Case Report

Upper and lower urinary tract infection caused by *Klebsiella pneumoniae* serotype K2 and CTX-M-15 β-lactamase-producing serotype K1: a case report and characterization of serum killing resistance

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CTX-M-15 β-lactamase-producing *Klebsiella pneumoniae* serotype K1 was isolated from a patient with fatal upper urinary tract infection (UTI) complicated by sepsis caused by *K. pneumoniae* serotype K2. Transfer of a CTX-M-15 β-lactamase plasmid from the K1 to the K2 strain was observed. However, plasmid acquisition by the K2 strain did not occur *in vivo*, suggesting that the K1 strain might not have contributed directly to the upper UTI. In addition, effects of K serotypes and plasmid acquisition on *K. pneumoniae* serum resistance were examined.

Case report

A 73-year-old male patient was admitted to our medical centre with a 39 °C fever, chills, abdominal distension and ambulatory difficulties. This patient had a long history of alcoholism resulting in liver cirrhosis. On admission, the following measurements were made: leukocyte count, 35 700 μl^{-1} (significantly elevated); C-reactive protein level, 4.49 mg dl^{-1}; aspartate aminotransferase, 174 IU l^{-1}; alanine aminotransferase, 73 IU l^{-1}; alkaline phosphatase, 881 IU l^{-1}; blood urea nitrogen, 21 mg dl^{-1}; and creatinine, 2.36 mg dl^{-1}. His urine was cloudy with a pH of 5.5, and had the following characteristics: protein content, 200 mg dl^{-1}; red blood cells, 120 per high power field; white blood cells, >200 per high power field; and bacteria, +++ per high power field. He was diagnosed with hepatic failure associated with alcoholic liver cirrhosis and urinary tract infection (UTI), while *Klebsiella pneumoniae* serotype K2 susceptible to broad-spectrum cephalosporins was isolated from arterial blood and urine taken upon admission and from arterial blood 34 days after admission. An ultrasonographic examination revealed hyperechoic cystic lesions in the right kidney superior border, indicative of a perinephric abscess. Biopsy cultivation revealed *K. pneumoniae* K2 on day 35. A dynamic computed tomography scan on day 8 showed a prostatic abscess. On day 17, multiresistant *K. pneumoniae* K1 was isolated from a urethral discharge culture presumably derived from the prostatic abscess. All three cultures from venous blood showed no growth, including the one collected at admission. Despite imipenem–cilastatin treatment, disseminated intravascular coagulation followed *K. pneumoniae* K2 sepsis and led to death on day 41.

Microbiological methods

Capsular K serotypes (K1–K6) were determined with Denka Seiken antisera. MICs were determined by a microdilution broth method using a WalkAway-96 SI System (NEG Combo 5 J, NEG MIC 5 J and ESBL plus panels; Dade Behring) with an inoculum of 10^4 c.f.u. per well. Susceptibility categories were determined according to the Clinical and Laboratory Standards Institute criteria (CLSI, 2007). The MICs for multiresistant *K. pneumoniae* K1 strain FM039343 are shown in Table 1. MICs of cefotaxime and ceftazidime significantly decreased from >128 μg ml^{-1} to ⩽0.12 μg ml^{-1} and from 64 μg ml^{-1} to ⩽0.12 μg ml^{-1}, respectively, in the presence of 4 μg clavulanic acid ml^{-1}, suggesting the production of class A extended-spectrum β-lactamase (ESBL) in this strain. In contrast, four *K. pneumoniae* K2 strains, including FM038943, derived from cultures of arterial blood taken upon admission were characterized by colonies with sticky consistency and were susceptible to broad-spectrum cephalosporins and aztreonam (Table 1). To investigate the genetic relationship among the isolates, genome typing was carried out as described previously (Nagano *et al.*,

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Abbreviations: ESBL, extended-spectrum β-lactamase; UTI, urinary tract infection.

Table 1. Antibiotic susceptibilities of *K. pneumoniae* clinical isolates and transconjugants

Values are the MIC (μg ml^{-1}).

