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Characteristics of Transmissible CTX-M- and CMY-Type β-Lactamase-Producing *Escherichia coli* Isolates Collected from Pig and Chicken Farms in South Korea

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The rapid dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has significantly contributed to public health hazard globally. A total of 281 *E. coli* strains recovered from pigs and chickens between 2009 and 2015 in South Korea were analyzed for ESBL production. ESBL phenotypes were recognized in 14 *E. coli* isolates; ten and three ESBL-producing isolates carried only $bla_{\text{CTX-M}}$ and bla_{CMY} genes, respectively, and one isolate harbored both genes. The predominant CTX-M and CMY types were CTX-M-15 (n = 8) and CMY-2 (n = 3). We also detected ESBL-producing isolates harboring $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CMY-6}}$, $bla_{\text{DHA-1}}$, and $bla_{\text{TEM-1}}$ genes. All ESBL-producing isolates showed resistance to the extent of the fourth generation cephalosporins, along with multidrug resistance. CTX-M-15-producing isolates showed higher MIC values than CTX-M-14- and CTX-M-65-producing isolates. The $bla_{\text{CTX-M}}$ and bla_{CMY} genes have the potential to be transferable. The spreading of bla_{CMY} and $bla_{\text{CTX-M}}$ genes was arbitrated mainly via Frep and IncI1 plasmids. Our isolates showed clonal diversity in PFGE analysis. This is the first report of *E. coli* isolates carrying $bla_{\text{CMY-6}}$ in chicken from South Korea. The emergence of CMY-6 ESBLs in a population of poultry suggests that extensive screening with long-term surveillance is necessary to prevent the dissemination of ESBL from chicken to human.

Keywords: Swine, poultry, extended-spectrum β-lactamase (ESBL), CTX-M, CMY, conjugative transfer, public health

Introduction

From studies of the last several decades, it is generally accepted that the imprudent application of antimicrobials has promoted the emergence of antimicrobial-resistant bacteria in both humans and animals [1, 2]. Food-producing animals may play an enormous role in transmission of antimicrobial resistance between animals and humans, serving as a reservoir. Thus many research studies have been concerned about the role of food-producing animals in the possible transfer of resistance to humans with regard to public health [3, 4], and have described the biological mechanism as being the transfer of resistance genes mediated by mobile genomic elements [5].

Globally, the emergence of extended-spectrum β-lactamases (ESBLs) resulted in the resistance of extended-spectrum cephalosporins, which are detected most commonly in *Enterobacteriaceae* [6]. The mechanism of β-lactamases is to provide protection from the lethal effect of the β-lactam class of antimicrobials on cell wall synthesis [7]. The vast majority of ESBLs belongs to SHV- and TEM-type families, which are common plasmid-mediated β-lactamases of *E. coli.* The family of CTX-M-type β-lactamases, which is a derivative of TEM or SHV, is frequently being described in

gram-negative bacteria that consist of as many as 170 CTX-M subtypes (http://www.lahey.org/studies/webt.asp) that are categorized in accordance to the subgroups of CTX-M 1, 2, 8, and 9 [8]. CTX-M enzymes possess a clinical significance of the high levels of hydrolytic activity against cefotaxime. Similarly, various families of plasmid-mediated AmpC (pAmpC) β-lactamases have been identified to date, and CMY-type β-lactamase produced by *E. coli* is the most frequently reported one [9]. These enzymes typically confer antimicrobial resistance to cephamycins.

The focus of previous studies was generally to assess the presence and transmission of ESBLs and pAmpC β-lactamase-producing *E. coli* isolates recovered from clinical cases [8, 10, 11]. Moreover, several studies have reported *E. coli* strains producing ESBLs and pAmpC β-lactamase [12, 13]. Recently, we reported extended-cephalosporin resistance among *Enterobacteriaceae* isolates from farm animals in South Korea [14] and anticipated that isolates from pigs and chickens in South Korea would demonstrate ESBL genes. Several reports on ESBL-producing *E. coli* strains from food-producing animal populations have been published by national surveillance [12, 15, 16]. However, much of them focused on the prevalence of resistant strains and resistance gene. Therefore, this work aimed to study the prevalence of ESBL-producing *E. coli* isolates recovered from pigs and chickens that had been collected in South Korea, and also to analyze the transfer of ESBL-associated genes and resistance among *E. coli* strains.

