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Characterization of Extended-Spectrum β-Lactamase Genes Found among *Escherichia coli* Isolates from Duck and Environmental Samples Obtained on a Duck Farm

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In this study, we focused on evaluating the occurrence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in fecal samples of healthy ducks and environmental samples from a duck farm in South China. Duck cloacal swabs and pond water samples were cultivated on MacConkey agar plates supplemented with ceftiofur. Individual colonies were examined for ESBL production. Bacteria identified as *E. coli* were screened for the presence of ESBL and plasmid-borne AmpC genes. The genetic relatedness, plasmid replicon type, and genetic background were determined. Of 245 samples analyzed, 123 had *E. coli* isolates with ceftiofur MICs higher than 8 μg/ml (116 [50.4%] from 230 duck samples and 7 [46.7%] from 15 water samples). bla_{CTX-M}, bla_{SHV-12}, bla_{CMY-2}, and bla_{DHA-1} were identified in 108, 5, 9, and 1 isolates, respectively. The most common bla_{CTX-M} genes were $bla_{CTX-M-27}$ ($n = 34$), $bla_{CTX-M-55}$ ($n = 27$), $bla_{CTX-M-24e}$ ($n = 22$), and $bla_{CTX-M-105}$ ($n = 20$), followed by $bla_{CTX-M-14a}$, $bla_{CTX-M-14b}$, $bla_{CTX-M-24a}$, and $bla_{CTX-M-24b}$. Although most of the CTX-M producers had distinct pulsotypes, clonal transmission between duck and water isolates was observed. bla_{CTX-M} genes were carried by transferable IncN, IncF, and untypeable plasmids. The novel CTX-M gene $bla_{CTX-M-105}$ was flanked by two hypothetical protein sequences, partial IS*Ecp1* upstream and truncated IS*903D*, *iroN*, *orf1*, and a Tn*1721*-like element downstream. It is suggested that the horizontal transfer of bla_{CTX-M} genes mediated by mobile elements and the clonal spread of CTX-M-producing *E. coli* isolates contributed to the dissemination of bla_{CTX-M} in the duck farm. Our findings highlight the importance of ducks for the dissemination of transferable antibiotic resistance genes into the environment.

Escherichia coli is a Gram-negative, rod-shaped bacterium commonly found in the lower intestinal tract of warm-blooded organisms. It can be easily disseminated in different ecosystems through the food chain and water supply, and it has been widely used as an indicator of fecal contamination (16, 25, 27). While most strains are harmless, many *E. coli* strains are harmful pathogens in humans and animals. Broad-spectrum cephalosporins are frequently used to treat serious infections caused by this bacterium. Resistance to broad-spectrum cephalosporins in *Enterobacteriaceae* has posed considerable and serious challenges for effective medical treatment (23). The main mechanism for *Enterobacteriaceae* resistance to cephalosporins is the production of extended-spectrum β-lactamases (ESBLs) or plasmid-encoded AmpC β-lactamases (pAmpC) (28). Food animals colonized with ESBL-producing *E. coli* have been considered potential sources of resistant *E. coli* causing infection in the community. These ESBL-producing *E. coli* isolates have been increasingly detected in food animals in different countries since 2002 and have gained considerable attention worldwide (28).

Researchers have revealed growing concerns about the release of antibiotic-resistant bacteria and their resistance genes from animal production facilities to natural environments, including groundwater and agricultural soils (12). This release of resistance genes into natural environments may pose significant challenges to human health and the evolution of environmental microbial populations (22). The presence of ESBL genes has been reported in wastewater, surface water, sewage, and sediment samples (8, 21). Despite extensive studies surveying ESBLs in pig, chicken, and cattle (15, 20), the diversity of ESBLs in duck and its living environments has seldom been investigated. According to the data from the Food and Agricultural Organization of the United Nations in 2005, 72% of the world's duck population was fed in mainland China (32). This percentage continues to increase annually. In the People's Republic of China, domestic ducks raised in the traditional free-range system frequently share water with wild waterfowl and are often in close contact with poultry, livestock, and humans from the same farm or village. To prevent and control the spread of infectious diseases, antimicrobial prophylaxis at the flock level was commonly used on duck farms, which could facilitate the selection of antimicrobial-resistant bacteria. Therefore, domestic ducks can act as potential vessels for resistant bacteria and may play an important role in the dissemination of resistant genes (32).