Antibiotic	*K. pneumoniae* K2 FM038943	*K. pneumoniae* K1 FM039343 (CTX-M-15 and TEM-1b)	*K. pneumoniae* K2 FM038943 Rif^r transconjugant* (CTX-M-15 and TEM-1b)	*E. coli* χ1037 Rif^r transconjugant† (CTX-M-15 and TEM-1b)	*E. coli* χ1037 Rif^r
Ampicillin	>16	>16	>16	>16	⩽2
Amoxicillin/CLA	⩽1/0.5	8/4	8/4	8/4	⩽1/0.5
Piperacillin	>64	>64	>64	>64	⩽8
Cefazolin	⩽1	>16	>16	>16	⩽1
Cefotiam	⩽8	>16	>16	>16	⩽8
Cefoperazon	⩽16	>32	>32	>32	⩽16
Cefoperazon/SUL‡	⩽4/2	8/4	8/4	8/4	⩽4/2
Cefotaxime	⩽0.5	>128	>128	>128	⩽0.5
Cefotaxime/CLA§	⩽0.12	⩽0.12	⩽0.12	⩽0.12	⩽0.12
Ceftazidime	⩽0.5	64	32	64	⩽0.5
Ceftazidime/CLA§	⩽0.12	⩽0.12	⩽0.12	⩽0.12	⩽0.12
Ceftriaxone	⩽0.5	>64	>64	>64	⩽0.5
Cefpirome	⩽1	>16	>16	>16	⩽1
Cefepime	⩽1	>32	16	32	⩽1
Cefozopran	⩽1	>16	>16	>16	⩽1
Cefaclor	⩽2	>16	>16	>16	⩽2
Cefpodoxime	⩽0.5	>64	>64	>64	⩽0.5
Cefoxitin	⩽2	4	⩽2	⩽2	⩽2
Cefmetazole	⩽0.5	1	1	⩽0.5	⩽0.5
Cefotetan	⩽0.5	⩽0.5	⩽0.5	⩽0.5	⩽0.5
Flomoxef	⩽1	⩽1	⩽1	⩽1	⩽1
Imipenem	⩽0.5	⩽0.5	⩽0.5	⩽0.5	⩽0.5
Meropenem	⩽0.5	⩽0.5	⩽0.5	⩽0.5	⩽0.5
Aztreonam	⩽0.5	64	64	32	⩽0.5
Gentamicin	⩽0.5	>8	>8	>8	⩽0.5
Amikacin	⩽2	16	16	16	⩽2
Minocycline	2	>8	8	8	⩽1
Levofloxacin	⩽0.5	2	⩽0.5	⩽0.5	⩽0.5
Fosfomycin	⩽4	>16	⩽4	⩽4	⩽4

*Frequency 3.0×10^{-4} transconjugants per recipient cell.
†Frequency 2.1×10^{-6} transconjugants per recipient cell.
‡SUL, sulbactam.
§CLA, clavulanic acid at a fixed concentration of 4 μg ml^{-1}.

2003). All four *K. pneumoniae* K2 strains showed exactly the same *Xba*I-digested genomic DNA pattern, despite being isolated from different clinical sources or at different times. The restriction profile of multiresistant *K. pneumoniae* K1 was distinguishable from that of the four K2 strains (Fig. 1).

Conjugal transferability of the resistance determinants was noted by using *K. pneumoniae* K1 as the donor, while a rifampicin-resistant (Rif^r) mutant of *Escherichia coli* χ1037 (Iyobe *et al.*, 1981) and an *in vitro*-generated Rif^r *K. pneumoniae* K2 were the recipients, resulting in a frequency of 2.1×10^{-6} and 3.0×10^{-4} transconjugants per donor cell, respectively. Susceptibility testing revealed that the β-lactam MICs for the transconjugants were similar to those for the donor strain (Table 1).

