MICROBIOLOGY AND FOOD SAFETY

The occurrence of CTX-M–producing *E. coli* in the broiler parent stock in Korea

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ABSTRACT A large number of antimicrobials are used for the treatment of bacterial infections, and the emergence of antimicrobial-resistant *Escherichia coli* (***E. coli***) in livestock and the transfer of resistant isolates to humans poses a serious potential risk to public health. In particular, broiler parent stock produce thousands of eggs for commercial broiler chickens and can transfer antimicrobial-resistant bacteria and drug-resistance genes to chicks. This study was conducted to investigate the prevalence and characteristics of third-generation cephalosporin-resistant and extended-spectrum β-lactamases (**ESBL**)-producing *E. coli* isolated from the broiler parent stock in Korea. Among 51 cefotaxime-resistant *E. coli* isolates, 45 (88.2%) isolates were identified as multidrug resistant and 21 isolates showed phenotypic and genotypic characteristics of CTX-M–producing *E. coli*. The CTX-M genes CTX-M-14, CTX-M-15, CTX-M-1, and CTX-M-1 were detected in 10, 7, 3, and 1 isolates, respectively. IS*Ecp1* or IS*26* + IS*Ecp1* were identified upstream of all CTX-M–type genes, and orf*477* and IS*903* were detected downstream of 9 and 10 CTX-M–type genes, respectively. Thirteen (61.9%) of the 21 CTX-M–producing *E. coli* isolates harbored class 1 integrons with 4 different gene cassette arrangements. Among the plasmid replicons, CTX-M-1 was located on I1, F, and FIB; CTX-M-14 on F and FII; CTX-M-15 on FII, FIA, and FIB; and CTX-M-65 on FIB. This is the first study to investigate the presence and distribution of third-generation cephalosporin-resistant and CTX-M–producing *E. coli* isolated from the broiler parent stock level in Korea, and the results indicate that comprehensive surveillance and persistent monitoring systems in broiler parent stock farms are necessary to prevent the dissemination of resistant isolates.

Key words: *Escherichia coli*, antimicrobial resistance, broiler parent stock, third-generation cephalosporin, CTX-M

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INTRODUCTION

Escherichia coli (***E. coli***) is easily recognized as noninvasive and harmless commensal bacteria (Barguigua et al., 2013). But antimicrobial resistance in *E. coli* plays a vital role in global dissemination because it is the most common pathogen in humans and livestock (Szmolka and Nagy, 2013). A large number of antimicrobials are used for treatment of bacterial infections, and of these, β-lactams are one of the most commonly prescribed drug classes with numerous clinical indications in both humans and livestock (Li et al., 2007; Bush and Bradford, 2016). In particular, the emergence of β-lactam–resistant *E. coli* in livestock and the transfer of resistant isolates to humans pose a serious potential risk to public health (Szmolka and Nagy, 2013).

One of the most important resistance mechanisms in Enterobacteriaceae including *E. coli* is by an enzyme called extended-spectrum β-lactamases (**ESBL**) that inactivate β-lactam antimicrobials including third-generation cephalosporins by hydrolyzing their β-lactam ring (Frère et al., 1992). Extended-spectrum β-lactamases are classified into different families, such as TEM-type, SHV-type, OXA-type, and CTX-M–type, according to their primary sequences and substrate profiles (Bush and Jacoby, 2010). Among them, the CTX-M type is currently the most prevalent extended-spectrum enzymes worldwide (Naseer and Sundsfjord, 2011). CTX-M β-lactamases can also be divided into 5 groups according to their amino-acid sequence identities (CTX-M-1, M-2, M-8, M-9, and M-25), and different

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CTX-M genotypes have different hydrolysis reactions to β-lactams (Bonnet, 2004; Pitout et al., 2004; Shi et al., 2015). But CTX-M β-lactamases are resistant to most β-lactams, including penicillins, narrow-spectrum cephalosporins, and the oxyimino-cephalosporins cefotaxime and ceftriaxone (Nathisuwan et al., 2001; Pitout and Laupland, 2008; Singleton, 2019).

