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ORIGINAL RESEARCH

Genomic Characteristics of Extended Spectrum β-Lactamase Producing *Escherichia coli* Isolates Recovered from a District Hospital in China

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Purpose: The isolation rate of extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* is increasing, posing a challenge to clinical anti-infective therapy. This study aims to provide new insight into the genomic characteristics and antimicrobial resistance mechanisms of extended spectrum β-lactamase producing *E. coli* isolates recovered from a district hospital in China.

Methods: A total of 36 ESBL-producing *E. coli* isolates were collected from body fluid samples from a Chinese district hospital. All isolates were subjected to whole genome sequencing to identify their antimicrobial resistance genes, virulence genes, serotypes, sequence types, and phylogenetic relationships by BacWGSTdb 2.0 webserver.

Results: Among these isolates, all were resistant to cefazolin, cefotaxime, ceftriaxone, ampicillin, 24 (66.7%) were resistant to aztreonam, 16 (44.4%) were resistant to cefepime, and 15 were resistant (41.7%) to ceftazidime. The $bla_{\text{CTX-M}}$ gene was detected in all ESBL-producing *E. coli* isolates. Two isolates carrying two different types of $bla_{\text{CTX-M}}$ genes simultaneously. The carbapenem resistance gene $bla_{\text{KPC-2}}$ was detected in one (2.8%) isolate. A total of 17 sequence types (STs) were found, with ST131 accounting for the majority (n =13; 36.1%). The most common serotype was O16:H5 associated with seven ST131 strains, followed by O25:H4/ST131 (n = 5) and O75:H5/ST1193 (n = 5). Evaluation of clonal relatedness revealed that all $bla_{\text{CTX-M}}$ gene-carrying *E. coli* had a difference of SNPs range from 7 to 79,198, which could be divided into four clusters. Only 7 SNPs could be found between EC266 and EC622, indicating that they are variants of the same clonal lineage.

Conclusion: This study investigated the genomic characteristics of ESBL-producing *E. coli* isolates recovered from a district hospital in China. Continuous surveillance of ESBL-producing *E. coli* infections is imperative to create efficient strategies for controlling the transmission of these multi-drug resistant bacteria in clinical and community settings.

Keywords: *Escherichia coli*, ESBLs, antimicrobial resistance, whole genome sequencing

Introduction

Currently, the emergence of bacterial resistance poses a significant challenge to public health worldwide. With the widespread use of antimicrobial agents in recent years, the isolation of multidrug-resistant *Escherichia coli* has increased yearly, particularly among Extended Spectrum β-lactamases (ESBLs) producing strains, which can cause not only multi-site infections but also epidemic outbreaks of hospital-acquired infections. A previous study found that 55.5% of community-onset bloodstream infections in China were caused by ESBL-producing *E. coli*.[1] ESBL-producing strains can cause not only bloodstream infections, but also high-mortality and difficult-to-treat diseases like pneumonia and urinary tract infections.[2]

ESBLs are serine β-lactamases, most of which belong to class A of the Ambler classification of β-lactamases.[3] They have the ability to hydrolyze oxyimino-β-lactam antibiotics, and can be effectively inhibited by ESBL inhibitors, including clavulanic acid, sulbactam, and tazobactam.[4] Most ESBLs are carried by conjugative plasmids, which can additionally harbor genes that provide resistance to other broad-spectrum antibiotics, such as aminoglycosides, macrolides, and quinolones.[5] There are multiple genotypes of ESBLs, of which CTX-M, TEM and SHV are the most common ones. Since the early 21st century, the CTX-M type has gradually replaced the TEM and SHV types as the most common genotype of ESBLs.[6] The

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initial CTX-M variant displays efficient hydrolysis of cefotaxime and ceftriaxone, but exhibits limited activity against ceftazidime.[7] Since then, additional variants have been identified, with roughly 200 types currently classified into at least five subspecies or groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25). Among these groups, CTX-M-15 and CTX-M-27 from the CTX-M-1 and CTX-M-9 groups respectively, feature a single amino acid alteration at position 240 (Asp to Gly), resulting in an increased capacity for hydrolysis of ceftazidime.[8,9] The extensive dissemination of $bla_{\text{CTX-M}}$ has been found to have a significant association with the clonal dissemination of ST131 *E. coli*, whereby $bla_{\text{CTX-M-15}}$ is the most frequently detected ESBL allele in this strain.[10] In Australia, 73% of ST131 isolates were found to harbor at least one ESBL gene, with $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ being the most frequently identified.[11] According to a recent study, CTX-M type is presently the most prevalent in China (97.33%), predominating CTX-M-55 (48.47%) and CTX-M-1 (17.94%).[12] The most common sequence type was ST131, followed by ST1193.[13]

Although multiple studies on ESBL-producing *E. coli* strains have been reported in China, there is a lack of such studies conducted in district hospitals. Therefore, the purpose of this study is to elucidate the molecular epidemiological and genomic features of ESBL-producing *E. coli* strains obtained from a local hospital.

Materials and Methods

Bacterial Isolates

Between January and December 2021, a total of 230 *Escherichia coli* strains were isolated from patients at the third people's Hospital of Xiaoshan, Hangzhou, China. Date of isolation, sample type, gender, age, department of hospitalization and clinical diagnosis were recorded. Detection of ESBL-producing isolates was performed by Double Disc Synergy Test (DDST) method following CLSI guidelines. Among them, a total of 36 non-duplicated ESBL-positive *E. coli* strains were recovered from clinical samples including blood, urine, cerebrospinal fluid, and bile.