Materials and Methods

Bacterial Isolates

Overall, 281 non-duplicate *E. coli* isolates were collected in this study. Among the 281 isolates, 206 isolates were isolated from necropsied pigs that had clinical symptoms of respiratory and digestive disorders between 2009 to 2015, and 75 isolates of avian pathogenic *E. coli* collected from 2011 to 2015 were kindly provided by Chung Ang Vaccine Laboratory (CAVAC, Korea). These isolates were re-confirmed by using a biochemical test (IMViC test) and/or Vitek 2 system (bioMérieux, France). Conjugation assay was performed using *E. coli* J53 Az^R strain as a recipient [14]. *E. coli* ATCC 25922, a quality control strain, was included in the antibiotic susceptibility testing and MIC tests. *Salmonella* serotype Braenderup strain (H9812) was selected as a standard universal size marker [17].

Antimicrobial Susceptibility Test and Detection of ESBL-Producing Isolates

Bacterial strains were assessed for antimicrobial susceptibility using a disc diffusion assay, according to the Clinical and Laboratory Standards Institute (CLSI) procedure [18]. The antimicrobial agents (Oxoid, UK) were as follows: ampicillin, 10 μg; ceftazidime, 30 μg; cefotaxime, 30 μg; ciprofloxacin, 5 μg; gentamicin, 10 μg; nalidixic acid, 30 μg; tetracycline, 30 μg; and trimethoprim-sulfamethoxazole, 1.25/23.75 μg. The antibiotic susceptibility testing results were interpreted in accordance to the guidelines set forth by CLSI [10]. After interpretation of the results, a double-disc synergy method was performed for all isolates showing resistance to ampicillin, cefotaxime, and ceftazidime, to determine the production of ESBL, as previously described [18]. Briefly, antimicrobial discs (BD, USA) containing cefotaxime/clavulanate (30/10 μg) and ceftazidime/clavulanate (30/10 μg) were placed with ceftazidime and cefotaxime on a plate inoculated with the resistant isolates. The production of ESBL was determined by measuring the difference of zone diameter in a plate incubated overnight at 37°, which indicated the inactive effect of clavulanate to the test agents. Then, the ESBL-producing isolates were further screened for profiling antimicrobial resistance to the β-lactam class. The following agents were included: cefaclor, 30 μg; amoxicillin-clavulanic acid, 30 μg; ceftriaxone, 30 μg; cephalothin, 30 μg; cefixime, 5 μg; cefpirome, 30 μg; cefepime, 30 μg; imipenem, 10 μg; and ertapenem, 30 μg. Moreover, the MIC values of ceftazidime, cefotaxime, ceftriaxone, aztreonam, and cefoxitin were determined by the microbroth dilution method [18]. All antimicrobial agents employed in this study were assayed in 2-fold dilutions from 0.25 to 2,048 μg/ml. Antimicrobial susceptibility and MIC tests were performed in triplicates for each isolate.

Detection of β-Lactamase Determinants

For all ESBL-producing isolates, PCR amplification with primers that target bla_{TEM} [19], bla_{SHV} [19], bla_{CTM-M} [20], and pAmpC β-lactamase genes [21] were conducted. For positive isolates of bla_{CTX-M}, further PCRs were performed to confirm bla_{CTX-M} genes, using the bla_{CTX-M} group primers that specifically amplify the CTX-M-1, CTX-M-2, CTX-M-8, and CTX-M-9 groups [22]. DNA templates for PCR were obtained by centrifugation of bacterial colony suspensions grown on tryptic soy broth in 500 μl of distilled water, following boiling for 10 min at 100°C. Sequence alignment and comparison with known sequences were carried out by searching the GenBank database using the BLAST search tool available at the National Center for Biotechnology Information Website (http://www.ncbi.nlm.nih.gov/BLAST).

