Characterization of Beta-Lactamases in Bloodstream-Infection Escherichia coli: Dissemination of bla_{ADC-162} and bla_{CMY-2} Among Bacteria via an IncF Plasmid

Linlin Xiao{1,2,3,4}†, Xiaotong Wang{3}†, Nana Kong{3}, Long Zhang{3}, Mei Cao{3}, Muzhen Sun{3}, Quhao Wei{1,2,3}* and Weiwei Liu{4,5}*

1 Department of Laboratory Medicine, Affiliated Sixth People’s Hospital South Campus, Shanghai University of Medicine & Health Sciences, Shanghai, China, 2 Department of Laboratory Medicine, Southern Medical University Affiliated Fengxian Hospital, Shanghai, China, 3 Department of Laboratory Medicine, Affiliated Fengxian Hospital, Anhui University of Science and Technology, Anhui, China, 4 Central Laboratory, Department of Laboratory Medicine, Shanghai Tenth People’s Hospital, Tongji University, Shanghai, China, 5 Department of Laboratory Medicine, Shanghai Skin Disease Hospital, Tongji University, Shanghai, China

Objectives: To describe the molecular characteristics of beta-lactamases in bloodstream-infection Escherichia coli isolated from elderly patients, and to determine the genotypic patterns of bla_{CMY-2} and bla_{ADC-162}.

Methods: A total of 50 bloodstream-infection E. coli isolates were obtained from patients aged > 50 years at Shanghai Sixth People’s Hospital South Campus during 2015–2018. The isolates were subjected to beta-lactamase detection using phenotypic and molecular methods. Beta-lactamase genes were verified by sequencing and the phylogenetic relationships of the isolates were analyzed by multilocus sequence typing (MLST). The transferability of plasmids carrying bla_{CMY-2} and bla_{ADC-162} genes was verified by conjugation experiments and plasmid replicon typing.

Results: Eight beta-lactamase subtypes were detected in 50 isolates of bloodstream-infection E. coli. bla_{TEM-1} (21/50) was the most common beta-lactamase gene, followed by bla_{CTX-M-14} (8/50), bla_{OXA-27} (5/50), bla_{CTX-M-27} (3/50), bla_{CTX-M-65} (1/50), bla_{ADC-162} (1/50), and bla_{CMY-2} (1/50). Of these, bla_{ADC-162} (ST95-A) and bla_{CMY-2} (ST95-B2) have not previously been reported in bloodstream-infection E. coli. In 21 isolates, beta-lactamase genes were located on conjugative plasmids belonging to incompatibility groups FrepB (n = 7), FIA (n = 1), FIC (n = 2), K (n = 8), N (n = 1), and I (n = 1), and bla_{CTX-M} was associated with the common elements ISEcp1, IS903, and IS26, but with special sequences (region V, region Y, and region W) for ISEcp1 in 14 isolates.

Conclusion: To the best of our knowledge, this study provides the first molecular characterization of beta-lactamase genes in E. coli isolated from the bloodstream in
INTRODUCTION

*Escherichia coli* is commonly isolated from clinical bloodstream infections. It is referred to as extraintestinal pathogenic *E. coli* (Hung et al., 2019) and is usually multidrug-resistant, potentially leading to sepsis and even death of infected patients (van der Mee-Marquet et al., 2015). Antibiotic selection as a result of the extensive clinical application of broad-spectrum antibiotics, especially third-generation cephalosporins, has led to the generation of drug-resistant bacteria (Baron et al., 2014). Bloodstream infection by multidrug-resistant *E. coli* thus presents difficulties in clinical treatment and has become an important public health problem (Bartoletti et al., 2014). The main resistance mechanism of Gram-negative bacteria such as *E. coli* involves the production of a variety of hydrolytically active beta-lactamases, from broad- to extended-spectrum enzymes, and the enzymatic hydrolysis profile and host range are constantly changing from chromosome-mediated to plasmid-mediated AmpC beta-lactamases (Du et al., 2002; Razazi et al., 2012).

The major beta-lactamate resistance genes in *E. coli* are currently members of the *bla* 

| CTX-M | TEM (sugE) | ADC | CMY-2 | ETS |
|-------|-----------|-----|-------|-----|

| Genotype | Description | Occurrence |
|----------|-------------|------------|
| CTX-M-1 | Extended-spectrum beta-lactamase | Commonly isolated from clinical bloodstream infection. |
| TEM-1    | Broad-spectrum beta-lactamase | Frequently found in *E. coli*. |
| ADC      | AmpC beta-lactamase | Widely distributed in *E. coli*. |
| CMY-2    | AmpC beta-lactamase | Often associated with multidrug resistance. |
| ETS      | Extended-spectrum beta-lactamase | Rarely observed in clinical isolates. |

The study also provides the first report of ISAb-1*-blaADC-162-trpA* and IS*Ecp1-blaCTX-M-14-†*S903-blaCMY-2-blaCTX-M-1*-ISAb-1*-blaADC-162-trpA* and IS*Ecp1-blaCTX-M-14-†*S903-blaCMY-2-blaCTX-M-1* in *E. coli*, and demonstrates IncF plasmid-mediated *blaADC-162* and *blaCMY-2* gene dissemination among bacteria.

**Keywords:** beta lactamase, bloodstream infection, *Escherichia coli*, extraintestinal pathogenic, AmpC

MATERIALS AND METHODS

**Bacterial Strains**

A total of 1242 *E. coli* isolates were recovered from patient samples at Shanghai Sixth People’s Hospital South Campus, China, during 2015–2018. Among these, 50 strains of bloodstream-infection *E. coli* were isolated from elderly patients and further characterized with regard to extended-spectrum beta-lactamase (ESBL) and *ampC* genes. *E. coli* ATCC25922, J53, and *E. coli* DH5α were maintained in our laboratory.

**Antimicrobial Susceptibility Testing**

Antibiotic susceptibility was determined by disk diffusion or broth dilution, using *E. coli* ATCC25922 as a control strain. The tested antibiotics included: amikacin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole, chloramphenicol, meropenem, imipenem, ceftriaxone, ciprofloxacin, levofloxacín, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, cefazolin, ceftriaxone, and cefoxitin. The results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018).

**Phenotypic Characterization**

ESBL production was determined by double-disc synergy tests and confirmed by E-testing, using cefotaxime-cefotaxime-clavulanic acid and ceftazidime-ceftazidime-clavulanic acid strips, according to the CLSI guidelines. Similarly, phenotypic confirmation of plasmid-mediated AmpC production was
were mixed 1:4, and a 0.22 µl liquid medium without antibiotics. J53 and donor bacteria single colony was selected and enriched for 18 h in LB amplification system was 20 µL, containing 1 µL of genomic DNA template (> 50 ng/µL), 10 µL of Premix-rTaq PCR solution (TaKaRa, Japan), 0.4 µL of each primer (10 pmol), and 7 µL of distilled water. PCR was performed using a ProFlex Base Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Singapore). The template was denatured at 94°C for 4 min, followed by 35 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 40 s, with a final extension stage at 72°C for 5 min. The PCR product was verified by agarose gel electrophoresis and sequencing. All beta-lactamase gene sequencing results were aligned using the BLAST program.

