Preplanned Studies

High Prevalence and Genomic Characterization of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in the Yellow River and Source Water from A One Health Perspective — Henan Province, China, 2023–2024

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Summary

What is already known about this topic?

The Yellow River serves as a significant conduit for antibiotic resistance transmission from environmental reservoirs to human populations. However, the occurrence and transmission pathways of the clinically relevant extended-spectrum β-lactamase (ESBL) gene *bla*$_{\text{CTX-M-G9}}$ and ESBL-producing *Escherichia coli* (*E. coli*) within the Yellow River remain poorly characterized.

What is added by this report?

This study reveals the widespread prevalence of *bla*$_{\text{CTX-M-G9}}$ throughout the Yellow River and its associated water sources in Henan Province, and demonstrates the environmental dissemination and probable animal origin of the dominant *E. coli* sequence type (ST) 6802 harboring the *bla*$_{\text{CTX-M-14}}$ genotype.

What are the implications for public health practice?

The findings underscore the critical need to strengthen environmental surveillance and implement robust control measures targeting antibiotic resistance of animal origin. Additionally, we advocate for enhanced public awareness and education initiatives regarding antibiotic resistance to foster broader societal engagement and support for mitigation efforts.

ABSTRACT

Introduction: Understanding the prevalence and dissemination pathways of the clinically relevant extended-spectrum β-lactamase (ESBL) genes and ESBL-producing *Escherichia coli* (*E. coli*) within the Yellow River is essential from a One Health perspective to control antibiotic resistance dissemination from environmental reservoirs to human populations.

Methods: Water samples were collected from the Yellow River and two of its major tributaries in Henan Province during 2023 and 2024. TaqMan quantitative polymerase chain reaction (qPCR) was used to quantify the abundance of the ESBL gene *bla*$_{\text{CTX-M-G9}}$. Twenty-three *E. coli* isolates underwent whole-genome sequencing (WGS), and 167 publicly available *E. coli* genomes from diverse sources were incorporated into the comparative phylogenetic analysis.

Results: The *bla*$_{\text{CTX-M-G9}}$ gene was ubiquitous across all sampling sites, exhibiting significantly higher relative abundance during the dry season compared to the flat season. The multidrug-resistant *E. coli* sequence type (ST) 6802 carrying *bla*$_{\text{CTX-M-14}}$ emerged as the predominant clone. Strong positive correlations were observed between *bla*$_{\text{CTX-M-G9}}$ abundance and plasmids carried by *E. coli* ST6802 in the Yellow River, providing evidence for clonal expansion during the dry season. Furthermore, comparative phylogenetic analysis integrating human, animal, and environmental isolates demonstrated that ST6802 strains from this study were closely related to those previously identified in anaerobic digestion systems treating pig manure in China, suggesting an animal-to-environment transmission pathway.

Conclusion: These findings emphasize the urgent need to implement targeted interventions that prevent the transmission of antibiotic resistance from animal sources into aquatic environments, thereby protecting public health and preserving the integrity of critical water resources.

The emergence and spread of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) poses a substantial threat to public health worldwide. In 2019, antimicrobial resistance accounted for an estimated 145,000 deaths in China, with ESBL-producing *E. coli* ranking as the third leading cause (8,032 deaths) (*1*). Recognizing this growing concern, the World Health Organization launched the "ESBL *E. coli* tricycle AMR surveillance project" in 2017, which encompasses monitoring across human populations, the food chain, and environmental reservoirs. Environmental surveillance has historically been the weakest component of this integrated approach. However, recent years have witnessed increasing attention to ESBL-producing *E. coli* surveillance in environmental settings (*2*). Among the diverse ESBLs, the cefotaxime from Munich (CTX-M) has emerged as the most prevalent ESBL type globally. In China, *E. coli* strains carrying CTX-M group 9 variants, particularly the CTX-M-14 subtype, predominate, despite CTX-M group 1 subtype CTX-M-15 being the most widespread variant internationally (*3*). Surveillance data from Chinese hospitals revealed a high prevalence (62.8%) of CTX-M group genes in *E. coli* isolated from outpatients. The most common genotype was the CTX-M group 9 subtype $bla_{\text{CTX-M-14}}$ (57.7%), followed by $bla_{\text{CTX-M-55}}$ (23.4%) and $bla_{\text{CTX-M-15}}$ (15.4%) (*4*). Notably, *E. coli* isolates from community-acquired urinary tract infections across various regions demonstrated a high prevalence of $bla_{\text{CTX-M-14}}$ genotype, with an incidence rate of 31.8% (*5*). Our previous research identified $bla_{\text{CTX-M-14}}$ as the dominant genotype in *E. coli* from anaerobic digestion systems treating pig manure (*6*).

The Yellow River, China's second longest river at 5,464 km, plays a critical role in water supply and agricultural irrigation throughout northern China. Flowing through densely populated urban areas and extensive agricultural lands, the river basin receives environmental discharges exceeding 3,000 tons of antibiotics annually (*7*), thereby functioning as both a reservoir and transmission medium for antibiotic resistance genes (ARGs). Understanding the prevalence, transmission dynamics, and potential sources of CTX-M group 9 genes and CTX-M-producing *E. coli* in the Yellow River and its associated source waters is essential from a One Health perspective to effectively control ARG dissemination from environmental reservoirs to human populations. Despite this critical need, the abundance of CTX-M group 9 genes and the dissemination routes of CTX-M-producing *E. coli* in the Yellow River remain poorly characterized.

