Characterization and Comparative Genomics Analysis of IncFII Multi-Resistance Plasmids Carrying bla_{CTX−M} and Type1 Integrons From Escherichia coli

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This research aimed to investigate the presence and transferability of the extended-spectrum β-lactamase resistance genes to identify the genetic context of multi-drug resistant (MDR) loci in two Escherichia coli plasmids from livestock and poultry breeding environment. MICs were determined by broth microdilution. A total of 137 E. coli resistant to extended-spectrum β-lactam antibiotics were screened for the presence of the ESBL genes by PCR. Only two E. coli out of 206 strains produced carbapenemases, including strain 11011 that produced enzyme A, and strain 417957 that produced enzyme B. The genes were bla_{KPC} and bla_{NDM}, respectively. The plasmids containing bla_{CTX−M} were conjugatable, and the plasmids containing carbapenem resistance gene were not conjugatable. Six extended-spectrum β-lactamase resistance genes were detected in this research, including bla_{TEM}, bla_{CTX−M}, bla_{SHV}, bla_{OAX−1}, bla_{KPC}, and bla_{NDM}, and the detection rates were 94.89% (130/137), 92.7% (127/137), 24.81% (34/137), 20.43% (28/137), 0.72% (1/137), and 0.72% (1/137), respectively. Two conjugative IncFII multi-resistance plasmids carrying bla_{CTX−M}, p11011-fosA and p417957-CTXM, were sequenced and analyzed. Both conjugative plasmids were larger than 100 kb and contained three accessory modules, including MDR region. The MDR region of the two plasmids contained many antibiotic resistance genes, including bla_{CTX−M}, mph (A), dfrA17, aadA5, sul1, etc. After transfer, both the transconjugants displayed elevated MICs of the respective antimicrobial agents. A large number of resistance genes clusters in specific regions may contribute to the MDR profile of the strains. The presence of mobile genetic elements at the boundaries can possibly facilitate transfer among Enterobacteriaceae through inter-replicon gene transfer. Our study provides beta-lactam resistance profile of bacteria, reveals the prevalence of β-lactamase resistance genes in livestock and poultry breeding environment in Zhejiang Province, and enriches the research on IncFII plasmids containing bla_{CTX−M}.

Keywords: plasmid, β-Lactam antibiotics, bla_{CTX−M}, MDR, mobile elements
INTRODUCTION

Escherichia coli is the most common conditional pathogen in the culture environment, which can cause varying degrees of infection in livestock and poultry. These Escherichia coli strains, which contain a large number of antibiotic resistant genes, accumulate in humans and farmed animals, and are released into the environment via feces. They continue to contaminate the human food environments (e.g., agricultural, aquatic, and livestock products) as well as livestock and poultry breeding environment (Sajjad et al., 2011; Chajecka-Wierzchowska et al., 2014; Yano et al., 2014; Blaak et al., 2015; Liao et al., 2015). Finally, multidrug-resistant E. coli accumulate in the human body through the food chain (Wellington et al., 2013).

Extended-spectrum β-lactamase (ESBLs) is currently considered one of the world’s major public health problems. In Enterobacteriaceae, E. coli causes the most infections and is the main species producing ESBL (Bradford, 2001). Many genes are able to produce ESBLs that can hydrolyze cephalosporins, penicillin, and monocyclic β-lactam antibiotics aztreonam (Kanayama et al., 2010). β-lactamases have been divided into four major classes (A, B, C, and D) according to the amino acid similarity. β-lactamases of classes A, C, and D are serine β-lactamases, whereas those of class B are metallo-β-lactamases (Paterson and Bonomo, 2005). Initially, the most prevalent ESBLs were TEM or SHV variants possessing amino acid substitutions (Sivaraman et al., 2021). βlactam-M is one of the most important ESBL genes, which has been found in several conjugative plasmids of Enterobacteriaceae (Abera et al., 2016; Abraham et al., 2018). IncF plasmids are usually low copy number plasmids, > 100 kb in size, and typically carry fused replicons with multiple replication initiation genes (Laura et al., 2010). The multi-replicon status is described as a means by which a plasmid with a narrow host range can replicate in a wide host range. Integrons were initially discovered because they are often associated with drug-resistant genes (Stokes and Hall, 1989). Class 1 integrons are the most frequently identified integron classes within clinical environments. This class was the first integron found, which mainly distributed on plasmids of Gram-negative bacteria (Goldstein et al., 2001). βlactam-M gene was used as a query for BLAST search in the NCBI nucleotide sequence database, and it was found that it existed mainly in Enterobacteriales. However, it is still unknown how frequently the gene occurs in the E. coli in Zhejiang Province, and the number of related IncFII plasmids containing βlactam-M is not very high in the NCBI database. Moreover, the genetic background of βlactam-M also needs to be further studied.

