

## Case Report

# Development of ertapenem resistance in a patient with mediastinitis caused by *Klebsiella pneumoniae* producing an extended-spectrum $\beta$ -lactamase

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The aim was to study the clinical and microbiological features associated with a carbapenem-resistant *Klebsiella pneumoniae* isolate that had been selected *in vivo* by an ertapenem-containing regimen in a patient with mediastinitis despite high blood and mediastinal levels of ertapenem. Carbapenem resistance was characterized by conjugation, PCR, DNA sequencing and analysis of outer-membrane proteins. The isolates susceptible and resistant to the carbapenems were compared by ribotyping and PFGE. Resistance to all available  $\beta$ -lactams was most probably due to combined production of extended-spectrum  $\beta$ -lactamase (ESBL) CTX-M-15 and loss of OmpK36 porin. The results of ribotyping and PFGE suggest that the carbapenem-resistant strain was a derivative of the original mediastinal isolate rather than a superinfecting isolate. This observation stresses the risk of selection of pan-penem resistant strains of enterobacteria when ertapenem is used for the treatment of severe infections due to ESBL-producing enterobacteria.

Received 28 April 2009

Accepted 7 September 2009

## Introduction

Ertapenem, a parenteral broad-spectrum 1-beta-methyl-carbapenem (Shah & Isaacs, 2003), has received regulatory approval in the United States and the European Union. The molecule has good *in vitro* activity against many common aerobic and anaerobic Gram-positive and Gram-negative bacteria including members of the *Enterobacteriaceae* producing plasmid- or chromosomally mediated  $\beta$ -lactamases, encompassing AmpC and extended-spectrum  $\beta$ -lactamases (ESBLs) (Livermore *et al.*, 2003). Ertapenem is not active against enterococci, nonfermenters and methicillin-resistant staphylococci (Livermore *et al.*, 2003). It shares structural features with meropenem, including its stability to dehydropeptidase-1, allowing administration without a dehydropeptidase-1

inhibitor. Superiority in *in vitro* activity and a low potential for so-called ‘collateral damage’ such as acquisition of resistant bowel flora have been reported in several randomized controlled trials (DiNubile *et al.*, 2005; Roy *et al.*, 2003; Solomkin *et al.*, 2003; Yellin *et al.*, 2002, 2007). Thus, ertapenem appears to be an attractive treatment alternative for adults as well as for children with complicated aerobic and anaerobic mixed infections caused by ertapenem-susceptible bacteria (Yellin *et al.*, 2007). Ertapenem resistance in *Klebsiella pneumoniae* is rare. A recent multicentre Israeli study found 15 isolates with an ertapenem non-susceptible phenotype among 663 (2.3%) ESBL-producing *K. pneumoniae* isolates (Leavitt *et al.*, 2009). Usually, the molecular mechanism of ertapenem resistance is due to the presence of an ESBL gene combined with the absence of OmpK36 (Leavitt *et al.*, 2009; Doumith *et al.*, 2009). *In vivo* emergence of ertapenem resistance in a *K. pneumoniae* clinical isolate encoding an ESBL belonging to the CTX-M group 1 and which had lost the OmpK36 porin has also been described in a patient with pneumonia (Elliott *et al.*, 2006). However, ertapenem is still an important agent for treating ESBL *K. pneumoniae*

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Abbreviation: ESBL, extended-spectrum  $\beta$ -lactamase.

Figures showing PFGE and plasmid profiles of *K. pneumoniae* strains Kp5 and Kp6 are available with the online version of this paper.

infections, especially in hospitalized patients, as antimicrobial co-resistance among *K. pneumoniae* ESBL-producing isolates limits the number of drugs useful against these strains (Schwaber *et al.*, 2005), and as ertapenem, compared to the other carbapenems, is less likely to lead to carbapenem resistance in *Pseudomonas aeruginosa* (DiNubile *et al.*, 2005). Here, despite high concentrations of the drug in blood and mediastinal tissues, we report the clinical and microbiological features associated with a carbapenem-resistant *K. pneumoniae* isolate that has been selected *in vivo* by an ertapenem-containing regimen in a patient with mediastinitis. The resulting strain was resistant to all available  $\beta$ -lactams.