This study was conducted to investigate the prevalence of ESBLs among commensal *E. coli* isolates from duck and environmental samples from a duck farm and to characterize the phenotype and genotype of ESBL genes, the replicon types of bla_{CTX-M}-

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TABLE 1 Distribution of ceftiofur-resistant *E. coli* isolates from duck and water samples

Sample source	No. of samples	No. (%) of samples containing ceftiofur-resistant *E. coli* isolates	No. of ESBL producers
1-wk-old duck	60	32 (53.3)	31
3-wk-old duck	20	12 (60.0)	12
7-wk-old duck	70	40 (57.1)	40
10-wk-old duck	60	27 (45.0)	24
22-wk-old duck	20	5 (25.0)	5
All duck cloacal samples	230	116 (50.4)	112
Pool water	15	7 (46.7)	7
Total	245	123 (50.2)	119

carrying plasmids, and the surrounding genetic background of $bla_{\text{CTX-M}}$ genes.

MATERIALS AND METHODS

Sampling and isolation of bacteria. In December 2006, a total of 230 cloacal swab samples were randomly collected from ducks of different growth phases (1 week old, FW; 3 weeks old, TW; 7 weeks old, HD; 10 weeks old, RD; 22 weeks old, DD) on a large duck breeding farm located in south China (Table 1). In addition, 15 water samples (10 ml/sample) were collected in sterile bottles from selected sites in the pools inhabited by ducks. All of the tested samples were obtained at the same point in time. Different sheds and ponds were assigned to the various growth phases of the ducks. The distance between two ponds averaged 55 m. Samples were plated on MacConkey agar plates containing ceftiofur (8 μg/ml). Presumptive *E. coli* colonies were identified by classical biochemical methods (20). One colony per plate was selected. The production system of this farm was all in and all out, and all of the samples were independent. The antimicrobial usage records for this duck farm indicated that aside from the ceftiofur, which was used for the prophylactic injection of day-old ducklings in duck production, other antibiotics, such as amikacin, enrofloxacin, and florfenicol, are used commonly and extensively to treat illness of the ducks during production.

Antimicrobial susceptibility testing and ESBL confirmation. The antimicrobial susceptibilities of the *E. coli* isolates were determined by using the agar dilution and disk diffusion methods, and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations (4, 5). Eight β-lactam antibiotics, including ampicillin, ceftiofur, ceftriaxone, cefotaxime, ceftazidime, cefoxitin, meropenem, and amoxicillin-clavulanic acid, as well as six non-β-lactam antibiotics, including gentamicin, amikacin, ciprofloxacin, chloramphenicol, florfenicol, and tetracycline, were tested. ESBL producers were identified by the phenotypic confirmatory test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (5).

Detection of β-lactamase genes. The PCR amplification of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, bla_{CMY}, and bla_{DHA} genes was carried out by PCR as previously described (29). Purified PCR products were directly sequenced from both ends or cloned into pMD18-T and then sequenced. The DNA sequences and deduced amino acid sequences were compared to genes in GenBank or at the Lahey Clinic (www.lahey.org/studies/) to confirm the subtypes of β-lactamase genes.