Characterization of Beta-Lactamase Genes

The beta-lactamase genotype of each isolate was determined by polymerase chain reaction (PCR) amplification with specific primers for blaTEM, blaSHV, blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9, blaCTX-M-25, blaOXA-1, blaPER, blaSME, blaKPC, blaVIM, blaGES, blaVEB, bladHA, blaADC, blaDCC, blaGTT, and blaESC, with bacterially isolated DNA as an amplification template. The total volume of the PCR amplification system was 20 µL, containing 1 µL of genomic DNA template, 10 µL of Premix-rTaq PCR solution, 0.4 µL of each primer (10 pmol), and 7 µL of distilled water. PCR was performed using a ProFlex Base Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Singapore). The template was denatured at 94°C for 4 min, followed by 35 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 40 s, with a final extension stage at 72°C for 5 min. The PCR product was verified by agarose gel electrophoresis and sequencing. All beta-lactamase gene sequencing results were aligned using the BLAST program.

Conjugation Experiments

Conjugation experiments were performed using sodium azide-resistant E. coli J53 as a receptor. Transconjugants were selected on Luria-Bertani agar plates supplemented with sodium azide (200 µg/mL) (Sigma, Germany) and ampicillin (100 µg/mL). J53 and donor bacteria were resuscitated overnight, and a single colony was selected and enriched for 18 h in LB liquid medium without antibiotics. J53 and donor bacteria were mixed 1:4, and a 0.22 µm pore size filter was applied to the blood plate. Thereafter, 150 µL of the mixed bacteria solution was taken up and added to the filter membrane, and cultured overnight. The filter with the bacteria was then removed and washed in LB liquid medium, diluting 1:100 and 150 µL was then applied to the double-antibody plate, cultured overnight, and a single colony was picked for further experiments. The presence of the beta-lactamase gene in the transconjugant was examined using the primers given in Table 1, and the susceptibility of the strain was tested experimentally, as described above.

Plasmid Replicon Typing

Plasmid DNA was isolated from E. coli using a SanPrep Column Plasmid Mini-Preps Kit plasmid isolation system (Sangon Biotech, Shanghai, China) and stored at −20°C, according to the manufacturer’s instructions. Plasmid replicon typing of E. coli was performed using a multiplex PCR-based method with 18 pairs of primers, as described previously (Carattoli et al., 2005). The typing results were obtained by agarose gel electrophoresis and verified by sequencing.

### Table 1: Primers used for PCR amplification.

| Primer | Primer sequence (5′-3′) | References |
|--------|-------------------------|------------|
| TEMF   | TCGGGGAAGATGTGCG        | Velasova et al., 2019 |
| TEMR   | TGCTTAACAGTTAGAGCACC    | Velasova et al., 2019 |
| SHVF   | GCGTTTACCGCCCTCAGCAAG   | Velasova et al., 2019 |
| SHVR   | TTAQGTTGCGACTGTCGATCA   | Velasova et al., 2019 |
| PRE-F  | GTCGCCGATGAAGAACGCT     | Che et al., 2014 |
| PRE-L  | TCGGCTCTGACCTCGCTGA     | Che et al., 2014 |
| SME-F  | GAAAGACGATTTGATGGGAGAT  | Che et al., 2014 |
| SME-L  | TOCCCTAAGCGGGCGAGAAG    | Che et al., 2014 |
| CTX-M-1F | CAGAGATTTGGCGTCGATGA   | Xiao et al., 2019 |
| CTX-M-1R | GGCCCATGTTAAAAAATGACTG   | Xiao et al., 2019 |
| CTX-M-2F | TCTAGAGCGATTCCGCCTGCTGA | Xiao et al., 2019 |
| CTX-M-2R | CCGCGCGACGCGGAATACCTCC | Xiao et al., 2019 |
| CTX-M-8F | AACTTAGCGACGCGATTCA     | Xiao et al., 2019 |
| CTX-M-8R | CGAAGATGGCCTAGGCTTCT    | Xiao et al., 2019 |
| CTX-M-9R | GGCGATGTTGCGAAGAAGTGC  | Xiao et al., 2019 |
| CTX-M-9F | GGCGATGCGTGCAGAAGGAGGCA | Xiao et al., 2019 |
| CTX-M-25F | GACAGTATGGCGGCGGCTTGT | Xiao et al., 2019 |
| CTX-M-25R | AACCAGGATGTTGCTGAGCAG | Xiao et al., 2019 |
| OKA-1-F | GGACACGGATTTCAACTTTAAG | Che et al., 2014 |
| OKA-1-R | GACGCGGATTTCCGTGAAATG  | Che et al., 2014 |
| ADC-F  | GGTATGGCTGTCGTTGCTT    | This study |
| ADC-R  | CTAGACTTTGGCGAAGGAGT   | This study |
| KPC-F  | CGTCTAGCTTCTGGCTTGT    | This study |
| KPC-R  | CTGTCATCCTCGTTAGGCG    | This study |
| NDM-F  | GGTGGCGATCCTGTTTTCT   | This study |
| NDM-R  | CGAATGGCCTACGATCT      | This study |
| IMP-F  | GAATAGAAGCTTGCTTAYCTTC | This study |
| IMP-R  | GGTATTAAYAAAAAACACACC | This study |
| VIM-F  | GATGTTGTGTTGCTGCGATA   | This study |
| VIM-R  | CGAATGGCCTACGCGAGG    | This study |
| VEB-F  | GCGTTATTACCCGAGA       | This study |
| VEB-R  | GGCATGGCAGGGCTGT      | This study |
| GES-F  | GTTTTCTCAATGCTCOCAGC   | This study |
| GES-R  | TGGCATGACCAATGCGCTGTA  | This study |
| DHA-F  | AACCTTCAGGGCTGCTGGT   | This study |
| DHA-R  | CGTACAAGCATGCTCTGGCT   | This study |
| EBC-F  | TCGTAAAACGCGATGTTGCG   | This study |
| EBC-R  | CTTACACTCGGTGCTGAGT    | This study |
| ACC-F  | AACAGCCTCAAGCAGCTGGA  | This study |
| ACC-R  | TTGCGCGATCCGATCTGAGC   | This study |
| ATT-F  | TGGCGCAAGCTCGAGGCGA   | This study |
| ATT-R  | TTTCTCTGTCGCGCTGCTGC  | This study |
| chuA-F | GACQAACAAACAGCGTCAAAGAT | Clermont et al., 2000 |
| chuA-R | TGGCGGATAGGAAAAGACA   | Clermont et al., 2000 |
| yhaA-F | TGAAGTGTCGAGGAGCCTG   | Clermont et al., 2000 |
| yhaA-R | ATGGAAGATGGCTCTCCAAC  | Clermont et al., 2000 |
| tsp4E-C2-F | GAGTATGCGGCGATCCATCA | Clermont et al., 2000 |
| tsp4E-C2-R | CGCGCGAAACAAAGTATTACGA | Clermont et al., 2000 |
| IS26-F | TTACATTCCAAAAACTCTGCTTACC | This study |
| IS26-R | TTACATTCCAAAAACTCTGCTTACC | This study |
| SMEF1-F | CAAATATGCACCCCTCGTCAAC | This study |
| SME1-R | GGTAAATGAGCCAGCACTG   | This study |
| ORF477-R | CTTGCTTGCGTGCTGAATT  | This study |
| blc-R | TTAGGATACGCGATCGGAGA | This study |

