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Molecular diversity in mechanisms of carbapenem resistance in paediatric Enterobacteriaceae

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Abstract

Development of carbapenem resistance in Enterobacteriaceae has impacted Clinical and Laboratory Standards Institute (CLSI) guidelines, infection control approaches and treatment strategies. The clinical, phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae (CRE) infections at paediatric referral centres are not well described. CRE were identified through the clinical microbiology laboratory at Seattle Children's Hospital (Seattle, WA). Clinical data were retrieved from medical records. Resistance testing, polymerase chain reaction (PCR) for resistance determinants, and *Escherichia coli* transformation were carried out for each isolate. Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were used to characterise strain relatedness. PCR amplification and sequencing as well as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were used to investigate porin alterations. Six CRE isolates were identified between 2002 and 2010. Significant molecular diversity was documented in their mechanisms of resistance, including plasmid-mediated serine carbapenemase (KPC) and metallo- β -lactamase (IMP), chromosomally-encoded β -lactamase (SME) and porin alterations with extended-spectrum β -lactamases. Patients had underlying health conditions and were from geographically diverse regions. In one case, PFGE of serial isolates documented the development of resistance in a previously susceptible strain. Molecular investigation of this strain identified insertion of the genetic mobile element insertion sequence *ISEcp1* in the *ompK36* gene, conferring a functional porin alteration as demonstrated by SDS-PAGE. This is the first description of porin disruption by *ISEcp1* in a CTX-M-15-positive isolate. This is the largest report of paediatric CRE to date. This diverse description of demographic, phenotypic and molecular characteristics highlights the challenge of CRE infections in high-risk paediatric patients and that attention to emerging resistance mechanisms (including membrane alteration) at paediatric referral centres is essential.

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Competing interests

None declared.

Ethical approval

Approval was granted by the Ethical Review Board of Seattle Children's Hospital (Seattle, WA) (ref. no. 12633).

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Keywords

Carbapenem resistance; Enterobacteriaceae; Porin; *ISEc1*

1. Introduction

The rapid emergence and spread of carbapenem resistance among Gram-negative pathogens has challenged clinicians to devise effective empirical and definitive antibiotic strategies for individual patients as well as institutional protocols [1,2]. Enterobacteriaceae are responsible for a large proportion both of hospital- and community-acquired infections and affect a wide variety of hosts. The effect of increasing rates of carbapenem resistance among Enterobacteriaceae necessitates a comprehensive study of the clinical features and molecular epidemiology of these challenging infections [3,4]. These characteristics are poorly described for paediatric referral centres, which are institutions contending with high-risk patients and increasing numbers of antibiotic-resistant pathogens.

Carbapenem resistance can result from the acquisition of plasmid or chromosomal resistance genes encoding serine carbapenemase or metallo- β -lactamase enzymes and efflux pumps, or by alteration of porin expression in combination with an AmpC- or extended-spectrum β -lactamase (ESBL)-type enzyme [5]. The nature of the responsible resistance determinant(s) may affect the dynamics of spread, the ability to detect the associated resistance phenotype in the clinical laboratory and the organism's susceptibility to treatment with alternative antibiotics [6]. Defining the clinical and molecular characteristics of carbapenem-resistant isolates will elucidate the breadth of emerging resistance and provide data to help guide current efforts to detect these strains and control their spread. Focused study is important as new Clinical and Laboratory Standards Institute (CLSI) guidelines for identifying carbapenem resistance are released (CLSI M100-S21) and institutions implement changes that will affect infection control, surveillance and patient care.

There are few studies of paediatric infections due to carbapenem-resistant Enterobacteriaceae (CRE). Here we describe the clinical and molecular details of the largest paediatric case series to date, comprising six cases of carbapenem resistance in patients seen between 2002 and 2010 at Seattle Children's Hospital (SCH), a 250-bed tertiary care centre in Seattle, WA.

2. Case summaries

2.1. Case 1

A 16-year-old Native American male from Washington was admitted for treatment of acute myelogenous leukaemia (AML). On Day 13 of hospitalisation, while receiving chemotherapy, he developed neutropenic fever and hypotension. Empirical therapy with ceftazidime and gentamicin was initiated. Blood cultures revealed imipenem-resistant *Serratia marcescens* with retained sensitivity to meropenem and a range of other antibiotic agents (Table 1). On Day 20, gentamicin was replaced with ciprofloxacin owing to renal insufficiency and the patient completed a treatment course with no further positive cultures.

