

# The Characteristics of Extended-Spectrum $\beta$ -Lactamases (ESBLs)-Producing *Escherichia coli* in Bloodstream Infection

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**Background:** Bloodstream infection (BSI) is a common type of infection frequently diagnosed in clinics. The emergence and spread of ESBLs-producing *Escherichia coli* (*E. coli*) has emerged as one of the biggest challenges in global community health.

**Methods:** The production of ESBLs was determined by the composite disk diffusion method. The expression of the various resistance and virulence genes were detected by PCR and sequencing. Multi-locus sequence typing (MLST) and phylogenetic groups were used for the classification. The transfer of resistant plasmids was determined by conjugation assay. The statistical differences were analyzed using Statistical Product and Service Solutions (SPSS) version 23.0.

**Results:** A total of 60 strains of ESBLs-producing *E. coli* were collected. The resistance genes that were identified included *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub> and *mcr-I*. The most common one was the *bla*<sub>CTX-M</sub> including *bla*<sub>CTX-M-27</sub> (n = 16), *bla*<sub>CTX-M-14</sub> (n = 15), *bla*<sub>CTX-M-15</sub> (n = 11), *bla*<sub>CTX-M-55</sub> (n = 14) and *bla*<sub>CTX-M-65</sub> (n = 5). A total of 31 STs were detected, and the most abundant among which was ST131 (n = 16, 26.7%). Most of the *E. coli* (n = 46, 76.7%) belonged to the groups B2 and D. And some virulence genes were related to the classification of the *E. coli*. Among them, the detection rates of *hek/hra*, *kpsMII* and *papGII-III* in groups B2 and D were higher than those in groups A and B1. The detection rates of *cnfI*, *iucC* and *papGII-III* in ST131 were higher than those in non-ST131. And the distributions of *hek/hra*, *iroN*, *iucC*, *kpsMII* and *papGII-III* were related to the *bla*<sub>CTX-M</sub> subtypes. Finally, most bacterial (n = 32, 53.3%) resistance genes could be transferred between the bacteria by plasmids, especially IncFIB.

**Conclusion:** ESBLs-producing *E. coli* in BSI exhibited had high resistance rates and carried a variety of virulence factors (VFs). This is necessary to strengthen the monitoring of ESBLs-producing isolates in the medical environment.

**Keywords:** *Escherichia coli*, sepsis, beta-lactamases, *bla*<sub>CTX-M</sub>

## Introduction

A variety of pathogens, including *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus*, *Streptococcus*, and *Enterococcus*, constitute an important class of pathogenic bacteria that can cause BSI. Among them, *E. coli* has been recognized as the most common pathogen.<sup>1</sup> Clinically, common *E. coli* includes five kinds of enteropathic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC). The pathogenicity of these *E. coli* strains was different. Therefore, rapid and simple identification of the type of *E. coli* could be very useful both for the clinical diagnosis and treatment. According to the literature, DNA microarray technology could be potentially used to detect and identify different pathogenic *E. coli* strains, especially urogenic *E. coli* (UPEC), by designing a series of effective and suitable long oligonucleotide microarray probes.<sup>2</sup> This could significantly improve the detection speed of pathogenic bacteria typing and aid in facilitating better hospital infection treatment.

At present, the clinical treatment drugs for *E. coli* mainly include trimethoprim-sulfamethoxazole (sulfonamide), ciprofloxacin (fluoroquinolones), levofloxacin (fluoroquinolones), penicillin and cephalosporins ( $\beta$ -Lactam).<sup>3</sup> However, *E. coli* has been found to be resistant to many different kinds of antibiotics including cephalosporins, quinolones and so on.<sup>4,5</sup> The most common was ESBLs-producing *E. coli*, which could effectively hydrolyze the various antibiotics to make them inactive, including penicillin, cephalosporins and monocyclic. Interestingly, 350 different ESBL variants have been identified so far. According to amino acid sequence, they could be divided into 9 independent families including TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA and OXA.<sup>6,7</sup> The prevalence of *bla*<sub>CTX-M</sub> has increased rapidly.<sup>8,9</sup> And the ESBLs-producing *E. coli* has spread all over the world in recent years.<sup>10,11</sup> In the United States, the ESBLs-producing bacteria were reported to cause about 26,000 drug-resistant infections and 1700 deaths every year. Moreover, in 2019 antibiotic resistance threat report of the Centers for Disease Control and Prevention (CDC), it was classified as an increasingly serious threat.<sup>12</sup> Among the hospitalized patients, ESBL producers accounted for 11.6% and 16.1% of *E. coli* primarily causing UTI and BSI, respectively.<sup>13</sup> In 2021, the bacterial resistance monitoring in China showed that the population of ESBLs-producing *E. coli* had reached up to 52.6%.<sup>14</sup> Besides, the role of Metallo- $\beta$ -lactamases (MBLs) in mediating the drug resistance of *E. coli* could not be ignored. At present, there are more than 2000  $\beta$ -lactamases (BLs) reported in the literature.<sup>15</sup> The BLs have been divided into four distinct categories: A, B, C and D according to different amino acid sequences. Among these, catalytic activities of the categories A, C and D mainly depended on the expression of serine at the active site of the enzyme, so they were termed as serine BLs. However, distinct from the classes A, C and D, the catalytic activity of class B relied primarily on  $\text{Zn}^{2+}$  of the active site, so it was named as MBLs.<sup>16</sup> MBLs have been identified in a variety of pathogenic bacteria since their discovery, including *Pseudomonas aeruginosa*, *K. pneumoniae*, *E. coli*, *Acinetobacter baumannii* and so on. The most widely disseminated were NDMs, IMPs, and VIMs.<sup>17,18</sup> MBLs have been found to be connected with the  $\beta$ -lactam ring in the process of amide bond hydrolysis to resist the activity of the different antibiotics,<sup>19,20</sup> and hydrolyzed a wide range of BLs, including carbapenems, cephalosporins, penicillin and so on.<sup>3</sup> This condition can make it more difficult for the patients to get effective and optimal treatments.

The pathogenesis of invasive diseases caused by ExPEC could be largely determined by the VFs. However, it is necessary to deeply examine the role of VFs in the specific clinical syndromes and host populations for preventing ExPEC infection and its related incidence rate, mortality, as well as increased health care costs.<sup>21</sup> *E. coli* contains a variety of VFs, including adhesins, toxins, protectors, iron absorption system, virulence islands and other virulence proteins.<sup>22,23</sup> The commonly found VFs include Cnf1, FimH, Sfa/Foc, IbeA, IroN, OmpA, HlyC, which can play distinct roles in the pathogenesis. For instance, Cnf1 can exert a key role in the regulation of invasion, dysfunction and apoptosis of human urinary tract epithelial cells. The toxin protein of HlyC can cause both adhesion and the growth of bacteria and promote the inflammation, dissolution and hemolysis of human urinary tract epithelial cells, whereas IbeA is involved in bacterial invasion.<sup>24,25</sup> These VFs can lead to various dangerous conditions in patients, such as bacteremia, septicemia, urinary sepsis, and even death, which can result in considerable difficulties in the treatment of clinical infection. In addition, a specific relationship between bacterial resistance and toxicity has been reported previously in the literature.<sup>26–28</sup> For example, Lee et al compared the distribution of VFs in *E. coli* and found that  $\alpha$ -Haemolysin, yersiniabactin receptor, serum resistance-associated outer membrane protein (TraT), and aerobactin receptor (IutA) could serve as independent predictors of pathogenicity. IutA and TraT were significantly more commonly found in groups *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-9</sub>, respectively.<sup>28</sup> These virulence and resistance genes might possibly be transmitted to other strains together to generate the virulent strains that could be more difficult to treat. Therefore, it is necessary to reveal the molecular relationship between the bacterial virulence and resistance to manage the transmission and treatment of infectious diseases. Based on this, we have collected ESBLs-producing *E. coli* from BSI and studied its molecular characteristics and the relationship between the bacterial virulence and resistance, which could provide a sound reference for the clinical infection control.