Conjugation experiments and plasmid analysis. Conjugation experiments were carried out with the $bla_{\text{CTX-M}}$-positive strains by the liquid mating-out assay using streptomycin-resistant *E. coli* C600 as the recipient strain as previously described (29). Transconjugants were selected on MacConkey agar containing cefotaxime (4 μg/ml) and streptomycin (2000 μg/ml). Selected transconjugants were further characterized for their antimicrobial susceptibility, ESBL phenotype, and the presence of $bla_{\text{CTX-M}}$ genes. Incompatibility (Inc) groups were assigned by the PCR-based replicon typing (PBRT) of plasmids (2) from transconjugants. The replicon sequence typing (RST) of IncF plasmids and plasmid multilocus sequence typing (pMLST) of IncN plasmids were determined according to the procedure described previously (10, 31), and alleles or sequence types (STs) were assigned by submitting the amplicon sequence to the plasmid MLST web site (www.pubmlst.org/plasmid).

Epidemiological typing. The phylogenetic group of all ceftiofur-resistant *E. coli* isolates was determined by multiplex PCR assays, using a combination of three DNA markers (*chuA*, *yjaA*, and TspE4.C2) as described by Clermont et al. (3). Sixty randomly selected $bla_{\text{CTX-M}}$-carrying *E. coli* isolates were also characterized by PFGE using the CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA) as described by Gautom (11). The results were interpreted according to the criteria of Tenover et al. (30).

Genetic background of the $bla_{\text{CTX-M}}$ gene. To characterize the genetic environment of the $bla_{\text{CTX-M}}$ gene, plasmid DNA was digested with EcoRI, ligated into pUC18 (TaKaRa Biotechnology, Dalian, China), and introduced into *E. coli* DH5α. Transformants were selected on LB agar plates containing 4 μg/ml cefotaxime. Sequencing was carried out by primer walking on both DNA strands. The sequences and the deduced amino acid sequences were analyzed and compared by using the NCBI BLAST program.

Nucleotide sequence accession numbers. The sequence of $bla_{\text{CTX-M-105}}$ and the genetic context of $bla_{\text{CTX-M-105}}$ on pHNDD81-1 have been deposited in GenBank under the accession numbers HQ833651 and JN232518, respectively.

RESULTS

***E. coli* isolation and antimicrobial susceptibility.** A total of 116 and 7 ceftiofur-resistant *E. coli* strains were isolated from 230 duck samples and 15 environment samples, respectively, from the same duck farm (Table 1). Using the double-disk synergy test, ESBL production was confirmed in 119 out of the 123 ceftiofur-resistant *E. coli* isolates.

Antimicrobial susceptibility tests showed that all of the 123 *E. coli* isolates were resistant to ampicillin, cefotaxime, and ceftriaxone, while 74.8, 20.0, and 16.0% of isolates showed resistance to ceftazidime, cefoxitin, and amoxicillin-clavulanic acid, respectively. In addition, some of these isolates showed resistance to other classes of antibiotics, including tetracycline (96.2%), gentamicin (90.2%), ciprofloxacin (84.8%), chloramphenicol (80.2%), florfenicol (59.2%) (MIC $>$ 16 μg/ml), and amikacin (52.0%). However, all of the isolates were susceptible to meropenem.

Identification of β-lactamase genes. bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{CMY}, and bla_{DHA} genes were found to be present in 68 (56.7%), 5 (4.1%), 108 (87.8%), 9 (7.5%), and 1 (0.8%) of the 123 ceftiofur-resistant *E. coli* isolates, respectively. $bla_{\text{CTX-M}}$ was shown to be dominant in these isolates. Sequence analysis revealed that the most common $bla_{\text{CTX-M}}$ type was $bla_{\text{CTX-M-27}}$ ($n = 34$), followed by $bla_{\text{CTX-M-55}}$ ($n = 27$), $bla_{\text{CTX-M-24e}}$ ($n = 22$), $bla_{\text{CTX-M-105}}$ ($n = 20$), $bla_{\text{CTX-M-14a}}$ ($n = 12$), $bla_{\text{CTX-M-14b}}$ ($n = 3$), $bla_{\text{CTX-M-24a}}$ ($n = 3$), and $bla_{\text{CTX-M-24b}}$ ($n = 1$) (Table 2). $bla_{\text{CTX-M-105}}$ was a novel variant of the CTX-M-9 group gene, specifying CTX-M-105 with two amino acids substitutions (A80V and A208E) compared to CTX-M-27. This new $bla_{\text{CTX-M-27}}$-like gene, $bla_{\text{CTX-M-105}}$, has been deposited in GenBank under the accession number HQ833651. Multiple types of $bla_{\text{CTX-M}}$ genes ($bla_{\text{CTX-M-55}}$ in combination with the CTX-M-9 group gene) were identified in 14 isolates. Seven *E. coli* isolates carried both $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ genes. All bla_{TEM} genes were found to be $bla_{\text{TEM-1}}$ by sequencing. In addition, $bla_{\text{SHV-12}}$ was identified in 5 isolates, of which 4 were CTX-M

