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Molecular Characterization of Plasmids Encoding CTX-M β-Lactamases and their Associated Addiction Systems Circulating Among *Escherichia coli* from Retail Chickens, Chicken Farms, and Slaughterhouses in Korea

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Extended-spectrum β-lactamases (ESBLs), particularly those of the CTX-M types, are the predominant resistance determinants of *Escherichia coli* that are rapidly spreading worldwide. To determine CTX-M types, *E. coli* isolates were collected from retail chickens (n = 390) and environmental samples from chicken farms (n = 32) and slaughterhouses (n = 67) in Korea. Fifteen strains harboring $bla_{\text{CTX-M}}$ genes were isolated from 358 *E. coli* isolates. The most common CTX-M type was eight of CTX-M-15, followed by six of CTX-M-1 and one of CTX-M-14. The $bla_{\text{CTX-M}}$ genes were identified in the isolates from retail chickens (n = 9), followed by feces, water pipes, floors, and walls. Conjugations confirmed the transferability of the plasmids carrying $bla_{\text{CTX-M}}$ genes to the recipient *E. coli* J53 strain. Furthermore, eight addiction systems carried by the replicons in CTX-M types were confirmed. The dominant system was identified as *ccdAB*, *vagCD*, and *pndAC* in donor strains and transconjugants. The clonal relationship between the two strains carrying $bla_{\text{CTX-M}}$ genes indicates that *E. coli* may transmit from the farm to retail chickens, suggesting a possible public health risk. Our findings demonstrate that the detection of CTX-M types in *E. coli* isolates is important for tracking ESBL production in animals, and suggest linkage of multiple addiction systems in plasmids bearing $bla_{\text{CTX-M}}$ genes.

Keywords: CTX-M types, *Escherichia coli*, addiction systems, retail chickens, chicken farms, slaughterhouses

Introduction

Extended-spectrum β-lactamases (ESBLs) are among the most important of acquired resistance determinants disseminated in the Enterobacteriaceae [3, 8]. ESBLs confer resistance to oxyimino-cephalosporins, which severely limits treatment options for a variety of illnesses, including urinary tract and gastrointestinal infections [8, 29]. Early ESBLs evolved from TEM- and SHV-type β-lactamases in *Klebsiella pneumoniae*, [20] whereas CTX-M-type ESBLs that are frequently associated with *Escherichia coli* have rapidly increased in the past decade [4, 17, 25]. The CTX-M family can be clustered into five groups (CTX-M-1, -2, -8, -9, and -25) based on their amino acid sequence similarities [29]. Recent studies have identified CTX-M-1 and CTX-M-9 family groups in Enterobacteriaceae from Korea [7, 12, 33]. In particular, the CTX-M family in *E. coli* is widespread worldwide and poses a major health concern in both communities and hospitals [25].

Currently, a genetic survey is being performed nearly worldwide to identify the dissemination of CTX-M β-lactamases [4, 17, 25]. Some studies have shown a relationship between $bla_{\text{CTX-M}}$ genes on conjugative plasmids and bacterial clones. Additional reports have suggested that such plasmids exploit the plasmid addiction system, which tracks toxin-antitoxin gene pairs. The addiction system may contribute to killing plasmid-free cells, allowing for the dissemination of intra- and inter-species plasmids [11,

13]. Researchers have revealed that *E. coli* plasmids bearing bla_{CTX-M} genes may contribute to the development of multiple addiction systems in their host [21]. In support of these findings, studies in European countries have demonstrated a high incidence of bla_{CTX-M} genes in *E. coli* strains among healthy animals [1, 14]. Furthermore, a Korean study has shown that *E. coli* clones with a high frequency of virulence determinants and addiction systems influence the prevalence of CTX-M β-lactamases among *E. coli* from cattle, farm workers, and the farm environment [30].

Although studies have identified the association of addiction systems and bla_{CTX-M} genes in *E. coli* among humans, it is necessary for researchers to perform such studies focused on animals, especially for animals that serve as a food source for human consumption. Therefore, the aim of this study was to characterize CTX-M β-lactamases in *E. coli* isolated from retail chickens, chicken farms, and slaughterhouses in Seoul, Gyeonggi-do and Jeollabuk-do, Korea and to investigate the association between *E. coli* CTX-M β-lactamases and their plasmid addiction systems.