Henan Province is located in the east-central region of China, encompassing the middle and lower reaches of the Yellow River. Although urban areas have primarily relied on the South-to-North Water Transfer Project since 2014, several regions in Henan continue to utilize water from the Yellow River as their primary or backup source for drinking water, irrigation, and landscaping. In this study, we collected water samples from the Yellow River at six cities in Henan Province (Lingbao, Luoyang, Jiaozuo, Xinxiang, Zhengzhou, and Puyang), an associated water source in Zhengzhou City, and two major tributaries (the Qin River and the Yiluo River) upstream of the water source. Sampling was conducted during three distinct hydrological periods: the wet season (August 2023), the dry season (December 2023), and the flat season (April 2024).

We employed TaqMan quantitative polymerase chain reaction (qPCR) to quantify the abundance of the $bla_{\text{CTX-M-G9}}$ gene and its associated mobile genetic elements (MGEs). The qPCR primers are provided in Supplementary Table S1 (available at https://weekly.chinacdc.cn/). During the dry season, we filtered 100 mL water samples from each sampling point through 0.45 μm microporous membranes for bacterial isolation. The filtered membranes were incubated on fuchsin sodium sulfite medium supplemented with 4 mg/L cefotaxime at 37 °C for 24 h. All colonies were isolated and purified. A total of 75 cefotaxime-resistant *E. coli* strains were recovered from Lingbao (*n*=10), Luoyang (*n*=7), Yiluo River (*n*=10), Jiaozuo (*n*=1), Qin River (*n*=4), Xinxiang (*n*=11), the water source in Zhengzhou City (*n*=17), and Puyang (*n*=15). All strains underwent polymerase chain reaction (PCR) detection of CTX-M group 9 genes and antibiotic susceptibility testing by disk diffusion methods as previously reported (6, *8*–*9*). We selected 23 *E. coli* isolates for whole-genome sequencing (WGS) based on their diverse antibiotic resistance profiles (Supplementary Table S2, available at https://weekly.chinacdc.cn/), ensuring representation of the range of resistance patterns observed. Additionally, we downloaded 167 *E. coli* genomes from the National Center for Biotechnology Information (NCBI) database (CTX-M-14-producing *E. coli*, *n*=162) and the EnteroBase database (*E. coli* ST6802, *n*=5) for comparative phylogenetic analysis.

WGS of 23 *E. coli* strains was performed using the Illumina NovaSeq PE150 platform (Sinobiocore Biotechnology Co., Ltd., Beijing, China). The genome sequences were deposited in the NCBI database under accession number PRJNA1306565. The assembled contig files were uploaded to the Centre for Genomic Epidemiology (CGE) platform (http://www.

genomicepidemiology.org/) for screening of ARGs, virulence factors (VFs), and plasmid replicon types, as well as multilocus sequence typing (MLST) analysis. Single nucleotide polymorphisms (SNPs) were determined on the CGE platform, and the SNP-based phylogenetic tree was visualized in iTOL online. The complete genome multilocus sequence typing (cgMLST) analysis was conducted using PubMLST (https://pubmlst.org/) and R (Version 4.4.1, R Foundation for Statistical Computing, Vienna, Austria). The cgMLST-based minimum spanning tree was generated using GrapeTree (Version 1.5.0, University of Melbourne, Melbourne, Australia).

The *bla*$_{\text{CTX-M-G9}}$ gene was ubiquitously detected across all sampling sites in the Yellow River, its tributaries, and the associated water source (Figure 1A). Relative abundance of *bla*$_{\text{CTX-M-G9}}$ was significantly higher during the dry season (10^{-5}-10^{-3} copies/16S rDNA) compared to the flat season (10^{-6}-10^{-4} copies/16S rDNA) (Figure 1B), with peak levels observed in the water source during the dry season. Antibiotic susceptibility testing results for all 75 cefotaxime-resistant *E. coli* isolates are presented in Supplementary Table S2. Among these isolates, 98.7%