2.2. Case 2

A 3.5-year-old Caucasian male from Alaska was hospitalised for induction chemotherapy for newly diagnosed AML. His early hospital course was complicated by neutropenic fever that was treated empirically with ceftazidime. Antibiotic therapy was changed to meropenem after 6 days owing to a presumed drug rash and the patient continued on empirical meropenem for a total course of 23 days. He had no positive blood cultures during

this time and had been afebrile for 11 days at the time of stopping antibiotics. A second course of induction chemotherapy was initiated on Day 28 owing to persistent evidence of leukaemic disease. On Day 32 he became febrile and meropenem was administered. On Day 42, a blood culture obtained from his central venous catheter grew carbapenem-resistant *Escherichia coli*; the pathogen remained susceptible to aminoglycosides. He was treated with gentamicin and no further positive cultures were recovered.

2.3. Case 3

An 11-year-old Hispanic male with relapsed acute lymphoblastic leukaemia (ALL) was admitted for severe nausea and vomiting of 5 days duration. Just prior to his admission he had received consolidation chemotherapy in Mexico. Upon hospitalisation, empirical therapy with ceftazidime, gentamicin and vancomycin was initiated. Cultures of blood and stool revealed carbapenem-resistant *E. coli*. Based on susceptibility results (Table 1), his treatment was changed to gentamicin and trimethoprim/sulfamethoxazole and he completed therapy with all subsequent cultures remaining negative.

2.4. Case 4

A 17-year-old Caucasian female from New Jersey was admitted for unrelated-donor haematopoietic cell transplantation for ALL. She had previously been hospitalised in New Jersey for chemotherapy and was transferred to SCH for transplantation evaluation after her second remission. On Day 5 post transplantation she developed neutropenic fevers and severe mucositis and was empirically treated with meropenem and gentamicin. On Day 9 post transplantation she became haemodynamically unstable. Gentamicin was changed to moxifloxacin and she was transferred to the Pediatric Intensive Care Unit (PICU). Blood cultures obtained while the patient was receiving meropenem revealed Gram-negative rods and tigecycline was added empirically. On Day 11 post transplantation, doxycycline was added to her regimen when the Gram-negative rod was identified as carbapenem-resistant *Klebsiella pneumoniae*, susceptible only to doxycycline and tigecycline. On Day 14 post transplantation the patient died of multi-organ failure secondary to *K. pneumoniae* septicaemia.

2.5. Case 5

A 17-year-old Asian male from Burma was admitted for chemotherapy for AML. Prior to hospitalisation at SCH, he had been hospitalised in Malaysia for two cycles of chemotherapy. On Day 15 of hospitalisation he developed neutropenic fever and rigors and was treated empirically with cefepime and gentamicin. Blood cultures revealed growth of an ESBL-producing *K. pneumoniae*. Based on the antibiogram, definitive therapy with meropenem and tobramycin was instituted and completed prior to discharge to the outpatient department (Day 35).

Then, 98 days following isolation of *K. pneumoniae*, the patient was re-admitted for chemotherapy. On Day 22 of the second hospitalisation, he developed neutropenic fever and rigors and was presumptively treated with meropenem. Blood cultures grew *K. pneumoniae* resistant to carbapenems and susceptible only to tobramycin, amikacin and tigecycline. His antibiotic therapy was changed to tobramycin and tigecycline. His course was complicated by prolonged fevers, persistent bacteraemia and typhlitis. He was managed in the PICU on Days 29 through 31 of hospitalisation owing to respiratory distress and severe sepsis syndrome. He was treated with a prolonged course of antibiotics despite no further positive cultures. He was discharged home on Day 52 with resolution of his symptoms.