Materials and Methods

Bacterial Strains

A total of 489 samples were obtained from Seoul, Gyeonggi-do and Jeollabuk-do, Korea in January, February, and July 2013, which included retail chickens ($n = 390$) and environmental samples from chicken farms (feces, feed, water pipe, and wall) ($n = 32$) and slaughterhouses (production line, floor, and guts) ($n = 67$). Retail chicken samples from 19 traditional markets and swab samples were collected from two farms in Gyeonggi-do and two farms in Jeollabuk-do. For *E. coli* isolation, chicken samples (25 g) were pre-enriched in 225 ml of buffered peptone water for 18–24 h at 37°C. In order to assay for bacterial growth, the environmental samples were streaked onto Eosin Methylene Blue agar (BBL; Becton Dickinson Micro System, Cockeysville, MD, USA) and incubated at 37°C for 16–18 h. Based on colony morphology and color, the bacterial strains were identified by using the Vitek 2 microbial identification system (bioMérieux Vitek, Hazelwood, MO, USA).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility was determined by using the disc diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [6]. The antimicrobial agents (BBL) and their corresponding concentrations were as follows: ampicillin (AM, 10 μg), piperacillin (PIP, 100 μg), amoxicillin-clavulanic acid (AmC, 20/10 μg), cephalothin (CF, 30 μg), cefazolin (CZ, 30 μg), cefoxitin (FOX, 30 μg), cefamandole (MA, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), cefepime (FEP, 30 μg), imipenem (IPM, 10 μg), amikacin (AN, 30 μg), aztreonam (ATM, 30 μg), streptomycin (S, 25 μg), gentamicin (GM, 10 μg), ciprofloxacin (CIP, 30 μg), nalidixic acid (NA, 30 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg), chloramphenicol (C, 30 μg), and tetracycline (TE, 30 μg). The double-disc synergy test was performed in order to confirm CTX-M-type isolates. The minimum inhibitory concentrations (MICs) of selected antimicrobials (CTX and CAZ) were determined using E-test strips (AB BIODISK; Solna, Sweden). *E. coli* strain ATCC 25922 was used as a quality control for the susceptibility test.

Identification of bla_{CTX-M} Genes

The bla_{CTX-M} gene was detected by polymerase chain reaction (PCR) using primers as previously described by Tofteland *et al.* [36]. To confirm the presence of bla_{CTX-M} β-lactamases, the sequences of the CTX-M-1, 8, 9, and 10 families were used for specific grouping. CTX-M-type families were detected by the PCR method. In order to subgroup the CTX-M isolates identified, sequence analysis was performed by Macrogen Inc. (Seoul, Korea). The sequences were confirmed to those in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) program available through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST).

Conjugations

The transferability of bla_{CTX-M} genes in *E. coli* isolates to *E. coli* J53 by conjugation was determined by broth-mating experiments at 37°C, as described in Tamang *et al.* [31]. Transconjugants were selected on MacConkey agar plates containing sodium azide (100 μg/l; Sigma) and cefotaxime (2 μg/ml). All the transconjugants were tested for antimicrobial susceptibility and the presence of β-lactamase genes, as described above.

PCR-Based Replicon Typing of Plasmids

Plasmid DNAs were isolated from transconjugants using a miniprep plasmid kit in accordance with the manufacturer's instructions (Life Technologies, USA). Plasmid replicon typing of *E. coli* strains carrying the CTX-M-type genes was conducted by PCR using 18 primer pairs (HI1, HI2, I1-Iγ, L/M, FIA, FIB, FIC, F, N, P, W, T, A/C, K, B/O, X, Y, and FII) [5]. Positive PCR products matching the amplified replicon sizes were sequenced and the sequences were confirmed by BLAST search [5].

Detection of Addiction Systems

For the donor strains and transconjugants bearing bla_{CTX-M} genes, the following eight plasmid addiction systems were detected and confirmed by PCR: PemK-PemI (*pemKI*) (plasmid emergency maintenance), CcdA-CcdB (*ccdAB*) (coupled to cell division), RelB-RelE (*relBE*) (relaxed control of stable RNA synthesis), ParD-ParE (*parDE*) (DNA replication), VagC-VagD (*vagCD*) (virulence-associated protein), Hok-Sok (*hok-sok*) (host killing), PndA-PndC (*pndAC*) (promotion of nucleic acid degradation), and SrnB-SrnC (*srnBC*) (stable RNA) [20].