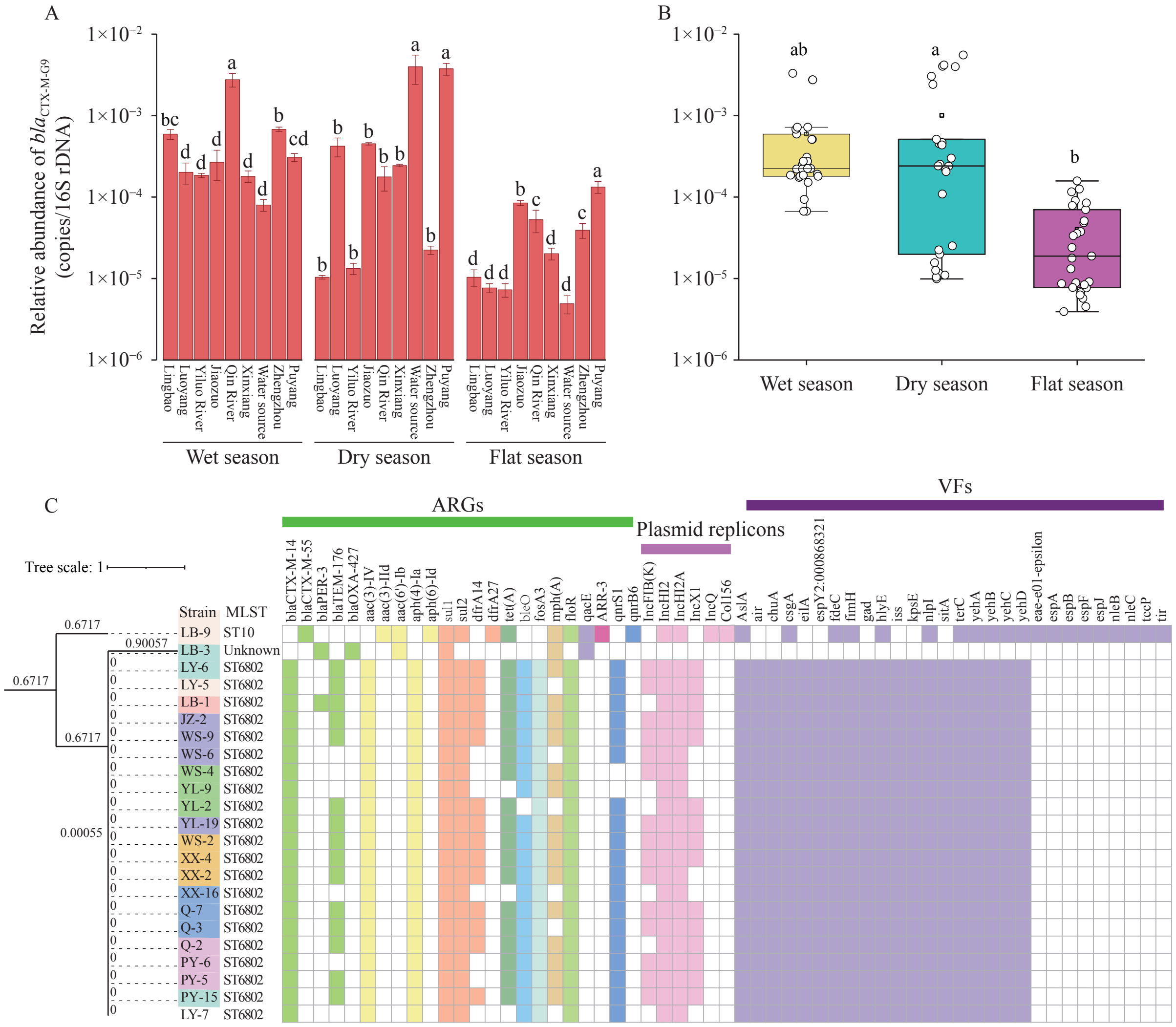

FIGURE 1. Prevalence of the *bla*$_{\text{CTX-M-G9}}$ gene and CTX-M-producing *E. coli* in the Yellow River and associated source water. Relative abundance of *bla*$_{\text{CTX-M-G9}}$ across different (A) cities and (B) seasons. (C) SNP-based phylogenetic tree of 23 CTX-M-producing *E. coli* isolates, displaying associated antibiotic resistance genes, virulence factors, and plasmid replicon types.

Note: For (A) and (B), one-way analysis of variance (ANOVA) with Tukey's *post-hoc* tests was employed to assess significant differences among groups.

Abbreviation: LB=Lingbao City; LY=Luoyang City; JZ=Jiaozuo City; XX=Xinxiang City; PY=Puyang City; WS=water source in Zhengzhou City; Q=Qin River; YL=Yiluo River; ARGs=antibiotic resistance genes; VFs=virulence factors; MLST=multilocus sequence typing; SNP=single nucleotide polymorphism.

(n=74) harbored the *bla*$_{\text{CTX-M-14}}$ genotype and demonstrated multidrug resistance phenotypes, with 78.7% (n=59) exhibiting resistance to seven or more of the tested antibiotics. Whole-genome sequencing revealed that 21 of 23 *E. coli* strains belonged to sequence type (ST) 6802, all of which carried *bla*$_{\text{CTX-M-14}}$ (Figure 1C). These ST6802 strains co-harbored 8–13 additional ARGs, 19 VFs, and two to four plasmid replicons (IncFIB, IncHI2, IncHI2A, and IncX1). SNP-based phylogenetic analysis demonstrated zero to four SNP differences among all ST6802 isolates, confirming clonal dissemination of ST6802 throughout the Yellow River. Moreover, during the dry season, *bla*$_{\text{CTX-M-G9}}$ abundance exhibited strong correlations with the plasmid replicons IncHI2 (R^2=0.86), IncHI2A (R^2=0.75), IncX1 (R^2=0.83), and IncFIB (R^2=0.67) in the Yellow River, providing additional evidence for clonal ST6802 spread (Figure 2).

Genome analysis identified 47 distinct STs among the 185 CTX-M-14-producing *E. coli* isolates examined (23 isolates from this study and 162 publicly available CTX-M-14-producing *E. coli* genomes), which were broadly distributed across human, animal, and environmental sources (Figure 3A and 3B). *E. coli* ST6802 has been previously detected in livestock and poultry feces, wild animals, animal-derived food products, anaerobic digestion systems, wastewater, and river water; however, no human isolates have been reported to date. cgMLST and SNP-based phylogenetic analyses demonstrated that the ST6802 isolates from this study were closely related to those recovered from anaerobic digestion systems treating pig manure, exhibiting 0–35 SNP differences (Figure 3C and 3D). Notably, ST6802 isolates from the drinking water source displayed zero SNP differences from the *E. coli* strain EFF60 (SAMN22853575) isolated from an anaerobic digestion system in China. These findings indicate environmental dissemination of CTX-M-14-producing *E. coli* ST6802 from a One Health perspective.