2.6. Case 6

An 18-year-old Israeli female was seen in the SCH Nephrology Clinic for consultation regarding management of recurrent urinary tract infections. One year prior to presentation she had had a renal transplant owing to end-stage renal failure due to congenital solitary right kidney and neurogenic bladder. The patient was medically managed in Tel Aviv (Israel) with a self-catheterisation regimen and daily cefalexin prophylaxis. The patient reported chronic infection with *K. pneumoniae* despite three 21-day courses of ceftriaxone. Urine culture from a specimen collected at SCH grew ertapenem-resistant *K. pneumoniae*. The patient returned to Israel for management and received no further care at SCH.

3. Methods

3.1. Case selection and clinical data

Patients from whom a CRE was isolated at SCH from 1999–2010 were identified by retrospective review of clinical microbiology data. All study isolates were obtained through routine clinical care. Clinical data, including medication history and details of the hospital course, were extracted from medical records. Collection and report of these data were approved by the SCH Institutional Review Board.

3.2. Antibiotic susceptibility testing

Specimen processing and phenotypic work-up was performed at the SCH Clinical Microbiology Laboratory according to CLSI standards. Minimum inhibitory concentration (MIC) values were determined using Etest and/or disk diffusion methodology. Breakpoints from the most recent CLSI publication (M100-S21) were used for final analysis. For tigecycline and colistin, European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2010 MIC breakpoints were used as no CLSI standards were available. The Modified Hodge test as described by the CLSI [7] was also performed for all isolates.

3.3. Resistance genotyping

Genotypic analyses, including polymerase chain reaction (PCR) screening for and sequencing of genes encoding the AmpC, ESBL and/or carbapenemase enzymes ACC, CMY, CTX-M, DHA, IMP, KPC, SHV and SME, were performed using previously described methodology and primers [8]. Transformation into *E. coli* was attempted using a QIAGEN PCR Cloning Plus Kit (QIAGEN Inc., Valencia, CA). Sequencing of the quinolone resistance-determining region (QRDR) of *gyrA* was performed as previously described [9].

3.4. Molecular epidemiology

Phylogroup determination for *E. coli* isolates was accomplished using the previously described rapid triplex PCR methodology [10]. Multilocus sequence typing (MLST) was performed for *E. coli* and *K. pneumoniae* isolates as previously described [11,12]. Sequence type (ST) assignments were obtained using the *E. coli* MLST database (<http://mlst.ucc.ie/mlst/dbs/Ecoli>) and the Institut Pasteur MLST database (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/>), respectively. Genetic relatedness was evaluated in serial *K. pneumoniae* isolates from Case 5 using standard pulsed-field gel electrophoresis (PFGE) by *Xba*I digestion [13].

3.5. Outer membrane porin (OMP) evaluation

The OMP genes *ompK35* and *ompK36* were amplified and sequenced for *K. pneumoniae* from Cases 5 and 6 using previously described primers [14]. Functional expression of membrane alteration was explored by previously described sodium dodecyl sulphate

polyacrylamide gel electrophoresis (SDS-PAGE) methodology [15,16]. Briefly, cells were lysed by sonication after synchronised growth in Mueller–Hinton broth. Membrane preparations were obtained by solubilisation using sodium *N*-lauroyl sarcosinate and ultracentrifugation. Preparations were run on a 12% SDS polyacrylamide gel and were stained with Coomassie blue.

4. Results

4.1. Antibiotic susceptibility testing

The six clinical isolates exhibited resistance to at least one carbapenem agent. MIC values and susceptibility patterns to other typical antibiotic agents as well as to an expanded panel of alternative agents (colistin, fosfomycin, tigecycline) varied between organisms (Table 1). Application of the Modified Hodge test demonstrated a positive result only in the Case 4 isolate.

4.2. Genetic relatedness of isospecific bacterial strains

There was no evidence of molecular relatedness between same-species isolates from any two cases. Although both *E. coli* isolates derived from phylogroup D, they demonstrated different ST profiles, precluding epidemiological relatedness (Table 2). Likewise, the *K. pneumoniae* isolates from Cases 4, 5 and 6 were unrelated to one another by MLST, but the *K. pneumoniae* isolate from Case 4 represented ST258, the lineage most clearly associated with the clonal spread of KPC resistance in the USA [17].