Phylogroup Determination and Plasmid Replicon Typing

All isolates producing ESBL were subjected to phylogenetic grouping to assign into one of the four phylogenetic groups (A, B1, B2, and D) using a multiplex PCR method [23]. This was performed by targeting two genetic determinants (*chuA* and *yjaA*) and an anonymous DNA fragment (TSPE4.C2). The finding of phylogenetic typing was applied to determine the pattern of antimicrobial resistance and the β-lactamase gene distributions among the ESBL-producing *E. coli* isolates investigated in this work. A multiplex PCR was performed on all isolates to type the

plasmid replicons using the previously described method [24].

Pulse-Field Gel Electrophoresis (PFGE)

PFGE analysis was performed on 14 ESBL-producing isolates, by digesting the genomic DNA using *XbaI* enzyme according to a standard protocol of the Center for Disease Control and Prevention (CDC), using a CHEF MAPPER apparatus (Bio-Rad Laboratories, USA), as stated previously [25]. Analysis of gel images were performed using GelCompar II software (Applied Maths, Belgium). Unweighted pair group method with arithmetic means analysis was employed to construct clustering based on the Dice similarity index.

Conjugation Assay

Conjugation assays were performed to determine the transferability of β-lactamase resistance genes on ESBL-producing isolates by using the broth mating method. As a recipient strain, *E. coli* J53 Az^R was used, and ESBL-producing isolates were used as the donors as previously described [26]. The PCR method used above was conducted to determine whether the β-lactamase resistance gene harbored by the donors could be transferred to the transconjugants. In addition, for all transconjugants, the antimicrobial susceptibility test, MIC test, and plasmid replicon typing were performed to assess the characteristics of transconjugants as conducted to the donors.

Results

Antimicrobial Resistance Profiling of ESBL-Producing Isolates

Of the 281 *E. coli* isolates, 20 of them showed resistance and/or intermediate resistance to cefotaxime and/or ceftazidime, as assessed by the disc diffusion method. Among these 20 isolates, 14 were confirmed to produce ESBLs using the double-disc synergy test. The ESBL-producing isolates investigated in this work have shown a concurrent resistance to nalidixic acid (n = 12), tetracycline (n = 11), ciprofloxacin (n = 9), gentamicin (n = 8), and trimethoprim/sulfamethoxazole (n = 7) (Table 1). As a result, 10 multidrug-resistant isolates displaying resistance to at least three classes of antimicrobial agents were observed.

The prevalence of antimicrobial resistance to β-lactam agents was as follows: ampicillin (n = 14); cephalothin (n = 14); cefaclor (n = 14); cefotaxime (n = 14); ceftriaxone (n = 14); cefixime (n = 13); ceftazidime (n = 11); cefpirome (n = 7); cefepime (n = 4); and amoxicillin/clavulante (n = 4). Resistance to ertapenem and imipenem was not observed by the investigated isolates (Table 1).

The MIC distribution of ceftazidime, cefotaxime, ceftriaxone, aztreonam, and cefoxitin for ESBL-producing isolates are shown in Table 1. There were various MIC values of all agents, ranging from 0.25 to 2,048 μg/ml. The MIC values of all agents for the isolates that showed a resistance by the disc diffusion method were higher than the breakpoint of each agent (ceftriaxone ≥ 4 μg/ml, cefotaxime ≥ 4 μg/ml, cefoxitin ≥ 32 μg/ml, ceftazidime ≥ 16 μg/ml, and aztreonam ≥ 16 μg/ml). Compared with the other three agents, a much higher average MIC value was observed for cefotaxime and ceftriaxone. Resistance to cefoxitin (MIC ≥ 32 μg/ml) was detected in four isolates, and these isolates had plasmid-mediated AmpC β-lactamase-encoding genes (CMY-6 or CMY-2) in common (Table 1).