TABLE 2 Distribution of β-lactamase genes among ceftiofur-resistant *E. coli* isolates[a]

Sample source (*n*)	No. of isolates carrying:												
	Any bla_{CTX-M}	$bla_{CTX-M-27}$	$bla_{CTX-M-55}$	$bla_{CTX-M-24e}$	$bla_{CTX-M-105}$	$bla_{CTX-M-14a}$	$bla_{CTX-M-14b}$	$bla_{CTX-M-24a}$	$bla_{CTX-M-24b}$	bla_{SHV-12}	bla_{CMY-2}	bla_{DHA-1}	bla_{TEM-1}
FW (32)	26	8	7	10	2	3	0	0	1	2	1		21
TW (12)	12	3	2	2	4	1	0	1	0		1		7
HD (40)	24	7	9	5	4	3	0	0	0		2		16
RD (70)	36	13	5	1	8	5	3	1	0	3	5	1	22
DD (27)	3	0	3	2	1	0	0	0	0				1
EV (7)	7	3	1	2	1	0	0	1	0				0
Total (123)	108	34	27	22	20	12	3	3	1	5	9	1	67

[a] FW, 1-week-old duck; TW, 3-week-old duck; HD, 7-week-old duck; RD, 10-week-old duck; DD, 22-week-old duck; EV, pool water.

producers. Genotypes of isolates of different origins are shown in Table 2.

Characterization of bla_{CTX-M} plasmids. Forty CTX-M-producing isolates were randomly selected for conjugation experiments to determine the transferability of cefotaxime resistance. Plasmids carrying bla_{CTX-M} from 33 isolates were successfully transferred to recipients. All of the transconjugants showed resistance to cefotaxime but were susceptible to cefoxitin, amoxicillin-clavulanic acid, and the other 6 non-β-lactam antibiotics, except 16 transconjugants exhibited resistance to ceftazidime and two transconjugants exhibited resistance to amikacin and gentamicin (Table 3).

The plasmid replicon typing of transconjugants revealed significant plasmid diversity. IncN, IncFII, and IncI1 replicons were detected in 10, 3, and 3 transconjugants, respectively (Table 3). Two plasmid replicons (IncN in combination with IncFII or IncI1) were present simultaneously in 2 transconjugants. Within FII replicons, F34 and F43 alleles were identified in 1 and 3 transconjugants, respectively. pMLST of the 10 IncN plasmids revealed that 4 of them were assigned to ST8. For the other 6 IncN plasmids, the *repA* allele was *repA4*, but these plasmids were negative for PCR amplifying *traJ* and *korA*.

Epidemiological typing. Phylogenetic group analysis showed that group A (50.4%; $n = 63$) was dominant among the *E. coli* isolates producing bla_{CTX-M} enzymes, followed by groups D (25.6%; 32) and B1 (21.6%; 27). Only one isolate belonged to the phylogenetic group B2.

