Genetic contexts related to the diffusion of plasmid-mediated CTX-M-55 extended-spectrum beta-lactamase isolated from Enterobacteriaceae in China

Xiaoxin Hu1†, Jianjun Gou1,2*†, Xiaobing Guo1,2, Zaiqiu Cao3, Yuan Li1, Hongjian Jiao4, Xiaohong He1, Yihui Ren1 and Fuyun Tian1

Abstract

Background: CTX-M-55 extended-spectrum beta-lactamases are being rapidly disseminated and transmitted in clinical practices around the world. The genetic contexts of the transferable plasmid-mediated blaCTX-M-55 gene in Enterobacteriaceae were detected and characterized in this study.

Methods: Isolates were obtained from the First Affiliated Hospital of Zhengzhou University between September 2015 and March 2016. Based on polymerase chain reaction and BLAST analysis, resistance genes and genetic context of the blaCTX-M-55 gene were investigated. Conjugation experiments and multilocus sequence typing were performed to demonstrate plasmid-mediated blaCTX-M-55 transmission.

Results: Thirteen blaCTX-M-55-positive isolates of Enterobacteriaceae were obtained. Seven isolates were Escherichia coli, 3 were Klebsiella pneumoniae, 1 was Citrobacter freundii, 1 was Morganella morganii and 1 was Serratia marcescens. The blaCTX-M-55 gene has not previously been identified from C. freundii and M. morganii. Four different blaCTX-M-55 genetic contexts were identified, and all of them harbored ISEcp1 in the region upstream of blaCTX-M-55 (in two cases, ISEcp1 was truncated by IS26, and in one case, it was truncated by IS1294), whereas ORF477 was detected downstream of the blaCTX-M-55 gene from 12 of 13 strains. The novel genetic context of ISEcp1Δ-blaCTX-M-55-ΔIS903 was firstly detected. A conjugation assay revealed that all blaCTX-M-55 plasmids were quickly and easily transferable to recipient E. coli, which then presented resistance to multiple antibiotics.

Conclusions: Numerous blaCTX-M-55-positive strains were isolated in a short period of 7 months. The findings indicate that blaCTX-M-55 was rapidly disseminated. The genetic context and conjugative transfer found in this study demonstrate that there is active transmission of blaCTX-M-55 among strains of Enterobacteriaceae in China, which could give rise to an urgent global public health threat.

Keywords: blaCTX-M-55, Enterobacteriaceae, ISEcp1

Background

Since the first reports of CTX-M extended-spectrum beta-lactamases (ESBLs) in 1989 [1], at least 26 bacterial species across the world have been referenced in the “CTX-M pandemic” [2]. More than 190 diverse variants of CTX-M have been recorded to date. Among these variants, CTX-M-55 pertains to the CTX-M-1 cluster, which is a variant of CTX-M-15 with only one amino acid substitution (Ala-80-Val) [3]. This variant was first reported in 2006 [4] and was identified in Thailand as well as in the UK [3–5]. Over the past decade, the isolate rate of CTX-M-55 in Escherichia coli from animals has been increasingly raised. However, CTX-M-55 was not identified...
in clinical practices in China until 2010, when it was detected from a person who traveled to China [6]. Since then, plenty of surveys have confirmed the emergence of \( \text{bla}_{\text{CTX-M-55}} \) among clinical pathogenic in China [7–11].

Conjugative plasmids are one of the most important mechanisms for the appearance and spread of \( \text{bla}_{\text{CTX-M}} \). These plasmids facilitate horizontal transfer to other isolates and even cross-species barriers [12]. Insertion sequences (ISs), which cause insertion mutations and genome rearrangements, are the smallest mobile elements (<2.5 Kb) independent transposition in an organism and competent to promote translocation, and the transferability of a resistance gene will largely increased under the mediated of ISs [13]. Various types of genetic platforms are associated with \( \text{bla}_{\text{CTX-M}} \) genes, and \( \text{ISEcp1} \) is frequently recorded upstream of \( \text{bla}_{\text{CTX-M}} \). \( \text{ISEcp1} \) can transpose the \( \text{bla}_{\text{CTX-M}} \) gene and act as a strong activator for the high expression of it [12, 14, 15]. In addition, other insertion sequences, including \( \text{IS26} \), \( \text{IS903} \) and \( \text{ORF477} \), are also frequently detected surrounding \( \text{bla}_{\text{CTX-M}} \) [16, 17].