Prevalence of β-Lactamase-Encoding Genes

Overall, 11 ESBL-producing *E. coli* isolates that harbored the bla_{CTX-M}-type genes were included in the CTX-M- or CTX-M-1 group. Following sequence analysis of isolates positive for the bla_{CTX-M} gene, we determined eight isolates carried $bla_{CTX-M-14}$, two isolates harbored $bla_{CTX-M-14}$, and one isolate had $bla_{CTX-M-65}$ (Table 1). Among these 11 bla_{CTX-M}-positive isolates, six isolates co-carried bla_{TEM-1}, and one isolate co-harbored bla_{CMY-2} and bla_{DHA-1}. All of the investigated *E. coli* strains were negative for the bla_{SHV} gene. Of the three isolates that were negative for bla_{CTX-M}, two isolates were shown to produce CMY-2, and one isolate to produce CMY-6 (Table 1).

Profile of Phylogenetic Grouping and Plasmid Replicon Typing

Among the 14 *E. coli* isolates producing ESBL, 10 were classified into groups A (n = 7) and B1 (n = 3), and four isolates into groups B2 (n = 2) and D (n = 2) (Table 1). Plasmid replicon typing revealed that four types of plasmid were detected in 14 ESBL-producing isolates. IncFrep was the predominantly detected replicon type in 13 ESBL-producing isolates. This was followed by IncFIB (n = 12), IncI1 (n = 6), and IncN (n = 2) (Table 1).

PFGE Analysis

All 11 CTX-M-producing strains and three CMY-producing strains showed PFGE profiles with a low similarity (<70% similarity), which suggests that these isolates are not likely to be originated from a single clone of *E. coli*. Nevertheless, strains EC085 and EC092 isolated from pigs in the same year and harboring both bla_{TEM-1} and $bla_{CTX-M-15}$ genes showed a high genetic homogeneity with 89.7% of similarity (Fig. 1).

Conjugation Assay

Horizontal transfer of the phenotypes and genotypes of the ESBL-producing isolates to the recipient strains (*E. coli* J53 Az^R) via the conjugation assay was found in nine

Table 1. Profile of antimicrobial resistance gene of ESBL-producing *Escherichia coli* isolated from pigs and chickens.

Strain	Origin	Phylo-genetic group	β-Lactamase genes		Plasmid replicon	Transfer	Antimicrobial resistance		MIC values (μg/ml)				
			CTX-M type	Other β-lactamase			β-Lactams	Others	CAZ	CTX	CRO	ATM	FOX
EC010	Pig	A	CTX-M-14	TEM-1	Frep, FIB, N	Positive	AMP, KF, CEC, CTX, CRO, CFM	NA, TE, SXT, GN	4	128	512	8	8
EC035	Pig	A	CTX-M-15	-	Frep, FIB	Positive	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO, FEP	CIP, NA, TE, SXT, GN	64	1,024	1,024	256	16
EC050	Pig	D	CTX-M-15	TEM-1	Frep, FIB	Positive	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO, FEP	GN	256	2,048	2,048	256	8
EC051	Pig	A	CTX-M-15	CMY-2	Frep, FIB	Positive	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM, CPO, FEP	CIP, NA, GN	512	512	1,024	256	512
EC065	Pig	B2	CTX-M-15	DHA-1	Frep	Negative	AMP, KF, CEC, CAZ, CTX, CRO, CFM	CIP, NA, TE, SXT, GN	32	128	256	32	16
EC085	Pig	A	CTX-M-15	TEM-1	Frep, I1	Positive	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO	CIP, NA, TE	128	1,024	1,024	128	16
EC092	Pig	A	CTX-M-15	TEM-1	Frep, FIB, I1	Negative	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO	CIP, NA, TE, SXT, GN	128	512	1,024	128	16
EC096	Pig	A	CTX-M-15	TEM-1	Frep, FIB, I1, N	Positive	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO	CIP, NA, TE, SXT, GN	32	2,048	2,048	64	8
EC105	Pig	B1	CTX-M-65	TEM-1	Frep, FIB, I1	Negative	AMP, KF, CEC, CTX, CRO, CFM	CIP, NA, TE, SXT, GN	2	512	512	16	8
EC240	Chicken	D	CTX-M-15	-	Frep, FIB	Positive	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO, FEP	-	32	1,024	1,024	64	8
EC243	Chicken	B1	-	CMY-2	Frep, FIB	Negative	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM	NA, TE	256	64	128	32	512
EC260	Chicken	A	-	CMY-6	FIB, I1	Positive	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM	CIP, NA, TE	512	128	256	128	1,024
EC262	Chicken	B2	-	CMY-2	Frep, FIB, I1	Positive	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM	NA, TE	1,024	128	256	128	512
EC264	Chicken	B1	CTX-M-14	-	Frep, FIB	Negative	AMP, KF, CEC, CTX, CRO	CIP, NA, TE, SXT, GN	2	256	256	4	4