In the poultry industry, the broiler operation system has a pyramidal structure in which the grandparent stock is at on the top, followed by parent stock (**PS**) that produces eggs for the production of commercial broiler chickens on the bottom of the pyramid. Because one PS produces thousands of eggs for commercial broiler chickens, antimicrobial-resistant bacteria and drug-resistance genes from the PS can be vertically transmitted to commercial broiler through hatcheries and chicks. Although many antimicrobial resistance studies have been reported at commercial-broiler level (Hussain et al., 2017; Mehdi et al., 2018), little is known about the prevalence and characteristics of third-generation cephalosporin-resistant and ESBL-producing *E. coli* at the PS level. Therefore, this study was conducted to investigate the prevalence and characteristics of third-generation cephalosporin-resistant and ESBL-producing *E. coli* isolated from the broiler PS in Korea.

MATERIALS AND METHODS

Sampling

Feces and dust were sampled from 9 broiler PS farms including 74 flocks (20 wk of age) between 2016 and 2018 in accordance with the standards set by the National Poultry Improvement Plan (USDA, 2012). Briefly, 15 different spots were swabbed per flock to collect 10 g of dust sample using a surgical gauze moistened with 12 mL of sterile double-strength skim milk (Fluka, Neu-Ulm, Germany). Approximately 10 g of feces was also sampled from 15 different locations. Samples were transported to the laboratory in a cooler and stored at 4°C until use.

Bacterial Identification

The samples were individually inoculated into 225 mL of modified *E. coli* broth with Novobiocin (Merck, Darmstadt, Germany) and incubated at 37°C for 20 to 24 h. Pre-enriched modified *E. coli* broth with Novobiocin was streaked onto MacConkey agar (BD Biosciences, Sparks, MD) plates and incubated at 37°C for 24 h. Five typical colonies selected from each sample were identified by PCR as previously described (Candrian et al., 1991), and plated on Mueller-Hinton agar (BD Biosciences) plates supplemented with 2-μg/mL cefotaxime to select third-generation cephalosporin-resistant *E. coli* (Shim et al., 2019). If the isolates from the same origin showed the same antimicrobial susceptibility patterns, only one isolate was randomly chosen and included in the analysis. As a result, a total of 51 cefotaxime-resistant *E. coli* were tested in this study (Table 1).

Antimicrobial Susceptibility Testing

All cefotaxime-resistant *E. coli* isolates were investigated for their antimicrobial resistance with the disc-diffusion test using the following discs (BD Biosciences): amoxicillin-clavulanate (20/10 μg), ampicillin (10 μg), cefepime (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg). Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (**CLSI**) (CLSI, 2015). The minimum inhibitory concentrations of third-generation cephalosporins (ceftazidime, cefotaxime, ceftiofur, and cefovecin) at concentrations ranging from 0.06 to 512 μg/mL were determined by standard agar dilution methods with Mueller-Hinton agar (BD Biosciences) according to the recommendations of the CLSI (CLSI, 2015). A double-disc diffusion method was performed with cefotaxime (30 μg)/cefotaxime-clavulanate (30 μg/10 μg; BD) and ceftazidime (30 μg)/ceftazidime-clavulanate (30 μg/10 μg; BD) to detect ESBL production according to the CLSI

Table 1. Distribution of 51 cefotaxime-resistant *E. coli* isolated from 9 broiler parent stock farms in this study.

Farm	No. of positive flocks/no. Of flocks tested (%)	No. cefotaxime-resistant *E. coli*[1]
A	5/6 (83.3)	7
B	3/9 (33.3)	6
C	4/11 (36.4)	6
D	4/19 (21.1)	10
E	5/8 (62.5)	8
F	2/8 (25.0)	4
G	2/5 (40.0)	5
H	2/3 (66.7)	3
I	1/5 (20.0)	2
Total	28/74 (37.8)	51

Abbreviation: *E. coli*, *Escherichia coli*.

[1]If several isolates from the same origin showed the same antimicrobial susceptibility patterns, only one isolate was included.

guidelines (CLSI, 2015). *Escherichia coli* ATCC 25922 was used as a control in the antimicrobial susceptibility tests. Multidrug resistance (MDR) was defined as acquired nonsusceptibility to at least one agent in 3 or more antimicrobial categories (Magiorakos et al., 2012).