Thus, this study intends to inquire into the prevalent trend of \( \text{bla}_{\text{CTX-M-55}} \) genes and their transferability and genetic contexts among clinical strains in Henan Province in central China.

Methods

Bacterial isolates, antimicrobial susceptibility testing and ESBLs confirmation

Total number of 227 unduplicated ESBL-positive Enterobacteriaceae [Escherichia coli (n = 93), Klebsiella pneumoniae (n = 86), Enterobacter cloaceae (n = 13), Enterobacter aerogenes (n = 6), Proteus mirabilis (n = 7), Citrobacter freundii (n = 13), Morganella morganii (n = 3), Serratia marcescens (n = 5), and Shigella flexneri (n = 1)] clinical isolates were obtained from the First Affiliated Hospital of Zhengzhou University in Central China between September 2015 and March 2016. All strains were confirmed by using Vitek 2 (bioMérieux, France). Antimicrobial susceptibility for the \( \text{bla}_{\text{CTX-M-55}} \)-Producing strains and transconjugants were determined using Vitek 2, followed by the measurement of minimum inhibitory concentrations (MICs) utilizing the broth microdilution method (for piperacillin–tazobactam, ampicillin–sulbactam, cefotaxime, ceftazidime, cefotetan, cepfime, imipenem, ertapenem, amikacin, gentamicin, levofloxacin, and ciprofloxacin). Microbroth and agar dilution methods were standardized following the protocols from the Clinical and Laboratory Standards Institute (CLSI) [18]. The MIC results were judged by 2014 CLSI criteria [18]. All isolates were confirmed to have the ESBL phenotype through the CLSI disc confirmatory test [18]. \( K. \ pneumoniae \) ATCC 700603 and \( E. \ coli \) ATCC 25922 were used as quality control strains.

Identification of resistance genes and the genetic contexts of \( \text{bla}_{\text{CTX-M-55}} \)

To verify the emergence of plasmid-mediated ESBL genes, all ESBL-positive strains were further characterized, and plasmid DNA was extracted utilizing a Tiangen Plasmid Purification Mini Kit (Tiangen Biotech, China) referring to the protocol of manufacturer. The primer sequences presented in Table 1 were used for the \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{CTX-M-1 Groups}} \) to determine the genetic context of \( \text{bla}_{\text{CTX-M-55}} \). Purified PCR productions were sequenced immediately from two ends and compared with genes in GenBank (http://www.ncbi.nlm.nih.gov/genebank/).

Multilocus sequence typing (MLST)

MLST for clinical \( E. \ coli \) and \( K. \ pneumoniae \) strains were detected basis on the assay discussed above [19, 20].

| Table 1 | PCR primers characteristics in this study |
|---------|-----------------------------------------|
| **PCR target** | **Primer name** | **Primer sequence** | **Annealing temperature (°C)** | **Product (bp)** | **Reference** |
| Upstream flanking region of \( \text{bla}_{\text{CTX-M-55}} \) | \( \text{ISEcp1-F} \) | CAAAATGATCCCCCTGCACA | 55 | Variable | [29] |
| | \( \text{ISEcp1-R} \) | ACTTTACTGGTACTGCACAT | | | |
| Downstream flanking region of \( \text{bla}_{\text{CTX-M-55}} \) | \( \text{ISEcp1-F} \) | TCTCGTGACRACAGTCCACCA | 55 | Variable | [29] |
| | \( \text{ISEcp1-R} \) | GATTTCCGTTGCCGCTTACG | | | |
| \( \text{bla}_{\text{CTX-M-1}} \) | \( \text{ORF477-F} \) | TGTTTTCGTGGTGCTGAATTT | 57 | 800 | [11] |
| | \( \text{ORF477-R} \) | GGCCATATTAAAAAATCGTCC | | | |
| \( \text{bla}_{\text{TEM}} \) | \( \text{ORF477-F} \) | CATTCCGCGTCGCCGCTTATC | 56 | 800 | [11] |
| | \( \text{ORF477-R} \) | CTGGTCTGTCATGTTGCTTGA | | | |
| \( \text{bla}_{\text{SHV}} \) | \( \text{ORF477-F} \) | AGCCCGTTGGCACAATCAAAC | 55 | 713 | [11] |
sequence types (STs) and allelic profiles were assigned after comparing them to an online database (http://bigdb.Pasteur.fr/ecoli/ecoli.html and http://bigdb Pasteur.fr/klebsiella/klebsiella.html).