## Case report

The patient was a 51-year-old man with a medical history of diabetes mellitus, ischaemic heart disease complicated by decreased ejection fraction (35%), and recent stroke. Because of his coronary heart disease, he underwent a triple coronary artery bypass graft, using both mammary arteries. The immediate post-operative course was marked by cardiogenic shock requiring catecholamine infusion. On post-operative day 2, he suffered from ventilator-associated pneumonia. A strain of *Klebsiella oxytoca* was isolated from a bronchoalveolar lavage (BAL) sample. The patient was treated with piperacillin–tazobactam, which was changed 2 days later to amoxicillin–clavulanic acid. The clinical course was marked by two other ventilator-associated pneumonias diagnosed on post-operative days 11 and 18, caused by *Escherichia coli* and *P. aeruginosa* strains, respectively, also isolated from BAL. The patient was successively treated with an initial course of cefotaxime changed to amoxicillin–clavulanic acid and then ceftazidime plus amikacin changed to piperacillin plus ciprofloxacin. Finally, after being tracheostomized, the patient was removed from the ventilator on day 31 post cardiac surgery.

The sternal wound became rapidly inflamed afterwards. Since the patient had numerous risk factors for mediastinitis (i.e. diabetes, multiple coronary bypass grafting), a sternal puncture was performed on post-operative day 12, which did not yield any micro-organism upon culture. The appearance of the wound remained unchanged until day 44 with the occurrence of a bloody and purulent discharge at the upper section of the sternal wound. Growth from a swab was positive for ESBL-producing *K. pneumoniae* (strain Kp1). Because the patient's condition was stable and the sternum solid, this was considered to be a superficial infection of the wound requiring no antibiotic treatment. However, the purulent discharge persisted despite local care and a surgical drainage was performed on day 50. Direct smear examination of the surgical samples was negative but cultures confirmed the presence of ESBL-producing *K. pneumoniae* (strain Kp2) together with *Streptococcus oralis*. Mediastinitis was thus diagnosed and treatment was initiated with ertapenem, 1 g per day, starting on day 54. Due to persistent positive cultures of

the mediastinal drainages on days 54, 61 and 66 (strains Kp3, Kp4 and Kp5), blood and mediastinal fluid concentrations of ertapenem were assayed by microbiological dosage on day 66. Residual and maximal concentrations were 0.5 and 88.9 mg l<sup>-1</sup> in the blood, respectively. The concentration of ertapenem was 9.4 µg ml<sup>-1</sup> in the mediastinal fluid collected through the drainage. The MIC of ertapenem against the *K. pneumoniae* strain isolated on day 66 (strain Kp5) was 0.094 mg l<sup>-1</sup> and the treatment was thus maintained. On day 70, cultures of the mediastinal drainage were still positive for ESBL-producing *K. pneumoniae* (strain Kp6) as was a faecal sample (strain Kp7), but the susceptibility to carbapenems of isolates Kp6 and Kp7 was strongly reduced with MICs of >32, 12 and 8 µg ml<sup>-1</sup> for ertapenem, meropenem and imipenem, respectively, for both strains. Treatment with ertapenem was changed to imipenem, at a dose of 4 g per day. The fourth injection of the antibiotic was followed by a general seizure, despite a 1 h infusion rate. The treatment was changed to co-trimoxazole and gentamicin for 15 days, which led to sterilization of the mediastinal drainage cultures and a normalization of white blood cell counts. However, faecal samples were still positive for ESBL-producing *K. pneumoniae* on days 76, 85, 90 and 97 (strains Kp8, Kp9, Kp10 and Kp11). Unfortunately, the patient had a massive aspiration complicated by pneumonia and septic shock leading to multiple organ dysfunction and died on day 99. Treatment and clinical course are summarized in Fig. 1.

