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Characterization of ST 38 CTX-M-producing *Escherichia coli* isolated from urine at a tertiary hospital in Daejeon, Korea

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Introduction and Purpose

ESBL-producing *E. coli* isolates are the most important causes of hospital-acquired (HA) and community-onset (CO) urinary tract infections and CTX-M-type ESBLs have spread rapidly in the past few decades. According to several studies in Korea, it is reported that 10-17% of *E. coli* isolates produce ESBLs, and CTX-M producing *E. coli* isolates has increased 17-92 % since the first report of CTX-M producing isolate in 2001. In this study, we aimed to investigate the prevalence of CTX-M types, integrons, and IS*CR*s among CTX-M-producing *E. coli* isolates in urine over a 4-year period, and to determine the genetic relationships between these strains.

Material and Methods

1. Bacterial isolates and definitions

- Consecutive non-duplicate ceftazidime and/or cefotaxime resistant clinical isolates of *E. coli* were obtained from urine collected at Chungnam National University Hospital from September 2011 to July 2014. All isolates were identified using the Vitek 2® automated ID system (bioMèrieux Vitek Inc., Hazelwood, MO, USA).

2. Molecular characterization of CTX-M producing *E. coli* isolates

All isolates were screened for the presence of bla_{CTX-M} by PCR and sequencing as described previously. For strains harboring bla_{CTX-M}, integrons and IS*CR* were detected by PCR and sequencing as described previously. Class 1 integrons were amplified using the primers hep58 (5′-TCATGGCTTGTTATGACTGT-3′) and hep59 (5′-GTAGGGCTTATTATGCACGC-3′). Class 2 integrons were amplified using the primers hep51 (5′-GATGCCATCGCAAGTACGAG-3′) and hep74 (5′-CGGGATCCCGGACGGCATGCACGATTTGTA-3′). Class 3 integrons were amplified using the primers Int3F (5′-GCCTCCGGCAGCGACTTTCAG-3′) and Int3R (5′-ACGGATCTGCCAAACCTGACT-3′). For detection of all IS*CR*s, the following primers were used: CRF (5′-CACTWCCACATGCTGTKKC-3′) and CRFF-r (5′-CGCTTGAGSCGTTGCRYCC-3′).

3. Molecular typing for CTX-M producing *E. coli* isolates

Molecular typing for CTX-M-producing *E. coli* isolates was performed by MLST, Phylogenetic analysis, rep-PCR, and PFGE which were previously described

Results

1. Characteristics of *E. coli* isolates from urine

A total of 471 *E. coli* isolates were obtained from urine, of which 84 (17.8%) isolates were ESBLs. Among these isolates, 79 (94.0%) isolates were positive for bla_{CTX-M}, of which 44 (55.7%) isolates had CTX-M-14, 32 (40.5%) isolates contained CTX-M-15, and 3 (3.8%) isolates carried both CTX-M-14 and -15. Other types of CTX-M ESBL were not found (Table1).

		2011 (10)	2012 (16)	2013 (19)	2014 (34)	Total (79)
bla_{CTX-M} gene	CTX-M-14	7	7	12	18	44
	CTX-M-15	2	9	6	15	32
	both	1		1	1	3
ST types	ST131	3	9	6	16	34
	ST38	1	2	7	10	20
	ST405	2	1	2	3	8
	ST69	2	1	1	1	5
	ST95	0	1	0	2	3
	ST493	0	1	1	0	2
	ST1488	1	0	0	0	1
	ST1177	1	0	0	0	1
	ST44	0	1	0	0	1
	ST354	0	0	1	0	1
	ST648	0	0	1	0	1
	ST2329	0	0	0	1	1
	ST1193	0	0	0	1	1