Detection and Characterization of β-Lactamase–Encoding Genes

Polymerase chain reaction amplification was performed using primers for the β-lactamase genes $bla_{\text{CTX-M}}$ (Pitout et al., 2004, Ogutu et al., 2015), bla_{TEM} (Briñas et al., 2002), bla_{SHV} (Briñas et al., 2002), and bla_{OXA} (Briñas et al., 2002). The PCR products were purified using GFX PCR DNA and the Gel Band Purification Kit (Amersham Bioscience, Freiburg, Germany) and sequenced using an automatic sequencer (Cosmogenetech, Seoul, Korea). The sequences were confirmed with those in the GenBank database using the Nucleotide Basic Local Alignment Search Tool (nBLAST) available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST). The presence of IS*Ecp1*, IS*26*, orf*477*, and IS*903* sequences surrounding the CTX-M–type genes was analyzed by PCR using primers and conditions as previously described (Eckert et al., 2006; Sun et al., 2010).

Plasmid Replicon Typing and Detection of Integrons and Gene Cassettes

For plasmid replicon typing and detection of integrons and gene cassettes, PCR was performed using DNA extracted from CTX-M–producing *E. coli* isolates. The primers used in this study targeted 18 different replicons (Johnson et al., 2007) and class 1 and 2 integrons (Ng et al., 1999; Sáenz et al., 2004). Gene cassettes were tested for integron-positive isolates (Ng et al., 1999; Sáenz et al., 2004). The PCR products of the gene cassettes were sequenced as described above.

Transfer of Resistance Genes by Conjugation

To determine the transferability of β-lactamase resistance genes, conjugation assays were performed using the broth mating method, with *E. coli* J53 used as the recipient as previously described (Tamang et al., 2012). Transconjugants were selected on MacConkey agar (BD Biosciences) plates containing sodium azide (100 μg/mL; Sigma, ST Louis, MO) and cefotaxime (2 μg/mL). All transconjugants were tested for the presence of β-lactamase genes, as described above.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (**PFGE**) was performed on CTX-M–producing *E. coli* isolates by digesting the genomic DNA using the *Xba*I (Takara Bio Inc., Shiga, Japan) enzyme according to the standard protocol of the Center for Disease Control and Prevention and CHEF MAPPER apparatus (Bio-Rad Laboratories, Hercules, CA), as previously described (Liu et al., 2007). Gel images were analyzed using InfoQuest FP software, version 4.5 (Bio-Rad). The dice coefficient was used to calculate similarity, and the similarity matrix was expressed graphically by an unweighted average linkage.

RESULTS

Antimicrobial Resistance Profile

The antimicrobial resistance patterns of cefotaxime-resistant *E. coli* isolated from broiler PS are shown in Figure 1. All cefotaxime-resistant *E. coli* isolates showed the highest resistance to penicillins (100.0%), followed by quinolones (90.2%), tetracyclines (78.4%), fluoroquinolones (66.7%), folate pathway inhibitors (60.8%), β-lactam/β-lactamase inhibitor combinations (56.9%), phenicols (49.0%), aminoglycosides (13.7%), and carbapenems (3.9%). Forty-five (88.2%) cefotaxime-resistant *E. coli* isolates were identified as having MDR, and the rate of resistance against 8 antimicrobial classes was the highest at 23.5%.

Characteristics of CTX-M–Producing E. coli

The phenotypic and genotypic characteristics of the 21 CTX-M–producing *E. coli* isolates among the 51 cefotaxime-resistant isolates are shown in Table 2. Regarding the distribution of CTX-M type, CTX-M-14 was the highest, being identified in 10 (47.6%) isolates, followed by CTX-M-15 (7 isolates, 33.3%), CTX-M-1 (3 isolates, 14.3%), and CTX-M-65 (1 isolate, 4.8%), and 14 (57.1%) isolates carried both TEM-1 and CTX-M-type genes. Thirteen (61.9%) transconjugants also showed a transferability of β-lactamase genes. In analysis of the surrounding regions of the CTX-M–type genes, IS*Ecp1* or IS*26* + IS*Ecp1* were identified upstream of all CTX-M–type genes, and orf*477* and IS*903* were detected downstream of 9 and 10 CTX-M–type genes, respectively. Thirteen (61.9%) of the 21 CTX-M–producing *E. coli* isolates harbored class 1 integrons, and the following 4 different gene cassette arrangements were identified in 10 isolates: *dfrA1* (n = 4), *aadA1* (n = 2), *dfrA17* (n = 2), and *aadA1*+*dfrA1* (n = 2). In plasmid replicon typing, CTX-M-1 was located on I1, F, and FIB; CTX-M-14 on F and FII; CTX-M-15 on FII, FIA, and FIB; and CTX-M-65 on FIB. All CTX-M–producing *E. coli* isolates had high minimum inhibitory concentrations for most third-generation cephalosporins.