DISCUSSION

Understanding the occurrence and genetic characteristics of ESBL-producing *E. coli* in the Yellow River is essential for tracking the origin and dissemination of clinically relevant antimicrobial

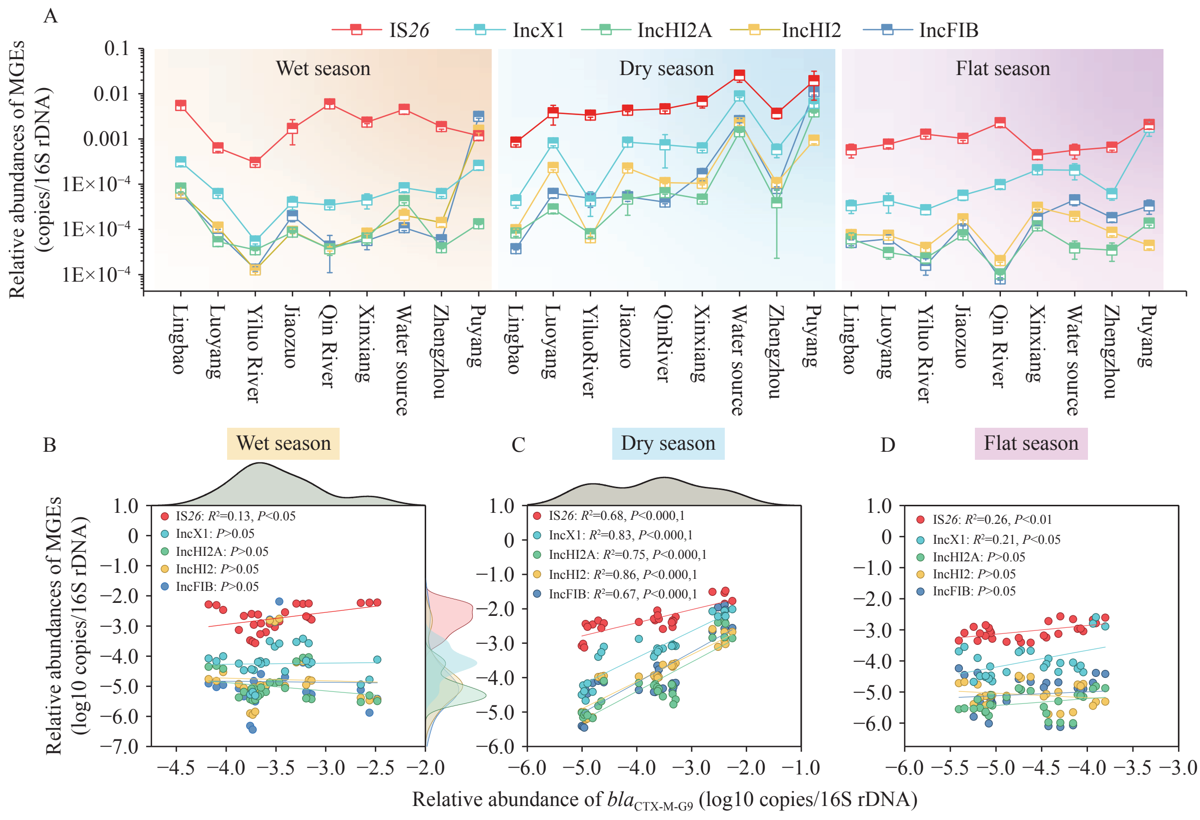

FIGURE 2. Relative abundances of plasmids and IS*26* carried by *E. coli* ST6802 in (A) the Yellow River and their correlations with *bla*$_{\text{CTX-M-G9}}$ during the (B) wet, (C) dry, and (D) flat seasons.

