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Original Article

Comparisons of CTX-M-Producing *Escherichia coli* Isolates from Humans and Animals in South Korea

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To investigate the possibility of transmission of CTX-M-producing *Escherichia coli* isolates among humans and animals, we compared CTX-M-producing *E. coli* isolates showing the same genotype from humans and dogs in Korea. Sixteen CTX-M-producing *E. coli* isolates from animals were selected and their genotypes were identified using MLST. Among clinical CTX-M-producing *E. coli* isolates from humans, which have been identified in previous studies, 12 isolates showing the same STs with those of *E. coli* isolates from animals were selected. For these 28 CTX-M-producing *E. coli* isolates, identification of bla_{CTX-M} genes and their genetic environments, antimicrobial susceptibility testing, extended MLST, and PFGE were performed. Some CTX-M-producing *E. coli* isolates from humans showed the same genotypes, such as ST10, ST38, ST58, and ST95, but different CTX-M enzymes and PFGE patterns. Thus, it can be concluded that dissemination of ESBL-producing *E. coli* isolates between humans and animals is rare so far.

Key Words: *Escherichia coli*, Extended spectrum β-lactamase, CTX-M-type, Transmission

INTRODUCTION

Escherichia coli is the one of the most common pathogens of bacterial infections worldwide. In particular, infections by extended spectrum β-lactamase (ESBL)-producing *E. coli* isolates have recently increased both in humans and animals (1). Among ESBLs, the global spread of CTX-M-producing *E. coli* isolates in humans has been reported, and has been termed a 'CTX-M pandemic' (2). Recently, there has been a rapid spread of the CTX-M-15-producing ST131 clone of *E. coli* (3). Since the first detection of an ESBL-producing *E. coli* isolate from a laboratory dog in Japan in 1998, a variety of ESBLs, including CTX-M-type ESBLs, have been isolated from various animals (4). As in humans, highly virulent CTX-M-15-producing ST131 isolates were also reported mainly in European countries (5). The dissemination of CTX-M-type ESBLs among *E. coli* isolates may be due to horizontal transfer of IncFII-type plasmid as well as clonal expansion (5~7).

E. coli is a commensal bacterium in animals such as poultry and cattle, and food from animal origins can be contaminated with *E. coli* (1). Since the late 1990s, ESBL-producing *E. coli* isolates have been found in retail meat and production animals worldwide (1). Several recent studies

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have reported CTX-M-harboring *E. coli* from companion animals, and the emergence of CTX-M-15-producing ST131 *E. coli* isolates has also been reported (8). In Korea, CTX-M-type ESBLs were identified in *E. coli* isolates from stray dogs (9). In that study, several features, such as CTX-M type, antimicrobial susceptibility, transferability of plasmid, plasmid replicon type, genetic environment of bla_{CTX-M} gene, phylogenetic group, and sequence type (ST) in multilocus sequence typing (MLST), were investigated. However, a comparison among CTX-M-producing *E. coli* isolates from humans and animals was not performed.

To discern the possible transmission of CTX-M-producing *E. coli* isolates among humans and animals, we compared CTX-M-producing *E. coli* isolates showing the same STs from humans and animals in Korea through the use of extended MLST and other techniques.

MATERIALS AND METHODS

Bacterial strains

Sixteen *E. coli* isolates from intestinal samples of animals taken during 2004-2008 in Korea were included in this study. All of the isolates produced CTX-M-type ESBLs (9). An additional 12 *E. coli* isolates showing the same sequence types (STs) of those animals were selected among 88 CTX-M-producing *E. coli* isolates from patients with bacteremia in a Korean hospital (10).

Characterization of β-lactamase-encoding genes

β-lactamase genes, including bla_{TEM}, bla_{SHV}, and bla_{CTX-M}, were detected by gene amplification and sequencing using specific primers as described previously (11~13).

In vitro antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by a broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guideline (14). Ten antimicrobial agents were tested: amikacin, ampicillin, aztreonam, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin /tazobactam, and trimethoprim/sulfamethoxazole. The results were interpreted based on susceptibility interpretive criteria established in the CLSI standard M100-S21 (14, 15).