PFGE Analysis

In determination of the epidemiological genetic relationships by PFGE (Figure 2), 17 PFGE patterns showing 85% similarity were observed in 21 CTX-M–producing *E. coli* isolates. In particular, isolates that

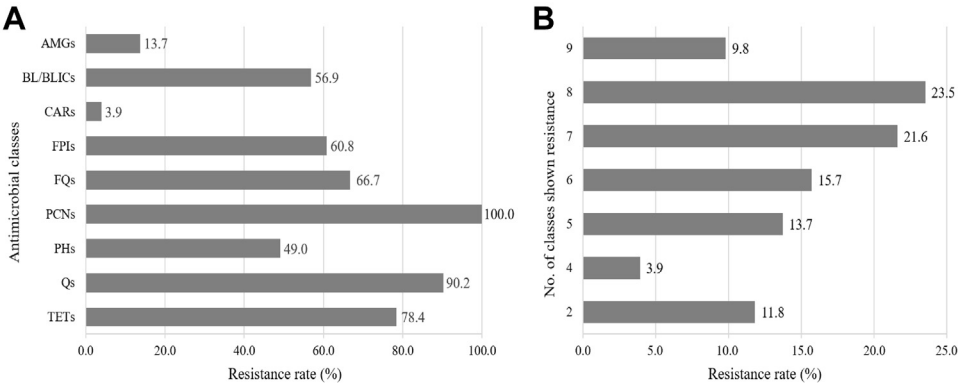

Figure 1. Antimicrobial resistance classes (A) and spectrum (B) against 51 cefotaxime-resistant *E. coli* isolated from broiler parent stock in Korea. Abbreviations: *E. coli, Escherichia coli*; AMGs, aminoglycosides; BL/BLICs, β-lactam/β-lactamase inhibitor combinations; CARs, carbapenems; FPIs, folate pathway inhibitors; FQs, fluoroquinolones; PCNs, penicillins; PHs, phenicols; Qs, quinolones; TETs, tetracyclines.

included 3 PFGE patterns (PE013, PE015, and PE016) were originated from the same broiler PS farm with the same antimicrobial resistance genes and plasmid replicon types and showed similar antimicrobial resistance patterns.

DISCUSSION

Because of the increased usage of antimicrobials in livestock, including third-generation cephalosporins, the emergence and dissemination of ESBL-producing Enterobacteriaceae has been reported in the world (Pitout et al., 2004; Ewers et al., 2012; Ogutu et al., 2015). In particular, ESBL-producing *E. coli* isolated from the poultry production pyramid are considered a significant risk to both poultry production and human health because of the possibility of the transmission of resistance genes (Nilsson et al., 2014). In this study, 51 cefotaxime-resistant *E. coli* isolates from 9 broiler PS farms showed high coresistance to penicillins (100.0%), quinolones (90.2%), tetracyclines (78.4%), fluoroquinolones (66.7%), and folate pathway inhibitors (60.8%), and 45 (88.2%) isolates expressed a typical MDR phenotype with antimicrobial resistance to 4-9 antimicrobial classes, including cephems. These results indicate that third-generation cephalosporin-resistant *E. coli* show an alarming trend in coresistance to other classes of antimicrobials and a high MDR rates (Bartoloni et al., 2013).