Analysis of Genetic Relatedness by Pulse-Field Gel Electrophoresis (PFGE)

The clonality of the CTX-M-producing *E. coli* isolates was determined by performing PFGE using XbaI-digested (enzyme was purchased from Takara Bio Inc., Shiga, Japan) genomic DNA. The DNA fragments were separated on a 1.4% agarose gel (pulsed-field certified agarose (Bio-Rad)) in accordance with the Centers for Disease Control and Prevention PulseNet standardized protocol [9]. Electrophoresis was performed using 0.5× Tris-borated-EDTA buffer under the following conditions: gradient, 6 V/cm; run time, 18 h; pulse times, 2.2–54.2 sec; temperature, 14°C; and angle, 120°. In order to identify the similarities between CTX-M types, restriction fragment length polymorphism analysis was performed and results were analyzed using InfoQuest FP software ver. 4.5 (Bio-Rad) to construct a dendrogram. For comparison, a cluster analysis was performed using the Pearson correlation similarity index. The relationship was calculated by the unweighted-pair group method using average linkages.

Results

Antimicrobial Resistance

In total, 358 *E. coli* strains were isolated from retail chicken (n = 302) and environmental samples from Korean chicken farms (n = 20) and slaughterhouses (n = 36). Among the strains, 15 CTX-M-producing *E. coli* isolates were identified. All of the $bla_{\text{CTX-M}}$-positive isolates were resistant to AM, CF, CZ, MA, and CTX, whereas 93.3%, 93.3%, 53.3%, and 46.7% were resistant to FEP, ATM, CAZ, and IPM, respectively. Moreover, some of $bla_{\text{CTX-M}}$-positive strains were resistant to other classes of antibiotics, including PIP (100%), NA (86.7%), CIP and TE (80%), S (60%), and C and SXT (46.7%). However, all the strains were susceptible to AN, AmC, and FOX.

Detection of $bla_{\text{CTX-M}}$ Genes

A total of 15 *E. coli* isolates carrying $bla_{\text{CTX-M}}$ genes were determined. The predominant CTX-M type identified was CTX-M-15 (n = 8), followed by CTX-M-1 (n = 6) and CTX-M-14 (n = 1). Table 1 shows the prevalence of the 15 isolates carrying $bla_{\text{CTX-M}}$ genes from the sources in retail chickens, chicken farm, and slaughterhouses. The $bla_{\text{CTX-M}}$ genes were identified predominantly in *E. coli* isolates from retail chickens (n = 9), followed by feces (n = 2) and water pipes (n = 2), floor (n = 1), and wall (n = 1).

Transferability of $bla_{\text{CTX-M}}$ Genes

The transfer of $bla_{\text{CTX-M}}$ gene by conjugation was confirmed in the recipient strain *E. coli* J53 from 15 $bla_{\text{CTX-M}}$ gene-positive isolates. PCR analysis verified that the respective $bla_{\text{CTX-M}}$ genes were present in all the transconjugants. Table 2 shows the characteristics of the conjugation-positive *E. coli* isolates carrying $bla_{\text{CTX-M}}$ genes.

Table 1. Prevalence of *E. coli* isolates producing $bla_{\text{CTX-M}}$ genes identified from multiple sources.

Sources	No. of *E. coli* isolates carrying			Total (%)
	CTX-M-1	CTX-M-14	CTX-M-15	
Chicken	4	1	4	9 (60)
Guts				
Feces			2	2 (13.3)
Feed				
Water				
Wall	1			1 (6.7)
Floor			1	1 (6.7)
Production line[a]	1		1	2 (13.3)
Total	6	1	8	15 (100)

[a]Stage of processing conveyer belt.

Analysis of the $bla_{\text{CTX-M}}$ Plasmids

Plasmids from $bla_{\text{CTX-M}}$-positive *E. coli* J53 transconjugants revealed a conjugative plasmid. Four of 18 plasmid replicon types, including Inc I1-Iγ, Inc FIA, IncF, and IncY, were present either alone or in combination. All the plasmids expressing $bla_{\text{CTX-M-1, -15}}$ genes were either positive within two replicon types or were non-typeable, whereas plasmids harboring the $bla_{\text{CTX-M-14}}$ gene showed three replicon types, Y, FIA, and F (Table 2). The predominant replicon type was confirmed as F (66.7%). However, only one strain carrying $bla_{\text{CTX-M-1}}$ was determined to express I1-Iγ without the F replicon type. Among the 15 conjugation-positive plasmids, four were non-typeable for the 18 incompatibility groups. All of them harbored CTX-M-1 and CTX-M-15, except for one harboring CTX-M-14.