Conjugation experiments
Conjugative assays were performed using the methods discussed above [7]. The bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates served as donors, and \textit{E. coli} C600 functioned as a recipient. Transconjugants were screened on Mueller–Hinton agar containing 750 μg/ml rifampin and 100 μg/ml ampicillin. The existence of bla\textsubscript{CTX-M-55}\textsuperscript{+} in the transconjugants was identified through antimicrobial susceptibility, PCR and DNA sequencing.

Results
Identification of bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates and their antimicrobial susceptibility and resistance determinants
Based on the results of this study, among 227 ESBL-positive \textit{Enterobacteriaceae}, 13 [13/227 (5.73%)] were identified as bla\textsubscript{CTX-M-55}\textsuperscript{+}, including 7/93 \textit{E. coli}, 3/86 \textit{K. pneumoniae}, 1/13 \textit{C. freundii}, 1/3 \textit{M. morganii}, and 1/5 \textit{S. marcescens}, which were collected from blood (n = 6), urine (n = 3), and sputum (n = 3) samples (Table 2). The antimicrobial susceptibility analyses of the 13 bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates are presented in Table 3. All strains were insusceptible to third-generation cephalosporins (ceftazidime and cefotaxime), fluoroquinolones (levofloxacin and ciprofloxacin), and gentamicin. In addition, 100% susceptibility to amikacin was found.

| Isolate | Specimen | Department | ESBL | MLST |
|---------|----------|------------|------|-------|
| EC30    | Blood    | Urology    | TEM/SHV | ST156 |
| EC32    | Blood    | Gastroenterology | – | ST305 |
| EC44    | Urine    | Respirations | – | ST182 |
| EC45    | Sputum   | ICU        | TEM | ST305 |
| EC52    | Blood    | Urology    | – | ST381 |
| EC54    | Blood    | ICU        | – | ST446 |
| EC67    | Blood    | Gastroenterology | TEM | ST2 |
| KP26    | Sputum   | Thoracic surgery | TEM | ST148 |
| KP37    | Blood    | General surgery | TEM/SHV | ST269 |
| KP146   | Urine    | Urology    | TEM | ST37 |
| CF547   | Urine    | Urology    | – | – |
| MM556   | Drainage fluid | Anus and intestine surgery | TEM | – |
| SM554   | Sputum   | Neurosurgery | – | – |

Table 2. Characteristics of bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates

Genetic contexts of bla\textsubscript{CTX-M-55}
The flanking region of bla\textsubscript{CTX-M-55}\textsuperscript{+} is presented in Fig. 1. Four different architectures [type I (9 isolates), type II (2 isolates), type III (1 isolate), and type IV (1 isolate)] were identified regarding the genetic contexts of the plasmid-mediated bla\textsubscript{CTX-M-55}\textsuperscript{+} genes. Type I architecture (ISEcp1\textsuperscript{Δ}-bla\textsubscript{CTX-M-55}\textsuperscript{+}-ORF477) was the most common and identified in 9 (69.23%) of 13 bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates; the occurrence of type II (IS26-\textsuperscript{Δ}ISEcp1-bla\textsubscript{CTX-M-55}\textsuperscript{+}-ORF477) and type III architecture (ISEcp1\textsuperscript{Δ}-IS1294\textsuperscript{Δ}ISEcp1-bla\textsubscript{CTX-M-55}\textsuperscript{+}-ORF477) was similar to type I architecture, although ISEcp1 was disrupted by IS26 in type II and by IS1294 in type III. Type IV (ISEcp1\textsuperscript{Δ}-bla\textsubscript{CTX-M-55}\textsuperscript{+}-ORF477) was characterized by the existence of IS903, which was detected mainly downstream of bla\textsubscript{CTX-M-55}\textsuperscript{+}.

Discussion
Since the CTX-M-55 firstly reported in 2006, it has been identified in \textit{E. coli}, \textit{K. pneumoniae}, \textit{S. flexneri} and \textit{Salmonella enteritidis} [3, 7, 10]. For all we know, bla\textsubscript{CTX-M-55} in \textit{C. freundii} and \textit{M. morganii} is firstly detected in this study. In addition, 13/227 isolates were identified as bla\textsubscript{CTX-M-55}\textsuperscript{+} positive in just 7 months. This rate far surpasses other ESBLs [21–23], which demonstrates the rapid dissemination of bla\textsubscript{CTX-M-55}\textsuperscript{+}. Notably, all bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates were identified as multiple drug-resistant (MDR) bacteria that are strongly resistant to cefotaxime and ceftazidime (MIC > 256 μg/ml). More significantly, molecular characterization also revealed that most of the bla\textsubscript{CTX-M-55}\textsuperscript{+} positive isolates harbored bla\textsubscript{TEM}. In addition, some isolates contained bla\textsubscript{SHV}. These results imply that the spreading of bla\textsubscript{CTX-M-55}\textsuperscript{+} over many different genera of \textit{Enterobacteriaceae} is activated in hospitals.
Table 3 Antibiotic susceptibilities of blaCTX-M-55-positive and their transconjugants