1http://www.ncbi.nlm.nih.gov/BLAST

(Continued)
### Phylogenetic Grouping and Sequence Type (ST) Determination

The major phylogenetic group of each *E. coli* strain was determined by multiplex PCR, using the primers listed in Table 1 (Clermont et al., 2000). Multilocus sequence typing (MLST) was performed according to the Pasteur protocol\(^2\). Eight conserved housekeeping genes were amplified by PCR using primer sets for *dinB, icdA, pabB, polB, putB, trpA, trpB*, and *uidA*, and sequenced (Table 1). Allele profiles and ST assays were performed according to the *E. coli* MLST website\(^2\) protocol.

### RESULTS

#### Antimicrobial Susceptibility

Fifty bloodstream-infection *E. coli* isolates were obtained from elderly patients (average age, 70.86 years, range 51–92 years; 30% men, 70% women). Most isolates came from the intensive care unit (76%, 38/50), and others from the hematology medical ward (14%, 7/50) and other wards (10%, 5/50). *In vitro* antimicrobial susceptibility testing showed that most isolates were sensitive to gentamicin (32%), trimethoprim/sulfamethoxazole (38%), chloramphenicol (46%), ciprofloxacin (48%), levofloxacin (50%), ampicillin (82%), 

---

\(^2\)https://bigsdb.pasteur.fr/ecoli/

---

**TABLE 1** | Continued

| Primer   | Primer sequence (5′–3′) | References          |
|----------|------------------------|---------------------|
| sug-R    | GGGCTGTTCTCCTGAATGAT   | This study          |
| ISAba1-F | TGGCACTTGGCTTTAATAAACCCTG | This study         |
| tnpA-F   | CATCACCCGQATAAAGQACC   | This study          |
| tnpA-R   | GGCTCAAAGQCAATACQACC   | This study          |
| M9R-F    | GAATCTCTTCTTAGCTACCTAA | This study          |
| M9R-R    | CGTTACGTAAAGCTAGCTAGAT | This study          |

---

**FIGURE 1** | Schematic diagram of the genetic environment surrounding the beta-lactamase genes. *GenBank Accession numbers. (Sequences of PCR products were analyzed with BLAST to identify target homologous sequences and their GenBank accession numbers; https://blast.ncbi.nlm.nih.gov/Blast.cgi).
aztreonam (36%), cefepime (54%), cefotaxime (54%), ceftazidime (24%), ceftazolin (54%), ceftriaxone (21%), and cefoxitin (9%). Moreover, all of the isolates were sensitive to imipenem, amikacin, tobramycin, and meropenem.

**Genotypes of Beta-Lactamase Genes**

Of the 50 strains, 33 were positive for the beta-lactamase genotype [21 ESBL phenotypes/beta-lactamase genotype positive (Table 2); 12 beta-lactamase genotype positive/phenotype negative (Table 3)]. Of these 33 strains, one (3%, 1/33) contained three beta lactamase genes, eight (24%, 8/33) contained two beta lactamase genes, and 24 (73%, 24/33) contained only one beta lactamase gene. Among the beta-lactamase-producing strains, 21 isolates were positive for bla<sub>TEM</sub>, one for bla<sub>SHV</sub>, one for bla<sub>CTX</sub>, two for the bla<sub>CTX</sub>-M-1 group, 13 for the bla<sub>CTX</sub>-M-9 group, and five for the bla<sub>OXA</sub>-1 group. Nucleotide sequence analysis showed that 21 bla<sub>TEM</sub>-positive isolates carried bla<sub>TEM</sub>-1 and both bla<sub>CTX</sub>-M-1 group-positive isolates carried bla<sub>CTX</sub>-M-55. Of 13 bla<sub>CTX</sub>-M-9 group-positive isolates, one had bla<sub>CTX</sub>-M-65, three had bla<sub>CTX</sub>-M-27, and nine carried bla<sub>CTX</sub>-M-14. The only bla<sub>SHV</sub> sequenced was bla<sub>SHV</sub>-42, the only bla<sub>ADC</sub> sequenced was bla<sub>ADC</sub>-162, the only bla<sub>CTX</sub> sequenced was bla<sub>CMY</sub>-2, and all 5 bla<sub>OXA</sub>-1 group-positive isolates carried bla<sub>OXA</sub>-30. Meanwhile, all 50 isolates were negative for bla<sub>SME</sub>, bla<sub>PER</sub>, bla<sub>SHV</sub>, bla<sub>ACC</sub>, bla<sub>REC</sub>, bla<sub>CTX</sub>-M-2 group, bla<sub>CTX</sub>-M-8 group, and bla<sub>CTX</sub>-M-25 group (Tables 2, 3).

**Conjugation Experiments**

All 50 E. coli isolates were tested by conjugation and 39 strains were successfully transferred. Thirty-two of the 33 strains carrying beta-lactamase genes were successfully conjugated, but EC-35 was not successfully conjugated. Cefotaxime- and ceftazidime-resistance phenotypes were simultaneously transferred to sodium azide-resistant E. coli J53 recipients by conjugation in beta-lactamase-positive E. coli isolates, respectively.

The conjugate was detected by amplifying the beta lactamase gene primer and the results showed that most of the beta lactamase genes were transferred. However, four OXA-1-group (EC-4, EC-31, EC-35, EC-48), three TEM-group (EC-7, EC-32, EC-49), and four CTX-M-group (EC-4, EC-7, EC-27, EC-41) genes did not transfer and were not amplified in the corresponding transconjugants. Resistance to non-beta-lactamase antimicrobials was also co-transferred in some cases, in addition to the transfer of extended-spectrum cephalosporin resistance (Clasen et al., 2019). The characteristics of the E. coli J53 transconjugants carrying beta-lactamase genes are shown in Table 4.

**Plasmid Analysis**

Replicon-typing data for the clinical isolates carrying beta-lactamase genes (Table 2) revealed 10 different replicon types. Among these, two or more plasmid replicon types were detected simultaneously in the 19/21 strain, no plasmid replicon type was detected in the EC-4 strain, and only one plasmid replicon (IncFIC) was carried in the EC-38 strain. The IncF plasmid replicon type was the most common replicon among both types of isolates. Among the ESBL-phenotype-negative strains, plasmid replicon types were not detected in three strains, and the remaining nine strains included only four plasmid replicon types, but all contained IncK plasmid replicons (Table 3).