Distribution of Addiction Systems

Upon further analysis of the *E. coli* strains, eight kinds of addiction systems were identified: *pemKI*, *ccdAB*, *relBE*, *parDE*, *vagCD*, *hok-sok*, *pndAC*, and *srnBC* (Table 3). In total, 85 plasmid addiction systems were confirmed in the 15 CTX-M-producing *E. coli* donor strains. However, 30 addiction systems were detected among the transconjugants. Among the donor strains, the mean number of addiction systems was highest in CTX-M-15-producing *E. coli* isolates (5.4), followed by those producing CTX-M-1 (4.6) and CTX-M-14 (0.7). Although eight addiction systems were detected in donor strains, four systems (*ccdAB*, *parDE*, *vagCD*, and

Table 2. Characterization of 15 *E. coli* transconjugants carrying bla_{CTX-M} genes, from retail chickens, chicken farms, and slaughterhouses.[a]

CTX-M type	Isolate	Site[b]	Origin	Etest MIC (mg/l)		Plasmid Inc type	Addiction system(s)							
				CT	TZ		*pemKI*	*ccdAB*	*relBE*	*parDE*	*vagCD*	*hok-sok*	*pndAC*	*srnBC*
CTX-M-15	13-ECO-FC-200	GY/GG	Chicken	16	1	NT	+	+	+	-	+	+	+	+
	13-ECO-FC-201	GY/GG	Chicken	>32	1.5	NT	+	-	-	+	+	+	+	+
	13-ECO-FC-271	BC/GG	Chicken	>32	1.5	F	+	+	-	-	+	-	+	-
	13-ECO-FC-287	BC/GG	Chicken	>32	1.5	NT	-	-	-	+	+	+	+	+
	13-ECO-VE-333	HS/GG	Water pipe	>32	4	F	+	-	-	-	-	+	+	+
	13-ECO-VE-338	HS/GG	Floor	>32	4	F	+	+	-	+	+	+	+	+
	13-ECO-VE-339	HS/GG	Feces	>32	4	F	-	+	-	+	+	+	+	+
	13-ECO-VE-355	IS/JB	Feces	>32	2	F	-	+	-	-	+	+	+	+
CTX-M-14	13-ECO-FC-136	YJ/GG	Chicken	>32	1	Y, FIA, F	-	+	-	-	+	+	+	+
CTX-M-1	13-ECO-FC-83	UJ/GG	Chicken	>32	1.5	NT	+	+	-	-	+	+	+	+
	13-ECO-FC-254	GR/GG	Chicken	>32	2.5	F	+	-	-	-	+	+	+	+
	13-ECO-FC-270	BC/GG	Chicken	>32	1.5	F	+	+	-	-	+	+	+	+
	13-ECO-FC-297	GY/GG	Chicken	>32	1	F, I1-Iγ	+	+	-	+	+	+	+	+
	13-ECO-VE-334	HS/GG	Water pipe	>32	2	I1-Iγ	+	+	-	-	+	+	+	+
	13-ECO-VE-337	HS/GG	Wall	>32	1.5	F, I1-Iγ	+	+	-	+	+	+	+	+

[a]Etest, Epsilometer test; NT, not typeable by PCR-based replicon typing; CT, cefotaxime; TZ, ceftazidime.

[b]GG, Gyeonggi-do; JB, Jeollabuk-do; BC, Bucheon-si; GR, Guri-si; GY, Goyang-si; HS, Hwaseong-si; IS, Iksan-si; UJ, Uijeongbu-si; YJ, Yangju-si.

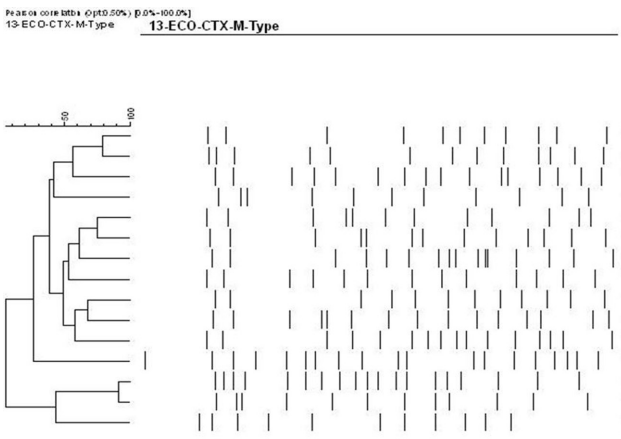
Table 3. Donor strain and transconjugant numbers of addiction systems in accordance with bla_{CTX-M} genes detected in *E. coli* isolates.