Culture, Identification and Antimicrobial Susceptibility Testing

Fresh samples were inoculated on Columbia blood agar plates at 35 °C for 18–48 h. The identification and antibiotic susceptibility testing of *E. coli* was performed using the VITEK 2 COMPACT automatic analysis system (BioMérieux, France), employing the GN and AST GN13 panels (BioMérieux, France), respectively. These included, ampicillin-sulbactam (SAM), cefotetan (CTT), nitrofurantoin (NIT), tobramycin (TOB), piperacillin-tazobactam (TZP), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), cefazolin (CZO), cefoxitin (FOX), ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), aztreonam (ATM), cefepime (FEP), imipenem (IPM), ertapenem (ETP), levofloxacin (LVX), ciprofloxacin (CIP), gentamicin (GEN), amikacin (AMK). The breakpoints that were used were those recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for isolates subsequently obtained.[14] The quality control strain for antimicrobial susceptibility testing was *Escherichia coli* (ATCC 25922). To assess the ESBLs phenotype, isolates identified as *E. coli* were tested for antimicrobial susceptibility by agar dilution method for ceftazidime, cefotaxime, ceftriaxone, and cefepime. The range of MICs for the above antimicrobial agents was set with reference to the quality control strain *E. coli* ATCC 25922. The bacterial solution was inoculated on MH agar plates containing the antimicrobials using a semi-automatic spotter, left to dry at room temperature, incubated overnight at 35°C and the MIC was read the following day. Minimum inhibitory concentrations (MICs) of the antimicrobial agents were interpreted using Clinical and Laboratory Standards Institute (CLSI) 2020. Species identification was also confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Billerica, MA, United States).[15]

Whole-Genome Sequencing (WGS)

Genomic DNA was extracted from the ESBL-positive *Escherichia coli* strains using a a Genomic DNA Purification Kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's recommendations. DNA purity and concentration were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The library was initially quantified using Qubit 2.0, then the fragment size distribution was calculated using an Agilent 2100. Quality control of short reads from Illumina sequencing using FastQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The INNUca pipeline v4.2.2 (https://github.com/B-UMMI/INNUca) was used for pruning (using Trimmomatic, v0.39) and

assembly (using SPADes, v3.14.0, and Pilon v1.23).[16–18] Default parameters were used. The Illumina HiSeq sequencing platform was applied to construct a 150 bp Paired-End library of each ESBL-positive *Escherichia coli* genome for sequencing at a depth of >100×.[19] The sequencing data were assembled using Unicycler v0.4.8 software.[20] The genome annotation using the NCBI Prokaryotic Annotation Pipeline (PGAP) to obtain complete annotation files for all types of bioinformatics analysis.[21] The genomic multilocus sequence typing (MLST) and bacterial core genome single nucleotide polymorphism (cgSNP) were performed by BacWGSTdb 2.0 (http://bacdb.cn/BacWGSTdb/Tools.php).[22–24] Predicted SNPs in the core genome and removed recombinant regions from SNP alignments using Snippy v4.4.5. Phylogenetic trees were constructed using RAxML v8.2.12 and pairwise distances of SNPs were determined using SNP-dist v0.6.3.[25] Antimicrobial resistance determinants were identified using ABRicate 1.0.1 program (https://github.com/tseemann/abricate) based on ResFinder 4.1 database (http://genomicepidemiology.org/).[26,27] Bacterial virulence factors were identified via virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/).[28] SerotypeFinder 2.0 (https://cge.food.dtu.dk/services/SerotypeFinder/) was used to predict the serotype.[29] The Interactive Tree Of Life (iTOL, https://itol.embl.de/) v5 website visualized and annotated the phylogenetic tree and highlight the existence of antibiotic resistance genes.[30] PlasmidFinder 2.1 (http://cge.cbs.dtu.dk/services/PlasmidFinder/) and ISfinder 1.0 (https://www-is.biotoul.fr/) were used to predict plasmid replicon types and insertion sequence (IS) elements.[31,32] The genome sequences of the 36 ESBL-producing *E. coli* isolates have been deposited in the NCBI GenBank database as BioProject accession numbers PRJNA874366.

Results

Antimicrobial Susceptibility Profiles of the ESBLs-Producing *Escherichia coli*

From January 2021 to December 2021, a total of 36 *E. coli* isolates derived from clinical samples were identified as ESBL producers through phenotypic screening at a Chinese district hospital (Table 1). The average age of the patients was 74.6±13.6 years. Of the 36 patients, 10 (27.8%) were male and 26 (72.2%) were female. Most isolates (n=19; 52.7%) were derived from urine samples, followed by blood samples (n=16; 44.4%). The resistance to the tested antibiotics in these *E. coli* strains ranged from high to low as cefazolin (36/36; 100.0%), cefotaxime (36/36; 100.0%), ceftriaxone (36/36; 100.0%), ampicillin (36/36; 100.0%), aztreonam (24/36; 66.7%), ciprofloxacin (24/36; 66.7%), levofloxacin (20/36; 55.6%), cefepime (16/36; 44.4%), ceftazidime (15/36; 41.7%), and gentamicin (9/36; 25.0%). The ESBL positive strains exhibited 100% resistance to cefazolin, ceftriaxone, cefotaxime, and ampicillin. On the other hand, the resistance rate to imipenem and ertapenem was low (1/36; 2.8%). However, we also found several strains intermediate to amikacin (1/36; 2.8%), ampicillin/sulbactam (1/36; 2.8%), imipenem (1/36; 2.8%), aztreonam (1/36; 2.8%), nitrofurantoin (2/36; 5.6%), piperacillin-tazobactam (2/36; 5.6%), ciprofloxacin (4/36; 11.1%), tobramycin (6/36; 16.7%), ceftazidime (7/36; 19.4), levofloxacin (14/36; 38.9%) and cefepime (18/36; 50%) (Table 2).