Chromosomal DNA of 54 isolates was available for PFGE typing, and the 54 isolates displayed 43 different PFGE profiles. No PFGE fragment pattern was obtained from the other 6 isolates. A majority of isolates showed unique, unrelated PFGE profiles and were unlikely to be considered the cause of an epidemic. However, 11 *E. coli* isolates carrying different bla_{CTX-M} type genes (7 $bla_{CTX-M-27}$, 3 $bla_{CTX-M-105}$, and 1 $bla_{CTX-M-24}$) belonged to the same PFGE pattern (Table 3). Of the 11 isolates, 8 isolates were obtained from ducks and 3 were obtained from environmental samples, which suggested that the water of the duck swimming pool was an important vehicle for the clonal dissemination of resistant *E. coli*.

Genetic background of the $bla_{CTX-M-105}$ gene. DNA of three plasmids carrying $bla_{CTX-M-105}$ was digested with EcoRI and cloned into pUC18 by selection for cefotaxime resistance. Only an 11.5-kb EcoRI-digested fragment from plasmid pHNDD81 carrying $bla_{CTX-M-105}$ was successfully cloned and then sequenced. In general, the genetic context of $bla_{CTX-M-105}$ on pHNDD81 was highly similar to that of $bla_{CTX-M-65}$ on pKC396 and pWCE307, with only minor variations (Fig. 1). As in the case of pKC396 and pWCE307, $bla_{CTX-M-105}$ was associated with partial IS*Ecp1*, including its right-hand inverted repeat (IRR). Downstream of $bla_{CTX-M-105}$, IS*903D* and a truncated gene, *iroN* (a virulence gene for *E. coli*, encoding an outer membrane iron receptor protein), were identified. This IS*Ecp1*-$bla_{CTX-M-105}$-IS*903D*-*iroN*Δ structure was part of a Tn*1721*-like element and was located adjacent to a fragment of *orfI*, which potentially encodes a methyl-accepting chemotaxis protein.

DISCUSSION

More than 50% of the duck fecal samples obtained in 2006 from a duck farm revealed ESBL-producing *E. coli* isolates. This percentage is similar to that recently found in fecal samples of healthy humans in China (19) but is higher than the percentages (10 to ~40%) of fecal carriage of ESBL-producing *E. coli* isolates detected in other countries (6, 13). Ceftiofur is approved for therapeutic parenteral use in swine, cattle, and poultry in China. In ducks, day-old poultry may be injected subcutaneously with ceftiofur to control colibacillosis, which may provide antibiotic selective pressure for the colonization of resistant bacteria in the gastrointestinal tract.

Similarly to findings of other studies from China (14, 19, 20, 29), bla_{CTX-M} alleles were the predominant genes encoding the ESBL phenotype among these isolates. Eight CTX-M gene types were detected, indicating a high diversity of bla_{CTX-M} genes in *E. coli* isolates from this duck farm. The main subtypes of bla_{CTX-M} ESBLs were similar to those of our previous reports (20, 29). However, unlike other studies in China where $bla_{CTX-M-14}$ was the most common bla_{CTX-M} variant (14, 19, 20, 29), $bla_{CTX-M-27}$ was found to be the most frequent type in this study. CTX-M-27 differs from CTX-M-14 by a single substitution of D240G that confers higher levels of resistance to ceftazidime than CTX-M-14 (1). It was only sporadically detected in some studies (14, 19). Previous reports showed that $bla_{CTX-M-27}$ was usually associated with duck isolates in China (GenBank accession no. GQ896550) (20), although it has also been detected in isolates of other origins, including humans and the environment (19, 21). Interestingly, a recent study of the diversity of ESBL-producing organisms in a sediment sample taken from a river located in Guangdong province showed that $bla_{CTX-M-27}$ was the most dominant of the ESBL genes (21). The basis for the successful dissemination of $bla_{CTX-M-27}$, which has increased resistance to ceftazidime on this duck farm, is unknown, but the high prevalence of $bla_{CTX-M-27}$ in commensal *E. coli* isolates from duck feces as well as duck pond water may pose a great threat to human medicine.