PFGE was used to characterise the genetic relatedness in five serially collected *K. pneumoniae* isolates from Case 5 (Fig. 1). The blood isolate collected during the first hospitalisation (06/09/2008) was identical by pulsotype to a stool surveillance isolate collected 8 weeks later (31/10/2008); both isolates were resistant to extended-spectrum β -lactams and were susceptible to carbapenems. A second pulsotype, with only minor band differences from the first, was observed in the carbapenem-resistant isolates collected from stool and blood later in the clinical course (25/12/2008 and 05/01/2009, respectively). These four isolates demonstrated a single MLST profile (ST336) and were thus genetically highly related. In contrast, an intervening bloodstream isolate (21/12/2008) was susceptible to extended-spectrum β -lactams and was genetically unrelated by PFGE to the four extended-spectrum β -lactam-resistant isolates recovered from this patient.

4.3. Extended-spectrum β -lactam resistance genotypes

In aggregate, the isolates in this case series encoded ESBL enzymes from Ambler classes A (SME, CTX-M, KPC), B (IMP) and C (CMY) (Table 2). Transformation into *E. coli* confirmed that the Case 4 KPC carbapenemase gene was plasmid-borne. The carbapenem resistance phenotype in Case 2 was spontaneously cured after passage in the laboratory with documented loss of the genotype, suggesting that the IMP determinant was plasmid-borne. Class A and C resistance genes (*bla*_{CTX-M} in Case 5 and *bla*_{CMY} in Cases 2 and 3, respectively) were transformed into *E. coli*; however, no carbapenemase locus was transformed. Transformation into *E. coli* of the *sme* gene from Case 1 was unsuccessful, consistent with its described chromosomal location [18].

4.4. Outer membrane porin evaluation

Because no carbapenemase enzymes were identified in the carbapenem-resistant *K. pneumoniae* isolates from Cases 5 and 6, we investigated whether OMP defects could have contributed to the resistance phenotypes. Previously published primers were used to amplify and sequence the two main porins (*ompK35* and *ompK36*) in the two highly related bloodstream isolates from Case 5 as well as the urine isolate from Case 6.

Notably, amplification of *ompK36* in the carbapenem-resistant isolate from Case 5 resulted in two bands (ca. 1.8 kb and 3 kb), both larger than expected (Fig. 2), whilst the carbapenem-susceptible isolate and the Case 6 isolate produced a single amplicon of expected size (ca. 1.2 kb). Sequencing of the respective amplicons revealed the presence of insertion sequence *ISEcp1* after nucleotide 505 in the carbapenem-resistant isolate but not the carbapenem-susceptible ESBL isolate. In Case 6, sequencing of *ompK35* demonstrated a 4-nucleotide duplication (GCGT) at nucleotide position 253, producing a downstream frameshift that would be expected to alter porin function. Both alterations occurred in strains carrying a plasmid-borne extended-spectrum enzyme (CTX-M-15 in Case 5 and CMY-2 in Case 6).

4.5. *gyrA* sequencing

Sequencing of the QRDR of the chromosomally located DNA gyrase *gyrA* was performed in the isolates from all six cases. In the *K. pneumoniae* isolates from Cases 4, 5 and 6, well described polymorphisms associated with fluoroquinolone resistance [9] were observed (Table 2).

5. Discussion

In this investigation, we present the largest paediatric case series of CRE to date. Molecular diversity in the mechanisms of resistance was demonstrated, including plasmid-mediated serine carbapenemase (KPC) and metallo- β -lactamases (IMP) enzymes, a chromosomally-encoded β -lactamase (SME), and AmpC or ESBL enzymes with OMP alterations. The wide range of clinical and molecular characteristics in these infections highlights the challenges in identifying and addressing carbapenem resistance.

The emergence of carbapenem resistance has been notable for its rapid dissemination. The infections in this study occurred in patients that were medically complicated and ethnically and geographically diverse. In paediatrics, high-level subspecialty care is provided by a limited number of institutions, resulting in the risk of importing new resistance mechanisms both to home and referral institutions. In Case 4, a patient transferred to Seattle for oncology treatment was likely colonised with KPC-2-carrying *K. pneumoniae* while receiving care in New Jersey. This isolate was documented by MLST analysis to be a member of the ST258 clone that has driven the spread of carbapenem-resistant *K. pneumoniae* throughout the world, reaching endemic levels not only in the Northeastern USA but now described across Europe, Israel and Asia [17,19–22]. With patients from Malaysia, Mexico and Israel represented in this series, this study reinforces the notion that resistance patterns are no longer ‘local’ and that providers must consider the travel history and corresponding molecular epidemiology of individual patients.