Genetic Characterization of ESBL Producing *E. coli* Isolates

The CTX-M gene was present in all ESBL-producing *E. coli* strains. Among them, nine (25.0%) isolates had $bla_{\text{CTX-M-27}}$, nine (25.0%) had $bla_{\text{CTX-M-55}}$, eight (22.2%) had $bla_{\text{CTX-M-14}}$, six (16.7%) had $\text{bla}_{\text{CTX-M-15}}$, three (8.3%) had $bla_{\text{CTX-M-174}}$, two (5.5%) had $bla_{\text{CTX-M-3}}$, and one (2.8%) had $bla_{\text{CTX-M-65}}$. One (0.9%) isolate harbored both $bla_{\text{CTX-M-15}}$ and $bla_{\text{KPC-2}}$ genes simultaneously. In this study, 47 different antibiotic resistance genes were identified from all ESBL-producing isolates. Resistance to aminoglycosides was mediated by fourteen genes, the most common of which was *aph (6)-Id*, which was found in 44.4% (n=16) of isolates. Individually or together, the acquired sulfonamide resistance genes *sul1, sul2*, and *sul3* were found in 24 (66.7%) isolates. Trimethoprim resistance genes *dfrA17, dfrA12*, or *dfrA14* were found in 19 (52.8%) isolates. Among these genes, *dfrA17* (n = 14; 38.9%) was the most prevalent, followed by *dfrA14* (n = 3; 8.3%) and *dfrA12* (n = 2; 5.5%). Tetracycline resistance was found in 25 (69.4%) isolates, with *tet(A)* found in 21 of them. The quinolone resistance gene *qnrS1* was detected in 7 (19.4%) isolates. The macrolide resistance gene *mdf(A)* was present in all strains (100%), and *mph(A)* was present in 24 (66.7%) strains. Furthermore, we discovered genes that were resistant to erythromycin (5.5%), phenol (8.3%), and fosfomycin (16.7%) in these strains (Figure 1).

All the isolates contained a variety of virulence factors. 77.8% (28/36) of the isolates had the iron regulatory gene *irp2*, which made them substantially superior biofilm formers. 69.4% (25/36) of the isolates that produced CTX-M had

Table 1 Clinical Characteristics of ESBL-Producing *Escherichia coli* Isolates

Patient	Isolate Number	Age (Years)	Gender	Sample Origin	Collection Date
Pa108	EC108	51	Female	Urine	2021/4/4
Pa117	EC117	78	Female	Blood	2021/4/5
Pa139	EC139	76	Female	Urine	2021/4/17
Pa170	EC170	75	Female	Blood	2021/4/26
Pa193	EC193	89	Female	Urine	2021/5/5
Pa237	EC237	69	Male	Blood	2021/5/19
Pa247	EC247	87	Female	Urine	2021/5/22
Pa262	EC262	85	Male	Urine	2021/5/28
Pa266	EC266	76	Female	Urine	2021/5/29
Pa322	EC322	81	Female	Blood	2021/6/15
Pa333	EC333	70	Female	Blood	2021/6/17
Pa349	EC349	69	Male	Blood	2021/6/20
Pa352	EC352	45	Female	Blood	2021/6/21
Pa353	EC353	74	Female	Urine	2021/6/22
Pa376	EC376	76	Female	Urine	2021/6/29
Pa391	EC391	91	Male	Blood	2021/7/2
Pa402	EC402	85	Male	Urine	2021/7/6
Pa416	EC416	84	Male	Urine	2021/7/10
Pa457	EC457	66	Female	Blood	2021/7/22
Pa488	EC488	84	Male	Urine	2021/8/4
Pa501	EC501	87	Female	Blood	2021/8/8
Pa506	EC506	90	Male	Blood	2021/8/9
Pa537	EC537	92	Female	Blood	2021/8/19
Pa563	EC563	54	Female	Urine	2021/8/31
Pa576	EC576	80	Female	Blood	2021/9/4
Pa586	EC586	77	Female	Bile	2021/9/8
Pa593	EC593	79	Male	Blood	2021/9/11
Pa266	EC622	77	Female	Urine	2021/9/22
Pa635	EC635	59	Female	Blood	2021/9/25
Pa644	EC644	76	Female	Urine	2021/9/27
Pa649	EC649	87	Female	Urine	2021/10/1
Pa650	EC650	42	Female	Urine	2021/10/1
Pa663	EC663	53	Female	Urine	2021/10/10
Pa664	EC664	83	Female	Blood	2021/10/10
Pa714	EC714	88	Male	Urine	2021/11/1
Pa717	EC717	49	Female	Urine	2021/11/2

the extra-enteric pathogenic *Escherichia coli* (ExPEC)-associated virulence gene *pap*. Of the 36 isolates, 69.4% (25/36) exhibited the polysaccharide transporter protein *kps*. Ferrobactin receptor *iutA* was found in 63.9% (23/36) of the isolates (Supplementary Table 1).

