Molecular Epidemiology and Characteristics of CTX-M-55 Extended-Spectrum β-Lactamase-Producing Escherichia coli From Guangzhou, China

Shihan Zeng1†, Jiajun Luo2†, Xiankai Chen2, LiShao Huang2, Aiwu Wu1*, Chao Zhuo3* and Xiaoyan Li2*

1 KingMed School of Laboratory Medicine, Guangzhou Medical University, Guangzhou, China, 2 Department of Clinical Laboratory, Fifth Affiliated Hospital, Southern Medical University, Guangzhou, China, 3 State Key Laboratory of Respiratory Disease, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

In recent years, the CTX-M-55 extended-spectrum β-lactamase (ESBL)-positive rate has gradually increased in the clinic. To identify the molecular epidemiology and characteristics of blaCTX-M-55-positive isolates, a total of 374 non-repetitive ESBL-producing Escherichia coli strains were collected from patients in two hospitals in Guangzhou, and 89 blaCTX-M-55-positive isolates were selected by CTX-M-1-group PCR amplification and confirmed by DNA sequencing. Whole-genome sequencing was used to analyze the resistance phenotype, plasmid types, phylogenetic relationships and genetic environment of the blaCTX-M-55 gene. Conjugation experiments and PCR were performed to confirm whether the plasmid harboring blaCTX-M-55 gene could be transferred. The results showed that all blaCTX-M-55-positive isolates were resistant to ceftriaxone, and 88.76 and 76.40% were resistant to ceftazidime and cefepime, respectively. The resistance rates to levofloxacin and sulfamethoxazole were 66.29 and 59.55%, respectively. However, the sensitivity rate of piperacillin/tazobactam, amoxicillin/clavulanate, and amikacin exceeded 90%. All blaCTX-M-55-positive isolates were sensitive to carbapenems. Thirty-two STs were detected in the blaCTX-M-55-positive isolates, among which the detection rate of ST1193 was relatively high (19.10%, 17/89), and other ST types were scattered. It remains to be seen whether ST1193 carrying the blaCTX-M-55 gene can become a popular clone strain in this region in the future. The plasmid types carrying the blaCTX-M-55 gene included IncI1, IncFII, IncFIC, IncFIB, IncHI2, IncI2, and IncX/Y, among which the IncI1 and IncFII plasmids were the main plasmids, accounting for 37.80 and 28.09%, respectively. Among them, 11 strains of the IncI1 plasmid existed in ST1193 strains. The blaCTX-M-55 gene was found on chromosomes of 13 isolates, and seemed to be increasing annually. Up to five distinct types of genetic environments surrounding the blaCTX-M-55 gene were analyzed. The
most common structure was type II “ISEcp1-bla\textsubscript{CTX-M−55}-ORF477.” In conclusion, whether ST1193, which carries bla\textsubscript{CTX-M−55} gene, will be an epidemic clone of this region in the future remains to be concerned. The plasmids IncI1 and IncFII, and mobile elements such as ISEcp1 and IS26 may be the main factors leading to the spread and prevalence of CTX-M-55 genotypes.