Plasmid replicon analysis was carried out in the 32 strains with beta-lactamase genes and successful conjugation. Interestingly, no plasmid replicons were detected in 12 transconjugants, and the other 20 transconjugants carried only one plasmid. A total of three plasmid replicon types were detected: IncF (9/19), IncK (9/19), and IncN (1/19) (Table 4). Nine strains were negative for both ESBL genotypes and phenotypes, with no conjugation result and no detection of any plasmid replicon type (Table 5).

**Genetic Environment of Beta-Lactamase Genes**

The genetic environment surrounding the beta-lactamase genes was verified. Overlapping PCR showed that all bla<sub>CTX</sub>-M groups were ISEcp1 upstream and orf477 or IS903 downstream. Both bla<sub>TEM</sub>-1 and bla<sub>OXA</sub>-30 were located between two transposes and bla<sub>ADC</sub>-162 was located between ISAba1 and tspA. Unexpectedly, the genetic environment surrounding EC-9 E. coli beta-lactamase genes was relatively unique; bla<sub>CTX</sub>-M-14 combined with bla<sub>CMY</sub>-2 through an insertion sequence (IS903), to constitute a composite structure of ISEcp1-bla<sub>CTX</sub>-M-14-IS903-bla<sub>CMY</sub>-2-blc-sug<sub>E</sub> (Figure 1).

**Phylogenetic Group and ST Designation**

Phylogenetic analysis of E. coli isolates carrying beta-lactamase genes showed that 24 belonged to virulent groups B2 (n = 19) and D (n = 5), and nine to non-toxic groups B1 (n = 3) and A (n = 6). Similarly, analysis of E. coli isolates with non-beta-lactamase genes showed that 13 belonged to virulent groups B2 (n = 9) and D (n = 4), and four belonged to non-toxic groups B1 (n = 3) and A (n = 1).

MLST analysis identified 12 unique STs among the 50 E. coli isolates (Tables 2, 3, 5): ST1 (n = 2), ST2 (n = 10), ST9 (n = 5), ST31 (n = 2), ST45 (n = 4), ST48 (n = 4), ST51 (n = 6), ST75 (n = 3), ST117 (n = 4), ST131 (n = 4), ST681 (n = 3), and ST730 (n = 3) strains.

**DISCUSSION**

In the present study, we characterized the ESBL and AmpC phenotypes and genotypes of beta-lactamase-producing E. coli blood isolates from patients in China from 2014 to 2018. These results provide the first extensive molecular report of plasmid-mediated ESBL and AmpC beta-lactamase-producing E. coli strains isolated from the bloodstream in elderly patients. Of the 50 E. coli isolates studied, 28 were positive for ESBL phenotypes, 33 were positive for beta-lactamase genes, 21 strains were positive for both, and 10 were negative for both. Thirteen (61.9%) strains had bla<sub>CTX</sub>-M-type ESBL genes and two (9.5%) produced ampC genes. bla<sub>CTX</sub>-M-type genes were more common than bla<sub>OXA</sub> and bla<sub>SHV</sub>, and ampC genes (bla<sub>ADC</sub> and bla<sub>CMY</sub>) were observed sporadically. A previous study reported that the bla<sub>TEM</sub> and bla<sub>SHV</sub> genes were the most prevalent while the detection
frequency of the CTX-M group was low among Escherichia coli isolated from China (Quan et al., 2017), however, the current study found a higher prevalence and variety of \( \text{bla}_{\text{CTX-M}} \) genes than previously reported (Shi et al., 2015; Zhao et al., 2016). The results of our study suggest that previous studies may have underestimated the frequency of \( \text{bla}_{\text{CTX-M}} \) gene transport in Escherichia coli samples isolated from blood, which may be related to increased selective pressure of cephalosporins in China.

The 50 Escherichia coli isolates in the current study carried a variety of \( \text{bla}_{\text{CTX-M}} \) genes (\( \text{bla}_{\text{CTX-M-14}}, 8/40; \text{bla}_{\text{CTX-M-27}}, 3/40; \text{bla}_{\text{CTX-M-55}}, 2/40; \text{bla}_{\text{CTX-M-65}}, 1/40 \)), and some also contained \( \text{bla}_{\text{TEM-1}} (21/40) \). Although these \( \text{bla}_{\text{CTX-M}} \) variants have previously been reported in Escherichia coli strains classified in many countries (Lambert et al., 2011), few studies have detected the \( \text{ampC} \) gene in bloodstream-infection Escherichia coli strains in China (Quan et al., 2017), and no previous studies have isolated Escherichia coli from blood samples from elderly patients. In particular, we detected and confirmed a case of the \( \text{ampC} \) gene \( \text{bla}_{\text{ADC-162}} \). Related studies to date have only detected the \( \text{ampC} \) gene \( \text{bla}_{\text{CMY-2}} \) in clinical bloodstream infections of Escherichia coli in