Serotyping and Multi-Locus Sequence Typing

All 36 ESBL-producing *E. coli* isolates had 11 H types, with H5 (n = 14; 38.9%) and H4 (n = 8; 22.2%) being the most common. The samples contained 18 different O types, O16 (n =7; 19.4%), O25 (n = 5; 13.9%), and O75 (n = 5; 13.9%) were the most prevalent O groups found. The ESBL-producing *E. coli* strains were linked to 17 distinct sequence types, with ST131 (n = 13; 36.1%) being the most prevalent. Most of the ST131 isolates belonged to serotypes O16:H5 (n = 7; 53.8%) and O25:H4 (n = 5; 38.5%), and only one isolate belonged to O107:H5 (n = 1; 7.7%). ST1193 (n = 5; 13.9%) was the next most common, with all of which are in serogroup O75:H5. The remaining 15 ST types are ST10, ST12, ST38, ST44, ST46, ST69, ST73, ST95, ST155, ST423, ST648, ST1196, ST1266, ST1421, and ST5150 (Table 3).

Table 2 Multidrug Resistance Profile of 36 ESBL-Producing *Escherichia coli* Isolates in This Study

Isolate	MIC (mg/L)																	
	AMK	SAM	CZO	CTT	CRO	ETP	IPM	NIT	TOB	AMP	ATM	FEP	CAZ	CTX	CIP	GEN	LVX	TZP	SXT
EC108	≤2	8	≥64	≤4	>512	≤0.5	≤1	≤16	≤1	≥32	16	>128	128	>128	≥4	≤1	≥8	≤4	≥320
EC117	4	8	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	8	4	8	64	≥4	≤1	≥8	≤4	≥320
EC139	≤2	≥32	≥64	≤4	256	≤0.5	≤1	≤16	≤1	≥32	32	64	8	128	≥4	≤1	≥8	≤4	≥320
EC170	≤2	≥32	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	32	16	32	128	≥4	≤1	≥8	≤4	≤20
EC193	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	16	16	16	64	0.5	≤1	1	≤4	≤20
EC237	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	8	≥32	16	8	8	64	0.5	≥16	1	≤4	≥320
EC247	≤2	16	≥64	≤4	256	≤0.5	≤1	≤16	≤1	≥32	32	32	16	128	1	≤1	1	≤4	≥320
EC262	≤2	≥32	≥64	≤4	512	≤0.5	≤1	≤16	≥16	≥32	≥64	128	128	>128	≥4	≥16	≥8	8	≤20
EC266	4	≥32	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	16	4	4	64	≥4	≤1	≥8	≤4	≤20
EC322	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	8	≥32	≥64	8	8	64	≥4	≥16	≥8	≤4	≥320
EC333	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	16	16	8	64	1	≤1	1	≤4	≤20
EC349	≤2	≥32	≥64	≤4	64	≤0.5	≤1	64	≤1	≥32	32	2	2	16	≥4	≤1	≥8	≤4	≤20
EC352	≤2	≥32	≥64	≤4	16	≤0.5	≤1	≤16	≥16	≥32	≤1	2	0.5	8	≥4	≥16	≥8	≤4	≥320
EC353	≤2	≥32	≥64	≤4	32	≤0.5	≤1	≤16	≤1	≥32	4	4	2	32	0.5	≤1	1	≤4	≥320
EC376	≤2	≥32	≥64	≤4	256	≤0.5	≤1	≤16	8	≥32	≥64	32	16	>128	0.5	≥16	1	≤4	≥320
EC391	≤2	≥32	≥64	≤4	32	≤0.5	≤1	≤16	4	≥32	2	4	1	32	≤0.25	≥16	1	≤4	≥320
EC402	≤2	8	≥64	≤4	32	≤0.5	≤1	≤16	≤1	≥32	16	4	4	32	≥4	≤1	≥8	≤4	≤20
EC416	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	4	8	2	32	≤0.25	≤1	1	≤4	≤20
EC457	≤2	≥32	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	4	8	2	32	≤0.25	≤1	≤0.25	≤4	≤20
EC488	≤2	≥32	≥64	≤4	128	≥8	≥16	≤16	≤1	≥32	≥64	32	32	128	≥4	≤1	≥8	64	≥320
EC501	≤2	8	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	4	8	8	64	≤0.25	≤1	≤0.25	≤4	≥320
EC506	≤2	8	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	16	8	16	64	1	≤1	1	≤4	≤20
EC537	≤2	≥32	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	4	16	4	64	≤0.25	≤1	1	≤4	≤20
EC563	≤2	≥32	≥64	≤4	256	≤0.5	≤1	≤16	≤1	≥32	16	32	16	128	≥4	≤1	≥8	≤4	≥320
EC576	≤2	8	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	4	4	4	64	≤0.25	≤1	1	≤4	≥320
EC586	≤2	≥32	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	≥64	16	32	128	≥4	≤1	≥8	≤4	≤20

(*Continued*)

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Table 2 (Continued).