In addition to ‘imported’ resistance that emerges in patients from endemic areas, this series also demonstrated the acquisition and/or expression of carbapenem resistance de novo (Case 5). Within 4 months of bacteraemia caused by an ESBL-producing *K. pneumoniae* strain, this patient developed a bloodstream infection with a highly related strain that was resistant to a carbapenem agent. Molecular investigation demonstrated alteration of the porin gene *ompK36* and no other identifiable carbapenem resistance genotype. Through examination of sequential isolates, it was possible to detect the presence of an insertion sequence (*ISEcp1*) in the *OmpK36*-encoding gene of the carbapenem-resistant, but not the carbapenem-susceptible, isolate. Found upstream of the *bla*_{CTX-M-15} gene [21], this genetic element is presumed to have participated in the initial mobilisation of this important ESBL enzyme from the chromosome of *Kluyvera* to a mobile plasmid where it has spread rapidly [23]. *ISEcp1* has also been previously implicated in OMP alteration-mediated carbapenem resistance by Cai et al. [24], who demonstrated the element upstream of the *ompK36* start

codon in a *K. pneumoniae* isolate also carrying ESBL and carbapenemase enzymes (CTX-M-14 and KPC-2, respectively). The current study is the first to document the development of carbapenem resistance due to *ompK36* disruption by *ISEcp1* in a CTX-M-15-positive isolate. Of concern, the resistance plasmid in this case provides the host strain with a broad-spectrum ESBL enzyme and a mobile selfish element capable of conferring carbapenem resistance via porin disruption.

The patients in this series were medically complicated and had challenging clinical courses, often necessitating prolonged hospitalisation and interruption in treatment for their underlying conditions. Particularly concerning in high-risk patients, we documented how porin alternations, working in combination with ESBL- and AmpC-type enzymes, may develop over a short period of time. Unfortunately, timely identification of carbapenem-resistant pathogens remains difficult and the frequency of porin alterations in particular is likely underestimated. We have documented some of the known challenges in identification: borderline MIC values; variability of susceptibility results within the carbapenem class; and negative Modified Hodge test results. Recently, the strategies and priorities for phenotypic detection have changed. The CLSI has recommended changes in the susceptibility breakpoints and reporting strategies for Enterobacteriaceae. Clinical laboratories are now guided to report susceptibility results for individual agents and to de-emphasise the use of the Modified Hodge test and the mechanism-based classification of ESBL pathogens. These changes will identify potentially resistant organisms while improving the provider's ability to treat with effective β -lactam agents, priorities supported by the current data. Even institutions with low carbapenem resistance rates are likely facing increased ESBL- and AmpC-type resistance with the emergence and continued dissemination of CTX-M (particularly CTX-M-15) and CMY-2 enzymes throughout Enterobacteriaceae. Patients with a previous history of ESBL- or AmpC-carrying organisms may be treated empirically with carbapenem therapy for febrile episodes, providing selective pressure for plasmid-mediated resistance and the development of porin alterations in colonising bacterial strains of the intestine and genitourinary tract. Large paediatric referral hospitals, which may unintentionally serve as catchment centres for antibiotic resistance, are important institutions to address and prevent the further spread of these infections through the adaption of new CLSI recommendations, provider education, infection control and antibiotic stewardship activities.

The global spread of carbapenem resistance has occurred quickly and is highly dynamic. As shown in this series, clinicians at paediatric referral centres must be attentive to a diverse array of resistance mechanisms in high-risk patients, including the possibility of development of carbapenem resistance in a patient infected by a previously susceptible isolate. Addressing these complex, potentially devastating, infections necessitates attention and continued investigation.

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Fig. 1.
Pulsed-field gel electrophoresis (PFGE) of sequential isolates from Case 5. ESBL, extended-spectrum β -lactamase; CR, carbapenem-resistant; Amp-R, ampicillin-resistant.

Fig. 2.