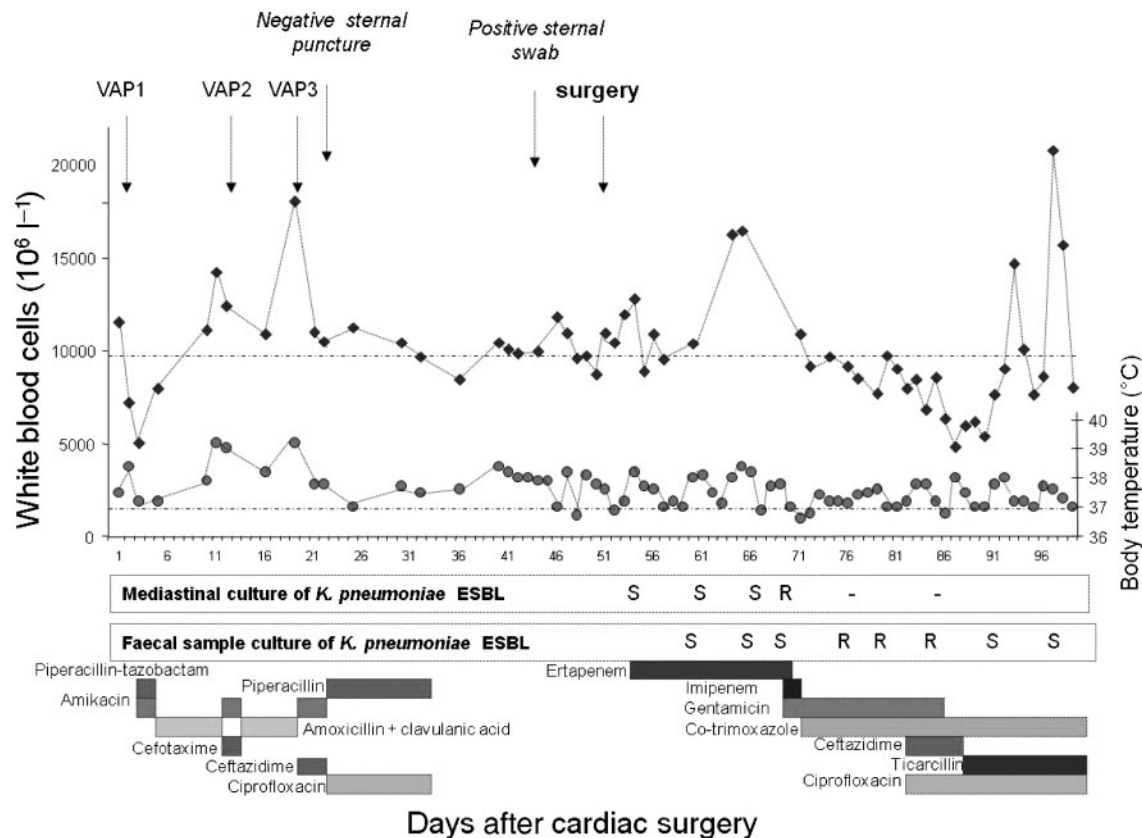
## Methods

### Identification and susceptibility testing of *K. pneumoniae*.

Bacterial identification was performed using API 20E strips (bioMérieux). Antibiotic susceptibility was determined by the disc diffusion method, as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie ([www.sfm.asso.fr](http://www.sfm.asso.fr)). ESBL production was detected by the double-disc synergy test using discs containing 30 µg cefpodoxime and 30 µg cefotaxime in combination with discs containing 20 µg amoxicillin plus 10 µg clavulanate. MICs of carbapenems were determined by the E-test method (AB Biodisk) with and without EDTA and in a plate with or without cloxacillin at 250, 500, 750 or 1000 mg l<sup>-1</sup>.