Bacterial contamination of surface water, particularly contaminated with feces-borne bacteria, has long been a water quality issue owing to the potential for disease transmission (9). As a

TABLE 3 Characteristics of $bla_{CTX\text{-}M}$-positive *E. coli* isolates and transconjugants

Strain	Sample source	Phylogenetic group	PFGE type[c]	β-Lactamase gene type(s)[b]	MIC (μg/ml) of transconjugants[e]		Non-β-lactam resistance phenotype[a]	Plasmid replicon type (pMLST or IncF allele)[d]
					CTX	CAZ		
FW15	1-wk-old duck	A	1	$bla_{CTX\text{-}M\text{-}105}$	>128	32	GEN, CIP, TET	UT
TW102	3-wk-old duck	B1	14	$bla_{CTX\text{-}M\text{-}105}$, $bla_{TEM\text{-}1}$	64	16	AMI, GEN, CIP, CHL, FFC, TET	IncI1, IncN (ST8)
DD81	22-wk-old duck	A	15	$bla_{CTX\text{-}M\text{-}105}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	128	32	CIP, CHL, FFC, TET	UT
HD99	7-wk-old duck	A	3	$bla_{CTX\text{-}M\text{-}105}$	128	32	AMI, GEN, CIP, CHL, FFC, TET	UT
RD125	10-wk-old duck	A	4	$bla_{CTX\text{-}M\text{-}105}$	128	4	GEN, CIP, CHL, FFC, TET	IncN
RD191	10-wk-old duck	A	4	$bla_{CTX\text{-}M\text{-}105}$	128	8	AMI, GEN, CIP, CHL, FFC, TET	UT
FW16	1-wk-old duck	A	2	$bla_{CTX\text{-}M\text{-}105}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	128	8	GEN, CIP, CHL, FFC, TET	IncN (ST8)
EV221	Pool water	B1	5	$bla_{CTX\text{-}M\text{-}105}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	128	32	GEN, CIP, CHL, FFC, TET	UT
HD96	7-wk-old duck	A	ND	$bla_{CTX\text{-}M\text{-}24e}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	128	8	AMI, GEN, CIP, CHL, TET	UT
HD101	7-wk-old duck	D	16	$bla_{CTX\text{-}M\text{-}55}$	32	8		UT
HD111	7-wk-old duck	B1	17	$bla_{CTX\text{-}M\text{-}55}$	128	8	CIP, CHL, FFC, TET	UT
FW122	1-wk-old duck	A	18	$bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	>128	64	AMI, GEN, CIP, CHL, FFC, TET	UT
FW51	1-wk-old duck	D	19	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	64	8	AMI, CIP, CHL, FFC, TET	UT
HD109	7-wk-old duck	D	6	$bla_{CTX\text{-}M\text{-}14}$	>128	64	GEN, CIP, CHL, FFC, TET	UT
RD187	10-wk-old duck	A	7	$bla_{CTX\text{-}M\text{-}14}$	32	4	GEN, CIP, CHL, FFC, TET	IncN
FW14	1-wk-old duck	D	8	$bla_{CTX\text{-}M\text{-}24b}$	32	4	AMI, GEN, CIP, CHL, TET	UT
FW11	1-wk-old duck	D	20	$bla_{CTX\text{-}M\text{-}24e}$	>128	16	AMI, GEN, CIP, CHL, TET	IncFII (F43)
FW20	1-wk-old duck	D	21	$bla_{CTX\text{-}M\text{-}24e}$, $bla_{TEM\text{-}1}$	64	4	AMI, GEN, CIP, CHL, FFC, TET	UT
RD189	10-wk-old duck	B1	22	$bla_{CTX\text{-}M\text{-}24e}$, $bla_{TEM\text{-}1}$	128	2	AMI, GEN, CIP, CHL, FFC, TET	IncI1
FW23	1-wk-old duck	B1	27	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	128	4	TET	IncN
FW41	1-wk-old duck	A	10	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	128	64	AMI, GEN, CIP, CHL, TET	UT
HD104	7-wk-old duck	A	ND	$bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1}$	128	64	AMI, GEN, CIP, CHL, TET	UT
HD141	7-wk-old duck	A	21	$bla_{CTX\text{-}M\text{-}27}$	32	4	CIP, CHL, FFC, TET	UT
HD77	7-wk-old duck	A	10	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	>128	64	AMI, GEN, CIP, CHL, TET	UT
RD175	10-wk-old duck	B1	24	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	>128	64	GEN, CIP, CHL, TET	UT
RD95	10-wk-old duck	A	11	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	>128	32	GEN, CIP, CHL, FFC, TET	IncN (ST8)
RD215	10-wk-old duck	A	11	$bla_{CTX\text{-}M\text{-}27}$	64	4	GEN, CIP, CHL, FFC, TET	IncN
EV224	Pool water	A	12	$bla_{CTX\text{-}M\text{-}27}$	>128	64	GEN, CIP, CHL, TET	IncN (ST8)
EV222	Pool water	A	1	$bla_{CTX\text{-}M\text{-}27}$	>128	64	GEN, CIP, TET	IncFII (F43)
EV220	Pool water	A	1	$bla_{CTX\text{-}M\text{-}27}$	128	4	GEN, CIP, CHL, FFC, TET	IncN
TW144	3-wk-old duck	A	1	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	32	4	AMI, GEN, CIP, CHL, FFC, TET	IncN
HD149	7-wk-old duck	A	26	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	128	16	AMI, GEN, CHL, TET	IncI1
HD163	7-wk-old duck	B1	25	$bla_{CTX\text{-}M\text{-}55}$	128	8	CIP, CHL, TET	IncFII (F34)