**Keywords:** CTX-M-55, IncI1 plasmid, ISEcp1, ST1193, E. coli, IncFII plasmid

**INTRODUCTION**

Extended-spectrum β-lactamases (ESBLs), including TEM, SHV, CTX-M, and OXA enzymes, are the main resistance mechanism of Enterobacteriaceae against β-lactam antibiotics. Among them, CTX-M type β-lactamase, which was first found in 1990 by Bauernfeind et al. (1990), could preferentially hydrolyze cefotaxime (CTX) compared with TEM- and SHV-type enzymes. The CTX-M type has been reported to be the main type of ESBL in Enterobacteriaceae spreading worldwide and is also widely distributed in zoonotic pathogens (Bernard et al., 1992; Rossolini et al., 2008). CTX-M-14 and CTX-M-15 have been reported to be the most common genotypes in China in recent years. However, the CTX-M-55 positive rate has gradually increased in China, especially in the area of South China (Zhang et al., 2014). In Zhang et al. (2014), Cao et al. (2011), and Zhao and Hu (2013), the detected positive rate of CTX-M-55 was even higher than that of its derivative type, CTX-M-15. CTX-M-55 is a variant of CTX-M-15 with only one amino acid substitution (Ala-80-Val). Both CTX-M-15 and CTX-M-55 belong to the CTX-M-1 group, but CTX-M-55 exhibits high hydrolytic activity to ceftazidime (Kiratisin et al., 2007). Since CTX-M-55 was first reported in Thailand in 2006, it has been identified in *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella*, and *Morganella morganii* (Kiratisin et al., 2007; Kim J. S. et al., 2017; Xia et al., 2017; Hu et al., 2018). Plasmids are known as an important reason for the rapid spread of *bla*\textsubscript{CTX-M}. Recently, CTX-M-55 has been reported to appear on the IncI1, IncF, IncP, and IncA/C plasmids (Kim J. S. et al., 2017; Xia et al., 2017; Hounmanou et al., 2021). Additionally, the transmission of *bla*\textsubscript{CTX-M-55} is also related to many mobile genetic elements. For example, ISEcp1, IS26, and IS903 are often detected around *bla*\textsubscript{CTX-M-55} (Lartigue et al., 2006; Poirel et al., 2008; Hu et al., 2018). In this study, 89 *bla*\textsubscript{CTX-M-55}-producing *E. coli* isolates isolated from patients in two hospitals in Guangzhou in recent years were selected, and the epidemiology and characteristics of these isolates were analyzed.

**MATERIALS AND METHODS**

**Bacterial Strains and Antimicrobial Susceptibility Testing**

A total of 374 non-repetitive ESBLs-producing *E. coli* strains isolated from patients in two hospitals in Guangzhou were identified by the advanced expert system (AES) of the VITEK-2 COMPACT Automatic Microbial Identification System (bioMérieux, Marcy-l’Étoile, France). Minimal inhibitory concentrations (MICs) including the ESBLs-resistant phenotype were determined using the VITEK-2 Automated Susceptibility System (Spanu et al., 2006). The MICs of the isolates against amoxicillin/clavulanic acid (AMC), piperacillin/tazobactam (TZP), amikacin (AMK), compound sulfamethoxazole (SXT), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ETP), imipenem (IPM), and levofloxacin (LVX) were determined by agar dilution method, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI/NCCCLS M100-S30) [Clinical and Laboratory Standards Institute [CLSI], 2021]. All isolates were collected in two tertiary hospitals from 2012 to 2017 and 2020, while the isolates from 2018 to 2019 were not collected, so they were not included in this study. *E. coli* ATCC 25922 was used as a quality control strain. *E. coli* C600 was used as the recipient strain in the conjugation experiments.

**Detection of the CTX-M-1-Group β-Lactamase Gene by PCR Assays**

Primers for detecting all CTX-M-1-group type genes were designed are shown in Supplementary Table 1. Bacterial genomic DNA was extracted for PCR amplification and the positive PCR products were subsequently sequenced and to confirm their phenotype. Only *bla*\textsubscript{CTX-M-55}-positive isolates were selected for subsequent testing.

**Conjugation Experiments**

The transferability of the *bla*\textsubscript{CTX-M-55} gene was determined by conjugation experiments with rifampin-resistant *E. coli* C600 as the recipient strain. Transconjugants were selected on Luria–Bertani agar plates containing rifampin (100 μg/mL) and CRO (4 μg/mL). PCR using CTX-M-1-group primers and sequencing were used to confirm whether the transconjugants carrying the *bla*\textsubscript{CTX-M-55} gene were successfully acquired. Antimicrobial susceptibility testing and plasmid replicon typing (primers are in Supplementary Table 1) were conducted on transconjugants. The presence of resistant genes from transconjugants was also investigated by PCR (primers showed in Supplementary Table 1).