A unique plasmid, approximately 87 kb, was isolated from the donor and transconjugants by rapid alkaline lysis (Takahashi & Nagano, 1984). Preliminary PCR analysis (Nagano *et al.*, 2003, 2004) on plasmid DNA isolated with the Plasmid Midi kit (Qiagen) revealed that the transconjugants showed amplification products for $bla_{\text{CTX-M-1}}$ and bla_{TEM} genes. Each of these two structural genes and flanking regions were subsequently amplified (Dutour *et al.*, 2002; Mabilat & Goussard, 1993) and sequenced on both strands. A BLAST search (www.ncbi.nlm.nih.gov/BLAST/) revealed 100 % identity to $bla_{\text{CTX-M-15}}$ (GenBank accession no. DQ302097) and $bla_{\text{TEM-1b}}$ (GenBank accession no. DQ058146).

To determine the contribution of each β-lactamase to antibiotic resistance, a cloning strategy using the pBC

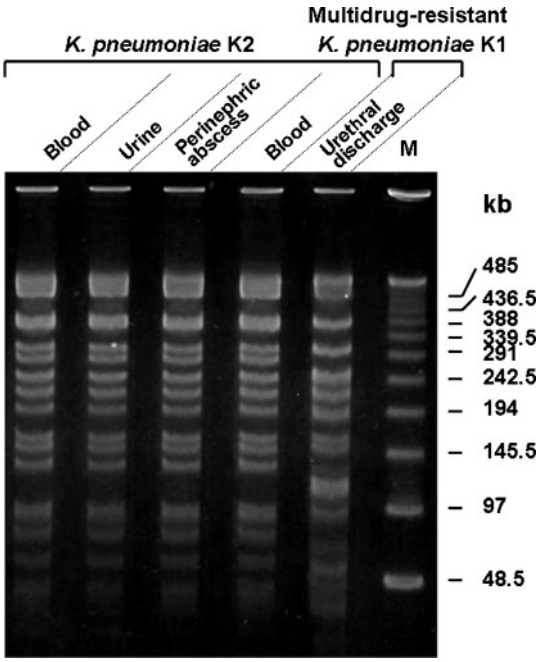

Fig. 1. Agarose gel electrophoresis of *Xba*I-digested genomic DNA from five *K. pneumoniae* isolates. Restriction profiles obtained for four *K. pneumoniae* K2 isolates from blood (FM038943), urine, perinephric abscess aspiration and blood (left to right) were identical, suggesting a clonal lineage. These patterns were clearly different from that of the *K. pneumoniae* K1 strain (FM039343) isolated from urethral discharge. Lane M, lambda DNA ladder.

SK(+) phagemid vector (Stratagene) and *E. coli* XL1-Blue strain (Stratagene) was adopted. The resulting CTX-M-15 β-lactamase-producing *E. coli* and the parental strain shared the same profiles of resistance to β-lactams, whereas TEM-1b β-lactamase-producing *E. coli* was susceptible to cefotaxime and ceftazidime (data not shown).

Sensitivity of the *K. pneumoniae* strains to normal human serum was evaluated as described previously (Hughes *et al.*, 1982). Bacterial suspension (500 µl) in 0.9 % NaCl (approx. 10^6 c.f.u. ml^{-1}) was mixed with 1500 µl of undiluted serum (final concentration of 75 %, v/v) with or without 30 µg cefotaxime ml^{-1} corresponding maximum plasma concentration after 1 g intravenous infusion. Viable counts were determined at the beginning and after 1, 2 and 3 h of 37 °C incubation. The assay was performed in triplicate for each strain, and the results showed intermediate resistance of *K. pneumoniae* K2 (Fig. 2b), whereas *K. pneumoniae* K1 was rapidly killed within 2 h of incubation with human serum (Fig. 2a). It is noteworthy that among two *K. pneumoniae* K2 strains, an ESBL producer showed a higher level of serum resistance by growing equally well in normal and heat-inactivated serum. The ESBL-producing K2 strain lost its serum resistance in combination with cefotaxime, even at subinhibitory concentrations (Fig. 2b, c).