## Methods

### Bacteria and Identification

We collected *E. coli* from the First Affiliated Hospital of Anhui Medical University in BSI from January 2020 to January 2021 randomly and eliminated the duplicate strains from the same patient. All the bacteria were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, BioMerieux Inc, France).

### Phenotypic Detection of ESBLs Production

The phenotypic detection of ESBL production was conducted by a combined disc test. Briefly, the discs of cefotaxime (30µg, OXOID, UK), cefotaxime/clavulanic acid (30µg/10µg, OXOID, UK) and ceftazidime (30µg/10µg, OXOID, UK), cefotaxime/clavulanic acid (30µg/10µg, OXOID, UK) were used for the test. The positive control strain was taken from the characteristic strain of the laboratory, and the negative control strain was ATCC25922. The production results of ESBLs were thereafter analyzed according to the Clinical and Laboratory Standards Institute (CLSI 2022) guidelines.

### AST

23 kinds of antibacterial drugs were tested by VITEK@2 compact system (BioMerieux Inc., France) and the K-B method. These included, amtreonam, ertapenem, gentamicin, tobramycin, compound simoxazole, cefotetan, amikacin, ampicillin, ampicillin/sulbactam, cefepime, ceftriaxone, ciprofloxacin, imipenem, levofloxacin, piperacillin/tazobactam, ceftazidime, cefazolin, cefotaxime (30µg, OXOID, UK), cefuroxime (30µg, OXOID, UK), meropenem (10µg, OXOID, UK), minocycline (30µg, OXOID, UK), piperacillin (100µg, OXOID, UK) and tetracycline (15µg, OXOID, UK). The results of all antibiotics except tetracycline were interpreted according to the guidelines of CLSI 2022, and the results of tetracycline were interpreted according to the break point recommended by the Food and Drug Administration (FDA) in 2022.

### Detection of ESBLs Genes and the Virulence Genes

The encoding genes of ESBLs were as follows: *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub> (variant), *bla*<sub>GES</sub>, *bla*<sub>CARB</sub>, *bla*<sub>PER</sub>, *bla*<sub>VEB</sub> and *bla*<sub>OXA</sub>, as well as carbapenemase genes (*bla*<sub>KPC</sub> and *bla*<sub>NDM-1</sub>) and colistin resistance gene *mcr-1*. The virulence genes included *cnf1*, *fimH*, *hek/hra*, *hlyC*, *ibeA*, *iroN*, *iucC*, *kpsMII*, *nlpI*, *papGII-III*, *sfa/foc* and *ompA*. The standard PCR method was used to detect the expression of all the above genes, and the specific primers used have been listed in Table 1. The PCR reaction was carried out under the following conditions: 30 cycles of 10s of denaturation at 98°C, 10s of annealing at specific temperature (Table 1), 20s of extension at 72°C, and a final additional 5 min at 72°C. The amplified products were observed by electrophoresis in 2% agarose gel and then sequenced (Shanghai Biotechnology, China) commercially. Finally, DNA sequences were analyzed by NCBI-BLAST program.

### MLST

The STs of ESBLs-producing *E. coli* were determined by MLST (Achtman). The internal parts of seven different housekeeping genes (*adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*) of *E. coli* were amplified with specific primers (the primer sequences have been shown in Table 1) after extracting *E. coli* DNA by the thermal lysis. Similarly, the amplification products of these genes were observed by electrophoresis in 2% agarose gel and sequenced (Shanghai Sangong Biotechnology, China).

### DRY LAB

The dry lab annotated the bioinformatics part of the nuclear acid-based technology.<sup>32</sup> Bioinformatics-related information was often obtained through online and/or offline tools, software, databases as well as other network services. In this study, the determination of STs was primarily based on the genetic variation of seven housekeeper genes. Seven housekeeper genes were amplified and then sequenced by special primers and compared with the data in the online database (<https://pubmlst.org/organisms/escherichia-spp>) to obtain the specific sites of each gene. The STs of bacteria

**Table 1** The Sequences of Primers and Corresponding PCR Programs Used in This Study

Genes	Function	Primers <sup>a</sup>	DNA Sequences (5'-3')	Annealing Temperature (°C)	Amplicon Size (bp)	References
<i>bla</i> <sub>TEM</sub>	Encoding TEM-type BL	TEM F TEM R	ATAAAATTCCTTGAAGACGAAA GACAGTTACCAATGCTTAATC	55	1080	[29]
<i>bla</i> <sub>SHV</sub>	Encoding SHV-type BL	SHV F SHV R	TGGTTATGCGTTATATTGCGC GGTTAGCGTTGCCAGTGCT	60	865	[29]
<i>bla</i> <sub>CTX-M-1</sub> GROUP	Encoding CTX-M-type BL	CTX-1 F CTX-1 R	CCGTCACGCTGTTGTTAGGAA GGTGACGATTTTAGCCGCC	60	818	This study
<i>bla</i> <sub>CTX-M-2</sub> GROUP		CTX-2 F CTX-2 R	CGACGCTACCCCTGCTATT CCAGCGTCAGATTTTTCAGG	60	552	This study
<i>bla</i> <sub>CTX-M-8</sub> GROUP		CTX-8 F CTX-8 R	TCGCGTTAAGCGGATGATGC AACCACGATGTGGGTAGC	60	666	[29]
<i>bla</i> <sub>CTX-M-9</sub> GROUP		CTX-9 F CTX-9 R	ATGGTGACAAAGAGAGTGCA TTACAGCCCTTCGGCGATGA	60	893	[29]
<i>bla</i> <sub>CTX-M-25</sub> GROUP		CTX-25 F CTX-25 R	GCACGATGACATTCGGG AACCACGATGTGGGTAGC	55	327	This study
<i>bla</i> <sub>VEB</sub>	Encoding VEB-type BL	VEB F VEB R	GCGTAATTTAACCAGA GCCTATGAGCCAGTGTC	55	961	This study
<i>bla</i> <sub>GES</sub>	Encoding GES-type BL	GES F GES R	ATGCGCTTCATTACGCAC CTATTTGTCCGTGCTCAGG	55	846	[29]
<i>bla</i> <sub>PER</sub>	Encoding PER-type BL	PER F PER R	AGTCAGCGGCTTAGATA CGTATGAAAAGGACAATC	55	978	[29]
<i>bla</i> <sub>OXA-1</sub>	Encoding OXA-type BL	OXA-1 F OXA-1 R	CTGTTGTTGGGTTTCGCAAG CTTGGCTTTATGCTTGATG	55	440	[29]
<i>bla</i> <sub>OXA-2</sub>		OXA-2 F OXA-2 R	CAGGCGCYGTTGCGATGAGTT GCCYTCTATCCAGTAATCGCC	60	233	[29]
<i>bla</i> <sub>OXA-10</sub>		OXA-10 F OXA-10 R	GTCTTTCRAGTACGGCATT GATTTTCTTAGCGGCACTTA	55	822	[29]
<i>bla</i> <sub>KPC</sub>	Encoding carbapenemase KPC	KPC F KPC R	CATTCAAGGGCTTTCTTGCTGC ACGACGGCATAGTCATTTGC	60	538	This study
<i>bla</i> <sub>NDM-1</sub>	Encoding carbapenemase NDM-1	NDM-1 F NDM-1 R	GGTCGCGAAGCTGAGCACC GCAGCTTGTCGGCCATGCG	60	782	This study
<i>mcr-1</i>	Colistin resistance gene	mcr-1 F mcr-1 R	ATGATGCAGCATACTTCTGTGTGG TCAGCGGATGAATGCGGT	60	1626	This study
<i>cnfI</i>	Cytotoxic necrotizing factor I	cnfI F cnfI R	AAGATGGAGTTTCTATGCAGGAG CATTGAGAGTCTGCCCTCATTATT	60	498	This study
<i>fimH</i>	Type I fimbriae	fimH F fimH R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	60	508	[30]
<i>hek/hra</i>	Adhesin/hemagglutinin	hek/hra F hek/hra R	CAGAAAACAACCGGTATCAG ACCAAGCATGATGTCATGAC	55	260	[30]
<i>hlyC</i>	Hemolysin	hlyC F	AGGTTCTTGGGCATGTATCCT	60	556	[30]