**Whole-Genome Sequencing and Analysis**

The genomic DNA of *bla*\textsubscript{CTX-M-55}-positive *E. coli* was extracted by a bacterial genomic DNA extraction kit (Tiangen, Beijing, China) and sequenced by next-generation sequencing (NGS) on an Illumine platform (Nuohezhiyuan, Tianjin, China). The quality of the raw readings was controlled by the interactive program FastQC (Wingett and Andrews, 2018), and the genomes were assembled using SPAdes 3.13.1 (Bankevich et al., 2012).
and annotated using Prokka 1.14.5 (Seemann, 2014) on the Linux system. Multilocus sequence typing analysis of the E. coli isolates was executed using MLST 2.18.0 (Larsen et al., 2012). The core genome multilocus sequence typing (cgMLST) of 17 ST1193 isolates was performed using Ridom SeqSphere+ 4.1.9 (Junemann et al., 2013). The resistance genes and plasmids type were determined based on the CGE server (Thomsen et al., 2016), and the plasmid circle map illustrates with BRIG (Alikhan et al., 2011). In all second generation genome annotation files, consigs harboring the bla CTX−M−55 gene were analyzed, and the bla CTX−M−55 gene locations were roughly determined combined with BLAST. Representative isolates with unclear bla CTX−M−55 gene locations or different bla CTX−M−55-harboring plasmid types were selected and sequenced for long read sequencing on the Nanopore platform (Nuohazhijuan, Tianjin, China). Finally, the location and genetic environment of the bla CTX−M−55 gene in bla CTX−M−55-positive E. coli were analyzed based on second- and third-generation genomic data. The genetic environment of the bla CTX−M−55 genes was drawn by Easyfig (Sullivan et al., 2011).

RESULTS
bla CTX−M−55-Positive Escherichia coli Isolates
A total of 132 bla CTX−M−55-positive ESBLs-producing E. coli (35.29%) isolates were obtained from all 374 ESBLs-producing E. coli clinical isolates. Overall, 6 bla CTX−M−3-positive isolates (1.60%), 37 bla CTX−M−15-positive isolates (9.89%), and 89 bla CTX−M−55-positive isolates (23.80%) were identified. In this study, 89 bla CTX−M−55-positive isolates were further analyzed, and the distribution of their specimen source and collection year are shown in Figure 1. The bla CTX−M−55-positive isolates were derived mainly from patient urine (56%), while other sources included blood (14%), purulent secretion (10%), sputum (9%), wound secretion (9%), and abdominal drainage fluid (2%).

MLST Profile
Thirty-two MLST profiles were determined from the 89 bla CTX−M−55-positive isolates (Supplementary Table 2), including three novel STs (ST12284, ST12285, and ST12303), of which sequences have been submitted to the PubMLST database. In addition to the relatively high detection rate of ST1193 (19.10%, 17/89), other ST types were scattered. It is worth noting that ST1193 isolates mainly emerged in 2020 (10/17) and were distributed in different years (Supplementary Table 2). By cgMLST, among 17 ST1193, except for 3 pairs of strains that showed the same alleles, the rest ST1193 strains isolated from different years still had different alleles (Figure 2).

Antimicrobial Susceptibility Profiles
All of the bla CTX−M−55-positive isolates were resistant to CRO, and 88.76 and 76.40% were resistant to CAZ and FEP, respectively (Supplementary Table 3). The resistance rates to LVX and SXT were 66.29 and 59.55%, respectively. However, the bla CTX−M−55-positive isolates presented high susceptibility rates to AMC, TZP, and AMK, with sensitivity rates of 95.95, 95.40, and 94.38%, respectively. All bla CTX−M−55-positive isolates were sensitive to IPM and ETP. Meanwhile, we found that bla TEM (coding β-lactamase genes), sul, and dfrA (mediating sulfonamide resistance), tet (mediating tetracycline resistance), aph(3′)-Ib and aph(6′)-Ib (mediating aminoglycoside resistance) were abundant in bla CTX−M−55-positive isolates (Figure 3 and Supplementary Table 3). The 16S rRNA methylase gene rmtB (mediating aminoglycoside antibiotic resistance) was detected in five isolates, all of which were resistant to AMK (Supplementary Table 3). There were sixteen isolates containing genes mediating fluoroquinolone resistance, of which three were aac(6′)-Ib-cr and thirteen were qnrS (Figure 3 and Supplementary Table 3). dfrA or sul genes were detected in almost all SXT resistant isolates (52/53), and 46 isolates were detected in both of them.