Recently, ESBL-producing *E. coli*, especially CTX-M–producing *E. coli*, have been increasingly reported worldwide (Liu et al., 2007; Szmolka and Nagy, 2013; Belmahdi et al., 2016). In this study, 4 types of CTX-M gene were detected: CTX-M-1, CTX-M-14, CTX-M-15, and CTX-M-65 where CTX-M-14 was the most common. CTX-M-14 and CTX-M-15 have been reported as the dominant CTX-M type in livestock, including in healthy animals and retail meats in the United Kingdom (Watson et al., 2012), China (Liu et al., 2007), Japan (Hiroi et al., 2012), and Spain (Blanc et al., 2006), as well as from commercial broiler farms and chicken meat in Korea (Jo and Woo 2016; Seo et al., 2018). Although little is known about CTX-M types in various pathogens from broiler PS, this study indicates that CTX-M-14 and CTX-M-15 may already be extensively disseminated across the broiler operation system, including the PS level in Korea. CTX-M–producing *E. coli* that carry the CTX-M-1 gene have also been reported as one of the common CTX-M types among the Enterobacteriaceae and have been recovered from poultry in several European countries, including France (Girlich et al., 2007), Germany (Kola et al., 2012), and the Netherlands (Leverstein-van Hall et al., 2011) as well as in other animals across the world, including in the United States (Shaheen et al., 2011). In addition, CTX-M-65, with only a 2-substitution difference from CTX-M-14, has often been isolated from livestock and humans in other countries (Yin et al., 2009; Bush, 2013), in contrast to the less-frequent and presently sporadic isolations in Korea (Park et al., 2019).

In this study, the *TEM-1* gene, which codes for another enzyme that confers β-lactam resistance, was detected in 14 CTX-M–producing *E. coli* isolates. Although *TEM-1* is not an ESBL gene, it can be induced into ESBL by mutations that alter the amino acid sequence around the active site of these β-lactamases (Bajpai et al., 2017). The present study also revealed that CTX-M–producing *E. coli* isolates have high minimum inhibitory concentrations to third-generation cephalosporins and exhibit cross-resistance to other classes of antimicrobial agents, which is consistent with previous reports that CTX-M genes increase resistance to cephalosporins and cause MDR (Woerther et al., 2013; Shim et al., 2019).

To detect any potential genetic platforms able to mobilize *CTX-M* genes, we analyzed the genomic characteristics of the 21 CTX-M–producing *E. coli*. An IS*Ecp1* element upstream of the *bla* gene was detected in all isolates. IS*Ecp1* improves the expression of CTX-M, and its association in *E. coli* isolated from poultry has been extensively reported worldwide (Liao et al., 2015; Maamar et al., 2016). Moreover, another upstream IS*26* was detected in 5 CTX-M-15–producing *E. coli* isolates. Although IS*26* was reportedly associated with

Table 2. Molecular characteristics of the 21 CTX-M–producing *E. coli* isolated from the broiler parent stock in Korea.