<i>ibeA</i>	Invasion of brain endothelium A	hlyC R	TTGCTTTGCAGACTGCAGTGT			[30]
		ibeA F	AGGCAGGTGTGCGCCGCTAC	60	170	[30]
		ibeA R	TGGTGCTCCGGCAAACCATGC			[30]
<i>iroN</i>	Salmocheilin	iroN F	GAAAGCTCTGGTGGACGGTA	60	127	[30]
		iroN R	CGACAGAGGATTACCGGTGT			[30]
<i>iucC</i>	Aerobactin	iucC F	AAACCTGGTTTACGCAACTGT	60	269	[30]
		iucC R	ACCCGTCTGCAAATCATGGAT			[30]
<i>kpsMII</i>	Capsule synthesis	kpsMII F	GCGCATTTGCTGATACTGTTG	60	272	[30]
		kpsMII R	CATCCAGACGATAAGCATGAGCA			[30]
<i>nlpl</i>	New lipoprotein I	nlpl F	TTCGTTGCGACAGCACTTAC	60	120	[30]
		nlpl R	TTCCATACGTGCCAGAATCA			[30]
<i>ompA</i>	Outer membrane protein A	ompA F	GAGCCTGGGTGTTTCCTACC	60	390	[30]
		ompA R	TTGTCAAGGTGTTGCCAGT			[30]
<i>papGII-III</i>	P fimbriae	papGII-III-F	CTGTAATTACGGAAGTGATTTCTG	60	1070	[30]
		papGII-III-R	ACTATCCGGCTCCGGATAAACCAT			[30]
<i>sfa/foc</i>	S fimbriae	sfa/foc F	CTCCGGAGAACTGGGTGCATCTTAC	60	410	[30]
		sfa/focR	CGGAGGAGTAATTACAAACCTGGCA		498	[30]
<i>adk</i>	Adenylate kinase	adk F	ATTCTGCTTGGCGCTCCGGG	52	583	[31]
		adk R	CCGTCAACTTTCGCGTATTT			[31]
<i>fumC</i>	Fumarate hydratase	fumC F	TCACAGGTCGCGACGCGCTTC	52	806	[31]
		fumC R	GTACGCAGCGAAAAAGATTC			[31]
<i>icd</i>	Isocitrate/isopropylmalate dehydrogenase	icd F	ATGGAAGTAAAGTAGTTGTTCCGGCACA	52	878	[31]
		icd R	GGACGCAGCAGGATCTGTT			[31]
<i>purA</i>	Adenylosuccinate dehydrogenase	purA F	CGCGCTGATGAAAGAGATGA	54	816	[31]
		purA R	CATACGGTAAGCCACGCAGA			[31]
<i>gyrB</i>	DNA gyrase	gyrB F	TCGGCGACACGGATGACGGC	58	911	[31]
		gyrB R	ATCAGGCCTTCACGCGCATC			[31]
<i>recA</i>	ATP/GTP binding motif	recA F	CGCATTGCTTTACCCTGACC	58	780	[31]
		recA R	TCGTCGAAATCTACGGACCGGA			[31]
<i>mdh</i>	Malate dehydrogenase	mdh F	ATGAAAGTCGCACTCTCGGCGCTGCTGGCGG	58	932	[31]
		mdh R	TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT			[31]

**Note:** <sup>a</sup>Primers. The "F" meant the forward primer and the "R" meant the reverse primer.

were determined by combining the sites of the seven housekeeping genes. Finally, all STs were imported into goEBURST software to analyze the cloning relationship.

## Phylogenetic Group Detection

The main phylogenetic groups of all ESBLs-producing *E. coli* were detected by the multiple PCR as described previously by Clermont et al.<sup>33</sup> Briefly, the existence or deletion of three DNA marker genes (*chuA*, *yjaA* and *TspE4. C2*) were used to group the isolates into distinct phylogenetic groups. It was specifically divided into the following four groups: group A (*chuA* −, *TspE4. C2* −), group B2 (*chuA* +, *yjaA* +), group D (*chuA* +, *yjaA* −) and group B1 (*chuA* −, *TspE4. C2* +). The primer pairs used were *chuA*.F (5′-GACGAACCAACGGTCAGGAT-3′) and *chuA*.R (5′-TGCCGCCAGTACCAAAGACA-3′), *YjaA*.F (5′-TGAAGTGTCTCAGGAGACGCTG-3′) and *YjaA*.R (5′-ATGGAGAATGCGTTCCTCAAC-3′), and *TspE4.C2*.F (5′-GAGTAATGTCGGGGCATTCA-3′) and *TspE4.C2*.R (5′-CGCGCCAACAAAGTATTACG-3′), which generate 279-, 211-, and 152-bp fragments, respectively.

## Plasmid Conjugation Assay

Sodium azide-resistant *E. coli* J53 was used as a recipient, and ESBLs-producing *E. coli* was employed as the donor. We used liquid mating test to select the transfer complex on Luria and Bertani agar containing 100 (μg/mL) sodium azide and 100 (μg/mL) ampicillin, respectively.<sup>34</sup> The presence of ESBLs genes including *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>OXA-1</sub> were then detected by PCR and sequencing (Shanghai Sangong Biotechnology, China).

## PCR-Based Replicon Typing

PCR-based replicon typing was carried out for the plasmids isolated from the transconjugant isolates by using the plasmid extraction kit (Accurate Biology, China). The determination of the incompatibility (Inc) group was carried out by using the specific primers (the primer sequences have been shown in Table 2) as previously described by Caratoli et al in 2005.<sup>35</sup> Five multiplex- and three simplex-PCRs were performed with 18 pairs of primers to identify FIA, FIB, FIC, HI1, HI2, I1-I7, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons. All amplification products were then commercially sequenced (Shanghai Sangong Biotechnology, China).

## Statistical Analysis

Statistical significance was carried out by the chi-square test. Analyses were performed using SPSS version 23.0, and a *p*-value <0.05 was considered to be statistically significant.

## Results

### Antimicrobial Susceptibility

In this study, 60 distinct strains of ESBLs-producing *E. coli* of BSI were randomly collected, including 26 males and 34 females, with an average age of 65.84 ± 13.386 years old. The fingerprints of two strains of *E. coli* by MALDI-TOF MS have been depicted in Figure 1. These 60 strains of *E. coli* were resistant to more than two different types of antibiotics, namely, multidrug-resistant *E. coli*, which were all resistant to ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefuroxime and piperacillin, but were all sensitive to tetracycline, minocycline, ertapenem, meropenem and imipenem. The non-sensitivity rates of the remaining 12 antibiotics ranged from 6.7% to 98.3% (Table 3). As shown in Table 3, the non-sensitivity rates of *E. coli* to 13 antibiotics were observed to exceed 50%. In addition, the non-sensitivity rates of quinolones (levofloxacin and ciprofloxacin) were found to exceed 90%, whereas the non-sensitivity rates of piperacillin/tazobactam, cefotetan and amikacin were lower than 20%.

