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Short Communication

CTX-M Type Extended Spectrum β-Lactamases in *Escherichia coli* Isolates From Community Acquired Upper Urinary Tract Infections at a University in the European Part of Turkey

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Extended spectrum β-lactamase (ESBL) producing *Escherichia coli* has been an emerging etiologic agent in the community acquired infections. We investigated the occurrence of ESBL producing *E. coli* isolated from patients admitted with community acquired urinary tract infection (UTI) to the hospital of the Trakya University, Turkey during 2006. Eleven single patient isolates of *E. coli* harboring ESBL were identified among 30 *E. coli* isolated from patients admitted with symptoms corresponding to upper UTI. CTX-M type ESBLs were detected in all 11 ESBL-producers by isoelectric focusing and polymerase chain reaction screening. Sequence analysis revealed CTX-M-1 in one isolate, CTX-M-3 in three isolates and CTX-M-15 in seven isolates. ESBL-producing *E. coli* isolated from community acquired UTIs are widespread in the European part of Turkey.

KEYWORDS: community acquired urinary tract infection, extended spectrum ß-lactamase, *Escherichia coli*

Introduction

Community acquired urinary tract infections (UTIs) are generally caused by *Escherichia coli* susceptible to expanded-spectrum β-lactam. antibiotics Extended-spectrum β-lactamases (ESBLs) mostly disseminate and persist in hospital settings. During the last decade, however, CTX-M-type ESBLs have emerged in the community setting among *E. coli* isolates, on plasmids that frequently bear additional resistance determinants. Consequently, ESBL-mediated resistance has consistently increased in *E. coli* in the community setting.[1] This increase in resistance within the community is a public health concern. There are limited data available in Turkey regarding ESBLs isolated from community onset UTIs. Our aim was to investigate the ESBLs among *E. coli* isolates from community acquired UTIs in the European part of Turkey (the Thrace area).

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Materials and methods

Isolates and clinical data

ESBL-producing *E. coli* isolated from patients admitted with symptoms corresponding to upper UTIs to the hospital of the Trakya University, Turkey in 2006 were included in the study. Clinical data were collected retrospectively by chart review. Community acquired infection was defined as (1) infections that occurred less than 48 hours after admission to the hospital in patients who had not been previously been hospitalized within the preceding 30 days; (2) did not fulfill the criteria for hospital acquired infections as delineated by the Centers for Disease Control and Prevention; and (3) occurred in patients who were not transferred from an outside hospital. After performing urine and blood cultures, antibiotic treatment was started empirically on admission. Treatments were modified according to the culture results. On the 4th day of therapy, urine culture was performed as a control.

Antibiotic susceptibility testing and detection of ESBL-producing isolates

Identification, antibiotic susceptibility, and the minimal inhibitory concentration values of the strains were determined using the Vitek2 system (bioMerieux SA, Marcy-l'Etoile, France) and the results interpreted according to the guidelines of the Clinical and Laboratory Standards Institute. A double-disk synergy test was used to screen ESBL production in *E. coli* isolates.

Isoelectric focusing

Crude extracts of the isolates were prepared by sonication and analytic isoelectric focusing (IEF) performed using a Model 111 Mini IEF Cell (Bio-Rad, Hercules, CA, USA) as described previously.[2] Enzymes were detected by nitrocefin (0.1 nM) overlay (Calbiochem US and Canada) and pI values were estimated relative to the known enzymes (TEM-1, pI = 5.4: SHV-1, pI = 7.6; OXA-14, pI = 6.2) used in the experiment.

Polymerase chain reaction

DNAs for β-lactamase screening by polymerase chain reaction (PCR) were extracted by incubating dense bacterial suspensions at 95°C for 10 minutes and removing the debris by centrifugation for 10 minutes at 12,000 *g* as described.[3] PCR was accomplished in 50 µL reaction,with using 5 µL of DNA extract, 1 × buffer, 1.5 mM of $MgCl_2$, 0.8 mM of dNTPs, 50 pmol of each primers and 1.5 U of *Taq* polymerase (Fermentas, Lithuania). Amplification was accomplished after a 5-minute denaturation at 95°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, annualing at 55°C for 60 seconds and extension at 72°C for 60 seconds. The PCR products were run on a 1.5% agarose gel and results visualized using a UV lamp. A 496 bp internal sequence from the $bla_{CTX\text{-}M\text{-}1}$ phylogenetic subgroup was amplified using forward primer: 5′-ATG TGC AGC ACC AGT AAA GT-3′ and reverse primer: 5′-CCC CCA CAA CCC AGG AAG CA-3′.