| Isolate      | Antibiotic susceptibility (μg/ml) |
|--------------|----------------------------------|
|              | SAM    | Tzp | Ctx | CAZ | CTT | FEP | IPM | ETP | AMK | GEN | LVX | CIP |
| EC30         | > 256  | > 256 | > 256 | > 256 | > 256 | < 1 | 2   | < 2 | 64  | > 32 | > 32 |
| EC32         | > 256  | 8    | > 256 | > 256 | > 256 | < 1 | 1   | < 2 | 64  | 16  | 8   |
| EC44         | > 256  | 8    | > 256 | > 256 | > 256 | < 1 | < 0.5 | < 2 | 32  | 16  | 8   |
| EC45         | > 256  | 64   | > 256 | > 256 | 32   | > 256 | < 0.5 | < 2 | 32  | 16  | 8   |
| EC52         | > 256  | 8    | > 256 | > 256 | 8    | 2   | < 0.5 | < 2 | 64  | > 32 | 8   |
| EC54         | > 256  | 8    | > 256 | > 256 | 8    | 16  | < 0.5 | < 2 | 32  | 16  | 8   |
| EC67         | > 256  | > 256 | > 256 | > 256 | > 256 | < 1 | 2   | < 2 | 32  | > 32 | 8   |
| KP26         | > 256  | 64   | > 256 | > 256 | 32   | > 256 | < 1 | 1   | < 2 | 32  | > 32 | 8   |
| KP37         | > 256  | > 256 | > 256 | > 256 | > 256 | 8 | 2   | < 2 | 32  | 16  | 8   |
| KP146        | > 256  | 64   | > 256 | > 256 | > 256 | 8 | 2   | < 2 | 32  | > 32 | 8   |
| CF547        | > 256  | 8    | > 256 | > 256 | > 256 | 8 | < 0.5 | < 2 | 64  | 16  | 8   |
| MM556        | > 256  | 64   | > 256 | > 256 | > 256 | > 256 | > 256 | 8 | 2   | < 0.5 | 2 | 32 | 16 | 8 |
| SM554        | > 256  | 8    | > 256 | > 256 | 64  | 8 | > 256 | < 0.5 | 4 | 32 | 16 | 8 |

E. coli transconjugants

| Isolate      | Antibiotic susceptibility (μg/ml) |
|--------------|----------------------------------|
| EC30-C600    | > 256  | 64   | > 256 | > 256 | > 256 | > 256 | < 1 | 2   | < 2 | 64  | < 0.25 | < 0.25 |
| EC32-C600    | > 256  | 8    | > 256 | > 256 | > 256 | > 256 | < 1 | 1   | < 2 | 32  | < 0.25 | < 0.25 |
| EC44-C600    | > 256  | 8    | 128  | 64    | 4    | 4    | < 1 | < 0.5 | < 2 | 32  | < 0.25 | < 0.25 |
| EC45-C600    | > 256  | 32   | > 256 | > 256 | 32   | 16   | < 1 | < 0.5 | < 2 | 32  | < 0.25 | < 0.25 |
| EC52-C600    | > 256  | 8    | > 256 | 128  | 8    | 2    | < 1 | < 0.5 | < 2 | 32  | < 0.25 | < 0.25 |
| EC54-C600    | > 256  | 8    | 128  | > 256 | > 256 | > 256 | < 1 | 1   | < 2 | 32  | < 0.25 | < 0.25 |
| EC67-C600    | > 256  | > 256 | > 256 | > 256 | > 256 | > 256 | < 1 | 2   | < 2 | 16  | < 0.25 | < 0.25 |
| KP26-C600    | > 256  | 32   | > 256 | > 256 | 16   | 8    | < 1 | 1   | < 2 | 32  | < 0.25 | < 0.25 |
| KP37-C600    | > 256  | 64   | > 256 | 64    | 8    | 128  | > 256 | > 256 | 8 | 2   | < 2 | 16  | < 0.25 | < 0.25 |
| KP146-C600   | > 256  | 32   | > 256 | > 256 | > 256 | > 256 | 8 | 2   | < 2 | 64  | < 0.25 | < 0.25 |
| CF547-C600   | > 256  | 4    | > 256 | 64    | 8    | 32   | < 1 | < 0.5 | < 2 | 32  | < 0.25 | < 0.25 |
| MM556-C600   | > 256  | 64   | > 256 | > 256 | > 256 | > 256 | 8 | 2 | < 0.5 | < 2 | 16 | < 0.25 | < 0.25 |
| SM554-C600   | > 256  | 8    | > 256 | 64    | 8    | 32   | > 256 | > 256 | 8 | 2   | < 0.5 | < 2 | 16 | < 0.25 | < 0.25 |
| EC6-C600     | < 2    | < 4  | < 1  | < 1  | < 4  | < 1  | < 1  | < 0.5 | < 2 | 1   | < 0.25 | < 0.25 |