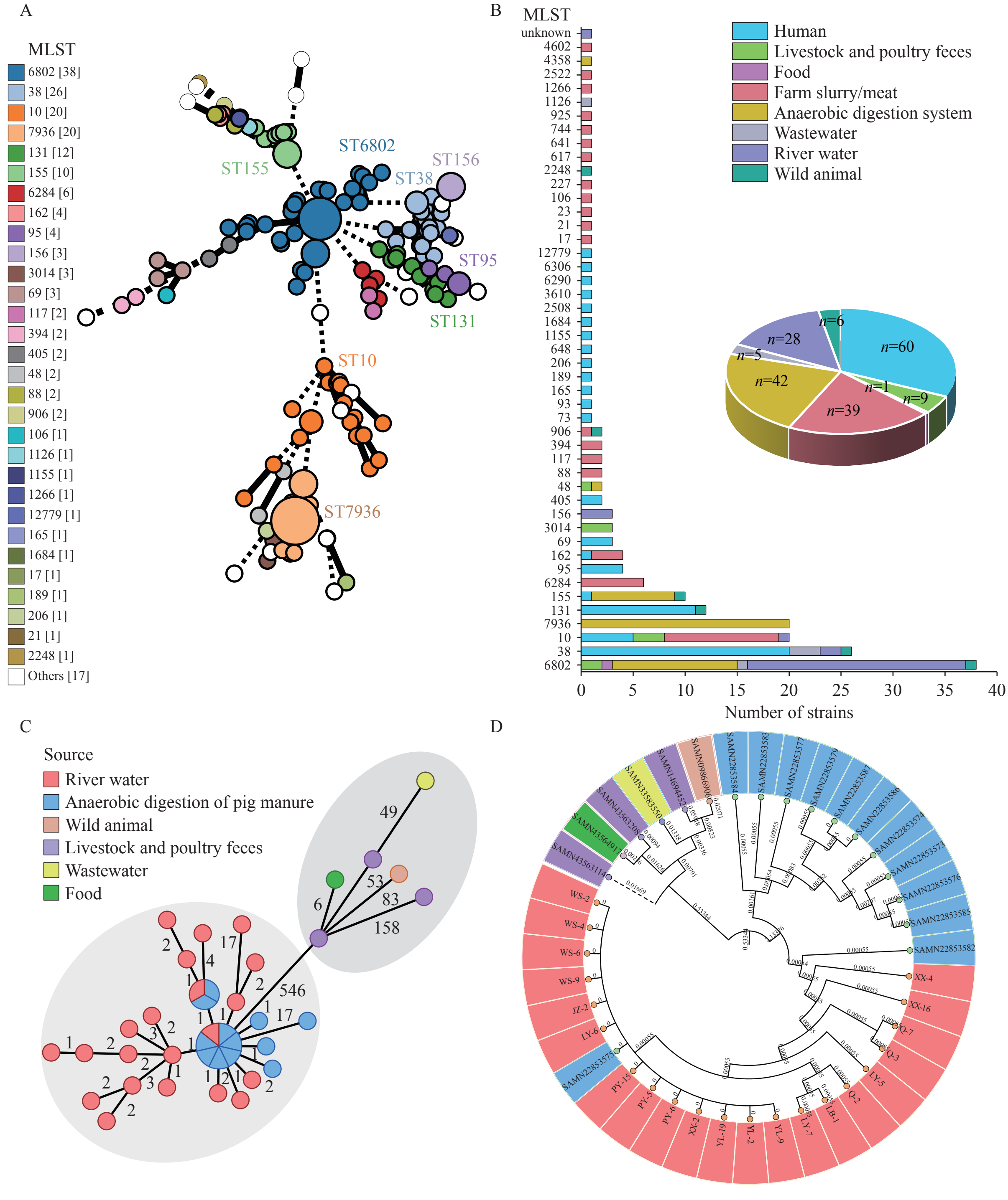

FIGURE 3. Genome-based phylogenetic analysis of CTX-M-14-producing *E. coli* and *E. coli* ST6802 from Yellow River water in this study, NCBI, and EnteroBase databases. (A) cgMLST-based minimum spanning tree of 190 *E. coli* isolates from diverse sources, comprising 185 CTX-M-14-producing *E. coli* and 5 *E. coli* ST6802 isolates. (B) Distribution of multilocus sequence types (MLSTs) among the 190 *E. coli* strains across different sources. (C) cgMLST-based minimum spanning tree of 39 ST6802 isolates from various sources. (D) SNP-based phylogenetic tree of 39 ST6802 isolates.

resistance in surface waters. This study investigated the abundance of the CTX-M group 9-type ESBL gene *bla*$_{\text{CTX-M-G9}}$ in the Yellow River across three seasons at nine sampling sites. We further characterized the genomic features of the dominant CTX-M-14-producing *E. coli* during the dry season and identified potential contamination sources through comparative genomic analysis. Our findings reveal critical insights into the environmental transmission pathways of ESBL-producing *E. coli* from a One Health perspective, demonstrating both the necessity of continuous ESBL surveillance in the Yellow River and the urgent need to prevent environmental dissemination of ESBL-producing *E. coli* from animal sources.

Despite significant progress in China over recent decades in regulating antibiotic use in livestock and in reducing environmental ARG dissemination (*10*–*11*), *bla*$_{\text{CTX-M-G9}}$ was detected in all water samples, with significantly a higher relative abundance during the dry season compared to the flat season. This seasonal pattern aligns with a previous study that reported peak ARG abundance in the Yellow River during winter (*12*). Three factors likely contribute to this observation. First, reduced water volume during the dry season concentrates ARGs. Second, increased antibiotic prescriptions for seasonal epidemic diseases during winter months elevate ARG abundance in aquatic environments through wastewater discharge (*13*). Third, low winter temperatures may slow microbial community turnover, favoring the survival and proliferation of specific ESBL-producing *E. coli* clones. Consistent with a previous report (*14*), Puyang City exhibited the highest *bla*$_{\text{CTX-M-G9}}$ abundance during both dry and flat seasons, likely reflecting its intensive livestock and poultry farming operations.

The number of cefotaxime-resistant *E. coli* isolates varied substantially across sampling sites. The water source (n=17) and the Puyang section (n=15) yielded the highest numbers of isolates, consistent with the elevated spatial abundance of the *bla*$_{\text{CTX-M-G9}}$ gene observed during the dry season (Figure 1A). These findings reflect the spatial distribution patterns of cefotaxime-resistant *E. coli* across the study region. Notably, only a single *E. coli* strain was isolated from the Jiaozuo section, likely due to either the selectivity constraints of the culture medium employed or the presence of alternative bacterial hosts harboring *bla*$_{\text{CTX-M-G9}}$.

The predominant genotype among cefotaxime-resistant *E. coli* from the Yellow River and its water source was *bla*$_{\text{CTX-M}}$. The emergence and dissemination of specific *E. coli* clones represent a key mechanism driving the spread of *bla*$_{\text{CTX-M}}$ genes. For instance, Falgenhauer et al. (*15*) documented the emergence of an *E. coli* ST949 clone harboring chromosomal *bla*$_{\text{CTX-M-15}}$ in German surface waters. In contrast, *bla*$_{\text{CTX-M-14}}$ typically resides on diverse plasmids, and CTX-M-14-producing *E. coli* in Chinese surface waters have demonstrated considerable genetic diversity, with no single clonal group predominating (*16*). This diversity is evident in Figure 3A, where the 167 CTX-M-14-producing *E. coli* strains retrieved from public databases belonged to 47 distinct ST types, underscoring the broad host range of *bla*$_{\text{CTX-M-14}}$. However, our study revealed a notable exception: the *E. coli* ST6802 clone carrying *bla*$_{\text{CTX-M-14}}$ was detected at all sampling sites across multiple cities, suggesting widespread clonal dissemination.