### Drug Resistance Genes

Multiple resistance genes were detected in 60 different strains of *E. coli*, including *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub> and *mcr-1* (Table 4), and no *bla*<sub>PER</sub>, *bla*<sub>GES</sub>, *bla*<sub>VEB</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>OXA-2</sub>, *bla*<sub>OXA-10</sub>. Among them, *bla*<sub>CTX-M</sub> was the most common one and found in all *E. coli*. However, we were able to only detect the groups *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-9</sub> but not



**Table 2** Primers Used for Amplifying 18 Major Replicons from Plasmids

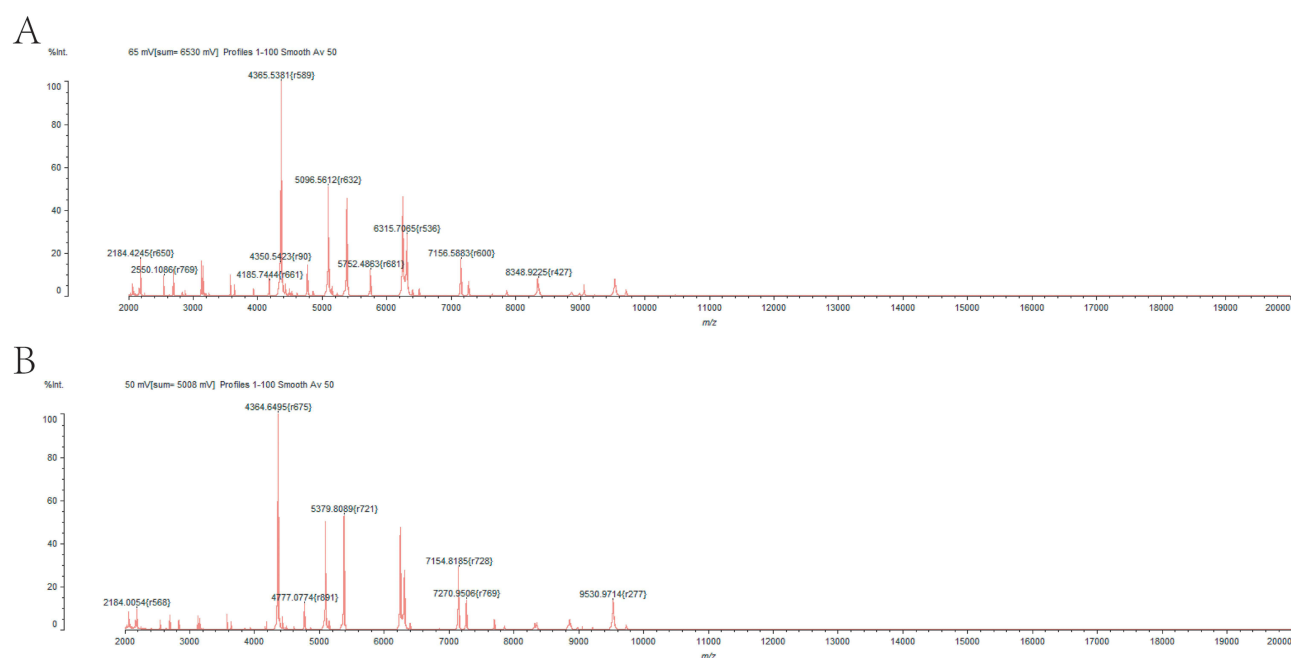
Primers <sup>a</sup>	DNA Sequences (5'-3')	Target Site	EMBL Accession No.	Annealing Temperature (°C)	Amplicon Size (bp)	References
HII F	ggagcgtggattacttctcagtac	parA-parB	AF250878	60	471	[35]
HII R	tgccgtttcacctcgtgagta					[35]
HI2 F	tttctcctgagtcacgtttaacac	iterons	BX664015	60	644	[35]
HI2 R	ggctcactaccgttgatcctc					[35]
II F	cgaagccggacggcagaa	RNAI	M20413	60	139	[35]
II R	tcgtcgttcgcgaagttcgt					[35]
X F	aaccttagaggctatttaagtgtctgat	ori $\gamma$	Y00768	60	376	[35]
X R	tgagagtcgaattttttctcatgttttagc					[35]
L/M F	ggatgaaaaactatcagcatctgaag	repA, B, C	U27345	60	785	[35]
L/M R	ctgcagggcgattctttaagg					[35]
N F	gtctaacgagcttaccgaag	repA	NC_003292	60	559	[35]
N R	gtttcaactctgccaagttc					[35]
FIA F	ccatgctgggtcttagaagagg	iterons	J01724	60	462	[35]
FIA R	gtatatccttactggcttcgcag					[35]
FIB F	ggagttctgacacagattttctg	repA	M26308	60	702	[35]
FIB R	ctcccgtcgttcaggcgatt					[35]
W F	cctaagaaacaacaaagccccc	repA	U12441	60	242	[35]
W R	gggtgcgcggcatagaaccgt					[35]
Y F	aattcaaaacactgtgagcctg	repA	K02380	60	765	[35]
Y R	gcgagaatggagattcaaaacttt					[35]
P F	ctatggccctgcaaacgcgcagaaa	iterons	M20134	60	534	[35]
P R	tcacgcgcagggcgagcc					[35]
FIC F	gtgaactggcagatgaggaagg	repA2	AH003523	60	262	[35]
FIC R	ttctcctcgtcgcacaaatagat					[35]
A/C F	gagaaccaaaagacaaagcctgga	repA	X73674	60	465	[35]
A/C R	acgacaaacctgaattgctcctt					[35]
T F	ttggcctgtttgtgcttaaacat	repA	K00053	60	750	[35]
T R	cgttgattacatttagctttggac					[35]
FIIS F	ctgtcgttaagctgatggc	repA	AE006471	60	270	[35]
FIIS R	ctctgccacaaacttcagc					[35]
FrepB F	tgatcgtttaaggaattttg	RNAI/repA	AY234375	52	270	[35]
FrepB R	gaagatcagtcacacatcc					[35]
K F	gcgggtccggaagccagaaaac	RNAI	M93063	60	160	[35]
K R	tctttcacgagccgcgcaaa					[35]
B/O F	gcgggtccggaagccagaaaac	RNAI	M28718	60	159	[35]
B/O R	tctgcgttcgcgaagttcga					[35]

**Note:** <sup>a</sup>Primers, The "F" meant the forward primer and the "R" meant the reverse primer.

groups *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-8</sub> and *bla*<sub>CTX-M-25</sub>. Figure 2A shows the partial electrophoretic results of 2% agarose gel of the different resistance genes. The sequencing results indicated that the *bla*<sub>CTX-M</sub> was distributed in five distinct subtypes, namely *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-65</sub>. Among them, *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-55</sub> were relatively common (n = 45, 75%), whereas *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-65</sub> were relatively rare (n = 16, 26.7%).

## Phylogenetic Group and MLST

The four phylogenetic groups were B2 (n = 27), D (n = 19), A (n = 11) and B1 (n = 3). The results of phylogenetic grouping depicted that the highly toxic groups B2 and D (n = 46, 76.7%) were more prevalent than the weakly toxic groups A and B1 (n = 14, 23.3%). In addition, we observed that the phylogenetic distributions of five *bla*<sub>CTX-M</sub> subtypes were significantly different (Table 5). And the result of *bla*<sub>CTX-M-55</sub> exhibited statistical difference. In addition, after grouping the bacteria according to their virulence, *bla*<sub>CTX-M-55</sub> still displayed unique difference, which were more common in groups A and B1 with weak toxicity, but the distributions of other subtypes were not different (Table 6). A total of 31 STs were found by MLST, among which the most common was ST131 (n = 16), and each other ST type was less than 3 strains (Table 7). The PCR results of seven housekeeper genes have been shown in Figure 2B. The results of



**Figure 1** The fingerprints (**A** and **B**) of two *E. coli* strains by MALDI TOF MS. The fingerprints were compared with the fingerprints in its own database of MALDI-TOF MS and it was found that the isolated bacteria were *E. coli*.

the single local variants (SLVs) of goEBURST (Figure 3) indicated that 31 STs were divided into 23 different groups, among which 10 STs were distributed in 3 clone groups, whereas the other 21 STs were distributed in a single group, which suggested that the cloning relationship of these 60 strains was far apart. The statistical analysis of the relationships