EC, E. coli; KP, K. pneumoniae; CF, C. freundii; MM, M. morganii; SM, S. marcescens

* SAM, ampicillin–sulbactam (1/0.5–256/128) (μg/ml) for each agent, and the numbers in parentheses indicate the test range; Tzp, piperacillin–tazobactam (0.5/4–256/4); Ctx, cefotaxime (0.03–256); CAZ, ceftazidime (0.03–256); CTT, cefotetan (0.03–256); FEP, cefepime (0.015–256); IPM, imipenem (0.06–32); ETP, ertapenem (0.004–32); AMK, amikacin (0.5–256); GEN, gentamicin (0.25–256); LVX, levofloxacin (0.008–32); CIP, ciprofloxacin (0.004–32)

Fig. 1 Surrounding the regions of blaCTX-M-55 gene in this study. Type I architecture (ISEcp1∆-blaCTX-M-55-ΔORF477) was found in isolates (EC32, EC44, EC52, EC67, KP26, KP146, CF547, MM556, SM554) (GenBank Accession Numbers: KX889071; KX889081; KX889072; KX889073; KX889074; KX889075; KX889076; KX889077; KX889078); Type II architecture (IS26-∆ISEcp1-blaCTX-M-55-ΔORF477) was found in isolates (EC45, KP37) (GenBank Accession Numbers: KX889079; KX889080); Type III architecture (ISEcp1∆-IS1294-∆ISEcp1-blaCTX-M-55-ΔORF477) was found in isolate (EC30) (GenBank Accession Number: KX889079); Type IV architecture (ISEcp1∆-blaCTX-M-55-ΔIS903) was found in isolate (EC54) (GenBank Accession Numbers: KX898438 and KX898439)
settings. Interestingly, our data indicate that some origins previously reported, in disruption by ISEcp1 IS1294 prevalence of ST131 has been observed [24]. Further, this study demonstrates the association of eight STs [ST305, ST182, ST381, ST446 and ST2 (E. coli) and ST148, ST269 and ST37 (K. pneumoniae)] with the products of CTX-M-55 first time, which means blaCTX-M-55 has been actively spreading among Enterobacteraeae in China. Given our focus on conjugative assays, the 13 transconjugants all exhibited resistance to cefotaxime and ceftazidime but sensitivity to fluoroquinolones, which was consistent with the original isolates. These results suggest that the plasmid-mediated blaCTX-M-55 gene is to answer for an ESBL phenotype with poor susceptibility to cefotaxime and ceftazidime and exhibits a strong transferability of resistance. This finding also indicates that fluoroquinolones should be used for the therapy of blaCTX-M-55-positive pathogen infections in clinical settings. Interestingly, our data indicate that some original isolates were resistant to cefepime, but the transconjugants were susceptible, which suggests that the original isolates may include other resistance genes that promote resistance to cefepime. We did not detect these genes in our study. These resistance genes cannot be transmitted through conjugative assays and are not located on the chromosome. Thus, this mechanism requires further study.