Gene sequencing

Sequence analysis was performed with the same primers on both strands of the PCR products, purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Basel, Switzerland). CTX-M types were further confirmed by amplification and sequencing on both sides of the full length *bla* genes (876 bp) using forward primer 5′-ATG GTT AAA AAA TCA CTG CGC-3′ and reverse primer 5′-TTA CAA ACC GTC GGT GAC GAT-3′. PCR amplification was accomplished as mentioned above, except that the extension time was 2 minutes.

Sequence analysis was done using the dye terminator cycle sequencing method and an ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA). The assay was carried out according to a standard protocol. Data were collected on an ABI 377 automated fluorescence sequencer (Applied Biosystems).

Results

During the study period, a total of 60 cases of community acquired UTI were admitted. An etiologic agent was isolated from 50 cases. Of all the isolates, 30 (60.0%) were *E. coli*. ESBL was detected in 11 (36.7%) of the *E. coli* isolates by the double-disk synergy test. Etiologic agents identified from urine cultures of the other patients were as follows: *Enterococcus faecalis* in 10 patients, *Enterococcus faecium* in four patients, and *Klebsiella pneumoniae* in six patients. The ESBL phenotype was not detected in the *K. pneumoniae* isolates. All the *E. coli* isolates that did not produce ESBL, along with *K. pneumoniae,* were susceptible to extended-spectrum cephalosporins.

CTX-M type ESBLs were detected in all 11 ESBL-producers by IEF and PCR screening. No other β-lactamases were detected. The primers used for screening were specific to the CTX-M-1 phylogenetic subgroup, and sequence analysis of the internal fragments amplified by these primers further suggested that the *bla* genes belonged to the same family. For the full-length amplification and sequencing of *bla* genes, we designed the outermost primers specific to the CTX-M-1 subgroup. Subsequently, sequence analysis revealed CTX-M-1 in one isolate, CTX-M-3 in three isolates and CTX-M-15 in seven isolates. CTX-M types, minimal inhibitory concentration values for antibiotics, blood culture results and the treatment data for the ESBL-producing *E. coli* are shown in the Table. None of the patients with ESBL-producing *E. coli* had a history of contact with health care centers, or admission to hospital, although five patients had a history of antibiotic exposure in the last 2 months. The antibiotics used by the five patients were second generation cephalosporins (3 patients) and fluoroquinolones (2 patients). None of the patients had recurrent UTIs.

ESBL-producing *E. coli* was isolated from both urine and blood from four patients. The bloodstream isolates had the same resistance phenotypes as the urine isolates. On admission to the hospital, the patients had been given ceftriaxone empirically. Treatment was then changed according to the culture results. The three patients with ciprofloxacin-susceptible isolates and a negative blood cultures were successfully treated with ciprofloxacin; otherwise, imipenem was antibiotic of choice. Urine cultures were negative in all patients obtained on the 4th day of the antibiotic treatment (control cultures). However, antibiotics were administered for 14 days, and all of the patients improved clinically.

Discussion

ESBL-producing *E. coli* has been detected among nosocomial infections worldwide, but in recent years this microorganism has also been reported in community acquired infections.[4–7] Although we do not know the exact prevalence of these organisms in our country, our findings suggest that ESBL producers have already begun to disseminate throughout the European part of Turkey. A recent publication reported that CTX-M is common among the ESBL-producing *E. coli* isolates obtained from community acquired UTIs in Izmir, a city in West Anatolia.[8] The rate of ESBL-producing *E. coli* was found to be 21% in that study. We also found this rate to be quite high (36.7%) and,