**Table 3** Non-Sensitivity Rates of 60 *E. coli* Strains to 23 Antibiotics

Antibiotics	Non-Sensitivity Rates, n(%)
Ampicillin	60 (100.0)
Ceftriaxone	60 (100.0)
Cefazolin	60 (100.0)
Cefotaxime	60 (100.0)
Cefuroxime	60 (100.0)
Piperacillin	60 (100.0)
Levofloxacin	59 (98.3)
Ciprofloxacin	55 (91.7)
Ampicillin/sulbactam	47 (78.3)
Compound sulfamethoxazole	46 (76.7)
Amtreonam	40 (66.7)
Tobramycin	32 (53.3)
Gentamicin	30 (50.0)
Ceftazidime	28 (46.7)
Cefepime	20 (33.3)
Piperacillin/tazobactam	8 (13.3)
Cefotetan	6 (10.0)
Amikacin	4 (6.7)
Ertapenem	4 (0.0)
Meropenem	4 (0.0)
Minocycline	4 (0.0)
Tetracycline	4 (0.0)
Imipenem	4 (0.0)



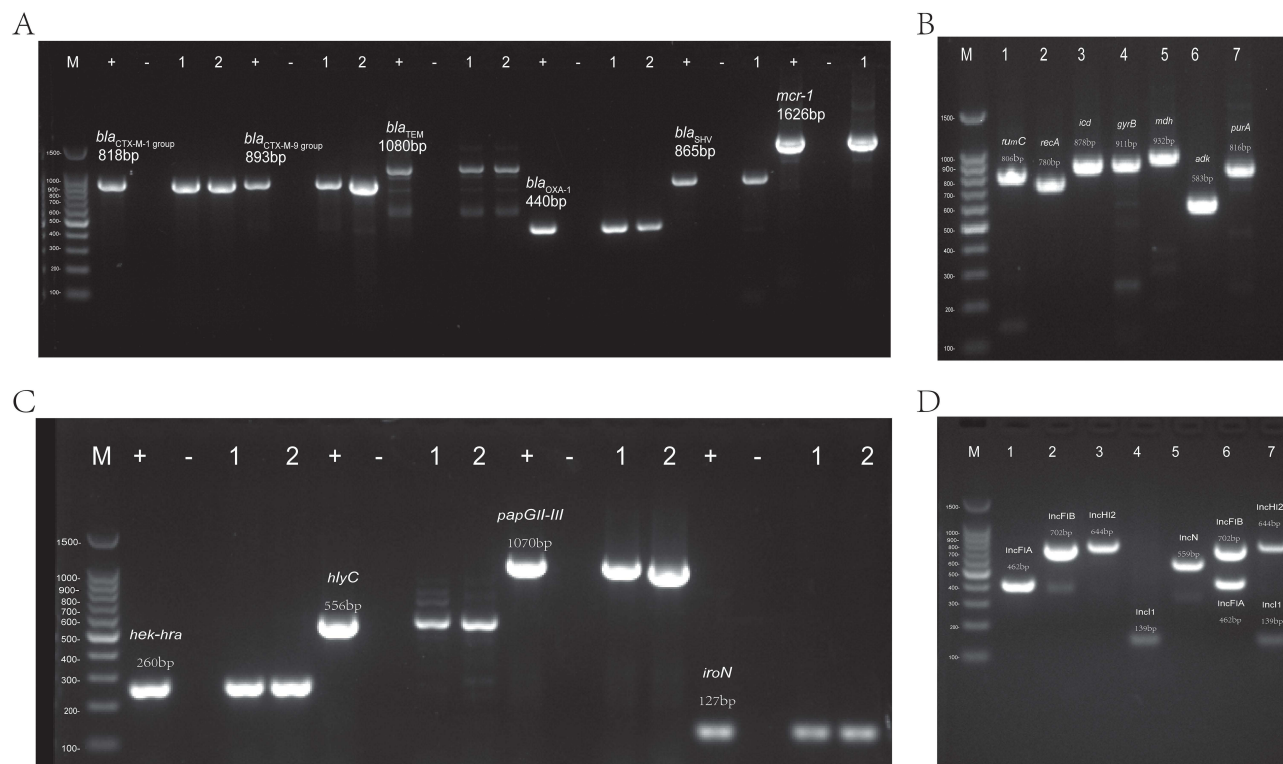
**Table 4** Distribution of Resistance Genes in 60 Strains of *E. coli*

Genes	N (%)
<i>bla</i> <sub>CTX-M-27</sub>	16 (26.7)
<i>bla</i> <sub>CTX-M-14</sub>	15 (25.0)
<i>bla</i> <sub>CTX-M-15</sub>	11 (18.3)
<i>bla</i> <sub>CTX-M-55</sub>	14 (23.3)
<i>bla</i> <sub>CTX-M-65</sub>	5 (8.3)
<i>bla</i> <sub>TEM</sub>	8 (13.3)
<i>bla</i> <sub>SHV</sub>	1 (1.7)
<i>bla</i> <sub>OXA-1</sub>	8 (13.3)
<i>mcr-1</i>	1 (1.7)

between eight positive resistance genes and ST131 indicated that there were significant differences in the distribution of *bla*<sub>CTX-M-27</sub> and *bla*<sub>CTX-M-55</sub> between the two types ( $P < 0.05$ , Table 8). The *bla*<sub>CTX-M-27</sub> was mostly found in ST131 strains, but *bla*<sub>CTX-M-55</sub> was only found in non-ST131 strains. In addition, statistical differences were observed between *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>CTX-M</sub> subtypes after classifying *E. coli* according to *bla*<sub>CTX-M</sub> subtypes (Table 9). *bla*<sub>TEM</sub> and *bla*<sub>OXA-1</sub> could only be found in *bla*<sub>CTX-M-27</sub> and *bla*<sub>CTX-M-55</sub> strains, respectively.

## Distribution of VFs

The detection rates of 12 VFs in *E. coli* ranged from 1.7% to 100%, among which *fimH*, *nlpI* and *ompA* were detected in all the strains (Table 10). The electrophoretic results of partial VFs have been shown in Figure 2C. Interestingly, the



**Figure 2** (A) The run products of resistance genes of *bla*<sub>CTX-M-1</sub> group, *bla*<sub>CTX-M-9</sub> group, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub> and *mcr-1* on 2% agarose gel electrophoresis. M, size marker; +, Positive control; −, Negative; 1, sample 1; 2, sample 2. (B) The run products of seven different housekeeper genes of *fumC*, *recA*, *icd*, *gyrB*, *mdh*, *adk*, *purA* on 2% agarose gel electrophoresis. M, size marker. (C) The run products of partial virulence genes of *hek-hra*, *hlyC*, *papGII-III*, *iroN* on 2% agarose gel electrophoresis. M, size marker; +, Positive control; −, Negative; 1, sample 1; 2, sample 2. (D) The run products of replicons of *IncFIA*, *IncFIB*, *IncHI2*, *IncI*, *IncN*, *IncFIA+IncFIB* and *IncHI2+IncI* on 2% agarose gel electrophoresis. M, size marker.

**Table 5** Distribution of Five *bla*<sub>CTX-M</sub> Subtypes in Phylogenetic Groups

<i>bla</i> <sub>CTX-M</sub> Subtypes	A (n = 11)	B1 (n = 3)	B2 (n = 27)	D (n = 19)	P <sup>a</sup>
<i>bla</i> <sub>CTX-M-14</sub>	2	0	6	7	0.544
<i>bla</i> <sub>CTX-M-15</sub>	4	0	6	1	0.142
<i>bla</i> <sub>CTX-M-27</sub>	1	0	11	4	0.157
<i>bla</i> <sub>CTX-M-55</sub>	5	3	1	5	<0.01
<i>bla</i> <sub>CTX-M-65</sub>	0	0	3	2	0.767