The bla CTX−M−55 Gene Location
The predicted plasmid components carrying the bla CTX−M−55 gene were of variable replicon types including IncI1, IncFIB, IncFII, IncFIC, IncHI2, IncI2, and IncX/Y (Figure 4A). The bla CTX−M−55 gene of 33 isolates was located on the IncI1 plasmid, among which 11 were carried by ST1193. The other bla CTX−M−55-positive plasmid was identified, 24 on the IncFII plasmid, 12 on the IncFIC plasmid, 6 on the IncFIB plasmid, 3 on the IncHI2 plasmid, 1 on the IncI2 or IncX/Y plasmid, and 1 simultaneously on the IncHI2 and IncFII plasmids (Supplementary Table 2). These plasmids were randomly present in different ST isolates. We also found that in thirteen of the 89 isolates containing the bla CTX−M−55 gene, BLSTA in NCBI suggested a chromosomal location (Figure 3 and Supplementary Table 2). Among them, the bla CTX−M−55 gene of three isolates was simultaneously located on both the chromosome and the IncI1 plasmid, and two were simultaneously located on the chromosome and the IncFIC plasmid. The isolates with the chromosome-encoding bla CTX−M−55 gene seemed to increase annually (Figure 4B). Our data (Figure 4B) showed that the total diversity of the plasmid types carrying the bla CTX−M−55 gene gradually increased between 2012 and 2020 (missing data from 2018 to 2019). Especially, the IncI1 and IncFII plasmids were continuously identified with higher detection rates. The IncFIB and IncHI2 emerged in the last 5 years, and the detection rate for these plasmids was still relatively low.

Circle diagrams were drawn for plasmid backbone comparison based on the known full-sequence plasmids in this study as reference. The IncI1 (Figure 5A), IncFIB (Figure 5B), IncI2 (Figure 5C), IncX/Y (Figure 5D), and IncHI2 (Figure 5E) plasmids had only one backbone type, while the IncFIC plasmid had two backbone types (Figures 5G,H). Most plasmids carrying bla CTX−M−55 with the same replicon were relatively conserved in this study, such as IncI1, IncFIB and IncHI2. In particular, almost all of the IncI1 plasmids were highly similar to p2474-3 (CP021208) in clinical E. coli isolated from a hospital in Anhui Province. Incidentally, we found that the sequence similarity between the IncI1 plasmid p628-CTXM (KP987217) isolated from K. pneumoniae, pST53-2 (CP050747.1) isolated from
Salmonella enteritidis and p2474-3 was higher than 95%, and there was only one resistance gene \( \text{bla}_{\text{CTX-M-55}} \) between them. In contrast, the backbones of the four IncFII plasmids (Figure 5F) were slightly different, with a distinct region of approximately 24 kbp containing 24 coding sequences (CDSs). This region included some mobile genes (\( \text{IS26} \), \( \text{ISEc36} \), \( \text{ISKpn19} \), \( \text{IS6100} \), \( \text{ISSBo1} \), \( \text{ISSO9R} \), and \( \text{Tn2} \)), the neo gene encoding aminoglycoside 3′-phosphotransferase, the hin gene encoding a specific recombinase, the \( \text{hac} \)b gene encoding a dehydrogenase, the \( \text{tet} \)(\( \text{M} \)) gene mediating tetracycline resistance, the \( \text{tap} \) gene encoding a multidrug efflux pump, and eight hypothetical proteins. Meanwhile, we found that the IncFII plasmid carrying the \( \text{bla}_{\text{CTX-M-55}} \) gene also contains other drug resistance genes, such as \( \text{bla}_{\text{TEM-1}} \), \( \text{fos(A)} \), \( \text{qnrS1} \), \( \text{tet} \), \( \text{dfrA} \), or \( \text{catA} \) genes by analyzing the long read sequence (Supplementary Tables 2, 3).