### Table 2: ESBL-positive bloodstream-infection Escherichia coli resistance phenotypes and genotypes.

| Strain | ESBL | MLST | PG | Plasmid replicon type | MIC | \( \text{bla} \) gene product | Transfer |
|--------|------|------|----|----------------------|-----|-----------------------------|----------|
|        |      |      |    |                      |     | ATM | FEP | CTX | CAZ | CZO | FOX |           |          |
| EC-2   | +    | ST51 | B2 | FIA,FIB,FrepB        | >16 | 16 | >32 | 16  | >16 | ≤  | OXA-30    | +         |
| EC-4   | +    | ST9  | A  | ND                   | 16  | 16 | >32 | 16  | >16 | 16 | OXA-30,CTX-M-65 | +         |
| EC-6   | +    | ST45 | D  | FIA,FIB,FrepB,N,K    | ≤ 2 | 16 | 16  | ≤ 1 | >16 | >8 | CTX-M-14   | +         |
| EC-7   | +    | ST48 | B2 | FIA,FIB1,K           | >16 | >16 | >32 | 8   | >16 | ≤  | TEM-1,CTX-M-55,CTX-M-14 | +         |
| EC-9   | +    | ST95 | B2 | FIC,K                | 8   | >16 | >32 | >16 | >32 | ≤  | CMY-2,CTX-M-14 | +         |
| EC-13  | +    | ST2  | B2 | FIA,FIB,FrepB,N,K    | >16 | >16 | >32 | >16 | >16 | ≤  | TEM-1      | +         |
| EC-20  | +    | ST730| B2 | FIB,FrepB,K          | 4   | >16 | >32 | ≤ 1 | >16 | ≤  | CTX-M-14   | +         |
| EC-24  | +    | ST131| B2 | FIB,FrepB,K          | 8   | >16 | >32 | 4   | >16 | 16 | CTX-M-27   | +         |
| EC-25  | +    | ST48 | B2 | FIB,FrepB,K          | >16 | >16 | >32 | >16 | >16 | ≤  | TEM-1      | +         |
| EC-27  | +    | ST131| B2 | FIA,FIB,FrepB,N,K    | >16 | >16 | >32 | >16 | >16 | ≤  | TEM-1,CTX-M-55 | +         |
| EC-29  | +    | ST31 | B2 | FIA,FIB,FrepB,N,K    | ≤ 2 | 16 | >32 | ≤ 1 | >16 | 16 | TEM-1      | +         |
| EC-31  | +    | ST9  | D  | FIB,FrepB,K          | ≤ 2 | 16 | >32 | ≤ 1 | >16 | 16 | OXA-30,CTX-M-14 | +         |
| EC-32  | +    | ST51 | D  | I1,Y,K               | >16 | >16 | >32 | 16  | >16 | ≤  | TEM-1      | +         |
| EC-36  | +    | ST131| B2 | FIA,FIB,FrepB,N,K    | >16 | >16 | >32 | >16 | >16 | ≤  | CTX-M-27   | +         |
| EC-37  | +    | ST2  | B2 | FIB,FrepB,K          | 4   | >16 | >32 | ≤ 1 | >16 | ≤  | TEM-1      | +         |
| EC-38  | +    | ST95 | A  | FIC                  | 8   | >16 | >32 | 2   | >16 | >32 | ADC-162    | +         |
| EC-39  | +    | ST681| B2 | FIA,FIB,FrepB        | >16 | >16 | >32 | 2   | >16 | ≤  | TEM-1,CTX-M-14 | +         |
| EC-41  | +    | ST131| B2 | FIB,FrepB,K          | 16  | >16 | >32 | 4   | >16 | 16 | TEM-1,CTX-M-27 | +         |
| EC-43  | +    | ST48 | D  | K,B                  | 8   | >16 | >32 | 2   | >16 | 16 | TEM-1,CTX-M-14 | +         |
| EC-48  | +    | ST9  | B2 | FIA,FIB              | >16 | >16 | >32 | >16 | >16 | 16 | TEM-1      | +         |
| EC-50  | +    | ST9  | D  | FIB,FrepB,K          | ≤ 2 | >16 | >32 | ≤ 1 | >16 | 16 | OXA-30,CTX-M-14 | +         |

PG, phylogenetic group; MIC, minimum inhibitory concentration.

### Table 3: ESBL-negative bloodstream-infection Escherichia coli resistance phenotypes and genotypes.

| Strain | ESBL | MLST | PG | Plasmid replicon type | MIC | \( \text{bla} \) gene product | Transfer |
|--------|------|------|----|----------------------|-----|-----------------------------|----------|
|        |      |      |    |                      |     | ATM | FEP | CTX | CAZ | CZO | FOX |           |          |
| EC-1   | –    | ST51 | B2 | FIA,FIB,K            | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-3   | –    | ST2  | B2 | ND                   | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-16  | –    | ST1  | B1 | FrepB,K              | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-18  | –    | ST51 | B2 | K                    | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-21  | –    | ST2  | A  | FrepB,K              | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-23  | –    | ST8  | B2 | FIB,FrepB,K          | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-26  | –    | ST2  | B2 | K                    | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-28  | –    | ST117| A  | FIB,FrepB,K          | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-33  | –    | ST45 | B2 | FIB,FrepB,K          | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |
| EC-35  | –    | ST730| A  | ND                   | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | OXA-30     | –         |
| EC-42  | –    | ST95 | A  | ND                   | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | ShV-42     | +         |
| EC-49  | –    | ST51 | B1 | K                    | ≤ 2 | ≤ 2 | ≤ 1 | ≤ 1 | ≤ 4 | ≤ 8 | TEM-1      | +         |

PG, phylogenetic group; MIC, minimum inhibitory concentration.
Europe, though genome-wide sequencing confirmed that certain strains of chicken carry \textit{bla\textsubscript{CMY-2}} with high homology (Pietsch et al., 2018; Ebmeyer et al., 2019; Seo et al., 2019). To the best of our knowledge, the current study provides the first evidence for \textit{bla\textsubscript{ADC-162}} in \textit{Escherichia coli} strains isolated from clinical samples from patients bloodstream infections. Overall, our results indicated that the diversity of ESBL and/or \textit{ampC} genes in \textit{E. coli} strains is increasing, constituting a potential public health problem.

Among the beta-lactamase-genotype-positive strains, most beta-lactamase genes could be transferred to the recipient \textit{E. coli} J53 strain by conjugation. Interestingly, 32 of the 33 beta-lactamase-genotype-positive strains were also carried conjugant plasmids, while EC-35 failed the conjugation test. EC-35 carried the \textit{bla\textsubscript{OXA-30}} gene, but no plasmid replicon was detected. Additional plasmid extraction experiments indicated that EC-35 carries a plasmid of approximately 15 kb in length, and the plasmid DNA amplified was positive for \textit{bla\textsubscript{OXA-30}} gene. EC-35 was shown to transmit the \textit{bla\textsubscript{OXA-30}} gene through a plasmid, consistent with previous reports (Carattoli, 2009). Eight of the remaining 32 conjugant strains partially or totally lost the beta-lactamase gene. No plasmid replicon was detected in J-EC-4 or J-EC-49, and the beta-lactamase gene was lost completely, while the other beta-lactamase genes were lost. All the plasmid replicons in J-EC-3, J-EC-7, J-EC-31, and J-EC-50 carried \textit{bla\textsubscript{CTX-M-14}} gene product Not detected genotype ESBL Resistance cotransferred