In this study, the relative abundance of the known ESBL genes in E. coli in livestock and poultry breeding environment was determined. This research provided beta-lactam resistance profile of bacteria isolated from livestock and poultry breeding environment in Zhejiang Province. Our study suggests that resistance to beta-lactam is too high in E. coli. Therefore, local farmers can possibly reduce the use of specific beta-lactam antibiotics, making a particular contribution to the reduction of local antibiotic resistance. Furthermore, two IncFII plasmids harboring βlactam-M along with other resistance genes were analyzed to clarify the basis for dissemination. This research enriched the study of plasmids containing βlactam-M resistance genes in the NCBI database.

MATERIALS AND METHODS

Bacterial Strains

A total of 206 non-duplicate E. coli isolates were collected from 6 livestock and poultry farms in Zhejiang Province, China, 2018. A list of collected samples from feces, urine, soil, and surrounding water environment was presented in Supplementary Table 1. Colonies with a green metallic sheen growing on Eosin Methylene Blue Agar plates were initially considered E. coli strains. The isolates were identified by VITEK-2 Compact and 16S rRNA gene identification using the universal 16s primers 27F and 1492R (Lane, 1991). EC600 served as the recipient strain in conjugal transfer experiments. EC600 was resistant to rifampicin, but sensitive to ampicillin, carbapenem, quinolones, and sulfonamides.

Antimicrobial Susceptibility Testing

E. coli strains were evaluated based on the size of the inhibition zones and classified as resistant, intermediate, or susceptible according to the CLSI criteria for Enterobacteriaceae as the breakpoints were shown in Supplementary Table 2 (Institute CLSI, 2018). E. coli strains exhibiting resistance to three or more classes of antimicrobials were considered to be multi-drug resistant (MDR). E. coli ATCC 25922 served as the control.

PCR Analysis

E. coli strains were screened for the presence of the ESBLs gene by PCR using the primers listed in Supplementary Table 3 (blaGES, blaKPC, blaSIM, blaNDM, etc.) and Supplementary Table 4 (blaCTX-M-1 universal, blaCTX-M-9, blaCTX-M-2, blaCTX-M-8, etc.), as described previously (Hong et al., 2012). PCR amplification consisted of denaturation at 95°C for 5 min followed by denaturation at 94°C for 30 s, annealing at their respective annealing temperature for 30 s, and polymerization at 72°C for 40 s for a total of 30 cycles, and a final extension at 72°C for 10 min. All the PCR products were subjected to Sanger sequencing.

The Enzyme-Producing Activity of Bacteria

The enzyme-producing activity was detected by a modified Carba NP method. The carbapenems producing types of Enterobacteriaceae mainly include A, B, and D, representing KPC, NDM, and OXA-48, respectively. According to the characteristics of the above enzymes, an improved Carba NP detection method was designed to detect specific enzyme production types. Class A carbapenems were at least partially inhibited by tazobactam, while class B carbapenems (metal-β-lactamases) were inhibited by divalent cationic chelators such as EDTA. No chemical inhibitors were available for class D carbapenems. The formulas for each system and the expected outcomes of different enzyme
production types were shown in Supplementary Table 5. 1705 was the control of enzyme A, and the well of substrate II and substrate IV turned yellow. 2146 was the control of enzyme B, and the wells of substrate II and substrate III turned yellow.