**Note:** <sup>a</sup>A *p* value of <0.05 was considered statistically significant by the Chi-square test.

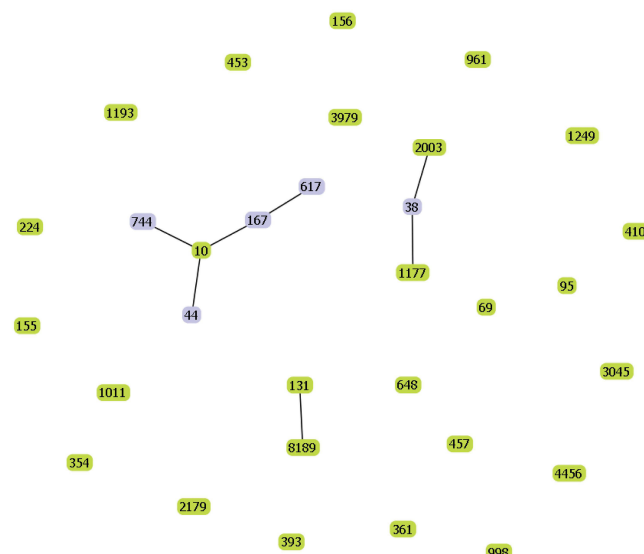
**Table 6** Distribution of Five *bla*<sub>CTX-M</sub> Subtypes in Groups A+B1 and B2+D

<i>bla</i> <sub>CTX-M</sub> Subtypes	Groups A+B1 (n = 14)	Groups B2+D (n = 46)	P <sup>a</sup>
<i>bla</i> <sub>CTX-M-14</sub>	2	13	0.483
<i>bla</i> <sub>CTX-M-15</sub>	4	7	0.264
<i>bla</i> <sub>CTX-M-27</sub>	1	15	0.086
<i>bla</i> <sub>CTX-M-55</sub>	8	6	0.003
<i>bla</i> <sub>CTX-M-65</sub>	0	5	0.329

**Note:** <sup>a</sup>A *p* value of <0.05 was considered statistically significant by the Chi-square test.

**Table 7** ST Distribution of 60 Strains of *E. coli*

STs	N
ST131	16
ST69	3
ST354	3
ST10	2
ST38	2
ST95	2
ST156	2
ST167	2
ST224	2
ST410	2
ST648	2
ST1177	2
ST2003	2
ST44	1
ST155	1
ST361	1
ST393	1
ST453	1
ST457	1
ST617	1
ST744	1
ST961	1
ST998	1
ST1011	1
ST1193	1
ST1249	1
ST2179	1
ST3045	1
ST3979	1
ST4456	1
ST8189	1



**Figure 3** The goEBURST results of the 60 distinct *E. coli* strains. The number of each point in the figure represented a ST, and the line between STs represented a housekeeper gene difference between the two STs. The figure showed that the 60 distinct *E. coli* strains were distributed in 31 STs. According to the SLV algorithm, goEBURST divided these STs into 23 groups, namely, clonal complexes. And most of these STs were scattered, indicated that they had a distant genetic relationship.

phylogenetic grouping suggested that groups B2 and D were more virulent in comparison to the groups A and B1. In addition, statistical analysis revealed that the three virulence genes (*hek-hra*, *kpsM* and *papGII-III*) in the groups B2 and D were substantially more than that in groups A and B1 (Table 11). Moreover, we found that four virulence genes were different between ST131 and non-ST131 strains ( $P < 0.05$ , Table 12). Among them, *cnf1*, *iucC*, *papGII-III* were mostly seen in ST131 strains, whereas *iroN* was predominantly observed in non-ST131 strains. Finally, we analyzed the relationship between the bacterial toxicity and resistance and found that there were no statistical differences in the distribution of VFs between the groups *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-9</sub> ( $P > 0.05$ , data not shown). However, it was found that the distribution of five virulence genes in the five *bla*<sub>CTX-M</sub> subtype strains exhibited statistical differences after classifying them according to the *bla*<sub>CTX-M</sub> subtypes ( $P < 0.05$ , Table 13), which were *hek/hra*, *iroN*, *iucC*, *kpsMII* and *papGII-III*.

**Table 8** Distribution of 8 Resistance Genes in ST131 and Non-ST131 *E. coli*

Resistance Genes	ST131 (n = 16)	Non-ST131 (n = 44)	P <sup>a</sup>
<i>bla</i> <sub>TEM</sub>	2	6	1
<i>bla</i> <sub>SHV</sub>	0	1	1
<i>bla</i> <sub>CTX-M-14</sub>	3	12	0.738
<i>bla</i> <sub>CTX-M-15</sub>	5	6	0.143
<i>bla</i> <sub>CTX-M-27</sub>	8	8	0.021
<i>bla</i> <sub>CTX-M-55</sub>	0	14	0.013
<i>bla</i> <sub>CTX-M-65</sub>	0	5	0.311
<i>bla</i> <sub>OXA-1</sub>	4	4	0.192

**Note:** <sup>a</sup>A *p* value of <0.05 was considered statistically significant by the Chi-square test.

**Table 9** Distribution of 3 Resistance Genes in Three *bla*<sub>CTX-M</sub> Subtypes of ST131

Resistance Genes	<i>bla</i> <sub>CTX-M-14</sub> (n = 3)	<i>bla</i> <sub>CTX-M-15</sub> (n = 5)	<i>bla</i> <sub>CTX-M-27</sub> (n = 8)	P <sup>a</sup>
<i>bla</i> <sub>TEM</sub>	0	0	2	0.667
<i>bla</i> <sub>SHV</sub>	0	0	0	/
<i>bla</i> <sub>OXA-1</sub>	0	4	0	0.005

**Note:** <sup>a</sup>A *p* value of <0.05 was considered statistically significant by the Chi-square test.

**Table 10** Distribution of 12 Virulence Genes in *E. coli*

Virulence Genes	N (%)
<i>fimH</i>	60 (100.0)
<i>nlpI</i>	60 (100.0)
<i>ompA</i>	60 (100.0)
<i>iucC</i>	44 (73.3)
<i>kpsM</i>	27 (45.0)
<i>papGII-III</i>	20 (33.3)
<i>hek-hra</i>	19 (31.7)
<i>hlyC</i>	16 (26.7)
<i>iroN</i>	15 (25)
<i>cnfI</i>	7 (11.7)
<i>ibeA</i>	3 (5.0)
<i>sfa-foc</i>	1 (1.7)

**Table 11** Distribution of 12 Virulence Genes in Groups A+B1 and B2+D

Virulence Genes	A+B1 (n = 14)	B2+D (n = 46)	P <sup>a</sup>
<i>cnfI</i>	0	7	0.184
<i>fimH</i>	14	46	- <sup>b</sup>
<i>hek/hra</i>	1	18	0.026
<i>hlyC</i>	3	13	0.74
<i>ibeA</i>	0	3	1
<i>iroN</i>	5	10	0.309
<i>iucC</i>	12	32	0.314
<i>kpsMII</i>	2	25	0.013
<i>nlpI</i>	14	46	-
<i>papGII-III</i>	1	19	0.023
<i>sfa/foc</i>	0	1	1
<i>ompA</i>	14	46	-

**Notes:** <sup>a</sup>A p value of <0.05 was considered statistically significant by the Chi-square test.

<sup>b</sup>Indicated that the above virulence genes were found in all 60 strains of *E. coli*, and the distribution differences of this virulence genes were not calculated.

**Table 12** Distribution of 12 Virulence Genes in ST131 and Non-ST131 *E. coli*

Virulence Genes	ST131 (n = 16)	Non-ST131 (n = 44)	P <sup>a</sup>
<i>cnfI</i>	6	1	0.001
<i>fimH</i>	16	44	- <sup>b</sup>
<i>hek/hra</i>	5	14	0.967
<i>hlyC</i>	7	9	0.1
<i>ibeA</i>	1	1	0.466
<i>iroN</i>	0	15	0.006
<i>iucC</i>	15	29	0.046
<i>kpsMII</i>	10	17	0.1
<i>nlpI</i>	16	44	-
<i>papGII-III</i>	11	9	<0.01
<i>sfa/foc</i>	0	1	1
<i>ompA</i>	16	44	-

**Notes:** <sup>a</sup>A p value of <0.05 was considered statistically significant by the Chi-square test.

<sup>b</sup>Indicated that the above virulence genes were found in all 60 strains of *E. coli*, and the distribution differences of this virulence genes were not calculated.