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; KF, cephalothin; CEC, cefaclor; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; CFM, cefixime; CPO, cefpirome; FEP, cefepime; CIP, ciprofloxacin; NA, nalidixic acid; TE, tetracycline; SXT, trimethoprim/sulfamethoxazole; GN, gentamicin.

transconjugants. The characteristics of the transconjugants are shown in Table 2. For the nine trasnsconjugants, PCR analysis for β-lactamase genes identified the transfer of $bla_{\text{TEM-1}}$, $n = 4$; $bla_{\text{CTX-M-15}}$, $n = 4$; $bla_{\text{CMY-6}}$, $n = 1$; $bla_{\text{CMY-2}}$, $n = 1$; and $bla_{\text{CTX-M-14}}$, $n = 1$ genes to the recipient strain, as detected in their donors. The antimicrobial susceptibility test on β-lactams of the nine transconjugants determined the transfer of β-lactam resistance. All transconjugants were resistant to cephalothin and ampicillin, except one transconjugant (EC096-Tc) that was susceptible to cephalothin. The two transconjugants producing only TEM-1-type β-lactamase were resistant to cephalothin and/or ampicillin. The other seven transconjugants producing CTX-M-type or CMY-type β-lactamases showed resistance to cefaclor and ceftriaxone. EC085-Tc and EC240-Tc that carried the $bla_{\text{CTX-M-15}}$ gene were resistant to cefpirome, which is included in the fourth-generation cephalosporins. In addition to the transfer of β-lactam resistance, resistance to non-β-lactams was also determined in four isolates. The resistance to gentamicin was commonly identified in four transconjugants. The MIC test for transconjugants revealed that the isolates producing only TEM-1-type β-lactamase were susceptible to five antimicrobial agents. Three transconjugants harboring $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-6}}$, and $bla_{\text{CMY-2}}$ genes showed resistance to cefoxitin with high MIC values, ranging from 512 to 1,024 μg/ml. The plasmid replicon typing results for the nine transconjugants revealed four different replicon types as their donors. Among these, three of nine transconjugants