Table1. Prevalence of CTX-M gene and distribution of ST types

2. Prevalence of integrons and IS*CR*s

Among CTX-M producing isolates, 45 (57 %) isolates contained class 1 integron, however no class 2 or class 3 integron were found. Class 1 gene cassette arrays found in this study were divided into 4 types. The type 1 amplicon, obtained in 37 isolates, carried *dfr*A17-*aad*A5 gene cassettes. The type 2 amplicon, found in 6 isolates, carried *dfr*A12-*aad*A2 gene cassettes. The type 3 amplicon, detected in one isolate, carried *aac*A4-*arr*3-*dfr*A27 gene cassettes. One isolates contained the type 4 amplicon carrying *dfr*A1-*aad*A1 gene cassettes. 4 types of ISCR were detected such as IS*CR*1, IS*CR*2, IS*CR*3, and IS*CR*14. IS*CR*s were detected in 9 (11.4 %) isolates, of which 7 isolates produced CTX-M-14 and others 2 isolates were CTX-M-15-producer (Table 3).

3. Diversity and comparison of molecular types

1) MLST

MLST experiments identified 13 unique STs. The most prevalent ST was ST131 (n = 34, 43.0%), followed by ST38 (n=20, 25.3%), ST405 (n = 8, 10.1%), ST69 (n = 5, 6.3%), ST95 (n = 3, 3.8%), and ST493 (n=2, 2.53%). The others, 7 each individual STs, were identified in 7 isolates.

2) Phylogenic analysis

Phylogenetic group B2 (n=37, 46.8%) included 23 CTX-M-15 and 14 CTX-M-14 producing isolates while, D (n=33, 41.8%) included 27 CTX-M-14 and 6 CTX-M-15 producing isolates.

3) rep-PCR

61 strains of *E. coli* showed mainly 3 band patterns according to rep-PCR. 20 of the 21 strains of A type were ST38, all 33 strains of B type were identified ST131, and the others (n=8), C type, were displayed ST405. These three type strains were epidemiologically-related, respectively.

4) PFGE

PFGE was performed on 34 ST131 isolates and 20 ST38 isolates, respectively. These isolates were genetically diverse on PFGE, comprising 28 different PFGE band patterns on 34 ST131 isolates and 15 on 20 ST38 isolates.

ST type	PG	Type of infections	CTX-M	rep-PCR	ISCR
ST131 (34)	B2 (34)	HO (24)	CTX-M-14 (7)	B (7),	
			CTX-M-15 (16)	B (15),L (1)	ISCR1 (1)
			CTX-M-14 & 15 (1)	B (1),	
		CO (10)	CTX-M-14 (4)	B (4)	
			CTX-M-15 (5)	B (5)	
			CTX-M-14 & 15 (1)	B (1)	
ST38 (20)	D (20)	HO (15)	CTX-M-14 (15)	A (15)	ISCR2 (1), ISCR14 (3)
		CO (5)	CTX-M-14 (4)	A (4)	ISCR2 (1)
			CTX-M-15 (1)	A (1)	
ST405 (8)	D (8)	HO (3)	CTX-M-14 (2)	C (2)	
			CTX-M-15 (1)	C (1)	ISCR14 (1)
		CO (5)	CTX-M-14 (2)	C (2)	ISCR3 (1)
			CTX-M-15 (3)	C (3)	
ST69 (5)	D (5)	HO (2)	CTX-M-14 (2)	I (1), J (1)	
		CO (3)	CTX-M-14 (2)	G (2)	ISCR14(1)
			CTX-M-15 (1)	A (1)	
ST95 (3)	B2 (3)	CO (3)	CTX-M-14 (3)	D (3)	

Table2. Characteristics of *E. coli* isolates in accordance with the ST types

Discussions

A total of 84 (17.8%) *E. coli* were found to be ESBL producers among which 94.0 % of *E. coli* isolates contained bla_{CTX-M}. In our study, however, CTX-M-14-producing isolates (55.7%) were more frequently observed than CTX-M-15 (40.5%). Interestingly, except ST131, ST38 showed higher prevalence than in other reports and the number of phylogenic group B2 including ST131 (n=34), ST95 (n=3), and ST493 (n=2) was similar to that of D including ST38 (n=20), ST405 (n=8), and ST69 (n=5) belonged to the phylogenetic group D. In conclusion, CTX-M-14-producing isolates, ST38, and phylogenetic group D are more widely spread than in other parts of Korea, which is considered as a unique characteristic of Daejeon area.