Addiction system	Donor strains				Transconjugants			
	CTX-M-1	CTX-M-14	CTX-M-15	Sum	CTX-M-1	CTX-M-14	CTX-M-15	Sum
pemKI	6		5	11				
ccdAB	5	1	5	11	5		4	9
relBE			1	1				
parDE	2		3	5			4	4
vagCD	6	1	7	14	4		6	10
hok-sok	6	1	7	14				
pndAC	6	1	8	15	6		1	7
srnBC	6	1	7	14				
n	6	1	8	15	6	1	8	15
Total	37	5	43	85	15		15	30
Mean	4.6	0.7	5.4	10.7	1.9		1.9	3.8

pndAC) were identified in the transconjugants. *vagCD* was the most common addiction system in both donor strains and transconjugants. In contrast to the donor strains, none of the addiction systems was detected in transconjugants expressing the $bla_{CTX-M-14}$ gene. The mean number of addiction systems for both $bla_{CTX-M-1}$- and $bla_{CTX-M-15}$-positive transconjugants was 1.9, none of which harbored *pemKI*, *relBE*, *hok-sok*, or *srnBC*.

PFGE Analysis

To determine the clonality of the bla_{CTX-M}-positive *E. coli* isolates, molecular typing analysis was carried out by



Strain	Site	Origin	CTX-M type	Non-β-lactam resistance pattern
13-ECO-FC-136	YJ/GG	Chicken	CTX-M-14	PIP S CIP NA SXT C TE
13-ECO-VE-333	HS/GG	Water pipe	CTX-M-15	PIP S GE CIP NA TE
13-ECO-FC-287	BC/GG	Chicken	CTX-M-15	PIP S CIP NA SXT C TE
13-ECO-VE-355	IS/JB	Feces	CTX-M-15	PIP S GE CIP NA TE
13-ECO-FC-201	GY/GG	Chicken	CTX-M-15	PIP S CIP NA SXT C TE
13-ECO-FC-270	BC/GG	Chicken	CTX-M-1	PIP S GE CIP NA SXT C TE
13-ECO-FC-254	GR/GG	Chicken	CTX-M-1	PIP S CIP NA SXT C TE
13-ECO-FC-200	GY/GG	Chicken	CTX-M-15	PIP S CIP NA SXT C TE
13-ECO-FC-271	BC/GG	Chicken	CTX-M-15	PIP S CIP NA SXT C TE
13-ECO-FC-297	GY/GG	Chicken	CTX-M-1	PIP TE
13-ECO-FC-83	UJ/GG	Chicken	CTX-M-1	PIP CIP NA
13-ECO-VE-334	HS/GG	Water pipe	CTX-M-1	PIP TE
13-ECO-VE-338	HS/GG	Floor	CTX-M-15	PIP CIP NA
13-ECO-VE-339	HS/GG	Feces	CTX-M-15	PIP CIP NA
13-ECO-VE-337	HS/GG	Wall	CTX-M-1	PIP CIP NA SXT TE

Fig. 1. Dendrogram showing PFGE analysis of XbaI-digested DNA from the 15 CTX-M-producing *E. coli* strains isolated from retail chickens, chicken farms, and slaughterhouses in Korea.

using XbaI-digested genomic DNA isolated from the original samples obtained from the following sites: 19 traditional markets, two chicken farms, and two slaughterhouses (each from Gyeonggi-do and Jeollabuk-do in Korea). PFGE patterns were analyzed for the 15 *E. coli* strains carrying $bla_{\text{CTX-M-15}}$ (n = 8), $bla_{\text{CTX-M-1}}$ (n = 6), and $bla_{\text{CTX-M-14}}$ (n = 1) genes (Fig. 1). The results revealed that 13 of 15 isolates were not genetically related, whereas the remaining two strains (13-ECO-VE-338 and 13-ECO-VE-339) isolated from the floor and chicken feces in the same farm showed high similarity to 90%. Furthermore, these two strains showed the same characteristics in CTX-M type and antimicrobial resistance pattern.

Discussion

In this study, 15/358 (4.19%) CTX-M-producing *E. coli* strains were isolated from retail chickens, chicken farms, and slaughterhouses. This percentage is higher than that shown in a previous study focused on CTX-M-type-producing *E. coli* isolated from chicken fecal samples, which revealed that 3.6% and 6.7% of single and pooled samples were CTX-M positive, respectively [26].