MLST genotyping and pulsed-field gel electrophoresis

MLST was performed using the protocols described at the website (http://mlst.ucc.ie/mlst/dbs/Senterica/documents/primersEnterica_html) (16). A ST based on the allelic profile of the seven amplicons (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was assigned to each strain. In addition, *Ec*MLST, in which internal fragments of 18 housekeeing genes (*arcA*, *aroE*, *aspC*, *clpX*, *cyaA*, *dnaG*, *fadD*, *grpE*, *icdA*, *kdsA*, *lysP*, *metG*, *mtlD*, *mutS*, *mdh*, *rpoS*, *torC* and *uidA*) were analyzed, was also carried out to investigate the further genetic divergence among *E. coli* isolates (17). Because of the many new allelic profiles in housekeeping genes, eSTs (STs in *Ec*MLST) were designated based on seven housekeeping genes at the MSU *Ec*MLST website (http://shigatox.net/new/tools/ecmlst.html). Pulsed-field gel electrophoresis (PFGE) analysis was used as previously described (18).

Genetic environments of bla_{CTX-M} genes

PCR mapping and sequencing were performed to investigate the genetic environment of the bla_{CTX-M} genes using previously described primers (19, 20). Sequences previously reported to be associated with the CTX-M group genes such as *tnpA* IS*Ecp1*, IS*Ecp1*, *tnpA* IS*26*, IS*26*, and ORF 513 were employed to investigate regions upstream of the bla_{CTX-M} genes. bla_{CTX-M} forward primer and reverse primers for ORF477, IS*903*, and *tnpA* IS*903* were used to characterize downstream segments of the bla_{CTX-M} genes. All the positive PCR products obtained were subjected to direct sequencing on both strands and the resulting nucleotide sequences were analyzed.

RESULTS

β-lactamase-encoding genes of *E. coli* isolates from animals

Among 16 CTX-M-producing *E. coli* isolates from animals, most (13 isolates) produced CTX-M-9 group ESBLs and only three were positive for CTX-M-group 1

Table 1. ESBL types, STs, and antimicrobial resistant profiles in ESBL-producing *E. coli* isolates from animals.

Strains	Origin	CTX-M	Other β-lactamases detected	ST	Susceptibility to other antibiotics[a]									
					AMK	AMP	AZT	CFX	CFZ	CIP	GEN	IMI	PIP/TZ	TMP/SMX
CTX-M-1 group														
E-6	Dog	CTX-M-3	TEM-1	95	S	R	S	R	S	S	S	S	R	R
06-D-22	Pig	CTX-M-15	TEM-1	58	S	R	R	R	R	S	S	S	R	R
E-33	Dog	CTX-M-55	−	125	S	R	R	R	R	S	S	S	R	R
CTX-M-9 group														
E-120	Dog	CTX-M-14	−	457	S	R	R	R	R	R	R	S	R	R
E-123	Dog	CTX-M-14	−	457	S	R	R	R	S	S	S	S	R	S
E-126	Dog	CTX-M-14	−	2,541	S	R	R	R	S	S	S	S	R	R
E-128	Dog	CTX-M-14	TEM-1	359	R	R	R	R	S	R	R	S	R	R
E-129	Dog	CTX-M-14	TEM-1	93	S	R	R	R	S	R	S	S	R	R
04-D-02	Dog	CTX-M-14	−	327	S	R	R	R	S	S	S	S	R	S
04-D-26	Cattle	CTX-M-14	TEM-1	2,197	S	R	R	R	S	R	R	S	R	R
05-D-37	Cattle	CTX-M-14	TEM-1	2,930	R	R	R	R	I	R	R	S	R	R
E-102	Dog	CTX-M-24	−	642	S	R	R	R	S	S	S	S	R	R
E-121	Dog	CTX-M-24	TEM-1	38	S	R	R	R	R	R	R	S	R	R
E-124	Dog	CTX-M-24	−	10	S	R	R	R	S	R	S	S	R	S
E-122	Dog	CTX-M-27	−	457	S	R	R	R	R	R	R	S	R	R
E-127	Dog	CTX-M-65	−	327	I	R	R	R	S	S	R	S	R	S

[a] AMK, amikacin; AMP, ampicillin; AZT, aztreonam; CFX, cefotaxime; CFZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; IMI, impenem; PIP/TZ, piperacillin/tazobactam; TMP/SMX, trimethoprim/sulfamethoxazole.