CTX-M type	Isolate	Farm	MIC (μg/mL)[1]				Other β-Lactamase	Self-transfer[2]	Insertion sequence		Replicon type	*Int*I1 gene	Cassette array	Pattern of non–β-lactam resistance[1]
			CAZ	CTX	EFT	CVN			Upstream	Downstream				
CTX-M-1	GAS-12-151	F5	1	≥512	≥512	512	TEM-1	+	IS*Ecp1*	Orf*477*	I1, F	−	-	TE, NA
	GAS-12-152	F5	1	≥512	512	≥512	TEM-1	+	IS*Ecp1*	Orf*477*	I1, F, FIB	−	-	TE, NA, CIP, C
	GAS-12-154	F5	2	≥512	512	≥512	TEM-1	+	IS*Ecp1*	Orf*477*	I1, F	−	-	TE, NA
CTX-M-14	SB-24-35	F1	2	≥512	≥512	≥512	TEM-1	+	IS*Ecp1*	IS*903*	F	+	*dfrA1*	TE, SXT, NA, CIP, C
	SB-39-121	F1	4	≥512	≥512	≥512	TEM-1	−	IS*Ecp1*	IS*903*	F	+	*dfrA1*	TE, SXT, NA, CIP
	SB-39-122	F1	4	≥512	≥512	≥512	TEM-1	−	IS*Ecp1*	IS*903*	F	+	*dfrA1*	TE, SXT, NA, C
	HM-21-150	F4	8	≥512	≥512	≥512	-	−	IS*Ecp1*	IS*903*	F	−	-	NA
	CN-24-176	F7	8	≥512	≥512	512	-	−	IS*Ecp1*	IS*903*	F, FII	+	*dfrA17*	TE, SXT, NA, CIP
	CN-24-179	F7	8	≥512	≥512	512	-	+	IS*Ecp1*	IS*903*	F, FII	+	*dfrA17*	TE, SXT, NA, CIP, IPM
	CI-6-200	F8	2	≥512	≥512	≥512	TEM-1	+	IS*Ecp1*	IS*903*	FII	−	-	TE, SXT, C
	CI-6-201	F8	2	≥512	512	≥512	TEM-1	+	IS*Ecp1*	-	-	−	-	TE, G, C
	ME-24-206	F9	2	≥512	≥512	≥512	TEM-1	+	IS*Ecp1*	IS*903*	FII	+	-	TE, SXT, NA, CIP, IPM, C
	ME-24-207	F9	4	≥512	≥512	≥512	TEM-1	−	IS*Ecp1*	IS*903*	FII	−	-	TE, SXT, NA, CIP, C
CT-M-15	CG-27-95	F2	32	≥512	≥512	512	TEM-1	+	IS*26*+IS*Ecp1*	Orf*477*	FII, FIB	+	*aadA1*	TE, NA, CIP, G
	GS-21-155	F6	8	≥512	512	≥512	-	−	IS*Ecp1*	Orf*477*	FII, FIA, FIB	+	*aadA1*+*dfrA1*	TE, SXT, NA, CIP, G, C
	CG-39-174	F2	64	≥512	≥512	≥512	TEM-1	+	IS*26*+IS*Ecp1*	Orf*477*	FII, FIB	+	*aadA1*	TE, NA, CIP, G
	GS-27-180	F6	4	≥512	≥512	≥512	-	+	IS*Ecp1*	Orf*477*	FII, FIA, FIB	+	*aadA1*+*dfrA1*	TE, SXT, NA, CIP, G, C
	JE-24-212	F10	8	≥512	512	512	-	−	IS*26*+IS*Ecp1*	Orf*477*	FII, FIA	+	-	NA, CIP
	JE-24-213	F10	16	≥512	≥512	≥512	-	+	IS*26*+IS*Ecp1*	Orf*477*	FII, FIA	+	-	NA, CIP
	JE-24-214	F10	4	≥512	≥512	≥512	TEM-1	−	IS*26*+IS*Ecp1*	-	FII	+	*dfrA1*	TE, SXT, NA, CIP
CT-M-65	JE-75-131	F3	16	≥512	≥512	≥512	TEM-1	+	IS*Ecp1*	IS*903*	FIB	−	-	TE, SXT, NA, CIP, C

Abbreviation: *E. coli*, *Escherichia coli*.

[1]MIC, minimum inhibitory concentrations; CAZ, ceftazidime; CTX, cefotaxime; EFT, ceftiofur, CVN, cefovecin; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin; C, chloramphenicol; SXT, sulfamethoxazole/trimethoprim; IPM, imipenem; G, gentamicin.

[2]Self-transfer of carrying β-lactamase genes in conjugation experiments.