The ST6802 isolates exhibited fewer than four SNP differences from one another, with highly similar ARG profiles, plasmid replicons, and VF patterns, confirming clonal dissemination. To validate this finding, we quantified the IncHI2, IncHI2A, IncX1, and IncFIB plasmids within ST6802, along with the IS*26* element, which typically flanks *bla*$_{\text{CTX-M-14}}$, in Yellow River samples. As anticipated, *bla*$_{\text{CTX-M-G9}}$ demonstrated strong correlations with these plasmids during the dry season, most notably with the IncHI2 plasmid (R^2=0.86). This correlation likely reflects the location of *bla*$_{\text{CTX-M-14}}$ on the IncHI2 plasmid in *E. coli* ST6802, as we previously reported (*6*). Remarkably, the ST6802 isolates harbored 19 distinct VF types, predominantly associated with adhesion and biofilm formation (fimH, csgA, fdeC, and yehA-D), iron acquisition (chuA and sitA), toxin production (hlyE), serum resistance (iss and kpsE), virulence gene expression (eilA), and stress response (gad, nlpI, and terC). The eilA gene has been identified in enteroaggregative *E. coli* strains that cause both acute and chronic diarrhea (*17*). The presence of VFs mediating bacterial adhesion and biofilm formation likely enhances the survival and environmental persistence of ST6802 isolates in the Yellow River ecosystem.

E. coli ST6802 has been documented in diverse sources globally, including chicken feces in China, a silver gull in Australia, wastewater in the United States, chicken and poultry products in Canada, and anaerobic digestion systems treating pig manure in China (Supplementary Table S3, available at https://weekly.chinacdc.cn/). Both cgMLST and SNP-based phylogenetic analyses demonstrated that the ST6802 isolates from this study were genetically identical to *E. coli* strain EFF60 (SAMN22853575) from an anaerobic digestion system and closely related to isolates from livestock and poultry feces. In contrast, these isolates showed substantial phylogenetic divergence from ST6802 strains recovered from urban wastewater. These findings strongly support an animal origin for *E. coli* ST6802 and its subsequent environmental dissemination. The zero-SNP difference between ST6802 isolates from river water and anaerobic digestion systems is particularly striking, as it underscores the public health risk posed by transmission of animal-origin-resistant bacteria into aquatic environments. This transmission pathway — from animal feces through aquatic environments to potential human exposure — identifies critical intervention points for preventing cross-environmental spread of antibiotic resistance.

Although ST6802 has not been detected in human clinical samples, the IncHI2 plasmid harboring *bla*$_{\text{CTX-M-14}}$ within *E. coli* ST6802 demonstrates broad host range, exceptional conjugative transferability, and remarkable genetic stability (*6*). These characteristics position ST6802 isolates as critical vectors for *bla*$_{\text{CTX-M-14}}$ dissemination across bacterial populations. Moreover, the co-occurrence of multiple virulence factors in *E. coli* ST6802 amplifies concerns regarding its environmental persistence and spread throughout the Yellow River system, presenting substantial public health risks. The potential for this multidrug-resistant, virulent clone to establish itself in drinking water sources and irrigation systems warrants immediate attention and enhanced monitoring strategies.

This study advances beyond previous investigations of ESBL-producing *E. coli* in animal manure waste treatment systems (*6*) by tracing the transmission pathways of animal-origin ST6802 isolates into aquatic environments through integrated whole-genome sequencing and phylogenomic analysis of 167 publicly available *E. coli* genomes from diverse animal, human, and environmental sources. These findings underscore the critical need for sustained surveillance programs and stringent source control measures to interrupt the transmission of ESBL-producing *E. coli* from agricultural settings to natural water systems. Furthermore, comprehensive public education initiatives regarding antibiotic resistance are essential to mobilize broader societal engagement and support for antimicrobial stewardship efforts.

Conflicts of interest: No conflicts of interest.

Funding: Supported by the National Key Research and Development Program of China (2022YFC3204703), the Natural Science Foundation of Henan Province of China (242300420335), and the Specialized Project of High-level Talents in Henan Agricultural University (30501469; 30501989).

doi: 10.46234/ccdcw2025.244

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Submitted: September 21, 2025
Accepted: November 09, 2025
Issued: November 14, 2025

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY TABLE S1. Primers used for quantitative polymerase chain reaction quantification of ARGs and MGEs.

Genes	Primers	Size (bp)	Reference	GenBank
bla$_{\text{CTX-M-G9}}$	F: ACCAATGATATTGCGGTGAT R: CTGCGTTCTGTTGCGGCT Probe: 6FAM-TCGTGCGCCGCTG-MGBNFQ	85	(*1*)	–
IS*26*	F: AGGAGATGCTGGCTGAACG R: GGCAAAGATCGGAAGGGTT	100	This study	NZ_CP130155.1
IncFIB	F: CCTTTGGTCTCGCTCTGGAA R: CGGATTTGACTCCCGTCGTT	105	This study	JN233704
IncHI2	F: GGCCAGTACATCCAACGGAA R: ACCAGAGAAGCCGGGATTTG	97	This study	BX664015
IncHI2A	F: CTTTAAGTGCTGGCTCGGGA R: TGCACCAGATGCGTTACTGT	228	This study	BX664015
IncX1	F: ATGGGCTGTATTCTGGCTGG R: TCAGCCAGCAGTCCCAAAAT	132	This study	EU370913

Abbreivation: ARGs=antibiotic resistance genes; MGEs=mobile genetic elements.

SUPPLEMENTARY TABLE S2. Antibiotic resistance profiles of 75 *Escherichia coli* isolates collected during the dry season.