**Transconjugants of the \( \text{bla}_{\text{CTX-M-55}} \)-Positive Isolates**

In total, 45 transconjugants were successfully obtained from 89 \( \text{bla}_{\text{CTX-M-55}} \)-positive isolates through conjugation experiments, and the transfer success rate was 50.56% (45/89). Combined with the \( \text{bla}_{\text{CTX-M-55}} \) gene location and the plasmid replicon typing results of transconjugants (Supplementary Table 2), it was found that most of the IncI1 (24/33, 72.72%) and IncFII plasmids (16/25, 64%) could be transferred into \( E. \) coli C600, while the transfer success rate of the IncFIC and IncFIB plasmids was lower than 33.33%. In this study, the IncHI2 (0/4), IncI2 (0/1), and IncX/Y (0/1) plasmids carrying the \( \text{bla}_{\text{CTX-M-55}} \) gene failed to transfer through conjugation experiments. Meanwhile, there is not only one plasmid in some transconjugants. All transconjugants were resistant to CRO. The transconjugants exhibited 95.56 and 82.22% resistance rates to CAZ and FEP, respectively. Only 4.44% of the transconjugants were resistant to AMC. In addition, the resistance rates of transconjugants to SXT, LEV, and AMK were 17.78, 6.67, and 2.22%, respectively. All transconjugants were sensitive to TZP, IPM, and ETP (Supplementary Table 3).

A total of 10 transconjugants were resistant to at least one antibiotic in LEV or SXT. Interestingly, the \( \text{bla}_{\text{CTX-M-55}} \) gene of seven transconjugants was located on IncFII, which means that in addition to the \( \text{bla}_{\text{CTX-M-55}} \) gene, IncFII in these transconjugants also carries genes that mediate LVX or SXT resistance (Supplementary Tables 2, 3). Analysis of drug resistance genes in transconjugants showed that the \( \text{dfr} \text{A} \text{17/dfr} \text{A} \text{14} \) gene are the main reason for the resistance of transconjugants to SXT. There were three transconjugants resistant to LEV, and \( \text{qnrS1} \) gene was detected in all of
them. Only one transconjugant carried the *rmtB* gene and was resistant to amikacin.

**Genetic Environment Surrounding the bla_CTX−M−55 Gene**

The genetic environment surrounding the *bla_CTX−M−55* gene is presented in Figure 6. Five structures were obtained by analyzing mobile elements around the *bla_CTX−M−55* gene and named type I to V. The mobile elements located upstream of *bla_CTX−M−55* mainly included IS*Ecp1* (complete or incomplete) and IS26. Downstream of the *bla_CTX−M−55* genes ORF477 was consistently found. Among them, type II “IS*Ecp1−bla_CTX−M−55−ORF477” was the predominant (63.16%, 60/95) genetic environment of the *bla_CTX−M−55* gene and plasmids containing this structure included IncI1, IncFIB, IncFIC, IncFII, IncHI2, and IncI2 (Figure 6). Likewise, the genetic environment of the *bla_CTX−M−55* gene on the chromosome (12/13) was almost type II, the other is type I. Compared with type II, only a large deletion (489 to 1140 bp) of IS*Ecp1* was found in type I. Moreover, the *bla_CTX−M−55* genes of isolate 75, 128, and 173 were found on both the chromosome and the IncI1 plasmid, and both of the genetic environments between them belong to type II. The *bla_CTX−M−55* gene of isolate N18 was found on both the chromosome and the IncFIC plasmid, among which the genetic environment on the chromosome was type II, and that on the IncFIC plasmid was type III “IS26-IS*Ecp1−bla_CTX−M−55−ORF477.” The occurrence of the type III structure was similar to that of the type II structure, but IS*Ecp1* of the type III structure was disrupted by IS26. Interestingly, IS26 mainly emerged upstream of the *bla_CTX−M−55* gene in the IncFIC and IncFII plasmids. Type IV “IS26-bla_CTX−M−55−ORF477” mainly exists in IncFII plasmids (15/17).

Notably, an IncFIC plasmid contained four copies of the *bla_CTX−M−55* gene (Type V) harbored in isolate 110, and the structure of the four-duplicated segment was “IS26-bla_CTX−M−55−ORF477.” The plasmid backbone was similar to that of IncFIC-2 (Figure 5H) and 151563 bp in size. This plasmid cannot be transferred through conjugation. The MIC of isolate 110 for CRO was higher than 256 µg/mL, and the MICs for CAZ and FEP were 64 µg/mL. Compared to the common *bla_CTX−M−55*-positive isolates with CRO ≥ 64 µg/mL, and the MIC50 for CAZ was 32 µg/mL, and the MIC50 for FEP was only 16 µg/mL.

