

Case Report



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Corresponding Author:

Cheol-In Kang, MD, PhD

Division of Infectious Diseases,
Department of Medicine, Samsung Medical
Center, Sungkyunkwan University School of
Medicine, Irwon-ro 81, Gangnam-gu,
Seoul 06351, Korea.

Tel: +82-2-3410-0324

Fax: +82-2-3410-0064

E-mail: collacin@hotmail.com

cikang@skku.edu

*These authors contributed equally to this article.

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ORCID iDs

Hyeri Seok

<https://orcid.org/0000-0002-2032-9538>

Min Kyeong Cha

<https://orcid.org/0000-0003-0211-977X>

Cheol-In Kang

<https://orcid.org/0000-0002-3835-8476>

Conflict of Interest

No conflicts of interest.

Failure of Ciprofloxacin Therapy in the Treatment of Community-Acquired Acute Pyelonephritis caused by *In-Vitro* Susceptible *Escherichia coli* Strain Producing CTX-Type Extended-Spectrum β -Lactamase

Hyeri Seok ^{1,*}, Min Kyeong Cha ^{2,*}, Cheol-In Kang ¹, Sun Young Cho¹, So Hyun Kim^{1,2}, Young Eun Ha¹, Doo Ryeon Chung^{1,2}, Kyong Ran Peck¹, and Jae-Hoon Song¹

¹Division of Infectious Disease, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

²Asia Pacific Foundation for Infectious Diseases, Seoul, Korea

ABSTRACT

While carbapenems are the drug of choice to treat extended-spectrum- β -lactamase (ESBL)-producing strains, some alternative carbapenem-sparing regimens are suggested for antibiotic stewardship. We experienced a case of ciprofloxacin treatment failure for acute pyelonephritis caused by an apparently susceptible *Escherichia coli*. A 71-year-old woman presented the emergency department with fever for 7 days and bilateral flank pain for 2 days. The laboratory results and abdominopelvic computed tomography finding were compatible with acute pyelonephritis. During 3-day ciprofloxacin therapy, the patient remained febrile with persistent bacteremia. After the change in antibiotics to ertapenem, the patient's clinical course started to improve. ESBL-producing *E. coli* isolates were identified in all three consecutive blood samples. Pulsed-field gel electrophoresis (PFGE) patterns, serotypes, and sequence types showed the three isolates were derived from the identical strain. The isolates produced CTX-M-14 type ESBL belonging to the ST69 clonal group. Despite *in vitro* susceptibility, the failure was attributed to a *gyrA* point mutation encoding Ser83Leu within quinolone resistance-determining regions. This case suggests that ciprofloxacin should be used cautiously in the treatment of serious infections caused by ciprofloxacin-susceptible, ESBL-producing *E. coli*, even in acute pyelonephritis because *in-vitro* susceptibility tests could fail to detect certain genetic mutations.

Keywords: Extended-spectrum beta-lactamase; *Escherichia coli*; Ciprofloxacin; Pyelonephritis; CTX-M

INTRODUCTION

Extended-spectrum- β -lactamase (ESBL)-producing *Enterobacteriaceae* have become an important issue in hospitals due to increased mortality from treatment failure [1]. As community-acquired ESBL-producing strains has been on the rise, to choose initial empirical

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Conceptualization: CIK. Data curation: HS, MKC. Formal analysis: CIK, HS, MKC. Investigation: SYC, YEH. Methodology: MKC, SHK, JHS. Project administration: CIK. Resources: HS, MKC, SYC, YEH. Software: MKC. Supervision: SHK, DRC, KRP, JHS. Validation: CIK, SHK, DRC, KRP, JHS. Visualization: CIK, HS, MKC. Writing - original draft: HS, MKC. Writing - review & editing: HS, MKC, CIK, SYC, SHK, YEH, DRC, KRP, JHS.

treatment became difficult as well. While carbapenems are the drug of choice to treat ESBL-producing strains, excessive use of carbapenems has led to carbapenem-resistant *Enterobacteriaceae* (CRE) [2]. For antibiotic stewardship, fluoroquinolones, beta-lactams, and aminoglycosides have been suggested as alternative carbapenem-sparing regimens [3-5]. Several studies have demonstrated successful treatment of ESBL-producing bacterial infections, such as urinary tract infections (UTIs), with non-carbapenem agents [6, 7].

Interestingly, we experienced a case of ciprofloxacin treatment failure for acute pyelonephritis (APN) caused by an apparently susceptible *Escherichia coli* strain and herein report the clinical presentation and microbiological characteristics. To identify the possible causes of treatment failure, we investigated the microbiological characteristics and *in vitro* susceptibility of various antimicrobial agents in clinical isolates.