(Table 1). Eight of the *E. coli* isolates from animals produced CTX-M-14 enzymes, and three CTX-M-24-producing isolates were found. Among the CTX-M-1 group, CTX-M-3, CTX-M-15, and CTX-M-55 were found in each one isolate. While TEM-1 β-lactamase was found in seven isolates, SHV-type β-lactamase was not found in any.

Results of MLST and antibiotic susceptibility testing of *E. coli* isolates from animals

Among 16 CTX-M-producing *E. coli* isolates from animals, 13 different STs were identified in MLST analysis. Only ST457 and ST327 were identified in multiple isolates: ST457 were found in two CTX-M-14-produing isolates and one CTX-M-27-producing isolates, and ST327 was found both in CTX-M-14- and CTX-M-65-producing isolate. All 16 isolates were resistant to ampicillin, cefotaxime, and piperacillin/tazobactam, and all but one was resistant to aztreonam. Five, seven, eight, and 12 isolates were resistant to ceftazidime, gentamicin, ciprofloxacin, and trimethoprim /sulfamethoxazole, respectively. Only two isolates were resistant to amikacin, and no isolates were resistant to imipenem. One isolate (05-D-37) showing ST2930 was non susceptible to all antimicrobial agents except imipenem.

Comparisons of β-lactamase-encoding genes and antibiotic susceptibility in *E. coli* isolates from humans and animals

In our previous study (10), 12 *E. coli* isolates from humans were found which showed the same STs as those from animals: ST10, ST38, ST58, and ST95 (Table 2). ST10

Table 2. ESBL types, STs, and antimicrobial resistant profiles in ESBL-producing *E. coli* isolates from human.

Strains	CTX-M	Other β-lactamases detected	ST	Susceptibility to other antibiotics[a]									
				AMK	AMP	AZT	CFX	CFZ	CIP	GEN	IMI	PIP/TZ	TMP/SMX
CTX-M-1 group													
K01-09028	CTX-M-3	TEM-1	58	R	R	R	R	R	R	S	S	R	R
B0707-025	CTX-M-15	TEM-1	38	S	R	R	R	R	R	R	S	R	R
CTX-M-9 group													
K01-07025	CTX-M-14	TEM-1	38	S	R	R	R	R	R	I	S	R	R
K01-08133	CTX-M-14	TEM-1	38	R	R	R	R	R	R	R	S	R	R
B0608-154	CTX-M-14	TEM-1	38	S	R	R	R	I	R	R	S	S	S
B0704-080	CTX-M-14	TEM-1	38	S	R	I	R	S	S	S	S	S	R
B0705-116	CTX-M-14	TEM-1	38	S	R	R	R	S	S	R	S	S	R
K01-09061	CTX-M-14	TEM-1	95	S	R	R	R	R	I	R	S	R	R
K01-12012	CTX-M-14	TEM-1	95	I	R	R	R	R	R	S	S	R	S
B0610-104	CTX-M-14	TEM-1	95	R	R	R	S	S	S	R	S	S	R
B0702-129	CTX-M-14	TEM-1	95	S	R	R	R	S	S	S	S	S	S
K01-11045	CTX-M-14	TEM-1	10	I	R	R	R	R	R	R	S	R	R

[a] AMK, amikacin; AMP, ampicillin; AZT, aztreonam; CFX, cefotaxime; CFZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; IMI, imipenem; PIP/TZ, piperacillin/tazobactam; TMP/SMX, trimethoprim/sulfamethoxazole.

and ST95 were found only in *E. coli* isolates producing CTX-M-14 (CTX-M-9 group), and ST58 was identified only in isolates producing CTX-M-3 (CTX-M-1 group). ST38 was found both in CTX-M-14- and CTX-M-15-producing *E. coli* isolates. The 12 *E. coli* isolates from humans produced TEM-1 β-lactamase, but no isolates produced SHV-type β-lactamase. Antimicrobial resistance profiles of *E. coli* isolates from humans were similar to those of *E. coli* isolates from animals. However, the rate of ceftazidime resistance was higher in *E. coli* isolates from humans (7/12 isolates), and the piperacillin/tazobactam resistance rate was lower in isolates from humans (7/12 isolates). As in *E. coli* isolates from animals, no imipenem-resistant isolates were found in humans. Two isolate (K01-08133 and K01-11045) was non susceptible to all antimicrobial agents except imipenem.