Isolate	MIC (mg/L)																	
	AMK	SAM	CZO	CTT	CRO	ETP	IPM	NIT	TOB	AMP	ATM	FEP	CAZ	CTX	CIP	GEN	LVX	TZP	SXT
EC593	≤2	≥32	≥64	≤4	512	≤0.5	≤1	≤16	≤1	≥32	≥64	64	32	>128	1	≤1	1	≤4	≥320
EC622	≤2	≥32	≥64	≤4	32	≤0.5	≤1	≤16	≤1	≥32	4	4	4	32	≥4	≤1	≥8	≤4	≤20
EC635	32	≥32	≥64	≤4	32	≤0.5	≤1	64	≥16	≥32	4	8	1	32	≥4	4	≥8	64	≤20
EC644	4	≥32	≥64	≤4	128	≤0.5	≤1	128	8	≥32	≥64	8	16	128	≥4	≥16	≥8	≤4	≤20
EC649	≤2	8	≥64	≤4	128	≤0.5	≤1	≤16	≤1	≥32	16	8	16	128	≥4	≤1	≥8	≤4	≤20
EC650	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	8	≥32	4	8	4	32	≥4	≥16	≥8	≤4	≥320
EC663	≤2	≥32	≥64	≤4	64	≤0.5	≤1	≤16	≤1	≥32	16	8	8	64	≤0.25	≤1	1	≤4	≥320
EC664	≤2	≥32	≥64	≤4	128	≤0.5	≤1	32	≤1	≥32	≥64	16	16	128	≥4	≤1	≥8	≤4	≤20
EC714	4	≥32	≥64	≤4	256	≤0.5	2	≤16	8	≥32	32	16	16	128	≥4	≥16	≥8	16	≥320
EC717	4	≥32	≥64	≤4	256	≤0.5	≤1	≤16	≤1	≥32	16	64	4	128	≤0.25	≤1	1	≤4	≤20

Notes: The resistance breakpoints for the antibiotics tested (mg/L) were: ampicillin-sulbactam, susceptible (S), ≤8, intermediate (I), 16, resistant (R), ≥32; cefotetan, S, ≤16, I, 32, R, ≥64; nitrofurantoin, S, ≤32, I, 64, R, ≥128; tobramycin, S, ≤4, I, 8, R, ≥16; piperacillin-tazobactam, S, ≤16, I, 32–64, R, ≥128; trimethoprim-sulfamethoxazole, S, ≤2/38, I, -, R, ≥4/76; ampicillin, S, ≤8, I, 16, R, ≥32; cefazolin, S, ≤2, I, 4, R, ≥8; ceftazidime, S, ≤4, I, 8, R, ≥16; cefotaxime, S, ≤1, I, 2, R, ≥4; ceftriaxone, S, ≤1, I, 2, R, ≥4; aztreonam, S, ≤4, I, 8, R, ≥16; cefepime, S, ≤2, susceptible-dose dependent (SDD), 4–8, R, ≥16; imipenem, S, ≤1, I, 2, R, ≥4; ertapenem, S, ≤0.5, I, 1, R, ≥2; levofloxacin, S, ≤0.5, I, 1, R, ≥2; ciprofloxacin, S, ≤0.25, I, 0.5, R, ≥1; gentamicin, S, ≤4, I, 8, R, ≥16; and amikacin, S, ≤16, I, 32, R, ≥64.

Abbreviations: SAM, ampicillin-sulbactam; CTT, cefotetan; NIT, nitrofurantoin; TOB, tobramycin; TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole; AMP, ampicillin; CZO, cefazolin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; ATM, aztreonam; FEP, cefepime; IPM, imipenem; ETP, ertapenem; LVX, levofloxacin; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin.

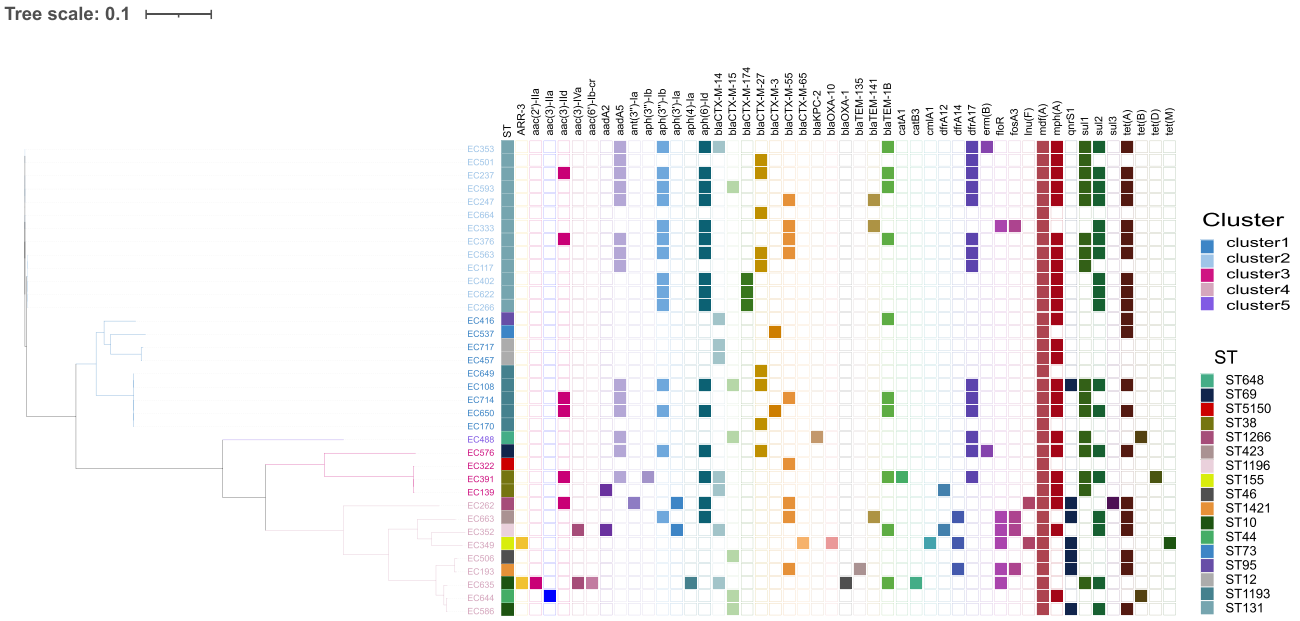

Figure 1 Recombination-filtered core genome phylogeny and the distribution of antimicrobial resistance genes in the 36 ESBL-producing *E. coli* isolates. The cell in different colors represents the presence of the gene, while the blank cell represents the absence of the gene.