**DISCUSSION**

Previous studies (Lartigue et al., 2004; Bevan et al., 2017) have shown that the isolation rate of Enterobacteriaceae producing CTX-M-55 in China has increased significantly in recent years, in aquaculture animals and in clinical patients, which demonstrates the rapid dissemination of *bla_CTX−M−55*, especially in South China (Zhang et al., 2014). In that study (Zhang et al., 2014), the CTX-M-55 (21/38, 55.26%) positive rate in clinical ESBLs-producing isolates from Guangdong Province (in South China) was much higher than that from other provinces in China (range from 2.56 to 31.25%). Previous study (Zhang et al., 2014)
have shown that the percentage of $bla_{CTX-M-55}$-positive $E. coli$ reported in China was 18.40% (47/256), exceeding the percentage of $bla_{CTX-M-15}$-positive $E. coli$ (31/256, 12.1%), which has been reported as the most widespread CTX-M gene in $E. coli$ in China. In this study, we obtained similar results with a more significant trend: the detection rate of $bla_{CTX-M-55}$-positive isolates reached 23.80% (89/374), which was higher than that of $bla_{CTX-M-15}$-positive isolates (37/374, 9.89%).

Most $bla_{CTX-M-55}$-positive isolates have been reported co-harboring $bla_{TEM}$ (Hu et al., 2018), and similar results were shown in this study. Except for $bla_{TEM}$, $aph\ (3')-Ib$, $aph(6')-Ib$, $dfrA$, $sul$, and $tet$ mediating different antibiotic resistances emerged frequently in the $bla_{CTX-M-55}$-positive isolates. All of the $bla_{CTX-M-55}$-positive isolates were resistant to CRO, and 88.76 and 76.40% were resistant to CAZ and FEP, respectively. The production of ESBLs is the main mechanism of resistance against cephalosporin in $E. coli$, and CTX-M-55 producing $E. coli$ are known to be resistant to the second and third generations of cephalosporin (Wang et al., 2013). Cefepime is the first fourth-generation cephalosporin approved for use in China, showing low toxicity and high activity against third-generation cephalosporin-resistant Enterobacteriaceae (Chapman and Perry, 2003). However, the resistance rates of $bla_{CTX-M-55}$-positive isolates to FEP reached 76.40% in this study, which showed that the resistance phenomenon of $bla_{CTX-M-55}$-positive isolates to cephalosporins became tougher. In addition, $bla_{CTX-M-55}$-positive isolates often carry $sul$ and $dfrA$ genes, which mediate their resistance to SXT up to 66.09%. Even though there are few $bla_{CTX-M-55}$-positive isolates carrying $qnrS$ or $aac(6')-Ib-cr$ gene, the resistance to fluoroquinolone reached 59.55%. Because fluoroquinolones are mainly mediated by plasmid mediated drug resistance genes (PMQRs) and mutations in quinolone resistance determining regions (QRDRs), and PMQR only confers low-level fluoroquinolone resistance (Robicsek et al., 2006; Strahilevitz et al., 2009; Zhang et al., 2019). Fortunately, the sensitivity of $bla_{CTX-M-55}$-positive isolates to AMC, TZP, and AMK was higher than 90%, and all $bla_{CTX-M-55}$-positive isolates were sensitive to IPM and ETP. Therefore, β-lactamase inhibitors, amikacin, and carbapenems can be used as a treatment strategy for $bla_{CTX-M-55}$-positive isolates. The results of resistance analysis indicate that the resistance genes carried by $bla_{CTX-M-55}$-positive clinical isolates are diverse and complex, which will bring great challenges to clinical treatment.