| Transconjugant | Donor strain | Plasmid replicon type | \textit{bla} gene product | Not detected genotype | ESBL | Resistance cotransferred |
|----------------|--------------|-----------------------|--------------------------|----------------------|------|------------------------|
| J-EC-1         | EC-1         | FIA                   | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-2         | EC-2         | FrepB                 | OXA-30                   |                      |      | ATM\textsuperscript{R}, FEP\textsuperscript{R}, CTX\textsuperscript{R}, CZO\textsuperscript{R}, CTX\textsuperscript{R} |
| J-EC-3         | EC-3         | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-4         | EC-4         | ND                    | OXA-30, CTX-M-55         | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-6         | EC-6         | K                     | CTX-M-14                 |                      |      | NT                     |
| J-EC-7         | EC-7         | K                     | CTX-M-14, CTX-M-55, TEM-1|                      |      | ATM\textsuperscript{R}, FEP\textsuperscript{R}, CTX\textsuperscript{R}, CZO\textsuperscript{R}, CRO\textsuperscript{R} |
| J-EC-9         | EC-9         | FIC                   | CMY-2, CTX-M-14          |                      |      | FEP\textsuperscript{R}, CTX\textsuperscript{R}, CAZ\textsuperscript{R}, CZO\textsuperscript{R}, CRO\textsuperscript{R}, FOX\textsuperscript{R} |
| J-EC-13        | EC-13        | FrepB                 | TEM-1                    |                      |      | ATM\textsuperscript{R}, FEP\textsuperscript{R}, CTX\textsuperscript{R}, CAZ\textsuperscript{R}, CZO\textsuperscript{R}, CRO\textsuperscript{R}, FOX\textsuperscript{R} |
| J-EC-16        | EC-16        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-18        | EC-18        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-20        | EC-20        | FrepB                 | CTX-M-14                 |                      |      | AMP\textsuperscript{R}  |
| J-EC-21        | EC-21        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-23        | EC-23        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-24        | EC-24        | K                     | CTX-M-27                 | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-25        | EC-25        | K                     | TEM-1                    | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-26        | EC-26        | ND                    | TEM-1                    | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-27        | EC-27        | FrepB                 | TEM-1, CTX-M-55          | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-28        | EC-28        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-29        | EC-29        | FrepB                 | TEM-1                    |                      |      | NT                     |
| J-EC-31        | EC-31        | K                     | CTX-M-14                 | OXA-30               | NT   | AMP\textsuperscript{R}  |
| J-EC-32        | EC-32        | I1                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-33        | EC-33        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |
| J-EC-36        | EC-36        | N                     | CTX-M-27                 | NT                   |      | FEP\textsuperscript{R}, CTX\textsuperscript{R} |
| J-EC-37        | EC-37        | K                     | TEM-1                    |                      |      | FEP\textsuperscript{R}, CTX\textsuperscript{R}, CAZ\textsuperscript{R}, CZO\textsuperscript{R}, CRO\textsuperscript{R}, FOX\textsuperscript{R} |
| J-EC-38        | EC-38        | FIC                   | ADC-162                 |                      |      | ATM\textsuperscript{R}, FEP\textsuperscript{R}, CTX\textsuperscript{R} |
| J-EC-39        | EC-39        | K                     | TEM-1, CTX-M-14          |                      |      | FEP\textsuperscript{R}, CTX\textsuperscript{R}, CAZ\textsuperscript{R}, CZO\textsuperscript{R}, CRO\textsuperscript{R}, FOX\textsuperscript{R} |
| J-EC-41        | EC-41        | FrepB                 | TEM-1, CTX-M-27          | NT                   |      | AMP\textsuperscript{R}  |
| J-EC-42        | EC-42        | ND                    | SHV-42                  |                      |      | AMP\textsuperscript{R}  |
| J-EC-43        | EC-43        | FrepB                 | TEM-1, CTX-M-14          |                      |      | AMP\textsuperscript{R}  |
| J-EC-48        | EC-48        | ND                    | TEM-1                    |                      |      | NT                     |
| J-EC-50        | EC-50        | K                     | CTX-M-14                 | OXA-30               |          | FEP\textsuperscript{R}, CTX\textsuperscript{R}, CZO\textsuperscript{R} |
| J-EC-49        | EC-49        | ND                    | TEM-1                    |                      |      | AMP\textsuperscript{R}  |

\textit{EC-35} had no transconjugants. ND, Not detected; NT, not transferred, donor strain ESBL phenotype positive but conjugant strain ESBL phenotype negative.
used to validate the genetic environment around bla\textsubscript{CTX-M-14}. Reverse PCR primers (M9R-F and M9R-R) were designed according to CTX-M-9, and sequencing and analysis of the reverse PCR amplification products revealed five and three base mutations in the ΔISEcp1 promoter regions of J-EC-6 and J-EC-31, respectively. This may have been due to an increase in the truncated length of ISEcp1, resulting in the loss of promoters in the primer regions and abnormal expression of \textit{bla}\textsubscript{CMY-1}

In the present study, only EC-9 and EC-38 strains, with \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162}, respectively, were resistant to fosfomycin. EC-9 carried FIC and K plasmid replicates, and EC-38 carried FIC plasmid replicates. Both strains were ST95 but belonged to different developmental groups, B2 and A, respectively. The genetic environments around \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162} were ISEcp1 (region\textsubscript{Y-42bp})-\textit{bla}\textsubscript{CTX-M-14}-IS903-\textit{bla}\textsubscript{CMY-2}-\textit{ble}-\textit{sug}E and ISABA1-\textit{bla}\textsubscript{ADC-162}-\textit{tnpA}, respectively. IS903 was inserted between two beta-lactamase genes in EC-9 to form a relatively complex tandem structure. Overlapping PCR demonstrated that ISEcp1 (region\textsubscript{Y-42bp})-\textit{bla}\textsubscript{CTX-M-14}-IS903-\textit{bla}\textsubscript{CMY-2}-\textit{ble}-\textit{sug}E could be completely transferred by conjugation. ISABA1 in ISABA1-\textit{bla}\textsubscript{ADC-162}-\textit{tnpA} is a specific insertion sequence found in \textit{Acinetobacter baumannii}, which can mediate the transfer of drug-resistance genes. The current study provides the first evidence for the existence of the same ISABA1-\textit{bla}\textsubscript{ADC-162}-\textit{tnpA} insertion sequence in \textit{E. coli}, suggesting that ISABA1 can be transmitted between \textit{E. coli} and \textit{A. baumannii} as a mobile genetic structural element.

Interestingly, \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162} were successfully detected in J-EC-9 and J-EC-38, respectively, and both carried only FIC plasmid replicons, indicating that \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162} were both transported by the IncF plasmid. Although only one IncF plasmid-mediated \textit{bla}\textsubscript{CMY-2} and one \textit{bla}\textsubscript{ADC-162}\textsuperscript{+} positive strain were detected in this study and no evidence of a cloning epidemic was found, the results suggested the need to remain highly vigilant. The IncF plasmid is known to contain three basic replicates: RepFIA, RepFIB, and RepFIC. The IncF plasmid is a narrow host plasmid with a specific region encoding multidrug-resistance genes. It is only prevalent in Enterobacteriaceae bacteria and can be used as a cloning agent (Yang et al., 2015). Multidrug resistance of the IncF plasmid is closely related to its mobile elements, including the insertion sequence, integron, and transposon, allowing it to capture or recombine resistance genes (Saul et al., 1989; Villa et al., 2010; Yang et al., 2015). It is therefore easy to conjugate and transfer, resulting in the dissemination of \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162} clones, with potentially adverse consequences. To the best of our knowledge, this is the first report of IncF-plasmid-mediated \textit{bla}\textsubscript{CMY-2} and \textit{bla}\textsubscript{ADC-162} in bloodstream-infection \textit{E. coli}, indicating the need to be vigilant.