[a] AMI, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CHL, chloramphenicol; FFC, florfenicol; TET, tetracycline. Patterns transferred by conjugation are underlined. The criterion for florfenicol is >16 μg/ml.
[b] Genes that were transferred by conjugation as determined by PCR are underlined.
[c] PFGE types were assigned 1, 2, 3, etc., by the visual inspection of the macrorestriction profile. ND, not determined.
[d] UT, untypeable; ST, sequence type.
[e] CTX, cefotaxime; CAZ, ceftazidime.

waterfowl, ducks usually deposit fecal material into the pond water and sediment, which facilitates the release of ESBL-producing organisms into the environment. In this study, the prevalence of ceftiofur-resistant *E. coli* isolates and the distribution of ESBL gene types in the pond water samples were similar to those of duck fecal samples. In addition, the clonal spread of CTX-M producers and the horizontal transfer of similar plasmids (IncN ST8) carrying $bla_{CTX\text{-}M}$ genes between duck isolates and pond water isolates were identified, which indicated that the feces-contaminated water was an important vehicle for the dissemination of the resistance genes.

The new variant of the $bla_{CTX\text{-}M\text{-}9}$ group gene, $bla_{CTX\text{-}M\text{-}105}$,

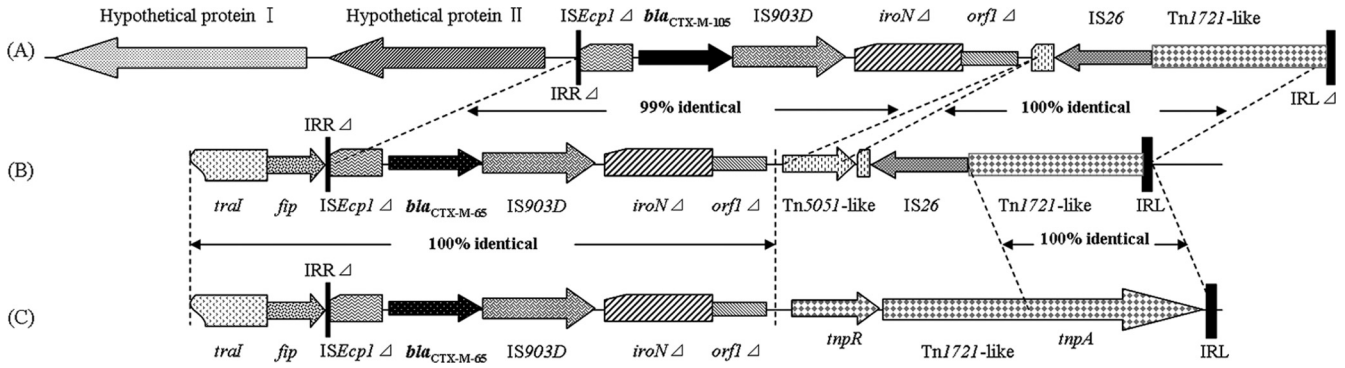

FIG 1 Genetic context of $bla_{\text{CTX-M-105}}$ and similar structures. (A) Genetic context of $bla_{\text{CTX-M-105}}$ on pHNDD81-1 (GenBank accession no. JN232518). (B) Genetic context of $bla_{\text{CTX-M-65}}$ on pKC396 (GenBank accession no. HM138653). (C) Genetic context of $bla_{\text{CTX-M-65}}$ on pWCE307 (GenBank accession no. HM440049).

was identified in 20 *E. coli* isolates of different origins (including pool water), which indicated that this gene has been circulating among *E. coli* isolates in the duck farm for a long time. Because most of the $bla_{\text{CTX-M-105}}$ producers were clonally unrelated and the $bla_{\text{CTX-M-105}}$ genes were located on different plasmids, we analyzed the genetic background surrounding it. $bla_{\text{CTX-M-105}}$ was located in a Tn*1721*-like element on pHNDD81-1 and is associated with an IS*Ecp1*-$bla_{\text{CTX-M}}$-IS*903D*-*iroN*Δ structure which has been seen in several cases associated with $bla_{\text{CTX-M-65}}$ (7, 33), $bla_{\text{CTX-M-24}}$ (26), $bla_{\text{CTX-M-19}}$ (24), and $bla_{\text{CTX-M-14}}$ (17). IS*Ecp1* has been proved to be able to recognize part of the *iroN* gene sequence as its alternative IRR and then mobilize $bla_{\text{CTX-M}}$, IS*903*, and the partial *iroN* gene (*iroN*Δ) into Tn*1721*. Since this IS*Ecp1*-mediated mobilization into the exact same position of Tn*1722* might have occurred only once, Zong et al. (33) speculated that $bla_{\text{CTX-M-14}}$ diverged to generate variants such as $bla_{\text{CTX-M-19}}$, $bla_{\text{CTX-M-24}}$, and $bla_{\text{CTX-M-65}}$ in this element. However, $bla_{\text{CTX-M-105}}$ differs from $bla_{\text{CTX-M-14}}$ by three nucleotide differences. In addition, there were two hypothetical protein sequences located upstream of the truncated IS*Ecp1*. In GenBank, there was no sequence similar to the two hypothetical protein sequences, except that a 507-bp sequence specific to avian pathogenic *E. coli* has 90% similarity to the first hypothetical protein nucleotide sequence (GenBank accession no. DQ643394) (18).

In conclusion, both the clonal transfer of ESBL producers and the horizontal transfer of IncN, IncF, or untypeable plasmids are contributing to the rapid dissemination of the $bla_{\text{CTX-M}}$ gene in the duck farm. The commensal *E. coli* isolates from ducks are an important reservoir of ESBLs genes. Just as critical is the pond water of the duck farm, which serves as an important vehicle for the spread of the resistant bacteria. These resistant bacteria may be transmitted to human beings through the food chain and strengthen human clinical resistance. Therefore, the hygiene of the animal ecosystem and prudent use of cephalosporin is necessary for the control of the persistence and the spread of resistant bacteria.

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