**Table 13** Distribution of 12 Virulence Genes in Five *bla*<sub>CTX-M</sub> Subtypes of *E. coli*

Virulence Genes	<i>bla</i> <sub>CTX-M-14</sub> (n = 15)	<i>bla</i> <sub>CTX-M-15</sub> (n = 11)	<i>bla</i> <sub>CTX-M-27</sub> (n = 16)	<i>bla</i> <sub>CTX-M-55</sub> (n = 14)	<i>bla</i> <sub>CTX-M-65</sub> (n = 5)	P <sup>a</sup>
<i>cnfI</i>	1	2	3	1	0	0.723
<i>fimH</i>	15	11	16	14	5	-. <sup>b</sup>
<i>hek/hra</i>	7	5	1	3	3	0.028
<i>hlyC</i>	2	5	7	2	0	0.082
<i>ibeA</i>	0	0	1	1	1	0.336
<i>iroN</i>	2	2	2	7	3	0.041
<i>iucC</i>	6	10	15	9	5	0.003
<i>kpsMII</i>	3	6	11	3	4	0.008
<i>nlpI</i>	15	11	16	14	5	-
<i>papGII-III</i>	1	8	7	2	2	0.002
<i>sfa/focDE</i>	0	1	0	0	0	0.267
<i>ompA</i>	15	11	16	14	5	-

**Notes:** <sup>a</sup>A *p* value of <0.05 was considered statistically significant by the Chi-square test. <sup>b</sup>Indicated that the above virulence genes were found in all 60 strains of *E. coli*, and the distribution differences of this virulence genes were not calculated.

## Plasmid Conjugation Assay

The results of the conjugative assays showed that 32 transconjugates grew on the plates containing sodium azide and ampicillin, whereas the donor and recipient bacteria were unable to grow. The plasmids of these transconjugates were found to contain resistance genes including *bla*<sub>CTX-M</sub> and part of *bla*<sub>OXA-1</sub> after PCR amplification and sequencing. The transconjugates of the remaining 28 strains of *E. coli* did not grow well on the dual-resistant plates. The results of PCR-based replicon typing showed that only one replicon was found in 18 among 32 transconjugates, namely, IncFIB (n = 12), IncFII (n = 5) or IncFHI2 (n = 1). In addition, 9 of 32 contained two replicons, and 5 of 32 contained three replicons. The partial PCR results of replicons have been shown in Figure 2D.

## Discussion

*E. coli* is a common pathogen found in hospital-acquired infections. In 2021, statistical data of bacterial drug resistance monitoring in China revealed that the detection rate of *E. coli* ranked first among the various detected pathogens.<sup>14</sup> The various drugs used to treat *E. coli* including penicillin and cephalosporin (β-Lactam), ciprofloxacin and levofloxacin (fluoroquinolones) and so on.<sup>3</sup> In addition, the resistance rates of *E. coli* to six diverse kinds of antibiotics had exceeded 50%, including ESBLs-producing *E. coli* (52.4%) in bacterial drug resistance monitoring in China in 2021.<sup>14</sup> And the monitoring of BSI pathogens in the hospital in 2020 showed that 60.9% of *E. coli* produced ESBLs.<sup>36</sup> The emergence of ESBLs has led to the inactivation of several types of antibiotics, including penicillins, cephalosporins, and monocyclics. This high drug resistance rate has made it difficult for the current clinical infection control. Therefore, ESBLs-producing *E. coli* has emerged as an important target for the treatment of BSI. In this study, the non-sensitivity rates of 60 *E. coli* strains to 13 antibiotics exceeded 50%, whereas the non-sensitivity rates of quinolones (91.7% for levofloxacin and 98.3% for ciprofloxacin) exceeded 90%. The high drug resistance rate effectively reduced the number of antibiotics that can be used for management of infection. Therefore, the high drug resistance of ESBLs-producing *E. coli* constitutes a major challenge in the treatment of BSI. In addition, *E. coli* was found to be sensitive to carbapenems (imipenem, meropenem, ertapenem), minocycline and tegacyclin, but these antibiotics were high-grade antibiotics, which are not commonly used as the first choice for treatment, while the non-sensitivity rates of piperacillin/tazobactam, cefotetan and amikacin were less than 20%. Thus, it could be recommended that the clinicians should carefully choose these three highly sensitive antibiotics for the treatment of ESBLs-producing *E. coli*. The sensitivity of bacteria to the drugs could be affected by many factors, including irrational use of antibiotics, transmission of the resistant plasmids and so on.<sup>37,38</sup> Therefore, it could be recommended that the doctors should use antibiotics reasonably to reduce the occurrence of drug-resistant bacteria and, at the same time, effectively aim to strengthen the control of hospital infection to reduce the spread of drug-resistant bacteria.

ESBLs, including TEM, SHV, CTX-M and OXA enzymes, have been identified as the main resistance mechanism to  $\beta$ -lactam antibiotics.<sup>39</sup> Among them, The CTX-M first discovered by Bauernfeind et al in 1990 which could preferentially hydrolyze cefotaxime compared to the TEM and SHV enzymes.<sup>40</sup> At present, there are more than 150 varieties of CTX-M ([www.lahey.org/studies](http://www.lahey.org/studies)), which have emerged as the most common ESBL. *bla*<sub>CTX-M</sub> has been reported to spread worldwide and could be divided into different groups: *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-8</sub>, *bla*<sub>CTX-M-9</sub> and *bla*<sub>CTX-M-25</sub>. Among these, the most common were groups *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-9</sub>, in which *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-15</sub> were the most common subtypes in recent years.<sup>41</sup> In this study, we detected a variety of drug resistance genes, and the most common was *bla*<sub>CTX-M</sub>, which was mainly distributed in five subtypes. These included *bla*<sub>CTX-M-27</sub> (n = 16), *bla*<sub>CTX-M-14</sub> (n = 15), *bla*<sub>CTX-M-15</sub> (n = 11), *bla*<sub>CTX-M-55</sub> (n = 14) and *bla*<sub>CTX-M-65</sub> (n = 5). It could be observed that at this stage, *bla*<sub>CTX-M-27</sub> had a high prevalence among the patients with BSI in the hospital. In addition, the SENTRY antibacterial monitoring study in 2016 showed that, compared with *bla*<sub>CTX-M-15</sub> (55.5%),<sup>13</sup> *bla*<sub>CTX-M-27</sub> (17.3%) was also important in *E. coli*-induced UTI and BSI. However, studies conducted 10 years ago indicated that *bla*<sub>CTX-M-14</sub> was the most common gene in China, followed by *bla*<sub>CTX-M-3</sub>.<sup>42</sup> *bla*<sub>CTX-M-3</sub> and *bla*<sub>CTX-M-15</sub> were two common genes in Europe.<sup>43</sup> *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-15</sub> were also considered to be two dominant genes in ESBLs-producing *E. coli* in Asia.<sup>44</sup> Therefore, the epidemiology of *bla*<sub>CTX-M</sub> might change according to place of origin. In addition, we found that the bacterium carried both *bla*<sub>CTX-M</sub> and *mcr-1*, *mcr-1* primarily mediated the resistance to colistin, which was the last line of defense in the treatment of *E. coli*.<sup>45</sup> The co-existence of the multiple resistance genes could make it more difficult to treat for infection. The spread of the bacterial resistance primarily depends on the transfer of the resistant plasmids, including the conjugated plasmids and non-conjugated plasmids. These plasmids could be transferred by means of separate transfer, co-transfer with the conjugated plasmids, and formation of the hybrid plasmids or homologous recombinant plasmids, which ultimately can lead to difficulties in treatment.<sup>46</sup> Interestingly, prior reports have indicated that *bla*<sub>CTX-M</sub> appeared on a variety of plasmids, including IncF, IncI, IncA/C, IncN and IncH, etc.<sup>47</sup> We found that 53% of the plasmids could be successfully combined and transferred, especially IncFIB. Thus, it was concluded that the IncFIB plasmid was an important cause of *bla*<sub>CTX-M</sub> transmission.