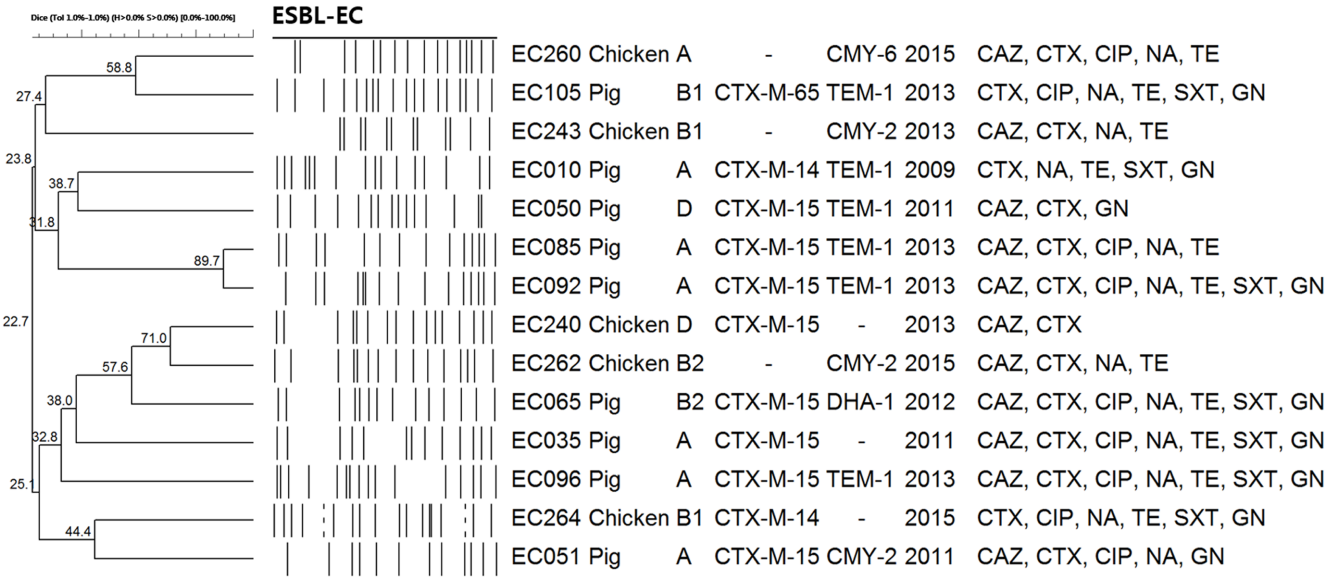

Fig. 1. Dendrograms generated showing the cluster analysis of *XbaI*-digested PFGE patterns of ESBL-producing *E. coli* strains isolated from pigs and chickens.

Similarity analysis was performed by using the Dice coefficient, and clustering was done by the unweighted-pair group method using average linkages. For 14 ESBL-producing *E. coli* strains, details given include the strain, origin, phylogenetic groups of each strain, β-lactamase-encoding genes, the sampling year of each strain, and antimicrobial resistance profiles.

Table 2. Characteristics of transconjugants of ESBL-producing *Escherichia coli* isolates.

Strain	Donor strains	Transferred β-lactamase genes		Plasmid replicon	Antimicrobial resistance		MIC values (μg/ml)				
		CTX-M type	Other β-lactamase		β-Lactams	Others	CAZ	CTX	CRO	ATM	FOX
EC010-Tc	PEC510	CTX-M-14	TEM-1	Frep, FIB, N	AMP, KF, CEC, CTX, CRO, CFM	-	4	64	64	8	16
EC035-Tc	PEC574	CTX-M-15	TEM-1	Frep, FIB	AMP, KF, CEC, CAZ, CTX, CRO, CFM	GN	64	512	512	128	8
EC050-Tc	PEC590	-	TEM-1	Frep	AMP, KF	GN	2	0.25	1	1	4
EC051-Tc	PEC591	CTX-M-15	-	Frep, FIB	AMP, KF, CEC, CAZ, CTX, CRO, CFM	GN	64	512	512	128	16
EC085-Tc	PEC705	CTX-M-15	-	I1	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO	-	128	1,024	1,024	256	16
EC096-Tc	PEC716	-	TEM-1	I1	AMP	SXT, GN	2	0.25	1	1	4
EC240-Tc	AEC34	CTX-M-15	-	Frep	AMP, KF, CEC, CAZ, CTX, CRO, CFM, CPO	-	128	1,024	512	8	1,024
EC260-Tc	AEC54	-	CMY-6	I1	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM	-	512	32	128	32	512
EC262-Tc	AEC56	-	CMY-2	I1	AMP, AMC, KF, CEC, CAZ, CTX, CRO, CFM	-	256	128	128	64	1,024