CASE REPORT

A 71-year-old woman presented the emergency department with fever and bilateral flank pain. The fever had lasted seven days, and the bilateral flank pain appeared two days before the visit. The initial vital signs showed blood pressure of 176/80 mmHg, pulse rate of 108 beats/min, respiratory rate of 25 breaths/min, and body temperature of 40°C. The results of the initial laboratory tests were as follows: white blood cell count 20,050 /mm³ with 91.3% neutrophils, hemoglobin 12.8 g/dL, platelet 101,000 /mm³, blood urea nitrogen 33.2 mg/dL, creatinine 2.32 mg/dL, erythrocyte sedimentation rate 67 mm/hr, C-reactive protein 30.73 mg/dL, and procalcitonin 27.17 ng/mL. The abdominopelvic computed tomography showed diffuse swelling of both kidneys with multifocal ill-defined low density regions compatible with APN (Fig. 1A). As she had no previous history of APN or prior exposure to antibiotics, ciprofloxacin (400 mg intravenously twice a day) was administered as an initial empirical antimicrobial therapy. The next day, gram-negative bacilli (GNB) were isolated in the two initial pairs of blood cultures and the urine culture. Three days after ciprofloxacin therapy, the patient was still febrile and the subsequent blood cultures showed GNB again. Ciprofloxacin was changed to ertapenem (1g intravenously once a day) and the patient's clinical symptoms and signs started to improve the following day. ESBL-producing *E. coli* isolates were identified in all three consecutive blood samples. All isolates showed *in vitro* susceptibility to ciprofloxacin according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, although the minimum

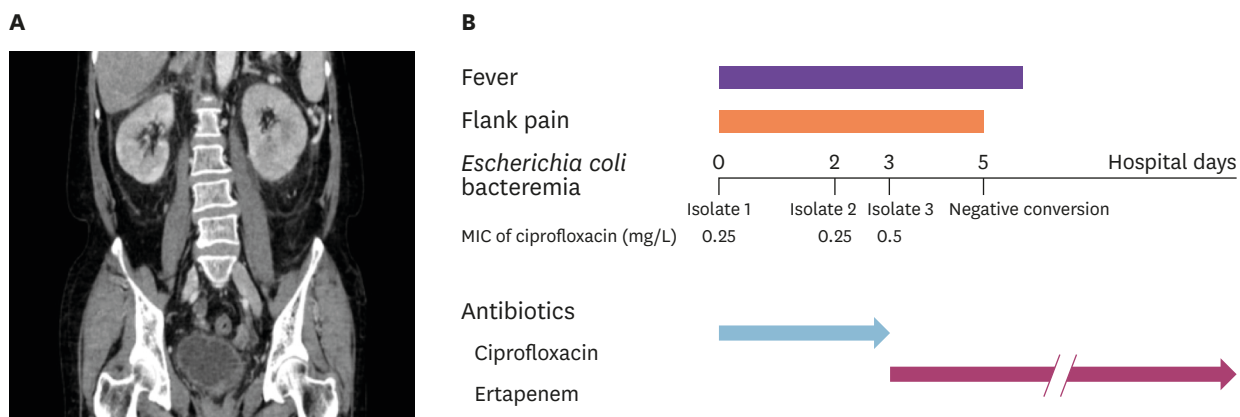


Figure 1. (A) Abdominopelvic computed tomography finding: diffuse swelling with multifocal ill-defined low density regions in bilateral kidneys (B) Clinical course and treatment of acute pyelonephritis with extended spectrum beta-lactamase-producing *Escherichia coli* bacteremia.

Table 1. Microbiological characteristics of *Escherichia coli* isolates during ciprofloxacin treatment

Isolates No.	Serotype	PFGE pattern	ST	Susceptibility to Ciprofloxacin	MIC (mg/L)								Amino acid substitution <i>gyrA</i>		
					AMP	SAM	AMC	TZP	CRO	CAZ	CTX	FEP		MEM	ATM
1	O15	A	69	0.25	0.25	>64/32	8/4	1/4	>32	2	>128	16	0.06	2	Ser83Leu
2	O15	A	69	0.25	0.25	64/32	8/4	1/4	>32	1	>128	16	0.06	2	Ser83Leu
3	O15	A	69	0.5	0.5	>64/32	8/4	2/4	>32	2	>128	32	0.06	4	Ser83Leu

*PFGE patterns are designated by an arbitrary single letter.

PFGE, pulsed-field gel electrophoresis; ST, sequence type; MIC, minimum inhibitory concentration; AMP, ampicillin; SAM, ampicillin/sulbactam; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; MEM, meropenem; ATM, aztreonam.

inhibitory concentration (MIC) levels of ciprofloxacin were slightly elevated, from 0.25mg/L in the first and second isolates to 0.5 mg/L in the third isolate (**Fig. 1B**).