Conjugal Transfer Experiments
Conjugal transfer experiments were performed with rifampin-resistant E. coli EC600 used as a recipient and each of the blaCTX-M-positive 11011 and 417957 isolates as a donor. Brain Heart Infusion (BHI) was supplemented with 2.5 mg/mL rifampicin and 200 µg/L ampicillin to screen transconjugants. After incubation at 37°C for 24 h, colonies growing on these selective plates were further confirmed by antimicrobial sensitivity experiments and VITEK-2 Compact.

Sequencing and Analysis
The two conjugatable plasmids of the transconjugants 11011-EC600 and 417957-EC600 were sequenced by the Illumina MiSeq platforms. The two plasmids DNA was extracted from the transconjugants 11011-EC600 and 417957-EC600 using the Qiagen Large-Construct Kit. After extraction, the mate-pair library was constructed. Then MiSeq (Illumina, CA, United States) was used to sequence the plasmids, and Newbler 2.6 was used to assemble the Illumina read sets straight off the MiSeq. Quality control and removing low-quality data were performed with TrimMomatic 0.36 (Bolger et al., 2014). Gapfiller V1.11 is used to fill gaps (Nederbragt, 2014). Cytoscape was used to splice the sequence to obtain the final cyclized plasmids. The plasmid sequences were submitted to Rast, Genemarks, Glimmer, and Prodigal library for preliminary gene prediction then submitted to ISFinder, Integrall, ResFinder, and TN Number Registry for mobile elements (Boetzer and Pirovano, 2012). Gene organization diagrams were drawn by running a gene alignment program in Perl language (Supplementary File) with Ubuntu 18.04 LTS1 and Inkscape 0.48.1.2 The complement sequences of the two plasmids p417957-CTXM and p11011-fosA have been submitted in GenBank under accession numbers NZ_MH454107.1 and NZ_MG764548.1, respectively.

RESULTS
Detection of the blaCTX-M Gene in Escherichia coli Strains
In this study, 206 strains of E. coli were identified by VITEK-2 Compact and the amplification of 16S rRNA gene

| Antimicrobial agent | Susceptible (strain) | Intermediate (strain) | Resistant (strain) |
|--------------------|----------------------|----------------------|-------------------|
| Cefazolin           | 35                   | 34                   | 137               |
| Ceftriazone         | 131                  | 0                    | 75                |
| Imipenem            | 201                  | 0                    | 5                 |
Replicon repA2-6-1-4 of the Backbone Area

Comparing the sequences in the database revealed that both plasmids p11011 fosA and p417957 CTXM belonged to multi-replicon IncFII plasmids, which both contained the main replicon repA2-6-1-4 (Figure 2). The sequence of this gene was identical to the replication initiation region of the reference plasmid pHN7A8. p417957 CTXM also contained a repFIA replicon and the backbone area, including core butler genes (ccdB, vagCD, sopAB, ygcA, klcA, and sst) responsible for maintaining the stability of the plasmid. In addition to the main replicon repA2-6-1-4 and the auxiliary replicon repFIA, plasmid p11011 fosA also contained a replicon repA2-6-1-4 structure. The sequence of this structure was different from that of pHN7A8. Therefore, the plasmid was likely to be hybridized by two IncFII plasmids. The backbone area of p417957 CTXM had a conjugal transfer region similar to the reference plasmid pHN7A8, considering their nucleic acid alignment and expressed proteins. The plasmid p11011 fosA contained two conjugal transfer regions. Conjugal transfer proteins and flagella proteins promoted flagella production, which collectively facilitated horizontal transfer of plasmids between bacteria.