Table. CTX-M types, minimal inhibitory concentration values for antibiotics of expanded-spectrum beta-lactam antibiotics-producing *Escherichia coli*, blood culture results and patient treatment data

Case	β-lactamase	AK	SAM	ATM	FEP	CAZ	CRO	CIP	GN	IPM	MEM	TZP	SXT	Blood culture	Treatment
1	CTX-M-1	<2	>32	4	32	<1	>64	<0.25	<1	<1	<0.25	64	<20	Negative	Ciprofloxacin
2	CTX-M-3	8	>32	>64	32	>64	>64	>4	>16	<1	<0.25	64	>320	Positive	Imipenem
3	CTX-M-3	8	>32	>64	32	16	>64	>4	2	<1	<0.25	8	>320	Negative	Imipenem
4	CTX-M-3	16	>32	<1	<1	<1	>64	0.50	4	<1	<0.25	64	>320	Negative	Ciprofloxacin
5	CTX-M-15	16	32	2	<1	4	>64	>4	<1	<1	<0.25	8	>320	Negative	Imipenem
6	CTX-M-15	16	>32	<1	<1	<1	>64	>4	4	<1	<0.25	<4	>320	Positive	Imipenem
7	CTX-M-15	8	>32	>64	8	16	>64	>4	>16	<1	<0.25	8	>320	Positive	Imipenem
8	CTX-M-15	16	>32	>64	4	16	>64	>4	2	<1	<0.25	8	>320	Negative	Imipenem
9	CTX-M-15	<2	32	16	>64	4	>64	<0.25	<1	<1	<0.25	<4	40	Negative	Ciprofloxacin
10	CTX-M-15	<2	>32	32	4	<1	>64	0.50	<1	<1	<0.25	<4	<20	Positive	Imipenem
11	CTX-M-15	16	>32	16	4	4	>64	>4	4	<1	<0.25	64	>320	Negative	Imipenem

AK=Amikacin; ATM=aztreonam; CAZ=ceftazidime; CIP=ciprofloxacin; CRO=ceftriaxone; FEP=cefepime; GN=gentamycin; IPM=imipenem; MEM=meropenem; SAM=sulbactam-ampicillin; SXT=co-trimoxazole; TZP=piperacillin-tazobactam.

among these ESBLs, the most prevalent type was CTX-M-15. Such a predominance of the CTXM-15 enzyme was also reported in the study from Turkey,[8] and from other European countries.[9–11]

Genes encoding ESBLs are usually located on plasmids carrying other resistance determinants for aminoglycosides, trimethoprim, sulfonamides, and quinolones. Resistance to quinolones was detected at a high rate (7/11) among our isolates. Some studies have reported a lower prevalence of resistance to quinolones and aminoglycosides,[12] whereas others have reported this rate as high as 50% among community acquired CTX-M-producing *E. coli* isolates.[10] There are concerns about the use of quinolones in an infection caused by ESBL-producing Enterobacteriaceae, even if it is susceptible to this class of antibiotics.[13,14] Therefore, high rates of quinolone and co-trimoxazole resistance among ESBL-producing urinary isolates is a significant complication that unavoidably increases carbapenem utilization, especially in the community setting. In this study, however, of the four patients with quinolone susceptible isolates, three were treated successfully with ciprofloxacin, signifying the use of quinolones in UTIs where the isolate is susceptible to this antibiotic. Carbapenems have been shown to be associated with the lowest mortality of any drug class when used against infections by ESBL-producing organisms. Mortality in patients with blood culture-positive, ESBL-producing organisms was less than 10% when patients received either imipenem or meropenem.[14] Non-β-lactam drugs, such as quinolones, trimethoprim-sulfamethoxazole, and aminoglycosides, are not adequate as a first line therapy for serious infections such as bacteremia.[15] We also selected carbapenem therapy for four patients with positive culture for both urine and blood. In conclusion, our results indicate that ESBL-producing *E. coli* isolated from community acquired UTIs are widespread in the European part of Turkey, and identify the predominant types as CTX-M β-lactamases. We can say that ESBL-producing bacteria are a problem not only for hospital acquired infections, but also for community acquired infections in the Thrace region of Turkey.

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