The current results demonstrated that the horizontal transmission of beta-lactamase genes in bloodstream \textit{E. coli} strains is mainly mediated by IncF and IncK plasmids. Despite the differences in plasmid skeleton and variety, few replicons were detected in bloodstream \textit{infection E. coli}, and the conjugates carried only one type of replicon at most. In addition, the plasmids and genetic environment of the \textit{bla}\textsubscript{CTX-M} group play an important role in regulating the expression, transfer, and transmission of resistance genes. Detection of ISEcp1

### TABLE 5 | ESB-genotype-negative bloodstream-infection \textit{Escherichia coli} resistance phenotypes and characterization of transconjugants.

| Strain | MLST | PG | PRT | ESBL | MIC | Transfer | Transconjugants |
|--------|------|----|-----|------|-----|---------|-----------------|
|        |      |    |     |      |     | ATM     | FEP CTX CAZ CZO FOX |
|        |      |    |     |      |     | ATM\textsuperscript{R}, FEP\textsuperscript{R}, CTX\textsuperscript{R}, CAZ\textsuperscript{R}, CZO\textsuperscript{R} |
| EC-5   | ST730 | B2 | ND  | -    | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-8   | ST1   | B2 | ND  | +    | >16 | >16     | >32             | >16             | >16            | >8             | +               |
| EC-10  | ST31  | B2 | FrepB, K | +  | >16 | >16     | >32             | >16             | >16            | >16            | +               |
| EC-11  | ST681 | B2 | ND  | +    | >16 | ≤8      | ≤2              | ≤4              | ≤8             | –               |
| EC-12  | ST51  | B2 | ND  | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-14  | ST51  | B2 | FrepB, K | +  | >16 | >16     | >32             | 8               | >16            | >8             | +               |
| EC-15  | ST117 | B2 | ND  | ≤8   | ≤8  | ≤2      | ≤1              | ≤4              | ≤8             | –               |
| EC-17  | ST45  | B2 | ND  | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-19  | ST48  | B2 | ND  | <2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-22  | ST2   | A  | ND  | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-30  | ST681 | B1 | ND  | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |
| EC-34  | ST2   | D  | ND  | ≤8   | ≤8  | ≤2      | ≤1              | ≤4              | ≤8             | –               |
| EC-40  | ST117 | D  | FIB, FrepBJ1, K | +  | >16 | >16     | >32             | >16             | >16            | >8             | +               |
| EC-44  | ST2   | D  | FIB, FrepB, K | +  | >16 | >16     | >32             | >16             | >16            | >8             | +               |
| EC-45  | ST2   | B2 | PI, FIB, K | +  | 16   | >16     | >32             | 4               | >16            | ≤8             | +               |
| EC-46  | ST2   | D  | FrepB, K | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | +               |
| EC-47  | ST45  | B2 | ND  | ≤2   | ≤2  | ≤1      | ≤1              | ≤4              | ≤8             | –               |

PG, phylogenetic group; PRT, plasmid replicon type; MIC, minimum inhibitory concentration.
upstream of bla_{CTX-M}, bla_{CMY-2}, and bla_{CMY-M} genes with different plasmids showed that ISEcp1 plays an important role in capturing, expressing, and continuously mobilizing bla_{CTX-M} group and bla_{CMY-2} genes. An ISEcp1 insertion sequence upstream of the gene could result in high levels of expression of bla_{CTX-M} resistance genes and carry these resistance genes between chromosomes and plasmids for transfer, leading to spreading among different strains. Inserted sequences such as IS26, IS903, and orf477 are also frequently associated with bla_{CTX-M} resistance genes. These mobile genetic elements can be distributed randomly upstream and downstream of bla_{CTX-M} resistance genes, forming different genomic components in different bla_{CTX-M} resistance genes, acting on drug-resistance genes either together or separately, regulating their expression, and mediating their transmission.

CONCLUSION

To the best of our knowledge, this study provides the first molecular characterization of beta-lactamase genes from bloodstream isolates of E. coli from elderly patients. Beta-lactamase genes, especially bla_{TEM-1}, bla_{CTX-M-14}, bla_{OXA-30-1}, bla_{CTX-M-3}, bla_{CTX-M-5}, and bla_{CTX-M-65}, were widely prevalent in bloodstream-infection E. coli from these patients. Interestingly, bla_{CMY-2} and bla_{ADC-162} were both transported by IncF plasmids, which are prone to conjugation, indicating the potential for outbreak epidemics related to these genotypes of bloodstream-infection E. coli.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found in GenBank under accession numbers: CO033400.1, CP027202.2, MH459020.1, CP032937.1, MH190863.1, MH917123.1, CP026943.1, and MH898876.1.

ETHICS STATEMENT

This study used strains obtained from patient blood. The Ethics Committee of Shanghai University of Medicine & Health Sciences Affiliated Sixth People’s Hospital South Campus waived the need for the study to be reviewed or approved by an ethics committee because none of the strains were cultured in primary culture, and no information could be traced directly to any individual patient.

AUTHOR CONTRIBUTIONS

LX and QW conceived the study, analyzed the data, and wrote the manuscript. WL coordinated the study. LX, XW, NK, MC, LZ, and MS performed the experiments. LX, QW, and WL revised the manuscript.

FUNDING

This study was supported by grants from the National Natural Science Foundation of China (Grant Nos. 81572061 and 81572034), and was partly supported by the Outstanding Academic Leaders Plan of Shanghai (Grant No. 2018BR07), the Shanghai University of Medicine and Health Sciences Seed Foundation (Grant No. SFP-18-20-15-003), and the Shanghai Municipal Health and Family Planning Commission Youth Project (Grant No. 20164Y0156), and Fengxian District Science and Technology Commission Youth Project (Grant No. 20181801).