Analysis of Multi-Drug Resistant Region in p11011 fosA and p417957 CTXM

As shown in Figure 3, the MDR-1 region of plasmid p11011 fosA consists of the following mobile elements: IS15DI mph (A)-IS15DI unit [containing macrolide resistance gene mph (A)], AICEVChNep1_virable region III (containing floreneric resistance gene flrO, streptomycin resistance gene strAB, sulfonamides resistance gene sul2), IS15DI erm (B)-IS15DI unit [macrolide resistance genes erm (B)], Tn2 remnant, IS26-fosA-IS26 unit (containing fosfomycin resistance gene fosA3), and IScep1 blaCTX M 55-orf77 transposition unit (containing β-lactam antibiotic blaCTX M 55). The MDR region of pHN7A8

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**TABLE 3** Major features of plasmids analyzed.

| Type         | Plasmid Name       |
|--------------|--------------------|
| Sequence Type| p11011 fosA        |
| Allele       | FIA_4, FII_36, FIA_33 |
| The total length (kb) | 217.4, 128.4, 128.4 |
| The number of ORF | 275, 151, 152 |
| Average GC content (%) | 53.92, 53.82, 54.52 |
| Accessory modules | MDR-1 region, rap-region, arc-related region |

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**TABLE 2** The result of MIC by using VITEK-2.

| Category | Antimicrobial agent | MIC (mg/L)/antimicrobial susceptibility |
|----------|---------------------|----------------------------------------|
|          |                    | 11011 | 11011 fosA-EC600 | 417957 | 417957 CTXM-EC600 | EC600 |
| Penicillin | Ampicillin         | ≥32/R | ≥32/R           | ≥32/R | ≥32/R           | 4/S |
| Penicillin/Subactam | ≥32/R | ≥32/R | ≥32/R           | ≥32/R | ≥32/R           | 4/S |
| Piperacillin | ≥128/R | ≥128/R | ≥128/R           | ≥128/R | ≤4/S |
| Piperacillin/Tazobactam | ≥128/R | ≥128/R | ≥128/R           | ≥128/R | ≤4/S |
| Cephalosporins | Cefazolin       | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | ≤4/S |
|                  | Cefuroxime        | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | 1/S |
|                  | Cefuroxime Axetil | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | 1/S |
|                  | Ceftriaxone       | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | 1/S |
|                  | Ceftizime         | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | 1/S |
| Monobactams | Aztreonam         | ≥64/R | ≥64/R           | ≥64/R | ≥64/R           | 1/S |
| Carbapenems | Imipenem          | ≥16/R | ≤1/S            | ≥16/R | ≤1/S            | 1/S |
|            | Meropenem         | ≥16/R | ≤0.25/S         | ≥16/R | ≤0.25/S         | ≤0.25/S |
| Aminoglycosides | Amikacin        | ≤2/S | ≤2/S            | ≤2/S | ≤2/S            | 2/S |
|                  | Gentamicin        | ≤1/S | ≤1/S            | ≤1/S | ≤1/S            | 1/S |
|                  | Tobramycin        | ≤1/S | ≤1/S            | ≤1/S | ≤1/S            | 1/S |
| Quinolones | Ciprofloxacin     | ≥4/R  | ≤0.25/S         | ≥4/R  | ≤0.25/S         | ≤0.25/S |
|                  | Levofloxacin      | ≥8/R  | ≤0.25/S         | ≥8/R  | ≤0.25/S         | 0.5/S |
| Nitrofurantoin | Nitrofurantoin   | 128/R | ≤16/S           | 128/R | ≤16/S           | 16/S |
| Sulfonamides | Trimethoprim/Sulfamethoxazole | ≥320/R | ≥20/S           | ≥320/R | ≥20/S           | 20/S |

S, Susceptible; R, Resistant; I, Intermediate.
Figure 1 | The complete sequence diagram of p11011-fosA and p417957-CTXM. Note: In the figure, the innermost ring represents \((\text{G-C})/(\text{G+C})\); the blue ring indicates GC content, and the outward depression indicates that the GC content is higher than the mean value. Black area in the outer circle represents the backbone area and gray area represents the accessory modules. The outermost circle shows the distribution of genes represented by colored arrows in the plasmid.