Among the strains identified in the current study, 14 (3.91%) belonged to the CTX-M-1 cluster and one (0.28%) belonged to the CTX-M-9 cluster [23]. ESBL-producing *E. coli* strains harboring $bla_{\text{CTX-M}}$ genes of CTX-M-1 or CTX-M-9 clusters were previously detected among food animals in China and Europe [2, 18, 35]. Moreover, a previous study in Korea demonstrated the prevalence of ESBL-producing *E. coli* among farm workers, where there was a rapid increase in human clinical *E. coli* isolates of the CTX-M-1 or CTX-M-9 clusters [32].

In this study, $bla_{\text{CTX-M-15}}$ was the predominant gene, followed by $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-14}}$. The $bla_{\text{CTX-M}}$ genes characterized in the samples obtained from healthy chickens, chicken farms, and slaughterhouses were previously observed in animals in Korea [16, 31, 32]. A previous study reported the detection of CTX-M-1 in *E. coli* isolated from food-producing poultry in France, which was consistent with our results [10]. Taken together, these results showed that diverse CTX-M types in food-producing animals are present in Korea.

All the strains bearing $bla_{\text{CTX-M}}$ genes were transferred to *E. coli* recipients by conjugation in this study. Plasmid replicon typing of the transconjugants revealed plasmid diversity, which was similar to $bla_{\text{CTX-M}}$ plasmids previously described in the United Kingdom [8]. In the current study, all replicon types demonstrated that IncF plasmids (4/8; 50%) were the most common among the $bla_{\text{CTX-M-15}}$-carrying plasmids. In agreement with a previous report [13], our results showed that the $bla_{\text{CTX-M-14}}$ gene was carried on IncY, IncF, and IncFIA plasmids. Moreover, IncF and IncI1-Iγ were the predominant replicon types in the plasmids carrying the $bla_{\text{CTX-M-1}}$ gene. It means that the spread of $bla_{\text{CTX-M}}$ genes were associated with IncF plasmids [20]. Therefore, these findings suggest that all the replicon types in the strains isolated from chickens were similar to the genetic structure of the plasmids.

Plasmid addiction systems can contribute to the stability and maintenance of plasmids in their hosts and may affect their distribution in the absence of antibiotic selection [8, 11, 24, 32]. In this study, the fact that the donor strains carried $bla_{\text{CTX-M}}$ genes confirmed the association between

the eight addiction systems and the IncF plasmid replicon type. A previous study has shown that the IncF plasmid replicon type has developed the ability to diffuse in *E. coli*, but its adherence in *E. coli* populations may contribute to the presence of addiction systems [20]. In addition, four addiction systems in the transconjugants would imply that addiction systems might be located on a non-conjugative plasmid or in the chromosome, suggesting that those located on conjugative plasmids harbor resistance markers [32]. Furthermore, among the transconjugants, the *ccdAB*, *vagCD*, and *parDE* systems were associated with the IncF, IncFIA, and IncFIB replicon types, but the *pndAC* system was associated with the IncI1-Iγ replicon type (data not shown here). Mnif *et al*. [21] reported the relation between the *vagCD* system and the IncFIA and IncFIB replicon types. Taken together, these findings suggest that there is a combination of specific plasmids with addiction systems.

PFGE analysis showed that most of the bla_{CTX-M}-positive isolates were highly diverse, with the exception of specific clonal strains. Two *E. coli* isolates (13-ECO-VE-338 and 13-ECO-VE-339) were genetically homogeneous, indicating the possibility that such similar strains may contribute to clonal expansion and horizontal transmission. Moreover, the CTX-M-15-producing *E. coli* isolate obtained from a chicken farm floor sample was related to an isolate from chicken feces (Fig. 1). These findings suggest that it is possible to transfer the *E. coli* isolates from the chicken farms environment to the chickens themselves, which represents a potential public health threat for human consumption of retail chickens.

In conclusion, this study has reported the identification and characterization of CTX-M β-lactamases and their associated addiction systems. Further analysis illustrated that the detection of CTX-M-producing *E. coli* is not only associated with the spread of a single clone but also is affiliated with various IncF replicon-type plasmids harboring multiple addiction systems. Our findings suggest that the linkage between addiction systems and plasmids carrying bla_{CTX-M} genes may contribute to the stability of their plasmids in host cells. It means that the spread of *E. coli* harboring CTX-M via foodborne transmission or environmental pathways can affect human health. Therefore, further analysis is required to assess the risk posed by these microorganisms to human health, with respect to a food safety perspective.

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