mL</td>
<td>Ertapenem</td>
<td>0.5</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>0.5</td>
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<tr>
<td></td>
<td>Imipenem</td>
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<td>0.5</td>
<td>0.125</td>
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<tr>
<td></td>
<td>Meropenem</td>
<td>(\leq 0.06)</td>
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<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime-clavulanate(^a)</td>
<td>1</td>
<td>16</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cefepime</td>
<td>64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Cefepime-clavulanate(^a)</td>
<td>0.25</td>
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<td>&gt;32</td>
<td>0.125</td>
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<td></td>
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<td>&gt;64</td>
<td>&gt;64</td>
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<tr>
<td></td>
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<td>16</td>
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<tr>
<td></td>
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<td>&gt;32</td>
<td>&gt;32</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Tigecycline</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</table>

\(^a\) Test was done with a fixed concentration of 4 \(\mu\)g/mL of clavulanate.
Figure 1. PFGE of XbaI-digested DNA from carbapenem-susceptible and carbapenem-resistant extended-spectrum β-lactamase–producing Klebsiella pneumoniae isolates. Lane M, λ ladder; lanes 1–4, DNA from isolates 2, 1, 4, and 3 respectively; lane 5, isolate 3 repeated; Kb, kilobase.

tance to ertapenem when they were introduced into a porin-deficient K. pneumoniae strain but only caused small reductions in susceptibility to imipenem (especially) and meropenem [3].

The impact of these differences on bacteriological and clinical outcome in patients is unknown. Munoz et al. [12] reviewed 45 cases in which ertapenem was used to treat infections caused by ESBL producers and recorded 2 cases, 1 of bacteremia and 1 of peritonitis, in which ertapenem-resistant K. pneumoniae was isolated after therapy, but did not establish whether these were super-infections or cases of selection that occurred during therapy.

In the present case, resistance unequivocally emerged in the original strain during ertapenem therapy, because findings of PFGE profiles were identical for all isolates. The susceptible and resistant organisms differed, however, in their lack of OmpK36, which is the major porin remaining in most ESBL-producing K. pneumoniae [9]. We cannot exclude the possibility that other mechanisms had been selected along with porin loss, but we consider this unlikely, because MICs of tetracyclines, which would be affected by up-regulation of chromosomal efflux, were unaltered. We suggest that the cross-resistance of the ertapenem-resistant isolates to cephalosporin–β-lactamase inhibitor combinations reflected restricted access to the clavulanate.

Curiously, the final isolate, obtained 10 days after ertapenem therapy was stopped but while the patient was still receiving imipenem, had the parental outer-membrane protein profile for K. pneumoniae and was as susceptible as the isolate obtained before therapy. It is possible but unlikely that reversion had occurred once ertapenem pressure was withdrawn; it is more probable that the Klebsiella infection was disseminated, with resistant variants becoming dominant at some body sites but not at others.

This case study prompts several questions. First, would imipenem or meropenem, with higher free drug levels and shorter dosage intervals than ertapenem, have been as selective for resistance in this manner? The likely answer is “no,” because these agents have been used for many years without such case reports and because imipenem remained sufficiently active to be an effective treatment against the ertapenem-resistant variant. Second, did this strain predicate a high risk for this type of selection by requiring (unusually for an ESBL-producing K. pneumoniae) a starting MIC of ertapenem (0.5 µg/mL) that exceeded MICs of imipenem (0.125 µg/mL) or meropenem (<0.060 µg/mL)? Third, might selection have been avoided by using a higher or more-frequent dosage of ertapenem (e.g., 2 g taken once daily or 1 g taken twice daily)? Finally, would the treatment failure have occurred if the isolate had been more fully susceptible to amikacin, with which it was coadministered?

This case is disturbing, as is the occurrence of ertapenem resistance in a small minority of other ESBL-producing K. pneumoniae [2, 11]. The additional occurrence of such cases needs to be monitored closely. The use of carbapenems is being driven by the accumulation of cephalosporin resistance and quinolone resistance to ertapenem when they were introduced into a porin-deficient K. pneumoniae strain but only caused small reductions in susceptibility to imipenem (especially) and meropenem [3].

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Figure 2. Outer-membrane proteins of carbapenem-susceptible and carbapenem-resistant extended-spectrum β-lactamase–producing Klebsiella pneumoniae. Lanes 1 and 8, molecular weight standards: 96,000 (phosphorylase b), 68,000 (bovine serum albumin), 45,000 (ovalbumin), 31,000 (carbonic anhydrase), 24,500 (soybean trypsin inhibitor), and 14,000 (lysozyme). Lanes 2–5, isolates, 1, 4, 2, and 3, respectively; lane 6, isolate 2 repeated; lane 7, K. pneumoniae ATCC 13883; lane 8, unrelated ertapenem-susceptible strain.
resistance in *Enterobacteriaceae*, and there is a need to define which groups of patients with infections due to resistant *Enterobacteriaceae* are best treated with ertapenem and which are better treated with imipenem, meropenem, or, in the future, doripenem. Although ertapenem seems to be more vulnerable to the mode of resistance described here, it is less likely to exert selection pressure on copresent nonfermenters, which are inherently resistant.

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**References**