REFERENCES

Andrea, D., Mm, Arena F, Pallecchi, L., and Rossolini, G. M. (2013). CTX-M-type β-lactamases: a successful story of antibiotic resistance. Int. J. Med. Microbiol. 303, 305–317. doi: 10.1016/j.ijmm.2013.02.008
Baron, S., Jouy, E., Larvor, E., Eono, F., Bougeard, S., and Kempf, I. (2014). Impact of third-generation-cephalosporin administration in hatcheries on fecal escherichia coli antimicrobial resistance in broilers and layers. Antimicrob. Agents Chemother. 58, 5428–5434. doi: 10.1128/AAC.03106-14
Bartoletti, M., Giannella, M., Caraceni, P., Domenicali, M., Ambretti, S., Tedeschi, S., et al. (2014). Epidemiology and outcomes of bloodstream infection in patients with cirrhosis. J. Hepatol. 61, 51–58. doi: 10.1016/j.jhep.2014.03.021
Carattoli, A. (2009). Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53, 2227–2238. doi: 10.1128/jac.01707-08
Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K. L., and Threlfall, E. J. (2005). Identification of plasmids by PCR-based replicon typing. J. Microbiol. Meth. 63, 219–228. doi: 10.1016/j.mimet.2005.03.018
Che, T., Bethel, C. R., Puszai-Carey, M., Bonomo, R. A., and Carey, P. R. (2014). The different inhibition mechanisms of OXA-1 and OXA-24 beta-lactamases are determined by the stability of active site carboxylated lysine. J. Biol. Chem. 289, 6152–6164. doi: 10.1074/jbc.M113.533562
Clasen, J., Birkegard, A. C., Graesboll, K., and Folkesson, A. (2019). The evolution of TEM-1 extended-spectrum beta-lactamases in E. coli by cephalosporins. J. Glob. Antimicrob. Resist. 19, 32–39. doi: 10.1016/j.jgar.2019.03.010
Clermont, O., Bonacorsi, S., and Bingen, E. (2000). Rapid and simple determination of the Escherichia coli phylogenetic group. Appl. Environ. Microbiol. 66, 4555–4558. doi: 10.1128/aem.66.10.4555-4558.2000
CLSI. (2018). Performance Standards for Antimicrobial Susceptibility Testing. Informational Supplement M100-s28, 28th Edn. Wayne, PA: Clinical Laboratory Standards Institute.
Du, B., Long, Y., Liu, H., Chen, D., Liu, D., Xu, Y., et al. (2002). Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae bloodstream infection: risk factors and clinical outcome. Intens. Care Med. 28, 1718–1723. doi: 10.1007/s00134-002-1521-1
Elmeyer, S., Kristiansson, E., and Larsson, D. G. J. (2019). CMY-1/MOX-family AmpC β-lactamases MOX-1, MOX-2 and MOX-9 were mobilized independently from three aeromonas species. J. Antimicrob. Chemoth. 74, 1202–1206. doi: 10.1093/jac/dkz025
Eckert, C., Gautier, V., and Arlet, G. (2006). DNA sequence analysis of the genetic environment of various blaCTX-M genes. J. Antimicrob. Chemoth. 57, 14–23. doi: 10.1093/jac/dkl398
Hung, W. T., Cheng, M. F., Tseng, F. C., Chen, Y. S., Shin-Jung, L. S., Chang, T. H., et al. (2019). Bloodstream infection with extended-spectrum beta-lactamase-producing Escherichia coli: the role of virulence genes. J. Microbiol. Immunol. Infect. doi: 10.1016/j.jmii.2019.03.005 [Epub ahead of print].

Frontiers in Microbiology | www.frontiersin.org 9 October 2019 | Volume 10 | Article 2175
Koh, T. H., Wang, G. C., Sng, L. H., and Koh, T. Y. (2004). CTX-M and plasmid-mediated AmpC-producing Enterobacteriaceae Singapore. Emerg. Infect. Dis 10, 1172–1174. doi: 10.3201/eid1006.030726
Lambert, M. L., Suetens, C., Saves, A., Palomar, M., Hiesmayr, M., Morales, I., et al. (2011). Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect. Dis. 11, 30–38. doi: 10.1016/S1473-3099(10)70258-9
Medeiros, A. A. (1984). Beta-lactamases. Br. Med. Bull. 40, 18–27.
Pietsch, M., Irrgang, A., Roschanski, N., Brenner Michael, G., Hamprecht, A., Rieber, H., et al. (2018). Whole genome analyses of CMY-2-producing Escherichia coli isolates from humans, animals and food in Germany. BMC Genomics 19:601. doi: 10.1186/s12866-018-4976-3
Quan, J., Zhao, D., Liu, L., Chen, Y., Zhou, J., Jiang, Y., et al. (2017). High prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae in community-onset bloodstream infections in China. J. Antimicrob. Chemother. 72, 273–280.
Razazi, K., Derde, L. P. G., Verachten, M., Legrand, P., Lesprit, P., and Brun-Buisson, C. (2012). Clinical impact and risk factors for colonization with extended-spectrum β-lactamase-producing bacteria in the intensive care unit. Intens. Care Med. 38, 1769–1778. doi: 10.1007/s00134-012-2675-0
Salverda, M. L., De Visser, J. A., and Barlow, M. (2010). Natural evolution of TEM-1 beta-lactamase: experimental reconstruction and clinical relevance. FEMS Microbiol. Rev. 34, 1015–1036. doi: 10.1111/j.1574-6976.2010.00222.x
Saul, D., Spiers, A. J., McAnulty, J., Gibbs, M. G., Bergquist, P. L., and Hill, D. F. (1989). Nucleotide sequence and replication characteristics of RepFIB, a basic replicon of IncF plasmids. J. Bacteriol. 171, 2697–2707. doi: 10.1128/jb.171.5.2697-2707.1989
Seo, K. W., Shim, J. B., and Lee, Y. J. (2019). Emergence of CMY-2-Producing Escherichia coli in Korean layer parent stock. Microb. Drug Resist. 25, 462–468. doi: 10.1089/mdr.2018.0254
Shi, H., Sun, F., Chen, J., Ou, Q., Feng, W., Yong, X., et al. (2015). Epidemiology of CTX-M-type extended-spectrum beta-lactamase (ESBL)-producing nosocomial Escherichia coli infection in China. Ann. Clin. Microbiol. Antimicrob. 14:4. doi: 10.1186/s12941-015-0063-7
van der Mee-Marquet, N. L., Blanc, D. S., Gbaguidi-Haore, H., Dos Santos Borges, S., Viboud, Q., Bertrand, X., et al. (2015). Marked increase in incidence for bloodstream infections due to Escherichia coli, a side effect of previous antibiotic therapy in the elderly. Front. Microbiol. 6:646. doi: 10.3389/fmicb.2015.00646
Velasova, M., Smith, R. P., Lemma, F., Horton, R. A., Duggett, N., Evans, J., et al. (2019). Detection of extended spectrum beta-lactam (ESBL), AmpC and carbapenem resistance in Enterobacteriaceae in beef cattle in Great Britain in 2015. J. Appl. Microbiol. 126, 1081–1095. doi: 10.1111/jam.14211
Villa, L., Garcia-Fernandez, A., Fortini, D., and Carattoli, A. (2010). Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. 65, 2518–2529. doi: 10.1093/jac/dkq347
Xiao, L., Wang, X., Kong, N., Cao, M., Zhang, L., Wei, Q., et al. (2019). Polymorphisms of gene cassette promoters of the class 1 integron in clinical proteus isolates. Front. Microbiol. 10:790. doi: 10.3389/fmicb.2019.00790
Yang, Q. E., Sun, J., Li, L., Deng, H., Liu, B. T., Fang, L. X., et al. (2015). IncF plasmid diversity in multi-drug resistant Escherichia coli strains from animals in China. Front. Microbiol. 6:964. doi: 10.3389/fmicb.2015.00964
Zhao, S. Y., Zhang, J., Zhang, Y. L., Wang, Y. C., Xiao, S. Z., Gu, F. F., et al. (2016). Epidemiology and risk factors for faecal extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) carriage derived from residents of seven nursing homes in western Shanghai. China. Epidemiol. Infect. 144, 695–702. doi: 10.1017/S0950268815001879
Zhao, W. H., and Hu, Z. Q. (2013). Epidemiology and genetics of CTX-M extended-spectrum beta-lactamases in gram-negative bacteria. Crit. Rev. Microbiol. 39, 79–101. doi: 10.3109/1040841X.2012.691460
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Xiao, Wang, Kong, Zhang, Cao, Sun, Wei and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.