### Molecular and biochemical characterization of *K. pneumoniae*

**Conjugation experiments.** Conjugation was performed in liquid medium with streptomycin-resistant *E. coli* HB101 (Boyer & Roulland-Dussoix, 1969) as a recipient. Selection of the transconjugants was performed on Brain Heart Infusion (BHI) agar supplemented with streptomycin (20 mg l<sup>-1</sup>) and ceftazidime (1 mg l<sup>-1</sup>). Randomly selected transconjugants were purified and their resistance phenotypes were determined. Standard PCR conditions were used on these transconjugants and on the clinical strains to amplify structure genes for ESBLs (TEM, SHV and CTX-M), oxacillinases (OXA-23, OXA-24, OXA-40, OXA-48 and OXA-58) and carbapenemases (IMP-1, IMP-2, KPC and VIM). The amplification products were purified with the QIAquick PCR Purification kit (Qiagen) and sequenced with a CEQ 2000 DNA Analysis System automatic sequencer (Beckman Coulter). Plasmid DNA of *K. pneumoniae* clinical strains Kp5 and Kp6 was obtained with NucleoSpin Plasmid (Macherey-Nagel).



**Fig. 1.** Treatment and clinical course. Diamonds, white blood cell count; circles, body temperature. VAP, ventilator-associated pneumonia; 'S', culture-positive with a *K. pneumoniae* isolate susceptible to penems; 'R', culture-positive with a *K. pneumoniae* isolate resistant to penems; '-', negative culture.

**Analysis of outer-membrane proteins.** Outer-membrane proteins were isolated by differential solubilization in *N*-lauroylsarcosine of cell envelopes obtained by sonication of bacteria grown in BHI broth. Samples were boiled for 2 min in Laemmli buffer. The proteins were separated by SDS-PAGE in a running buffer of 0.025 M Tris, 0.2 M glycine and 0.1 % SDS (pH 8.3) with a 12 % acrylamide, 0.12 % bis-acrylamide gel and stained with Coomassie blue.

**Ribotyping and PFGE.** Two ESBL-producing *K. pneumoniae* strains, one susceptible (Kp5) and one resistant (Kp6) to the carbapenems, were compared by amplification and sequencing of the *rpoB* gene and determination of the capsular type by restriction of the *cps* locus.

PFGE of *Xba*I (Roche Diagnostics)- and *Spe*I (Roche Diagnostics)-digested genomic DNA of Kp5 and Kp6 was carried out and the macrorestriction patterns were interpreted according to Tenover *et al.* (1995).

## Results and Discussion

### Antibiotic susceptibility of the *K. pneumoniae* isolates

All the ESBL-producing *K. pneumoniae* isolates were resistant to kanamycin, amikacin, tobramycin, netilmicin, tetracycline, nalidixic acid, ofloxacin and ciprofloxacin but

remained susceptible to gentamicin, co-trimoxazole and colistin.

Strains Kp6, Kp7, Kp8 and Kp9 were resistant to all the  $\beta$ -lactams including the carbapenems with MICs of  $>32$ , 12 and  $8 \mu\text{g ml}^{-1}$  for ertapenem, meropenem and imipenem, respectively. No decrease in the MICs was observed when plates with cloxacillin (inhibition of cephalosporinase activity) or E-test containing EDTA (inhibition of metallo- $\beta$ -lactamases) were used. These results exclude the production of an AmpC  $\beta$ -lactamase or of a metallo- $\beta$ -lactamase by these carbapenem-resistant clinical isolates.

The other ESBL-producing strains of *K. pneumoniae* were susceptible to carbapenems.

### Molecular and biochemical characterization of the *K. pneumoniae* isolates

The *E. coli* transjugants obtained were resistant to cephalosporins (and more so to cefotaxime than to ceftazidime), tetracycline, kanamycin, tobramycin, amikacin and netilmicin and found to harbour the *bla*<sub>CTX-M-15</sub> and *aac(6')**Ib* (which encodes an aminoglycoside 6'-*N*-acetyltransferase) genes by PCR and sequence analysis.

Both Kp5 (carbapenem-susceptible) and Kp6 (carbapenem-resistant) were group 1 *K. pneumoniae* with capsular type C51a and of ribotype A (P. A. D. Grimont, unpublished nomenclature) and the two strains were indistinguishable by PFGE (Supplementary Fig. S1 in JMM Online) and had identical plasmid profiles (Supplementary Fig. S2).