Previous studies (Russo and Johnson, 2003) showed that the population structure of CTX-M-producing $E. coli$ is dominated by the high-risk clone ST131, and other important epidemic clones included ST405, ST38, ST648, ST410, and ST1193 (Coque et al., 2008). However, the detection rate of ST131
in this study was only 4.94% (4/89). ST1193 (19.10%) was detected much higher than that of other strains. ST1193 was reported as a fluoroquinolone-resistant E. coli clone (Platell et al., 2012; Kim Y. et al., 2017; Tchesnokova et al., 2019) and it is commonly coresistant to sulfonamides, β-lactams, and tetracyclines (Tchesnokova et al., 2019). The blaCTX-M-55-positive E. coli isolates carried by patients with urinary tract infections was reported in China (Xia et al., 2017), ST1193 (18%) was also the most common ST. ST1193 is often associated with clinical isolates of urinary tract infections (Ding et al., 2021). The blaCTX-M-55-positive isolates were obtained from multiple clinical specimens in this study. Of the 17 ST1193 isolates, 10 were derived from urine specimens. The results of cgMLST analysis showed that, except for the three pairs of strains, the other blaCTX-M-55-positive ST1193 clones isolated from different time and departments exist allelic difference, which indicate that although the genetic relationship of these ST1193 strains were closely related, they still had different degrees of mutation over time. We also found that a total of 10 isolates of ST1193 were detected in 2020 and 7 isolates in 2012–2017 (Supplementary Table 2). Therefore, we speculate that ST1193 may become a potential epidemic clone among clinical isolates carrying blaCTX-M-55 in the future, but it still needs further observation. Furthermore, this study showed that the blaCTX-M-55 gene is located on the chromosome with an increasing trend, and a study of blaCTX-M-55 from Salmonella (Zhang et al., 2019) also showed that blaCTX-M-55 mainly existed in the chromosome (10/11), which suggests that we should pay close attention to the vertical transmission of blaCTX-M-55-positive strains.

Conjugative plasmids play a key role in the horizontal transfer of drug resistance genes among E. coli (Carattoli, 2013), which is an important reason for blaCTX-M transmission. In recent years, blaCTX-M-55 in China has mainly been located on the IncI1 plasmid and sporadically emerged on the IncF and IncP plasmids (Bevan et al., 2017; Xia et al., 2017). The plasmid types carrying the blaCTX-M-55 gene found in this study included IncI1, IncFII, IncFIC, IncFIB, IncHI2, IncI2, and IncX/Y. Among them, the detection rates of IncI1 and IncFII plasmids were the highest. Meanwhile, most IncI1 and IncFII plasmids can be transferred to E. coli C600, but the transfer success rate of other plasmids was low. We also found plasmids similar to IncI1 in K. pneumoniae and S. enteritidis (Fu et al., 2020), and the sequence identity was higher than 95%, indicating that the IncI1 plasmid carrying blaCTX-M-55 can be spread in different strains. The IncI1 plasmid carrying only one drug resistance gene of blaCTX-M-55 in Enterobacteriaceae is still relatively conserved after several years of transmission. In this study, 64.71% (11/17) of ST1193 contained IncI1 plasmids, while 88% (14/16) of the ST1193 isolates studied by Xia et al. (2017) also contained IncI1 plasmids. In addition, many IncI1
plasmids (22/33, 66.67%) were distributed in other ST types. Our results suggest that the dominant plasmid IncI1 may be one of the main reasons for the widespread dissemination of the \( \text{bla}_{\text{CTX-M-55}} \) gene in \( E. \) coli. Additionally, the IncFII plasmid can be spread in many ST isolates. Meanwhile, it should be noted that the IncFII plasmid carrying the \( \text{bla}_{\text{CTX-M-55}} \) gene also contains other drug resistance genes, such as \( \text{bla}_{\text{TEM-1}}, \text{fos(A)}, \text{qnrS1}, \text{tet}, \text{dfrA}, \) or \( \text{catA} \) genes by analyzing the long read sequence (Supplementary Tables 2, 3). Likewise, the analysis of the results of transconjugants showed that the coexistence of the \( \text{bla}_{\text{CTX-M-55}} \) gene and other resistance genes (\( \text{dfrA17}, \text{dfrA14}, \text{qnrS1}, \text{tet}, \) and \( \text{rmtB} \) seemed to be the most common on the IncFII plasmid. Therefore, isolates containing the IncFII plasmid carrying \( \text{bla}_{\text{CTX-M-55}} \) were more resistant to clinical antibiotics, and it should also be considered in the clinic.