Polymerase chain reaction (PCR) amplification of OmpK36 in Case 5. * Sequencing of a larger amplicon in lane 4 revealed insertion of *ISEcp1* after nucleotide position 505 in the OmpK36 locus, suggesting porin defect via gene disruption by the insertion sequence. Amp-R, ampicillin-resistant; ESBL, extended-spectrum β -lactamase; CR, carbapenem-resistant.

Fig. 3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of Case 5 isolates. * Loss of the OmpK36 band in a carbapenem-resistant (CR) isolate, demonstrating functional porin alteration consistent with sequencing results. ESBL, extended-spectrum β -lactamase.

Table 1

Resistance phenotype and minimum inhibitory concentrations (in mg/L) for carbapenem-resistant Enterobacteriaceae^a

Case	Species	MER [≥4]	IPM [≥4]	DOR [≥4]	ERT [≥1]	CAZ [≥16]	TZP [≥128]	CIP [≥4]	GEN [≥16]	AMK [≥64]	TOB [≥16]	SXT [≥4/76]	SUL [≥512]	FOS [≥256]	COL [≥2]	TIG [≥4]	MHT
1	<i>Serratia marcescens</i>	S (1)	R (>32)	S (2)	R (2)	S	S	S	S	S	S	S	S	S (12)	R (>256)	S (4)	–
2	<i>Escherichia coli</i>	R (16)	R (32)	R (16)	R (>32)	R	R	S	S	S	S	S	R	S (6)	S (0.38)	S (0.125)	–
3	<i>E. coli</i>	R (16)	R (32)	R (16)	R (>32)	R	R	R	S	I	R	S	R	S (4)	S (0.125)	S (0.5)	–
4	<i>Klebsiella pneumoniae</i>	R (32)	R (32)	R (32)	R (32)	R	R	R	R	R	R	R	R	R (256)	S (0.5)	S (1)	+
5	<i>K. pneumoniae</i>	R (16)	S (1.5)	R (4)	R (32)	R	R	I	R	S	S	R	R	R (1024)	S (1)	S (1.5)	–
6	<i>K. pneumoniae</i>	S (1.5)	S (0.25)	S (1)	R (12)	R	R	R	R	S	S	R	R	S (64)	S (1)	S (0.75)	–

MER, meropenem; IPM, imipenem; DOR, doripenem; ERT, ertapenem; CAZ, ceftazidime; TZP, piperacillin/tazobactam; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; SXT, trimethoprim/sulfamethoxazole; SUL, sulfamethoxazole; FOS, fosfomycin; COL, colistin; TIG, tigecycline; MHT, Modified Hodge test; S, susceptible; I, intermediate; R, resistant.

^a Reported interpretation (S, I and R) and minimum inhibitory concentration breakpoints [in brackets] are per Clinical and Laboratory Standards Institute (CLSI) document M100-S21, with the exception of colistin and tigecycline where European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2010 breakpoints were used.

Table 2

Molecular characteristics of carbapenem-resistant Enterobacteriaceae

Case	Species	MLST	GyrA ^a	Resistance gene	<i>Escherichia coli</i> transformable?	Porin defect
1	<i>Serratia marcescens</i>	N/A	N/A	SME	No	N/A
2	<i>E. coli</i>	ST1061	83: Ser (TCG); 87: Asp (GAC)	CMY-2; IMP-4	CMY-2	N/A
3	<i>E. coli</i>	ST648	83: Leu (TTG); 87: Asn (AAC)	CMY-2; IMP-4	CMY-2	N/A
4	<i>Klebsiella pneumoniae</i>	ST258	<u>83: Ile (ATC)</u> ; 87: Asp (GAC)	KPC-2	KPC-2	N/A
5	<i>K. pneumoniae</i>	ST336/STc17	83: Tyr (TAC); 87: Asp (GAC)	CTX-M-15; SHV-12	CTX-M	OmpK36: IS <i>LEcp1</i> insertion at position 504
6	<i>K. pneumoniae</i>	ST327	83: Phe (TTC); 87: Gly (GGC)	CMY-2	No	OmpK35: 4-nucleotide duplication at position 253

MLST, multilocus sequence typing; N/A, not available.

^aUnderlined GyrA residues are associated with increased fluoroquinolone minimum inhibitory concentrations [9].