Analysis of the outer-membrane proteins by SDS-PAGE revealed lack of a major outer-membrane protein of 37 kDa in the carbapenem-resistant isolate Kp6. This membrane protein could correspond to OmpK36, which is known to play a role in permeability to carbapenems (Martínez-Martínez *et al.*, 1999; Leavitt *et al.*, 2009; Doumith *et al.*, 2009) and is homologous to OmpC of *E. coli* (Fig. 2). In addition, both strains lacked OmpK35.

### Concluding remarks

We describe the emergence of resistance to all  $\beta$ -lactams, including imipenem, during ertapenem therapy of an infection due to *K. pneumoniae* producing an ESBL belonging to the *bla*<sub>CTX-M</sub> group I. Resistance was most probably due to the combination of synthesis of the ESBL with loss of the porin OmpK36. A recent study by Doumith *et al.* (2009) reported the role of both OmpK35 and OmpK36 in decreasing the MICs of carbapenems in *K. pneumoniae*, but in our study, OmpK36 seemed to be the only one specifically involved in the *in vivo* emergence of resistance to the carbapenems. It has been previously reported that clinical isolates of *K. pneumoniae* lacking expression of ESBLs express the two porins OmpK35 and

OmpK36, whereas most isolates producing these  $\beta$ -lactamases express only the porin OmpK36, while there is either very low expression of the OmpK35 porin or it is not expressed (Hernandez-Alles *et al.*, 1999). Confirming the specific role of OmpK36, particularly in carbapenem resistance, *K. pneumoniae* clinical isolates with reduced carbapenem susceptibility which produce OmpK35 but with no OmpK36 expression (Wang *et al.*, 2009) have just been described, and another study has just reported the particular role of OmpK36 in decreasing the MICs of carbapenems, even in KPC-producing strains (Landman *et al.*, 2009).

A similar pan-penem resistant *K. pneumoniae* isolate has also emerged during ertapenem therapy in a patient with pneumonia (Elliott *et al.*, 2006). We report, however, several additional features of these resistant strains. First, resistance emerged in spite of the fact that ertapenem concentrations were high at the site of infection as well as in the blood. Second, we showed that intestinal colonization persisted for a long period of time after the site of infection had been sterilized by use of co-trimoxazole to which the infecting strain was susceptible.

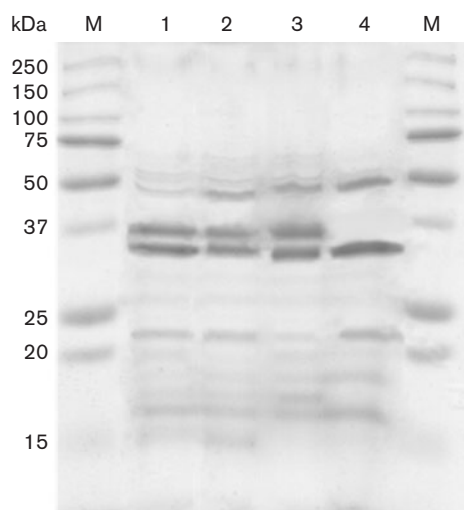
This observation stresses the risk of selection of pan-penem resistant strains of enterobacteria when ertapenem is used for the treatment of severe infections due to ESBL-producing enterobacteria. Strict isolation precautions should be implemented for these patients to prevent dissemination of the responsible micro-organisms by means of faecal contamination, even after sterilization of the primary focus of infection.

### Acknowledgements

This work was supported by the French National Reference Center for Antibiotic Resistance.

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**Fig. 2.** Outer-membrane proteins of carbapenem-susceptible and carbapenem-resistant ESBL-producing *K. pneumoniae*. Lanes: M, molecular mass standards; 1 and 2, ertapenem-susceptible control strains U2A2191 and U2A2192; 3, strain Kp5; 4, strain Kp6 lacking a prominent protein band with an estimated molecular mass of 37 kDa.

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