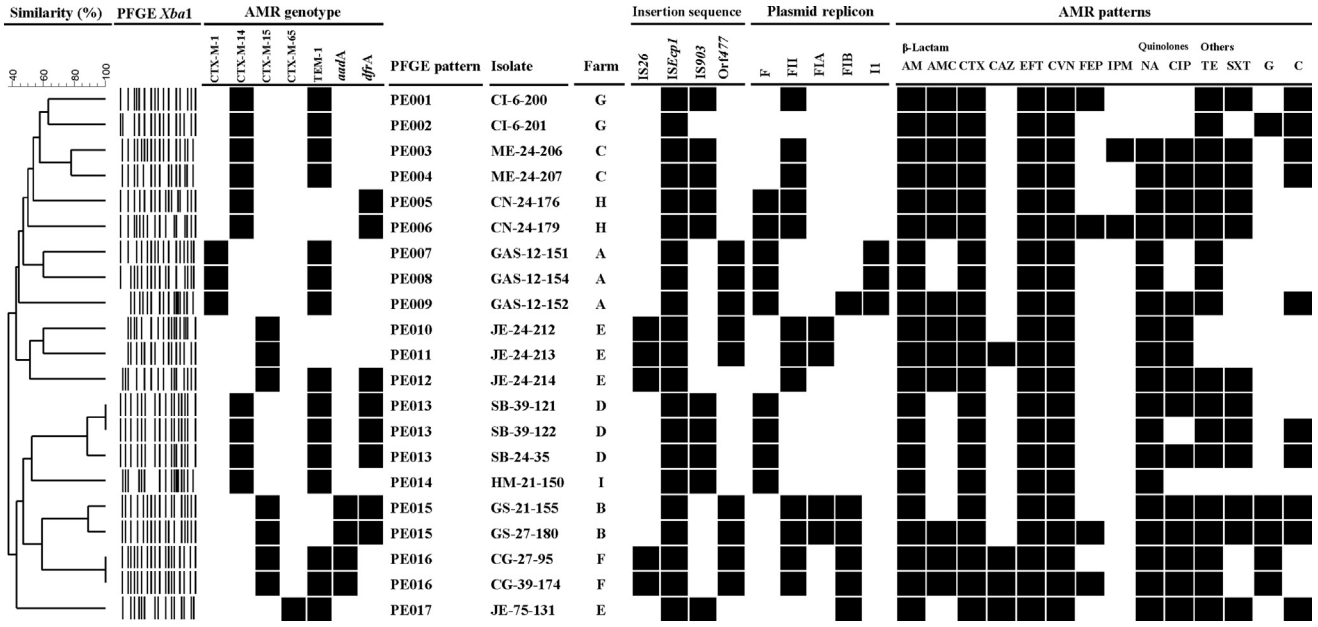

Figure 2. Pulsed-field gel electrophoresis patterns of *Xba*I-digested total DNA of 21 CTX-M–producing *E. coli* isolated from the broiler parent stock in Korea. Abbreviations: *E. coli, Escherichia coli*; AM, ampicillin; AMC, amoxicillin-clavulanate; CTX, cefotaxime; CAZ, ceftazidime; EFT, ceftiofur; CVN, cefovecin; FEP, cefepime; IPM, imipenem; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; SXT, sulfamethoxazole/trimethoprim; G, gentamicin; C, chloramphenicol.

different variants of CTX-M, CTX-M-15 with IS*26* is the most prevalent CTX-M–type gene in this study (Tacão et al., 2012; Jeon et al., 2019). Downstreams of the *bla* genes, Orf*477* and IS*903* were found in 19 CTX-M–producing *E. coli* isolates as described previously for Enterobacteriaceae isolates from livestock (Ramos et al., 2013; Maamar et al., 2016).

In this study, 13 CTX-M–producing *E. coli* isolates contained class 1 integrons. Ten isolates also contained at least one more cassettes. Although *aadA* and *dfrA* were the dominant gene cassette array in this study and have already been reported as the frequent genes in *E. coli* from the poultry industry (Kang et al., 2005), this is the first cassette reported in *E. coli* isolated from the broiler PS in Korea. In addition, 13 transconjugants identified in this study carried the same antimicrobial-resistant genes of the donor strains, demonstrating that β-lactamase–producing *E. coli* isolates may be clonally transmitted to humans through contaminated food products of poultry origin (Shaheen et al., 2011).

Plasmids act as delivery vectors in the spread of antimicrobial resistance through horizontal gene transfer (Thomas and Nielsen, 2005). In particular, the sharing of CTX-M plasmids between humans and livestock has been continuously reported (Shaheen et al., 2011; Maamar et al., 2016). In this study, most isolates (95.2%) among the CTX-M–producing *E. coli* isolates harbored IncF plasmids including F, FII, FIA, and FIB. IncF plasmids are considered to play a prominent role in the dissemination of MDR among Enterobacteriaceae worldwide (Yang et al., 2015, 59). In particular, *E. coli* carrying CTX-M–type genes on the IncF plasmid have previously been found in humans, meat, and livestock (Kim et al., 2011; Maamar et al., 2016; Irrgang et al., 2017). Pulsed-field gel electrophoresis determined epidemiological genetic relationships, revealed that 3 PFGE patterns exhibited the same antimicrobial resistance genes and plasmid replicon types, and originated from the same PS farm, respectively. The previous studies reported that similar PFGE patterns can indicate genetic homogeneity (Tamang et al., 2014; Jo and woo 2016). Thus, these results indicate that *E. coli* carrying the same genetic characteristics are circulating in the same PS farms, and these isolates may contribute to vertical transmission to their broiler farms. This is the first study to investigate the prevalence and characteristics of third-generation cephalosporin-resistant and CTX-M–producing *E. coli* isolated from the broiler PS level in Korea. These results indicate that comprehensive surveillance and persistent monitoring of the system in broiler PS farms are necessary to prevent the dissemination of resistant isolates.

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DISCLOSURES

The authors declare no conflict of interest.

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