Table 4 | Antibiotic resistance genes in sequenced plasmids.

| Plasmid     | Resistance gene | Resistance phenotype | Nucleotide position | Region located   |
|-------------|-----------------|----------------------|---------------------|------------------|
| p11011-fosA | mph(A)          | Macrolides resistance| 17065-17970         | MDR-1 region     |
|             | floR            | Florfenicol resistance| 25301-26515         |                  |
|             | strAB           | Streptomycin resistance| 27471-29110         |                  |
|             | sul2            | Sulfonamides resistance| 29171-29986         |                  |
|             | emr(B)          | Macrolides resistance| 31936-32673         |                  |
|             | blaTEM-18       | β-lactam resistance  | 36041-36091         |                  |
|             | fosA3           | Fosfomycin resistance| 40467-40883         |                  |
|             | blCTX-M-55      | β-lactam resistance  | 43548-44423         |                  |
|             | tetAR           | Tetracycline resistance| 113327-115182       | MDR-2 region     |
|             | dflA17          | Trimethoprim resistance| 119457-119930       |                  |
|             | aadA5           | Aminoglycosides resistance| 120016-120849       |                  |
|             | sul1            | Sulfonamides resistance| 121309-122335       |                  |
|             | mph(A)          | Macrolides resistance| 127546-128451       |                  |
| p417957-CTXM| blCTX-M-55      | β-lactam resistance  | 7839-8714           | MDR-1 region     |
|             | aacC2           | Aminoglycosides resistance| 11222-12083         |                  |
|             | tnrB            | Tunicamycin resistance| 12095-12637         |                  |
|             | dflA17          | Trimethoprim resistance| 27180-27653         |                  |
|             | aadA5           | Aminoglycosides resistance| 27784-28572         |                  |
|             | sul1            | Sulfonamides resistance| 29032-29958         |                  |
|             | mph(A)          | Macrolides resistance| 35269-36174         |                  |

was similar to the MDR-1 region of p11011-fosA, which contained fosfomycin resistance genes mediated by IS26 and aminoglycoside resistance genes mediated by Tn2.

The MDR region of p417957-CTXM contained integron In54, IS26-mph (A)-mrx-mphR (A)-IS6100 unit, ISEcp1-blaCTX-M-55-orf477 transposition unit (containing β-lactamase...
resistance gene \( \text{bla}_{\text{CTX-M-55}} \), and \( \text{aacC2-tmrB} \) region (containing aminoglycoside resistance gene \( \text{aacC2} \) and tunicamycin resistance gene \( \text{tmrB} \)).

The multi-drug resistance region MDR-2 of the plasmid p11011-fosA was composed of the following mobile elements in sequence: Tn5403, \( \Delta \text{Tn1721} \) (containing tetracycline resistance gene \( \text{tetAR} \)), IS26, In54 (containing trimethoprim resistance gene \( \text{drfA17} \), sulfonamides resistance gene \( \text{sul1} \)), IS26-\( \text{mph(A)} \)-\( \text{mphR} \)-(A)-IS6100 unit (containing macrolide resistance genes), Tn3 family transposon remnant, Iron acquisition gene cluster, IS1R and IS1A (Figure 4). The typical structure of type I integron included IRI, 5 prime -CS, GCA, 3 prime -CS, Tn402 tni module, and IRT. In54 was a type I integron in both plasmids p11011-fosA and p417957-CTXM. The 5 prime -CS region contained integrase \( \text{IntI1} \) responsible for the integration of capture resistance genes, and downstream of \( \text{IntI1} \) contained trimethoprim resistance gene \( \text{drfA17} \) and aminoglycoside resistance gene \( \text{aadA5} \). In addition, the 3 prime -CS region also contained sulfonamides resistance gene \( \text{sul1} \). Moreover, the \( \text{tni} \) region of In54 was replaced by the \( \text{chrA} \) region, which was responsible for chromate particle transport. \( \text{ChrA} \) region was usually located in IS26-\( \text{mph(A)} \)-\( \text{mphR} \)-(A)-IS6100 unit.