Mobile Genetic Elements

Except for EC586, which had no plasmid replicon, all isolates were found to have at least one plasmid replicon. A total of 33 different replicons were detected, with IncFIB (n = 29; 80.6%) is the most regularly discovered replicon type. Of these, IncFIB (AP001918) (n = 25; 69.4%) accounted for the majority. Only a small percentage of strains possess IncFIB (pHCM2) (n = 6; 16.7%) or IncFIB (K) (n = 1; 2.8%). EC376 and EC563 have both IncFIB (AP001918) and IncFIB

Table 3 Molecular Typing of ESBL-Producing *E. coli* Based on Comparative Genomic Analysis

Isolate Number	ESBL Genotype	Sequence Type	Serotype	Insertion Sequence (IS) Elements
EC108	$bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$	ST1193	O75:H5	Tn*3*, IS*903B*
EC117	$bla_{CTX\text{-}M\text{-}27}$	ST131	O25:H4	-
EC139	$bla_{CTX\text{-}M\text{-}14}$	ST38	O86:H18	IS*Ecp1*
EC170	$bla_{CTX\text{-}M\text{-}27}$	ST1193	O75:H5	IS*903B*
EC193	$bla_{CTX\text{-}M\text{-}55}$	ST1421	O9:H4	-
EC237	$bla_{CTX\text{-}M\text{-}27}$	ST131	O16:H5	-
EC247	$bla_{CTX\text{-}M\text{-}55}$	ST131	O16:H5	-
EC262	$bla_{CTX\text{-}M\text{-}55}$	ST1266	O88:H34	Tn*3*
EC266	$bla_{CTX\text{-}M\text{-}174}$	ST131	O25:H4	-
EC322	$bla_{CTX\text{-}M\text{-}55}$	ST5150	O1:H15	IS*Ecp1*
EC333	$bla_{CTX\text{-}M\text{-}55}$	ST131	O16:H5	-
EC349	$bla_{CTX\text{-}M\text{-}65}$	ST155	O9:H51	IS*903B*
EC352	$bla_{CTX\text{-}M\text{-}14}$	ST1196	O91:H28	-
EC353	$bla_{CTX\text{-}M\text{-}14}$	ST131	O16:H5	-
EC376	$bla_{CTX\text{-}M\text{-}55}$	ST131	O16:H5	IS*Ecp1*
EC391	$bla_{CTX\text{-}M\text{-}14}$	ST38	O102:H6	-
EC402	$bla_{CTX\text{-}M\text{-}174}$	ST131	O25:H4	-
EC416	$bla_{CTX\text{-}M\text{-}14}$	ST95	O18:H7	IS*Ecp1*
EC457	$bla_{CTX\text{-}M\text{-}14}$	ST12	O4:H5	IS*Ecp1*

(*Continued*)

Table 3 (Continued).

Isolate Number	ESBL Genotype	Sequence Type	Serotype	Insertion Sequence (IS) Elements
EC488	$bla_{CTX-M-15}$	ST648	O153:H6	IS*Ecp1*
EC501	$bla_{CTX-M-27}$	ST131	O107:H5	IS*903B*
EC506	$bla_{CTX-M-15}$	ST46	O8:H4	IS*Ecp1*
EC537	$bla_{CTX-M-3}$	ST73	O6:H1	IS*Ecp1*
EC563	$bla_{CTX-M-27}$, $bla_{CTX-M-55}$	ST131	O25:H4	-
EC576	$bla_{CTX-M-27}$	ST69	O17:H18	IS*903B*
EC586	$bla_{CTX-M-15}$	ST10	O101:H9	IS*Ecp1*
EC593	$bla_{CTX-M-15}$	ST131	O16:H5	IS*Ecp1*
EC622	$bla_{CTX-M-174}$	ST131	O25:H4	-
EC635	$bla_{CTX-M-14}$	ST10	O32:H9	IS*903B*
EC644	$bla_{CTX-M-15}$	ST44	O101:H4	Tn*3*
EC649	$bla_{CTX-M-27}$	ST1193	O75:H5	IS*903B*
EC650	$bla_{CTX-M-3}$	ST1193	O75:H5	IS*Ecp1*
EC663	$bla_{CTX-M-55}$	ST423	O8:H9	-
EC664	$bla_{CTX-M-27}$	ST131	O16:H5	-
EC714	$bla_{CTX-M-55}$	ST1193	O75:H5	IS*Ecp1*
EC717	$bla_{CTX-M-14}$	ST12	O4:H1	IS*Ecp1*

(pHCM2). IncFIB (AP001918) and IncFIB (K) are both present in EC664. In addition to IncFIB replicon type, Col156 (n = 23; 63.9%), IncFIA (n = 15; 41.7%), Col (MG828) (n = 14; 38.9%), ColRNAI (n = 8; 22.2%), IncFIC (FII) (n = 8; 22.2%), IncFII (n = 7; 19.4%), IncI1 (n = 7; 19.4%) and Col (BS512) (n = 7; 19.4%) type replicons being the most prevalent. Multiple replicons were present in most isolates (n = 32; 88.9%), with IncF family plasmids showing up in 30 (83.3%) isolates (Supplementary Table 2).