Strain	*bla*$_{\text{CTX-M-G9}}$	Antibiotics											No. of drugs
		AMC	AMP	CTX	FEP	GEN	KAN	TET	CIP	ERY	SXT	CHL	
LB-1	+	R	R	R	R	S	S	S	I	R	R	S	6
LB-2	+	R	R	R	I	S	S	R	R	R	S	S	6
LB-3	+	R	R	R	R	S	I	S	I	R	S	S	5
LB-4	+	R	R	R	I	S	S	R	I	R	R	S	6
LB-6	+	R	R	R	I	R	S	R	I	R	S	S	6
LB-7	+	R	R	R	I	R	I	I	I	R	R	S	6
LB-8	+	R	R	R	I	S	S	R	I	R	R	S	6
LB-9	-	R	R	R	R	R	I	R	I	R	R	R	9
LB-10	+	R	R	R	I	R	S	S	R	R	R	S	7
LB-11	+	R	R	R	I	S	S	R	R	R	R	S	7
LY-1	+	R	R	R	R	I	S	R	I	R	R	R	8
LY-3	+	R	R	R	R	I	S	R	I	R	R	I	7
LY-4	+	R	R	R	I	S	S	R	I	R	R	R	7
LY-5	+	R	R	R	R	I	S	R	I	R	R	R	8
LY-6	+	R	R	R	R	S	S	R	I	R	R	R	8
LY-7	+	R	R	R	R	I	S	R	I	R	R	R	8
LY-8	+	R	R	R	R	I	S	R	I	R	R	R	8
YL-1	+	R	R	R	I	R	S	S	I	R	S	S	5
YL-2	+	R	R	R	R	S	S	R	I	R	R	R	8
YL-3	+	R	R	R	I	R	S	S	R	R	R	S	7
YL-9	+	R	R	R	R	R	S	R	I	R	R	I	8
YL-11	+	R	R	R	R	S	S	S	I	I	S	S	4
YL-12	+	R	R	R	I	I	I	R	I	R	R	R	7
YL-15	+	R	R	R	I	S	S	S	I	I	S	S	3
YL-18	+	R	R	R	R	S	S	S	I	I	S	S	4
YL-19	+	R	R	R	R	S	S	R	I	R	R	I	7
YL-20	-	R	R	R	R	I	S	R	I	R	R	I	7

Continued

Strain	*bla*$_{CTX-M-G9}$	Antibiotics											No. of drugs
		AMC	AMP	CTX	FEP	GEN	KAN	TET	CIP	ERY	SXT	CHL	
JZ-2	+	R	R	R	I	S	S	S	I	R	I	S	4
Q-2	+	R	R	R	R	R	S	R	I	R	R	I	8
Q-3	+	R	R	R	R	R	I	R	I	R	R	R	9
Q-4	+	R	R	R	R	R	S	R	I	R	R	I	8
Q-7	+	R	R	R	R	R	I	R	I	R	R	I	8
XX-1	+	R	R	R	I	S	S	R	I	R	R	R	7
XX-2	+	R	R	R	R	S	S	R	I	R	R	R	8
XX-3	+	R	R	R	I	S	S	R	I	R	R	R	7
XX-4	+	R	R	R	R	I	S	R	I	R	R	I	7
XX-9	+	R	R	R	I	I	S	R	I	R	R	I	6
XX-10	+	R	R	R	I	I	S	R	I	R	R	R	7
XX-11	+	R	R	R	I	S	S	R	I	R	R	R	7
XX-13	+	R	R	R	I	S	S	R	I	R	R	R	7
XX-14	+	R	R	R	R	I	S	R	I	R	R	I	7
XX-16	+	R	R	R	R	S	S	R	I	R	R	R	8
XX-17	+	R	R	R	R	S	S	R	I	R	R	I	7
WS-1	+	R	R	R	R	S	S	R	I	R	R	R	8
WS-2	+	R	R	R	R	R	S	R	I	R	R	R	9
WS-3	+	R	R	R	R	S	S	R	I	R	R	R	8
WS-4	+	R	R	R	R	I	S	R	I	R	R	R	8
WS-5	+	R	R	R	I	S	S	R	I	R	R	R	7
WS-6	+	R	R	R	R	I	S	R	I	R	R	R	8
WS-7	+	R	R	R	R	I	I	R	I	R	R	I	7
WS-8	+	R	R	R	R	I	S	R	I	R	R	R	8
WS-9	+	R	R	R	R	S	R	I	R	R	R	R	9
WS-11	+	R	R	R	R	R	I	R	I	R	R	R	9
WS-12	+	R	R	R	R	R	S	R	I	R	R	I	8
WS-13	+	R	R	R	R	I	S	R	I	R	R	R	8
WS-14	+	R	R	R	R	I	S	R	I	R	R	R	8
WS-15	+	R	R	R	R	R	I	R	I	R	R	R	9
WS-16	+	R	R	R	R	R	S	R	I	R	R	R	9
WS-17	+	R	R	R	S	S	S	S	I	R	I	R	5
WS-18	+	R	R	R	I	S	S	R	I	R	R	I	6
PY-1	+	R	R	R	R	I	S	R	I	R	R	I	7
PY-2	+	R	R	R	I	S	S	R	I	R	R	R	7
PY-3	+	R	R	R	R	I	S	R	I	R	R	I	7
PY-4	+	R	R	R	I	I	S	R	I	R	R	I	6
PY-5	+	R	R	R	R	I	S	R	I	R	R	R	8
PY-6	+	R	R	R	R	I	I	R	I	R	R	R	8
PY-7	+	R	R	R	I	I	S	R	I	R	R	R	7
PY-9	+	R	R	R	R	S	S	R	I	R	R	I	7

Continued

Strain	*bla*$_{\text{CTX-M-G9}}$	Antibiotics											No. of drugs
		AMC	**AMP**	**CTX**	**FEP**	**GEN**	**KAN**	**TET**	**CIP**	**ERY**	**SXT**	**CHL**	
PY-10	+	R	R	R	R	I	S	R	I	R	R	R	8
PY-11	+	R	R	R	I	I	S	R	I	R	R	R	7
PY-12	+	R	R	R	R	R	S	R	I	R	R	I	8
PY-13	+	R	R	R	R	S	S	R	S	R	R	R	8
PY-14	+	R	R	R	I	I	S	R	I	R	R	R	7
PY-15	+	R	R	R	R	S	S	R	I	R	R	R	8
PY-16	+	R	R	R	R	I	S	R	I	R	R	R	8
Sum	74	75	75	75	48	17	1	63	5	72	66	41	

Note: Strains displayed in bold font were selected for whole-genome sequencing analysis. Different background colors represent different levels of drug resistance: red represents resistant, pink represents intermediate, and white represents susceptible."+" indicates positive PCR detection results for *bla*$_{\text{CTX-M-G9}}$. "-" indicates negative PCR detection results for *bla*$_{\text{CTX-M-G9}}$.