The sporadic existence of CTX-M-55-positive strains in mainland China has been occasionally detected. In some surveys, CTX-M-55 incidence has surpassed that of CTX-M-15 [25]. Heterogeneous genetic contexts may indicate the dissemination and mobilization of blaCTX-M-55. As shown in Fig. 1, all isolates were detected ISEcp1, locating upstream of blaCTX-M-55, this region contains the promoter sequence (−35 and −10) and act as a significant role in the expression and mobilization of the β-lactamase genes [12, 15, 26]. Moreover, the presence of ISEcp1 in this cross-species study indicates that the complete or partial insertion sequence was probably excised along with CTX-M-55 during horizontal transfer. Previous reports demonstrated that the disruption of the ISEcp1 element by IS26 was linked to the promotion of blaCTX gene dissemination [27, 28]. Interestingly, as previously reported, ISEcp1 disruption by IS1294 in blaCTX-M-55 was detected from a chicken in China, which may contribute to the mobilization of blaCTX-M-55 [29]. Remarkably, the two E. coli strains [EC30 (this study) and E. coli C21 [29]] shared the same MLST type (ST156), which suggests that these isolates are clonally related. This coincidence implies that blaCTX-M-55 is likely to be transferred from animals to the clinical setting. Fey et al. found that a 12-year-old boy acquired ceftriaxone-resistant Salmonella enterica serotype Typhimurium from cattle [30]. Jing Zhang et al. reported that CTX-M-55 had already been transmitted to humankind from animals and is distributed among both hospitals and community in China. The findings of our investigation and previous studies indicate that blaCTX-M-55 can be transmitted to humankind from food and can enhance clinical resistance. Notably, the novel arrangement ISEcp1−blaCTX-M-55−ΔIS903 is characterized by the element of IS903 which is detected downstream of blaCTX-M-55 first time and often identified by the context of other blaCTX-M genes [31]. The mechanism responsible for its presence remains unclear. According to Poirel et al., ISEcp1, blaCTX and IS903 form a putative transposon, and this block of genes could be disseminated by transposition [26, 32]. This finding implies that IS903 contributes to the dissemination of blaCTX-M-55, which requires further study. Therefore, our findings strongly suggest that genetic elements (ISEcp1, ORF477, IS26, IS1294, and IS903) are involved in the inter-species and intra-species mobilization and dissemination of blaCTX-M-55. Additionally, CTX-M-55-harboring isolates in animals may act as a potential storage of bacterial that is spread in clinical.

Conclusions

This investigation reminds a high occurrence rate of CTX-M-55-producing ESBLs in patients from different departments at the First Affiliated Hospital of Zhengzhou University in Henan Province. These plasmid-mediated blaCTX-M-55-positive isolates are contributed to the transmission of blaCTX-M-55 to new species and new hosts by conjugation. Data obtained in this study suggest that the genetic context of blaCTX-M-55, especially ISEcp1, act as a vital part in the mobilization, dissemination, and expression of drug resistance determinants. We also demonstrated a novel arrangement of blaCTX-M-55 (ISEcp1−blaCTX-M-55−ΔIS903). Thus, the presence of MDR Enterobacteraeae contains conjugative plasmids that co-harbor other IS elements, such as ISEcp1, should be surveilled worldwide because the active transfer and high prevalence of these pathogenic will significantly decrease our further selection of clinical therapies. Further studies on this issue should be performed to help us obtain a deeper understanding of the transmission and
dissemination of plasmid-mediated bla_{CTX-M-55} in different genetic platforms.

Nucleotide sequence accession number
The nucleotide sequences presence in this study have been submitted to GenBank under the following accession numbers: KX889070 (E. coli: EC30); KX889071 (E. coli: EC32); KX889072 (E. coli: EC52); KX889438 and KX889439 (E. coli: EC54); KX889073 (E. coli: EC67); KX889074 (K. pneumoniae: KP26); KX889075 (K. pneumoniae: KP146); KX889076 (C. freundii: CF547); KX889077 (M. morganii: MM556); KX889078 (S. marcescens: SM554); KX889079 (E. coli: EC45); KX889080 (K. pneumoniae: KP37); KX889081 (E. coli: EC44).

Authors’ contributions
JG and XH contributed to study design. XH, YR and YL collected the samples and performed the experiments. All authors contributed to data analysis. JG and XH contributed to study design. XH, YR and YL collected the samples and performed the experiments. All authors contributed to data analysis. JG and XH drafted the manuscript. All authors read and approved the final manuscript.

Author details
1 Department of Clinical Laboratory, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. 2 Key Laboratory of Laboratory Medicine Technology, Xinyang Vocational and Technical College, Xinyang, Henan, China. 3 School of Medicine, Pusan National University, Busan, Republic of Korea. 4 Department of Medical Laboratory Technology, Xinyang Vocational and Technical College, Xinyang, Henan, China.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Not applicable.

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Not applicable.

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