IS*Ecp1*, which has been reported to be the cause of antimicrobial resistance gene translocation from plasmid to chromosome,[33] was found in 13 (36.1%) isolates. The transposable element IS*903B* was found in 7 isolates (19.4%). The transposable element Tn*3* was found in 3 isolates (8.3%) (Table 3).

Single-Nucleotide Polymorphisms and Phylogenetic Assessment of *E. coli* Strains

Phylogenetic analysis revealed that all 36 ESBL-producing *E. coli* isolates were classified into five clusters. Cluster 1 has nine isolates (EC108, EC170, EC416, EC457, EC537, EC649, EC650, EC714, EC717), cluster 2 has thirteen isolates (EC117, EC237, EC247, EC266, EC333, EC353, EC376, EC402, EC501, EC563, EC593, EC622, EC664), cluster 3 has four isolates (EC139, EC322, EC391, EC576), cluster 4 has nine isolates (EC193, EC262, EC349, EC352, EC506, EC586, EC635, EC644, EC663), and the remaining isolate (EC488) is a singleton. The number of SNPs in the 36 ESBL-producing *E. coli* isolates ranged from 7 to 79,198 after the recombinant region was removed. Internal isolate differences in cluster 1, 3, and 4 ranged from 85 to 17,624 SNPs, 201 to 40,924 SNPs, and 239 to 41,729 SNPs, respectively. Cluster 2 internal isolates differed by 7 to 19,512 SNPs. Only 7 SNPs were found between EC266 and EC622 in this cluster, indicating that they are variants of the same clone (Figures 1 and 2).

Discussion

Since the beginning of the 21st century, ESBL-producing Enterobacteriaceae bacteria have become a serious global public health concern with CTX-M type *E. coli* is currently the most prevalent species linked with ESBLs worldwide.[34] Currently, there is a limited amount of research focused on ESBL-producing strains originating from district hospitals. Concerningly, our study indicated that some of the ESBL-producing *E. coli* isolates were resistant to carbapenems, aminoglycosides, and quinolones. Additionally, the *E. coli* isolates in our study demonstrated resistance to first-line antibiotics, such as cephalosporins, macrolides, quinolones, and aminoglycosides, which are commonly used in clinical practice. This suggests that the overuse and misuse of essential antibiotics may have a possible effect on clinical isolates. It is worth noting that third-

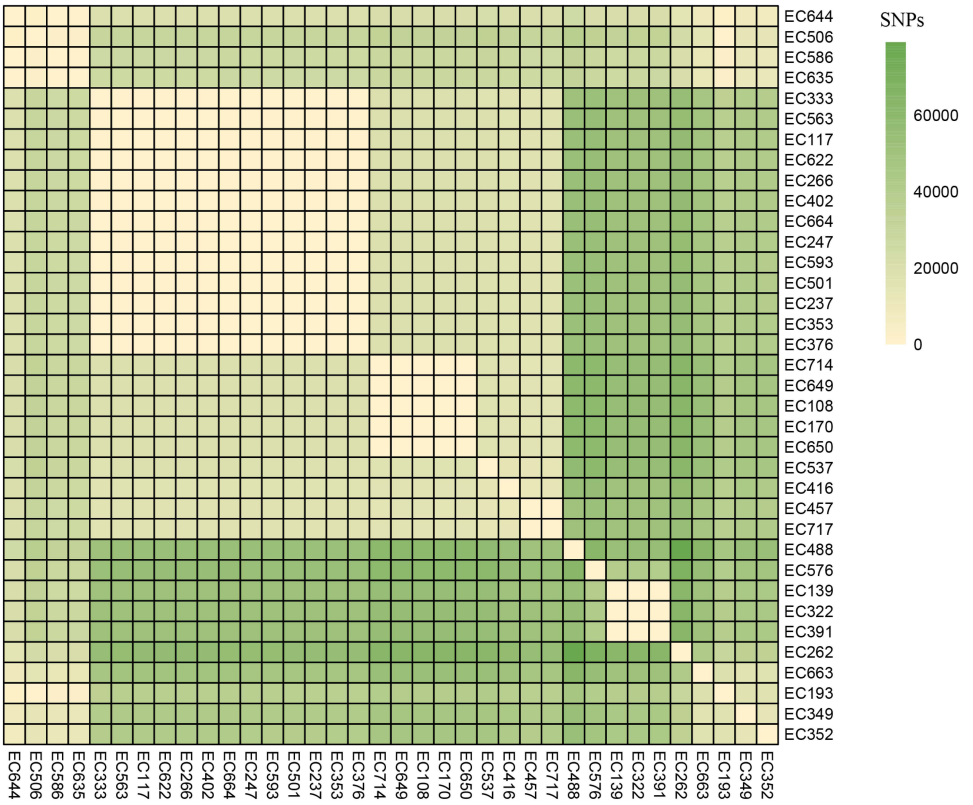

Figure 2 The single nucleotide polymorphisms (SNPs) numbers between each isolate.

generation cephalosporins, like ceftriaxone, are commonly used in clinical settings in many developing countries.[35] Ceftriaxone and cefotaxime have been reported to have similar antibacterial profiles, and are the most commonly used antibiotics against gram-negative bacteria.[36] All isolates were observed to have a high resistance rate in our study.