Mobile sequences such as insertion sequences (ISs) and transposons (Tns) are important elements that mediate the horizontal transmission of the \( \text{bla}_{\text{CTX-M}} \) gene (Cullik et al., 2010;
Ecp1 is often located upstream of the CTX-M gene and is responsible for the movement of all β-lactamases in E. coli. Five types of genetic structure were obtained by analyzing the environment surrounding blaCTX-M-55 in the isolates. The upstream elements were mainly ISeca1 and IS26. Similar to previous studies (Cao et al., 2011; Fu et al., 2020), the most common genetic environment for blaCTX-M-55 is type II “ISEcp1-blaCTX-M-55-ORF477.” Studies (Wang et al., 2013) have shown that ISEcp1 seems to be a strong activator of blaCTX-M-55 expression. Additionally, IS26, IS903, and ORF477 are also detected frequently around them (Lartigue et al., 2006; Poirel et al., 2008; Hu et al., 2018). In this study, we found that the blaCTX-M-55 gene existed on both chromosomes and plasmids of the same isolate. Interestingly, the genetic environments of isolates 75, 128, and 173 on the chromosome and on the IncI1 plasmid were the same, both of which were type II. When comparing the genetic environments of the chromosome and IncFIC plasmid of isolate N18, only one copy of ISEcp1 on the plasmid was disrupted by IS26. It has also been reported (Ensor et al., 2006; Zhang et al., 2019) that the disruption of the ISEcp1 element by IS26 is related to the transmission of the blaCTX-M gene. ISEcp1 can mobilize the CTX-M gene to different types of plasmids with IS26 (Canton et al., 2012). This result showed that under certain conditions, the mobile element may be able to mediate the transfer of the blaCTX-M-55 gene between the plasmid and the chromosome. Although IS903 was not detected, we found that ISEcp1 and IS26 were upstream of the blaCTX-M-55 gene. More interestingly, there were four copies of “IS26-blaCTX-M-55-ORF477” in isolate 110. Generally, the presence of multi-copy blaCTX-M-55 genes may be associated with increased cephalosporins resistance. The transposition mechanism of IS26 is generally regarded to involve replicative transposition and co-integrate formation (Jiang et al., 2020). We can speculate that IS26 mediated transposon unequal crossover, produced four copies of IS26 composite transposons in a row, which has also been reported in previous studies (Lee et al., 2012; Jiang et al., 2020). Therefore, we should strengthen the detection and analysis of mobile genetic element IS26 to monitor the transmission trend of plasmids carrying the blaCTX-M-55 gene with related elements in the clinical environment.

CONCLUSION

Most of the isolates carrying the blaCTX-M-55 gene are highly resistant to cephalosporins, but are still highly sensitive to amikacin, β-lactamase inhibitors and carbapenems, which can be used as the choice of clinical medication. In this study, prevalent ST clones were not detected. However, it remains to be seen whether ST1193 carrying the blaCTX-M-55 gene can become a popular clone in this region. Meanwhile, we should pay close attention to the spread of CTX-M-55-positives isolates. The epidemic plasmid Incl1 and IncFII, which have the highest detection rate and transfer efficiency, may play an important role in the spread of the blaCTX-M-55 gene. In particular, the IncFII plasmid usually carries more drug resistance genes. Mobile elements such as ISEcp1 and IS26 may be the main factors leading to the spread and prevalence of CTX-M-55 genotypes on chromosomes and plasmids.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and
accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, JAHSRN000000000-JAHSS00000000.

**AUTHOR CONTRIBUTIONS**

XL conceived and designed the study. CZ and AW provided the samples. XL and CZ funded the project. SZ and LH carried out the experiment. SZ, XC, and JL steered the literature search, data collection, and analysis. SZ drafted the manuscript. AW, XL, and CZ reviewed and approved the submission of the manuscript. All authors discussed the results and commented on the manuscript.

**FUNDING**

This project was funded by the Youth Foundation of the National Natural Science Foundation of China (81902104), the Medical Science and Technology Research Project of Foshan Science and Technology Innovation Program (1920001000717), and the International Cooperation and Exchange Program of the National Natural Science Foundation of China (81861138056).

**ACKNOWLEDGMENTS**

We thank Kai Zhou (The First Affiliated Hospital of Southern University of Science and Technology, China) and Yan Jiang (The Sir Run Shaw Hospital, College of Medicine, Zhejiang University, Hangzhou, China) for their suggestions and revisions to this study, and Jingjie Song for his technical assistance in the genome sequencing data analysis.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.730012/full#supplementary-material
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