DISCUSSION

In this study, \( \text{E. coli} \) strains isolated from the livestock and poultry breeding environment in Zhejiang Province showed high-level resistance (137/204) to extended-spectrum \( \beta \)-lactam antibiotics (Cefazolin, Ceftazidime, and Imipenem). These antibiotics are prevalently used for the treatment of infections caused by \( \text{Enterobacteriaceae} \). Essentially, all ESBL producers demonstrated
multi-drug resistance profiles and carried at least one ESBL resistance gene, especially \textit{bla}TEM (94.89%) and \textit{bla}CTX-M (92.7%). Among all the \textit{E. coli} strains investigated in the current study, the prevalence of ESBL producers (67.2%) is quite close to that previously reported in Henan Province (60.8%) from 2007 to 2008 (Yuan et al., 2009). However, it is still lower than those reported in northeast China (Jilin, Liaoning, and Heilongjiang Provinces (100%) from 2011 to 2013 (Tong et al., 2015).

In this study, six extended-spectrum β-Lactamase resistance genes were detected in all antibiotic resistant strains, and \textit{bla}CTX-M was the second common resistance gene. However, Tong et al. (2015) found that CTX-M-type ESBL resistance genes were the most common genotype of \textit{E. coli} isolated from chickens in China. These contradictories may be due to different regions where the strains were isolated and various poultry species. However, it might be related to mobile genetic elements such as plasmids and transposons as well. Previous reports showed that CTX-M-encoding genes in \textit{E. coli}, isolated from food animals, have a high diversity in China, and CTX-M-14 was the most widespread CTX-M enzyme, followed by CTX-M-55 (Zheng et al., 2012). \textit{bla}CTX-M-55 has been widely reported in food-producing animals and pets in China (Rao et al., 2014). In this study, the strains carrying CTX-M-55-type ESBL showed different MDR profiles. In order to further study the mobile genetic elements related to CTX-M gene transfer, we selected two plasmids of two strains for sequencing and analysis.

We identified two conjunctive IncFII multi-resistance plasmids carrying \textit{bla}CTX-M and analyzed the genetic context of \textit{bla}CTX-M and MDR region. Most CTX-M genes were identified to be associated with IncFII or IncI1 plasmids (Carattoli, 2009; Cao et al., 2011). The most common IncFII type plasmid was F2:A-B-, which had also been reported to be linked to \textit{bla}CTX-M genes in \textit{Enterobacteriaceae} strains isolated from other regions (Laura et al., 2010; Kim et al., 2011). In the

![FIGURE 4](image-url)
current study, the IncFII plasmid p11011-fosA contains another ESBL resistance genes \( \text{bla}_{\text{TEM-18}} \) and fosfomycin resistance gene fosA3, while the other IncFII plasmid p47957-CTX don’t have the two resistance genes. fosA3 is frequently co-transferred with \( \text{bla}_{\text{CTX-M}} \) genes, and the dissemination of fosA3 may be related to the co-selection of cephalosporins (Hou et al., 2012). In previous epidemic plasmids, it was observed that the fosA3 gene is frequently co-transferred with \( \text{bla}_{\text{CTX-M-14}}, \text{bla}_{\text{CTX-M-55}}, \text{bla}_{\text{CTX-M-65}}, \text{floR}, \text{and rmtB} \) genes. Thus, reducing the total use of antibiotics, especially cephalosporins, in livestock and poultry breeding environment contributes to controlling the dissemination of plasmid-mediated fosfomycin and \( \beta \)-Lactamase resistance genes (Yang et al., 2014).