All the isolates were identified by the VITEK2 system (BioMérieux, Lyon, France). *In vitro* antimicrobial susceptibility tests of *E. coli* isolates were performed using the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. We used the double-disk synergy test for phenotypic detection of ESBL producers, as per the CLSI standards. Amplification of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, and plasmid-mediated quinolone resistance *qnr* genes were previously performed using primers [8]. To investigate the presence of mutations in the quinolone resistance-determining regions (QRDRs), the chromosomal genes *gyrA*, *gyrB*, *parC*, and *parE* were amplified using polymerase chain reaction (PCR) [9]. All isolates were serotyped by O-type specific PCR and examined by pulsed-field gel electrophoresis (PFGE) after bacterial DNA digestion via *Xba*I. The genetic relationships were assessed using multilocus sequence typing (MLST).

The three isolates were derived from the identical strain as per their matching PFGE patterns, serotypes, and sequence types (STs) (**Table 1**). The MICs of ciprofloxacin by the broth microdilution method increased from 0.25 to 0.5 mg/L, but remained within the susceptible range. All of the isolates were resistant to all of the cephalosporins tested except ceftazidime, and all retained their susceptibility to carbapenems. The isolates exhibited the ST69 clonal group and produced CTX-M-14 ESBL along with TEM-1. The *qnr* genes (*qnrA*, *qnrB*, and *qnrS*) were not detected. A single mutation in *gyrA* (Ser83Leu) was found in all the three isolates, as compared with the corresponding sequences of the *E. coli* K-12 strain. No amino acid substitutions were identified in *gyrB*, *parC*, and *parE*.

DISCUSSION

In the current study, we demonstrated ciprofloxacin treatment failure in a case of community-acquired acute pyelonephritis which was caused by the apparently susceptible CTX-type ESBL-producing *E. coli* strain. We investigated the possible cause of this treatment failure despite *in vitro* susceptibility by identifying the genotypic resistance against fluoroquinolone.

UTIs are one of the most common bacterial infections affecting public health worldwide [10]. *Escherichia coli* is the most common causative pathogen, and treatment strategies have been modified with the advent of ESBL-producing *E. coli*. With this epidemiological change, antibiotic stewardship and the cost of treating UTIs have become major healthcare issues [11]. Several carbapenem-sparing regimens, including fluoroquinolone, have been recommended to treat UTIs caused by ESBL producers [6, 7]. Meanwhile, some previous

studies warned of the possibility of fluoroquinolone treatment failure due to ESBL-producing organisms. One study showed higher failure rates in the ciprofloxacin group compared to the imipenem group in the treatment of ESBL-producing *Klebsiella pneumoniae* bacteremia [12]. Another study characterized specific genetic determinants conferring low-level fluoroquinolone resistance *in vivo* in a murine model of pyelonephritis caused by *E. coli* [13]. In our case, a possible mechanism for ciprofloxacin treatment failure was the acquisition of a single point mutation within the QRDR which preceded phenotypic resistance. Although ciprofloxacin may be considered as a viable therapeutic option for GNB infections, including APN, ciprofloxacin should be used with caution in the treatment of serious infections caused by ESBL-producing *E. coli*, even in APN due to ciprofloxacin-susceptible isolates.

Although *E. coli* isolates were susceptible to ciprofloxacin *in vitro*, we found a single *gyrA* mutation in Ser83Leu, one of the most common modifications in fluoroquinolone resistance [14]. Two mutations within the *gyrA* gene encoding Ser83Leu and Asp87Asn have been proposed to confer decreased fluoroquinolone susceptibility. Mutations in *parC* and *gyrA* would lead to a decreased susceptibility of *E. coli* to fluoroquinolones; no amino acid substitutions were found within *parC* in this study [15]. CTX-M-14 was identified in this case, which was one of the most common types seen in a previous Korean study [16]. CTX-M-14 was associated also with ST69, which is known to be a common sequence type found in community-acquired ESBL-producing *E. coli*.

Two observations can be drawn from this case. First, although the antimicrobial susceptibility test showed that the isolates were susceptible to ciprofloxacin *in vitro*, an unexpressed phenotype resulted in treatment failure, particularly in ESBL-producing *E. coli* isolates. With the possibility of *in vivo* resistance of current antibiotics, modification of treatment regimens according to the expected clinical course should be considered when treating serious infections caused by ESBL-producing pathogens. Furthermore, current drug susceptibility criteria cannot reflect the genetic level of ciprofloxacin resistance; modification of these criteria might be warranted. Second, molecular and genotypic analysis of ESBL-producing strains may be necessary for effective treatment. These methods might help to identify resistance mechanisms more accurately than conventional methods and to enhance antimicrobial stewardship program in the treatment of APN caused by ESBL-producing *E. coli*.

In conclusion, this report showed the emergence of reduced susceptibility to ciprofloxacin in ESBL-producing *E. coli*, leading to therapeutic failure during ciprofloxacin therapy for APN. Our case suggests that we should closely monitor the clinical response of ciprofloxacin when to treat patients with infections caused by ciprofloxacin-susceptible, ESBL-producing *E. coli*.

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