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

Eugene Elliott,† Adrian J Brink,† Johan van Greune,‡ Zia Els,†
Neil Woodford,§ Jane Turton,¶ Marina Warner,* and David M Livermore**

†Department of Clinical Microbiology, Ampath Laboratories, Milpark Hospital, Johannesburg, South Africa; ‡Department of Clinical Microbiology, Ampath Laboratories, N1-City Hospital, and ¶Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency Colindale, London, United Kingdom; §Department of Clinical Microbiology, Milpark Hospital, Johannesburg, and **Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency Colindale, and *Laboratory of Healthcare Associated Infection, Centre for Infections, Health Protection Agency Colindale, London, United Kingdom

Four sequential extended-spectrum β-lactamase–producing isolates of *Klebsiella pneumoniae* were obtained from a patient after treatment with ertapenem and cultured. The first and fourth isolates were susceptible to ertapenem, whereas the second and third were resistant. All 4 isolates belonged to the same strain and produced a group 1 CTX-M enzyme; additionally, the resistant isolates had lost a porin.

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 MERKEL® test, using 30 μg of cefoxitin and 30 μg of cefotaxime 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 *blaCTX-M* alleles by PCR, initially with universal primers MA1 and MA2 (amplicon size, 554 base pairs), and then with primers specific for various *blaCTX-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 μg/mL. The second and third isolates also had intermediate resistance to meropenem (MICs, 4–8 μg/mL) and reduced susceptibility to imipenem (MICs, 0.5–1 μg/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 *blaCTX-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 kDa, 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 β-lactamases are mostly ≤1 μg/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-

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### Table 1. MICs for extended-spectrum β-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></td>
<td>20 January 2005</td>
<td>25 January 2005</td>
<td>1 February 2005</td>
<td>4 February 2005</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>
</tr>
<tr>
<td>MIC, μg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
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<td>&gt;16</td>
<td>&gt;16</td>
<td>0.5</td>
</tr>
<tr>
<td>Imipenem</td>
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<td>1</td>
<td>0.5</td>
<td>0.125</td>
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<tr>
<td>Meropenem</td>
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<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Cefotaxime</td>
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<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefotaxime-clavulanatea</td>
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<td>16</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Cefepime</td>
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<td>64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>64</td>
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<tr>
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<tr>
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<td>&gt;64</td>
<td>&gt;64</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
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<td>16</td>
<td>16</td>
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<tr>
<td>Gentamicin</td>
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<td>≥32</td>
<td>≥32</td>
<td>≥32</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*a Test was done with a fixed concentration of 4 μg/mL of clavulanate.
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 im-
ipenem 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 var-
iant. 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. pneu-
moniae [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

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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 ertape-
nem-susceptible strain.

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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.

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a λ ladder

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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 ertape-
nem-resistant isolates to cephalosporin–β-lactamase inhibitor
combinations reflected restricted access to the clavulanate.

Curiously, the final isolate, obtained 10 days after ertapenem

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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**