In this study, we found that \( \text{bla}_{\text{CTX-M-55}} \) was located on the IS\text{Ecp1-}\text{bla}_{\text{CTX-M-55-orf477}} \) transposition unit, which was similar to previous studies (Zheng et al., 2012). IS\text{Ecp1-}\text{bla}_{\text{CTX-M-55-orf477}} transposition unit is a significant element in the propagation of \( \text{bla}_{\text{CTX-M-55}} \). IS\text{Ecp1} is bounded by 14-bp inverted repeats, with \( \text{IR}_L \) at the left end of the transcription direction of the transposase gene and \( \text{IR}_R \) at the right end. In addition, IS\text{Ecp1} uses \( \text{IR}_R \) in conjunction with alternative sequences like these \( \text{IR} \) to mobilize adjacent regions, forming a 5-bp direct repeat (DR) on transposition (Poirel et al., 2005; Wachino et al., 2006). The MDR region in pHN7A8 and p11011-fosA is mainly formed by the insertion of mobile elements. The insertion of Tn2\text{-rmtB} element into the \( \text{insB} \) of IS\text{IR} results in only 214 bp residue in \( \text{insB} \). The insertion of the region of 9.4 kb \( \Delta \text{Tn6377}, \text{IS26-fosA3}-\text{IS26} \) unit (Ho et al., 2013) and \text{IS1294} between \( \text{tnpA} \) and \( \text{tnpR} \) of Tn2 leads to the loss of \text{res} site of Tn2. Tn2\text{-rmtB} element (Dai et al., 2008) and the variant of this element have been widely found in \textit{Enterobacteriaceae}. Tn6377 was originally identified on the plasmid pKP96 (Shen et al., 2008) in \textit{Klebsiella pneumoniae}. The transposon was formed by inserting Tn\text{1722} (Zong et al., 2011) into the IS\text{Ecp1-}\text{bla}_{\text{CTX-M-65-IS903D}} \) unit of the Tn3 family. Tn6377 in pHN7A8 is interrupted at both terminals by IS\text{26}.

The MGEs, such as transposons, play an essential role in the rapid spread of AMR by horizontal gene transfer due to the selective pressure imposed by antibiotics (Snesrud et al., 2018). Tn5403 found in MDR-2 region in plasmid p11011-fosA did not contain antibiotic resistance genes and was first identified in the plasmid of \textit{Klebsiella pneumoniae} (Rinkel et al., 1994). Another transposon Tn1721 belongs to the transposon of the Tn21 family and has three 38 bp repeats and two transposase genes \( \text{tnpA} \) with the same sequence. There are \( \text{tnpA}, \text{tnpR}, \text{res} \) sites, and the gene \( \text{mcp} \) that encodes methyl-chemotactic proteins at one terminal, with 38 bp reverse repeats on both sides of this terminal. This terminal is named Tn1722 and can be moved independently. The rest of Tn1721 contains the tetracycline resistance gene \( \text{tet}A \). Although Tn1721 in the MDR-2 region of p11011-fosA is interrupted by IS\text{26} and Tn5403, the key tetracycline resistance gene in this transposon remains intact. IS\text{26-mph} \( \text{(A)}-\text{mrx-mphR} \text{(A)}\)-IS\text{6100} unit is the carrier element for common macrolide resistance genes, which are usually located on resistant plasmids in \textit{E. coli} (Partridge, 2011).

CONCLUSION

The presence of multiple mobile elements in a \( \text{bla}_{\text{CTX-M}} \)-carrying multi-resistance plasmid makes it flexible. These elements aid its persistence and dissemination among \textit{E. coli} and potentially other Gram-negative bacteria. This study demonstrated the abundance of \( \beta \)-lactamase resistance genes in livestock and poultry breeding environment in Zhejiang Province. It also upgraded the research on IncFII plasmids containing \( \text{bla}_{\text{CTX-M}} \) antibiotic resistance genes in the NCBI database. This research also provided \( \beta \)-lactam resistance profile of bacteria isolated from farms in Zhejiang Province, indicating that local farmers can possibly reduce the use of specific \( \beta \)-lactam antibiotics to reduce the evolution of multi-resistant strains.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/nuccore/NZ_MH454107.1 and https://www.ncbi.nlm.nih.gov/nuccore/NZ_MG764548. 1NCBI, GenBank accession numbers NZ_MH454107.1 and NZ_MG764548.1.

AUTHOR CONTRIBUTIONS

WZ: visualization, data curation, software, and writing—original draft preparation. EZ: conceptualization, methodology, formal analysis, and writing—original draft preparation. JZ: visualization and investigation. ZH: software and writing—review and Editing. YZ: software and validation. JH: formal analysis and resources. DQ: conceptualization, resources, supervision, and writing—review and Editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.753979/full#supplementary-material
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