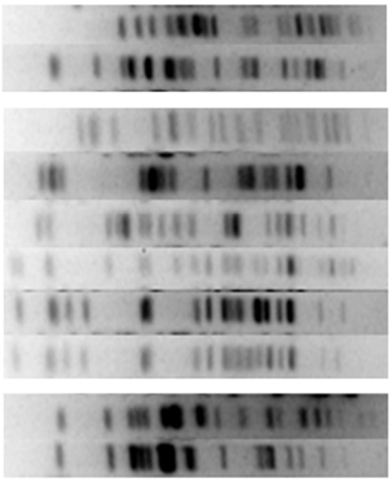
Results of clonality studies in *E. coli* isolates from human and animals

E. coli isolates with the same ST showed the same or similar genotypes in *Ec*MLST (Table 3). Both isolates with ST10 and 58 showed the same *Ec*MLST types, eST171 and eST230, respectively. *E. coli* isolates with ST38 showed two *Ec*MLST types, eST303 and eST861, which are single locus variants differing only in a single nucleotide of *icdA*. Four isolates with ST95 showed eST29 and one isolate from humans (B0702-129) showed eST1003, which have also a single locus variant of eST29 differing in *uidA* (data not shown).

We performed PFGE to determine the epidemiological relationship between CTX-M producing *E. coli* isolates from humans and animals. PFGE was used with *E. coli* isolates sharing the same $bla_{\text{CTX-M}}$ group and STs: two ST10 isolates with $bla_{\text{CTX-M-24}}$ and $bla_{\text{CTX-M-14}}$, six ST38 isolates with $bla_{\text{CTX-M-24}}$ and $bla_{\text{CTX-M-14}}$, and two ST58 isolates with

Table 3. Genotypic characterization of bla_{CTX-M} surrounding DNA

Strain	CTX-M	Origin	eST	Distance (kb) by PCR with the indicated primer								Genetic environment
				Upstream bla_{CTX-M}					Downstream bla_{CTX-M}			
				tnpA IS*Ecp1*	IS*Ecp1* 5'	*tnpA* IS*26*	IS26 5'	ORF 513	ORF 477	IS903 5'	*tnpA* IS*903*	
ST10												
E-124	CTX-M-24	Dog	171	0.8	1.7	−	−	ND[b]	−	0.3	−	B
K01-11045	CTX-M-14	Human	171	2.4	>3	−	−	ND	−	0.3	1.0	E
ST38												
E-121	CTX-M-24	Dog	303	0.8	1.7	−	−	ND	−	0.3	−	B
K01-08133	CTX-M-14	Human	303	0.8	1.7	−	−	ND	−	0.3	−	B
B0704-080	CTX-M-14	Human	303	0.8	1.7	−	−	ND	−	0.3	−	B
K01-07025	CTX-M-14	Human	861	0.8	1.7	−	−	ND	−	0.3	−	B
B0608-154	CTX-M-14	Human	861	−	−	1.1	1.7	ND	−	0.3	1.0	F
B0705-116	CTX-M-14	Human	861	0.8	1.7	−	−	ND	−	0.3	−	B
ST58												
06-D-22	CTX-M-15	Pig	230	0.8	1.7	−	−	ND	1.3	−	−	C
K01-09028	CTX-M-3	Human	230	0.8	1.7	−	−	ND	1.3	−	−	C
ST95												
E-6	CTX-M-3	Dog	29	−	−	−	−	−	1.3	−	−	A
K01-09061	CTX-M-14	Human	29	−	−	−	−	−	−	0.3	1	D
K01-12012	CTX-M-14	Human	29	0.8	1.7	−	−	ND	−	0.3	−	B
B0610-104	CTX-M-14	Human	29	−	−	−	−	−	−	0.3	1	D
B0702-129	CTX-M-14	Human	1,003	−	−	−	−	−	−	0.3	1	D