Abbreviations: LB=Lingbao City; LY=Luoyang City; JZ=Jiaozuo City; XX=Xinxiang City; PY=Puyang City; WS=water source in Zhengzhou City; Q=Qin River; YL=Yiluo River ; AMC=amoxicillin-clavulanic acid; AMP=ampicillin; CTX=cefotaxime; FEP=cefepime; GEN=gentamicin; KAN=kanamycin; TET=tetracycline; CIP=ciprofloxacin; ERY=erythromycin; SXT=sulfamethoxazole; CHL=chloramphenicol.

SUPPLEMENTARY TABLE S3. Genomic characteristics of *E. coli* ST6802 isolates from different sources.

Strain	ST	Source	ARG profiles	VFs	Plasmid replicons	GenBank accession number	References/ Database
E. coli ZB8C2M	6802	Chicken feces, Guangzhou, China	*bla*$_{\text{CTX-M-55}}$, *aac(3)*, *aadA*, *aph(3')*, *aph(6)*, *ARR*, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{TEM}}$, *cat*, *cmlA1*, *floR*, *dfrA*, *fosA3*, *mph*, *erm*, *qnr*, *sul*, *tet*	air, chuA, eilA, sitA, gad, kpsE, kpsMII_K5, terC, traT	IncFIB, IncFII, IncHI2, IncN, IncQ1, IncX1, p0111	SAMN14694452	NCBI
ESC_JA8833AA	6802	Wild animal, Silver Gull, Australia	*bla*$_{\text{CMY-2}}$, *bla*$_{\text{TEM-1C}}$, *sitABCD*	aslA, air, astA, chuA, cia, csgA, eilA, espY2, fdeC, fimH, gad, hha, hlyE, hra, kpsE, kpsMII_K5, nlpI, sitA, terC, traT, yehA, yehB, yehC, yehD, yghJ	IncI1-I(Alpha)	SAMN09866906	EnteroBase
ESC_DB8730AA	6802	Environment, wastewater, United States	*sitABCD*	aslA, air, astA, chuA, csgA, eilA, espY2, fdeC, fimH, hha, hlyE, kpsE, kpsMII_K5, nlpI, sitA, terC, yehA, yehB, yehC, yehD, yghJ	IncI1-I(Alpha)	SAMN33583550	EnteroBase
ESC_NB8187AA	6802	Poultry, chicken, Canada	*sitABCD*, *tetA*	aslA, air, chuA, cma, csgA, cvaC, eilA, espY2, fdeC, fimH, hha, hlyE, hlyF, iroN, iss, kpsE, kpsMII_K5, nlpI, ompT, sitA, terC, traJ, traT, yehA, yehB, yehC, yehD, yghJ	ColpVC, IncFIB, IncFII	SAMN43563114	EnteroBase
ESC_NB8461AA	6802	Food, chicken, Canada	*bla*$_{\text{CMY-2}}$, *sitABCD*, *tet(B)*	aslA, air, anr, chuA, cia, csgA, cvaC, eilA, espY2, etsC, fdeC, fimH, gad, hha, hlyE, hlyF, iroN, iss, iucC, iutA, kpsE, kpsMII_K5, mchF, nlpI, ompT, sitA, terC, traJ, traT, tsh, yehA, yehB, yehC, yehD, yghJ	Col440II, IncFIA, IncFIB, IncFIC, IncI1-I, p0111	SAMN43563208	EnteroBase
ESC_NB8748AA	6802	Poultry, poultry, Canada	*bla*$_{\text{CMY-2}}$, *sitABCD*, *tet(B)*	aslA, air, anr, chuA, cia, csgA, cvaC, eilA, espY2, etsC, fdeC, fimH, gad, hha, hlyE, hlyF, iroN, iss, iucC, iutA, kpsE, kpsMII_K5, mchF, nlpI, ompT, sitA, terC, traJ, traT, tsh, yehA, yehB, yehC, yehD, yghJ	Col440II, IncFIA, IncFIB, IncFIC, IncI1-I, p0111	SAMN43564917	EnteroBase
12 *E. coli* isolates	6802	Anaerobic digestion systems of pig manure, China	*bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{TEM-176}}$, *aac(3)-IV*, *aph(4)-Ia*, *sul1*, *sul2*, *dfrA14*, *tet(A)*, *fosA3*, *mph(A)*, *floR*, *mdf(A)*, *qnrS1*	air, chuA, eilA, gad, iss, kpsE, sitA, terC	IncFIB, IncHI2, IncHI2A, IncX1	PRJNA777386	NCBI
21 *E. coli* isolates	6802	River water and water source, China	*bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{TEM-176}}$, *aac(3)-IV*, *aph(4)-Ia*, *sul1*, *sul2*, *dfrA14*, *tet(A)*, *bleO*, *fosA3*, *mph(A)*, *floR*, *qnrS1*	aslA, air, chuA, csgA, eilA, espY2:000868321, fdeC, fimH, gad, hlyE, iss, kpsE, nlpI, sitA, terC, yehA, yehB, yehC, yehD	IncFIB, IncHI2, IncHI2A, IncX1	This study	This study

Abbreviation: ST=sequence type; ARG=antibiotic resistance gene; VFs=virulence factors.

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