Discussion

Bacterial infection of the prostate gland might have occurred from an ascending urethral infection or by reflux of infected urine into prostatic ducts emptying into the posterior urethra. Invasion of rectal bacteria by direct extension or by lymphogenous or haematogenous spread may also constitute other possible routes (Domingue & Hellstrom, 1998). Since the ESBL producer isolated from a purulent urethral discharge was not detected in other clinical samples including blood, urine, perinephric abscess and sputum, the prostatic abscess is the most probable source of this strain, while infected urine is the less likely source. *In vivo* transfers of ESBL-encoding plasmids have been previously reported (Neuwirth *et al.*, 2001). The

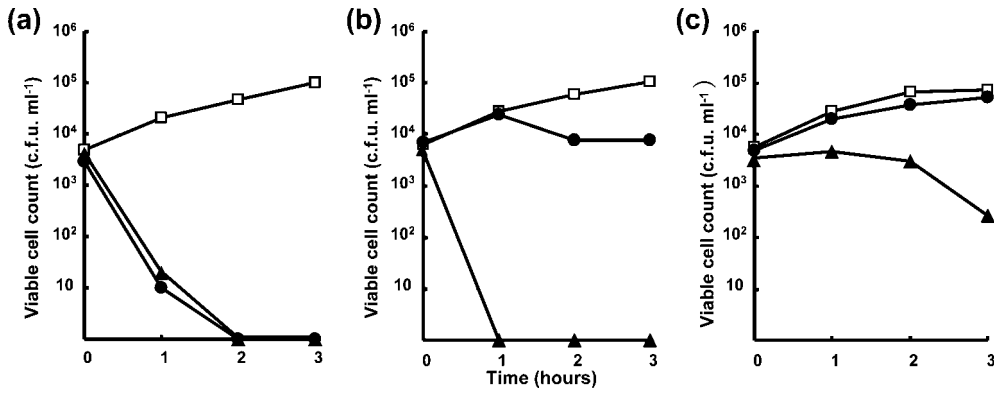

Fig. 2. Effects of K serotypes and acquisition of ESBL-encoding plasmid on *K. pneumoniae* serum resistance. K1 strain FM039343 (a), K2 strain FM038943 (b) and K2 transconjugant (c) were incubated in either 75 % (v/v) normal human serum (NHS, ●), NHS containing 30 µg cefotaxime ml^{-1} (▲) or heat-inactivated NHS (□). Results shown are the mean from three independent experiments.

mating assay showed that the plasmid transfer frequency from the *K. pneumoniae* K1 strain to the K2 strain was constant, about a hundred times reproducibly higher than transfer from the K1 strain to *E. coli* χ1037. However, the acquisition of the ESBL-encoding plasmid by the K2 strain did not occur *in vivo*, which also suggests that the K1 strain might not have contributed directly to the upper UTI in this case.

ESBL-producing *K. pneumoniae* has been increasingly reported worldwide. However, few studies have analysed the association between virulence and ESBL production in *K. pneumoniae* (Di Martino *et al.*, 1997; Sahly *et al.*, 2004). Mizuta *et al.* (1983) have previously shown that *K. pneumoniae* strains expressing capsular serotypes K1 and K2 are particularly virulent in mice. Moreover, it is well established that the K2 serotype is among the most common capsular serotypes detected from patients with UTI, pneumonia or bacteraemia (Podschun & Ullmann, 1998). In this report, the DNA restriction profiles were identical among all four *K. pneumoniae* K2 strains, suggesting a persistent infection of the upper urinary tract, despite their *in vitro* sensitivity to therapeutic agents. To assess an association of K serotypes and ESBL production with pathogenicity, serum sensitivity was taken as a measure of virulence for *K. pneumoniae*. The results indicate that serum killing resistance was significantly higher in *K. pneumoniae* K2 than in K1. In particular, acquisition of the ESBL-encoding plasmid enhanced K2 serum resistance. It is uncertain whether serum resistance in K2 ties in with clinical course in this case. However, the possible acquisition of ESBL-encoding plasmids among more virulent *K. pneumoniae* serotypes might result in severe therapeutic problems in immunocompromised hosts, while persistent infection may substantially increase the risk of progression to nosocomial spread.

This case is remarkable in that *K. pneumoniae* strains with different capsular types, susceptibility profiles and serum-mediated killing resistance were isolated from the same patient with severe UTI.

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