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; KF, cephalothin; CEC, cefaclor; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; CFM, cefixime; CPO, cefpirome; SXT, trimethoprim/sulfamethoxazole; GN, gentamicin; ATM, aztreonam; FOX, cefoxitin.

displayed more than one type of replicon. IncFrep was the most frequently detected replicon type, which was observed in five transconjugants, followed by IncI1 (n = 4), IncFIB (n = 3), and IncN (n = 1).

Discussion

From 206 isolates that originated in necropsied pigs, nine isolates (4.36%) were ESBL-producing *E. coli* strains. Five isolates (6.67%) from 75 isolates of avian pathogenic *E. coli* produced ESBLs. A total of 14 isolates (4.98%) were identified as ESBL-producing *E. coli* from 281 *E. coli* isolates. This prevalence is similar to previous studies that reported *E. coli* isolates from pigs in Denmark [27] and from chickens in Japan [28]. On the contrary, *E. coli* isolates recovered from pigs and chickens had shown a high prevalence of ESBL production as reported in Hong Kong [29], The Netherlands [30], and South Korea [12]. With respect to the β-lactamase type produced, out of 281 *E. coli* isolates tested, 11 isolates (3.91%) produced CTX-M-type β-lactamase and three isolates (1.07%) produced CMY-type β-lactamase. One isolate (EC051) produced a combined type of β-lactamases (CTX-M-15 and CMY-2). Although a similar frequency of CTX-M-type β-lactamase-producing *E. coli* isolates was detected in *E. coli* strains recovered from pigs and cattle in South Korea [16], the prevalence of CTX-M-type lactamase described in previous reports is much higher than that of our study [29, 30].

In this study we investigated ESBL-producing *E. coli* isolates harboring various types of β-lactamase genes. Three different types of $bla_{\text{CTX-M}}$, namely, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-14}}$ and two different CMY-type ($bla_{\text{CMY-2}}$ and $bla_{\text{CMY-6}}$) β-lactamase genes were observed. These resistance variants have been reported previously from *E. coli* isolates originated from food-producing animals in Asia [28, 29, 31]. The $bla_{\text{CTX-M-15}}$ gene, which is the most dominant CTX-M-type β-lactamase detected globally [32], was also identified most frequently in this study. Similarly, the $bla_{\text{CMY-2}}$ detected in this study has previously been detected in *E. coli* strains recovered from food-producing animals in Europe, the United States, and South Korea [19, 33, 34]. In South Korea, many studies reported the prevalence of $bla_{\text{CTX-M-14}}$ genes in *E. coli* strains isolated from pigs, cattle, and chickens [14–16] and *Salmonella* spp. from chickens [13]. Furthermore, in this study, we identified the $bla_{\text{CMY-6}}$ gene in one isolate from chickens. This variant has previously been described for *E. coli* isolates from only clinical cases in South Korea [35]. Thus, to the best of our knowledge, this is the first description of an *E. coli* strain harboring $bla_{\text{CMY-6}}$ from chickens in South Korea. These findings suggest that the emergence of $bla_{\text{CMY-6}}$ genes in *E. coli* isolates recovered from chickens may constitute an immense risk to public health, given the increased consumption of chicken in South Korea.