Strain	Origin	ST	CTX-M	Other β-lactamases
E-124	Dog	10 (171)	CTX-M-24	-
K01-11045	Human	10 (171)	CTX-M-14	TEM-1
E-121	Dog	38 (303)	CTX-M-24	TEM-1
K01-08133	Human	38 (303)	CTX-M-14	TEM-1
B0704-080	Human	38 (303)	CTX-M-14	TEM-1
K01-07025	Human	38 (861)	CTX-M-14	TEM-1
B0608-154	Human	38 (861)	CTX-M-14	TEM-1
B0705-116	Human	38 (861)	CTX-M-14	TEM-1
06-D-22	Pig	58 (230)	CTX-M-15	TEM-1
K01-09028	Human	58 (230)	CTX-M-3	TEM-1

Figure 1. Comparison of PFGE patterns of ST10, ST38, and ST58 CTX-M-producing *E. coli* isolates from humans and animals.

$bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}3}$ (Table 3). Three ST10, ST38, and ST58 *E. coli* isolates from animals showed different PFGE patterns from ST10, ST38, and ST58 *E. coli* isolates from human, respectively (Fig. 1).

Analysis of the regions surrounding $bla_{CTX\text{-}M}$ genes

Two ST10 isolates from dogs and humans showed different genetic environments, and two ST58 isolates from pigs and humans showed the same genetic environment, which is labeled C in Table 3. Four ST38 *E. coli* isolates from human showed the same genetic environment (labeled B) as an ST38 *E. coli* isolate from dog (E-121), while one (B0608-154) did not (Table 3).

DISCUSSION

A recent study showed an extreme low-level rate of (1.9%) of ESBL production among canine *E. coli* isolates from Korea (9). However, all of these isolates possessed the $bla_{CTX\text{-}M}$ gene, most of which was $bla_{CTX\text{-}M\text{-}14}$. In addition to $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$ was also found in an ESBL-producing *E. coli* isolate from pigs. $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ are the most widely distributed ESBLs among clinical *E. coli* isolates from humans worldwide, including Korea (10). Therefore, it is worthwhile to investigate the possibility that CTX-M-producing *E. coli* isolates have transferred from animals to humans or vice versa.

This study found no evidence of the spread of CTX-M-producing *E. coli* isolates between animals and humans in Korea. Although some isolates showed the same or similar genotypes even in *Ec*MLST, their $bla_{CTX\text{-}M}$ genes and PFGE patterns were different each other. In addition, they showed different antimicrobial resistance patterns. However, genotypes ST38 and ST58 showed identical genetic structures surrounding the $bla_{CTX\text{-}M}$ gene. This may indicate a common origin of the $bla_{CTX\text{-}M}$ genes. Notably, $bla_{CTX\text{-}M\text{-}24}$ and $bla_{CTX\text{-}M\text{-}3}$ genes differ from $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ in only one amino acid, respectively (21, 22).

Some reports have found similarities among CTX-M-producing *E. coli* isolates from animals, including isolates from meat, and humans (22~25). There have also been reports of dissemination of CTX-M-producing *E. coli* isolates in veterinary hospitals in Korea (26). In particular, ST10 isolates producing CTX-M-1 type β-lactamase were found in both chicken meat and human samples from rectal swabs in the Netherlands (24).

The ST10, ST38, and ST58 genotypes were not commonly found in CTX-M-producing *E. coli* isolates from humans in Korea (10). Although ST38 was found in six isolates out of 88 CTX-M-producing *E. coli* isolates, each one ST10 and ST58 isolate was identified. No ST131 or ST405, which are the most frequently isolated from humans in Korea, have been identified from ESBL-producing *E. coli* isolates from animals in Korea (27). It may be concluded that the dissemination of ESBL-producing *E. coli* isolates between humans and animals is thus far rare. However, increasing opportunities for close contact between animals, including between companion animals and humans, and the use of antimicrobial agents of the same class in treating infections may promote the dissemination of isolates or horizontal transfer of plasmids with antimicrobial genes between humans and animals. Thus, there should be continuous survey and comparison of genotypes and antimicrobial resistance determinants of pathogens from humans and animals.

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