Clermont et al<sup>33</sup> proposed a simple and rapid phylogenetic grouping technique based on the triple PCR earlier in 2000. According to this method, *E. coli* was divided into four major phylogenetic groups (A, B1, B2 and D). The extraintestinal virulent strains mainly belonged to groups B2 and D, whereas the commercial strains primarily belonged to groups A and B1. However, the emergence of complicated scientific research technology has shed light on additional MLST data and revealed novel strain nucleotide sequences. Therefore, Clermont et al were able to improve the above-described method in 2013 and further divided *E. coli* into distinct groups (A, B1, B2, C, D, E, and F) and class I.<sup>48</sup> In this study, we have classified *E. coli* according to the toxicity and discussed the relationship between VFs and toxicity, as well as the relationship between bacterial toxicity and resistance. Hence, the old Clermont typing method was more suitable for our study. The phylogenetic groups indicated that most *E. coli* belonged to the highly toxic groups B2 and D (n = 46, 76.7%), which was consistent with the previous reports that most of the *E. coli* strains that could cause human UTI and other parenteral infections belonged to group B2, or to a lesser extent, group D, and only a few belonged to groups A and B1.<sup>49</sup> The results of the statistical analysis showed that the phylogenetic grouping of *bla*<sub>CTX-M-55</sub> was statistically different, and it was found to be more common in groups A and B1. However, the distributions of other subtypes were not significantly different. This observation suggested that the strains containing *bla*<sub>CTX-M-55</sub> were generally less toxic. The toxicity of *E. coli* was controlled by VFs, and their roles in regulating toxicity have been discussed in the next section. In addition, previous studies have indicated that the population structure of *E. coli* harbouring *bla*<sub>CTX-M</sub> was mainly dominated by the high-risk clone ST131. The success of this clone could be attributed to the acquisition of antimicrobial resistance, especially the resistance to fluoroquinolones.<sup>50</sup> Similarly, the highest detection rate of ST131 in this study was 26.7% (16/60). However, other important epidemic clones, including ST38, ST69, ST410 and ST1193, ST10, were also detected in this report. These clones were usually resistant to the broad-spectrum cephalosporins and quinolones. The distribution of eight drug resistance genes in ST131 and non-ST131 indicated that *bla*<sub>CTX-M-27</sub> was mostly found in ST131, but *bla*<sub>CTX-M-55</sub> was only detected in non-ST131. Overall, combined with the above phylogenetic results, we were able to conclude that *bla*<sub>CTX-M-27</sub> and *bla*<sub>CTX-M-55</sub> were mostly found in highly toxic ST131 and weakly toxic non-ST131 strains, respectively.



*E. coli* possesses a variety of VFs to make it resist different defense mechanisms, including fimbriae, flagella, and toxins. Different VFs and hosts can effectively form complex interactions. And understanding the difference of VFs could aid in understanding the pathogenic mechanism of *E. coli*. In this study, 12 virulence genes were identified, and these VFs participated in the process of extraintestinal pathogenesis, of which FimH, Hek/hra, PapGII-II, and Sfa/foc belonged to adhesins. They could enable the bacteria to adhere to a variety of cells and mucosal surfaces and play important roles in the development of bacterial toxicity.<sup>51</sup> Iron absorption factors *iroN* and *iucC* were the typical VFs of ExPEC, which were essential for *E. coli* to survive in the urinary tract, because the iron availability in the urinary tract was extremely deficient, and all the bacteria must absorb iron from the host proteins (such as heme). They also participated in the biosynthesis and virulence formation of the bacteria. In addition, prior studies have found that UPEC could more easily breakthrough the urethral mucosal barrier, leading to urinary tract infection and even septicemia when Aerobacin was highly expressed.<sup>52</sup> The protectin *kpsM* encoded proteins are related to the capsular polysaccharide output. The capsular was identified as a VF related to immune escape of bacteria, which could be used by the bacteria to resist adverse external environment and adhere to the specific cell surfaces to attack the target cells.<sup>53</sup> OmpA was a transmembrane protein that could effectively combine with the N-glucosamine epitope of the glycoprotein Gp96 on the surface of human microvascular endothelial cells to effectively enhance the antiserum effect of *E. coli* and increase the invasion of the blood–brain barrier.<sup>54</sup> Finally, NlpI was involved in regulation of cell adhesion, invasion and division and can aid the bacteria escape the immune killing effect of the host.<sup>55</sup> The function of CnfI, HlyC and IbeA has been described in the introduction section.

In this study, the detection rates of 12 VFs varied from 1.7% to 100%. Interestingly, similar to our results, Johnson et al reported<sup>13,56</sup> that the detection rates of VFs varied from 0.4% to 98%. and the high-risk epidemic ST131 isolates carried more virulence genes in comparison to the non-ST131 isolates, especially *iha*, *hlyD*, *sat*, *iutA*, *fyuA*, *malX*, *ompT* and *traT*, *tsh* and *espC*.<sup>57</sup> In this report, *cnfI*, *iroN*, *iucC*, *papGII-III* were also related to ST131 ( $P < 0.05$ ). Among them, *cnfI*, *iucC*, *papGII-III* were mostly found in ST131, which suggested that these VFs might play significant roles in the toxicity of ST131. In addition, compared with the groups B1 and A, groups B2 and D with stronger toxicity possessed more VFs, and *hek-hra*, *kpsM*, *papGII-III* showed statistical differences between the two groups. Hence, these might play stronger roles in controlling the toxicity of *E. coli*. The potential relationship between the bacterial virulence and resistance remains a hotspot in pathogen research.<sup>49</sup> There are differences found in the resistance of bacteria with different toxicity. It was reported that the virulence genes appeared more frequently in carbapenemase producers than in the non-producers, and *fimA*, *iutA* and *cnfI* exhibited significant differences.<sup>13,56</sup> In this study, we found that *hek/hra*, *iroN*, *iucC*, *kpsM*, and *papGII-III* showed statistical differences among *bla*<sub>CTX-M</sub> subtypes. This finding suggested that there might be a possible relationship between the bacterial virulence and resistance. However, some limitations of our study include that the results might not be universal enough, and further studies were needed to evaluate a greater number of hospitals. The emergence of ESBL-producing strains encoding various VFs was a focus of infection treatment. Therefore, appropriate implementation of the monitoring plans could be crucial to limit their transmission.

## Conclusion

In conclusion, ESBLs-producing *E. coli* emerged as a common pathogen of BSI and was found to be resistant to many antibiotics. The common resistance genes included *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>OXA-1</sub>. Among them, *bla*<sub>CTX-M</sub> was the main resistance gene. The *bla*<sub>CTX-M</sub> subtypes mainly included *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-65</sub>. The pathogenicity of *bla*<sub>CTX-M</sub> had changed to a certain extent over time. Compared with the results of ten years ago in China, in which *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-3</sub> accounted for the majority, in recent years, *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-55</sub> have been identified as novel epidemic genes. The transmission of these resistant genes mostly depended on the transfer of the resistant plasmids. A variety of ESBLs-producing *E. coli* clones were prevalent in the hospitals, including ST131, as well as other common popular clones. VFs were pathogenic factors of *E. coli*, with the detection rates ranging from 1.7% to 100%. In addition, their distributions showed clear statistical differences among distinct types of *E. coli*. The emergence of highly resistant and highly virulent pathogens could lead to serious infection. Hence, real-time monitoring and limiting its transmission were essential for management of infection. At the same time, further research is needed to explore the specific role of VFs as pathogenic factors.



## Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

## Ethical Approval and Consent to Participate

This study has been approved by the ethics review committee of the First Affiliated Hospital of Anhui Medical University and was conducted in accordance with the Declaration of Helsinki. And the committee agreed to waive the informed consent of the participants (Quick-PJ 2023-01-25).

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This work was supported by the Anhui Provincial Natural Fund (Project's number: 9021138203) and the Subject Construction Fund of the First Affiliated Hospital of Anhui Medical University (Project's number: 9001001855).

## Disclosure

The authors declare that they have no competing interests in this work.

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