In this study, the MIC test for five β-lactam agents showed that the average MIC of each agent for isolates that produced CTX-M-15 was higher than that produced CTX-M-65 and CTX-M-14, especially for cefotaxime and ceftriaxone (data not shown). Although the number of samples tested was too low to have statistical significance, the data are consistent with a study conducted previously, which depicted that CTX-M group 1 showed higher MIC values than CTX-M group 9 for third- and fourth-generation cephalosporins [36]. Meanwhile, our results revealed that the isolates producing CMY-2 or CMY-6 β-lactamase had higher MICs for cefoxitin (ranging 512 to 1,024 μg/ml) than those without CMY-type β-lactamase (ranging 4 to 16 μg/ml), which is in agreement with the previous studies that showed the potential of CMY-type β-lactamases to resist against cefoxitin [34, 37]. Therefore, we suggest that the isolates that produced both CTX-M-15 and CMY-2 could have high MIC values for cefotaxime, ceftriaxone, and cefoxitin. The susceptibility test for β-lactam resistance determined that all ESBL-producing *E. coli* strains demonstrated extensive resistance, with a wider scope affecting the fourth-generation cephalosporins. This may have resulted from an increasing trend in the amount of usage of cephems, such as ceftiofur, annually from 2006 [38]. Usually, CMY-type β-lactamase presence imparts isolates to resist first-, second-, and third-generation cephalosporins, with the exception of fourth-generation cephalosporins [33]. Interestingly, in this study, all isolates producing CMY-2 and/or CMY-6 showed resistance to fourth-generation cephalosporins.

In this study, nine isolates transferred the β-lactamase-encoding genes by the conjugation assay to the recipient strain. No $bla_{\text{DHA-1}}$ and $bla_{\text{CTX-M-65}}$ genes were transferred to the recipient strains, which implies that these genetic determinants may not be situated in the plasmid that transferred the $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CMY-2}}$, $bla_{\text{CMY-2}}$, and $bla_{\text{TEM-1}}$ genes. Moreover, our findings suggest that the horizontal dissemination of bla_{CMY} and $bla_{\text{CTX-M}}$ genes in the *E. coli* strains tested in this study is on account of IncFrep and IncI1 plasmids, respectively. Some β-lactamase-encoding genes are situated within the mobile genetic elements consorted with other resistance genes, which paves the way for strains to resist the antimicrobials that could be broadly used in animals and humans (*e.g.*, fluoroquinolones and aminoglycosides), which could also play a tremendous role in the co-selection of these resistance genes [39]. Similarly, in this study, four isolates co-transferred gentamicin resistance with β-lactams to the recipient strain. Interestingly, the two isolates only transferred the $bla_{\text{TEM-1}}$ gene that had lost their ESBL-producing phenotype, and showed an

increased susceptibility to β-lactams in the disc diffusion test and MIC test.

In this study, three of the *E. coli* strains carrying bla_{CTX-M} genes and one isolate carrying bla_{CMY} genes belonged to phylogenetic groups B2 and D, known for their virulence. These results may raise interest concerning the transfer of CTX-M- or CMY-producing *E. coli* isolates that belong to the virulent phylogenetic groups from animals to humans, since humans and animals share the same environment and remain in close contact with each other [40]. Moreover, molecular typing by PFGE depicted that *E. coli* strains producing CTX-M and CMY were clonally highly diverse, which implies that the dissemination of the bla_{CMY} and bla_{CTX-M} genes in *E. coli* isolates among pigs and chickens may result mainly from horizontal transmission, instead of clonal expansion from a single clone of *E. coli*. In this context, there was no significant correlation between the phylogenetic groups and the groupings of isolates in each *Xba*I-digested PFGE cluster.

In conclusion, we have conducted a comprehensive study of recent dissemination of ESBL-producing *E. coli* strains among pigs and chickens in South Korea. Despite that the carriage of extended-spectrum cephalosporin resistance in animals is negligible, the spread of ESBL genes in these strains could arise among animal species, as well as humans, which can lead to therapeutic failures in both human and veterinary medicines. To our best knowledge, we have reported for the first time bla_{CMY-6} and $bla_{CTX-M-14}$ genes in *E. coli* isolates from chickens in South Korea. More studies are needed with a better long-term surveillance to trace the evolution and dissemination of CTX-M- and CMY-type β-lactamases between different food-producing animals.

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Conflict of Interest

The Authors have no conflicts of interest to declare.

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