Our findings indicated that the most prevalent CTX-M variants being CTX-M-27, CTX-M-55, CTX-M-14, and CTX-M-15. Among these, CTX-M-15 is the most found variant currently, followed by CTX-M-14 and CTX-M-27.[37] CTX-M-27 is a member of the CTX-M-9 group, a novel ESBL that has emerged in several countries including Europe, Japan, Korea, Vietnam, and China.[38,39] IncF plasmids and transposons are frequently associated with the transfer of $bla_{\text{CTX-M-27}}$ in *E. coli*, and antibiotic stress may facilitate the evolution of a $bla_{\text{CTX-M}}$ propagation mechanism. In terms of an extended-spectrum cephalosporin resistance phenotype, the CTX-M-27 enzyme mimics the historical CTX-M group 1 variant, CTX-M-15, carried by *E. coli* ST131.[40] In our study, it was found that the majority (8/9; 88.9%) of strains carrying the CTX-M-27 gene were not susceptible to ceftazidime. The high hydrolytic activity of CTX-M-27 against ceftazidime and cefotaxime was attributed to a decrease in ceftazidime Km caused by the substitution of D240G, most likely through altering the location of the 3 chain-residue during hydrolysis.[41] It differs from the widely distributed CTX-M-14 of the same group by only one amino acid residue (Asp-240-Gly), resulting in increased activity against ceftazidime.[38] Recent research has revealed that the CTX-M-1 group and CTX-M-9 group predominate in *E. coli* isolates with $bla_{\text{CTX-M}}$ found in Indonesia and Vietnam.[42] Both CTX-M-15 and CTX-M-55 belong to CTX-M-1 group. CTX-M-55 is derived from CTX-M-15 with only one amino acid substitution (Ala-77-Val) and exhibits greater anti-ceftazidime activity than CTX-M-15.[6] CTX-M-55 is primarily found in China and has become the predominant ESBL subtype in the region,[6] which is supported by a recent study indicating it as the most common and widespread subtype of CTX-M in food animals.[43]

According to MLST and phylogenetic analysis, ESBL-producing *E. coli* showed genetic diversity in the communities in this study. Following cgSNP-based phylogenetic analysis, 36 ESBL-producing *E. coli* isolates could be divided into five clusters, suggesting five independent transmission events. With the exception of EC266 and EC622, which were admitted to the same patient at different times, the SNP distances between the other strains were distant, suggesting that they were not

transmitted from the same clone and that intra-hospital transmission events may not occur. The most frequently observed lineage in our study was ST131, which is also the most common sequence type and high-risk clone of ESBL-EC worldwide.[44] It has been shown that ST10 *E. coli* was found to be one of the main vectors of $bla_{\text{CTX-M-27}}$ transmission in China,[45] but ST131 was predominant in our study. This lineage is known for its frequent demonstration of multidrug resistance. Most clinical isolates of *E. coli* belonging to phylogenetic group B2 are of serotype O25b:H4, including the ST131 lineage. Despite being a member of phylogenetic group B2, the dominant serotype among the *E. coli* isolates in our investigation was O16:H5, which exhibited different resistance rates compared to the common O25b:H4 serotype. Notably, the O16:H5 isolates showed a substantial increase in gentamicin resistance and a significant decrease in ciprofloxacin resistance.[46] Among the *E. coli* isolates in our investigation, 42.8% of the seven O16:H5 isolates produced CTX-M-55, and 28.6% produced CTX-M-27, whereas 60% of the O25b:H4 isolates produced CTX-M-174. Additionally, our study identified ST1193 as another crucial sequence type in ESBL-EC. Similar to *E. coli* ST131, *E. coli* ST1193 is also emerging as a successful high-risk clone for MDR *E. coli*.[47] Belonging to phylogenetic group B2, ST1193 has the serotype O75:H5 and has emerged from the clonal complex 14 (CC14), which is known for its potent mutagenic and biofilm-forming capabilities.[48] Since 2012, the worldwide incidence of ST1193 has been increasing steadily. Between 2010 and 2017, its prevalence has increased dramatically from 4.4% to 22.2%.[49] The alarming increase in the prevalence of ST1193 suggests that the burden of MDR *E. coli* clones on public health is escalating. This underscores the need for ongoing surveillance and increased attention to infections caused by *E. coli* ST1193. In our study, we also identified other lineages such as ST10, ST12, ST38, ST44, ST46, ST69, ST73, ST95, ST155, ST423, ST648, ST1196, ST1266, ST1421, and ST5150, all of which exhibited high rates of antibiotic resistance.

ESBL genes are commonly found to be linked with mobile genetic elements (MGEs) such as plasmid replicons, transposons, and integrative conjugative elements.[50] Our study found that plasmids belonging to the IncF family were present in 30 (83.3%) of the isolates. These plasmids have been identified as one of the key factors responsible for the dissemination of ESBLs, particularly the $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ genes.[37,51] IncFIB type plasmid is the most frequently discovered replicon type,[52] followed by Col156, IncFIA, Col (MG828), ColRNAI, IncFIC, IncFII, IncI1 and Col (BS512), which is consistent with other studies. IS*Ecp1* is a member of the IS*1380* insertion sequence family, which has been identified as one of several factors involved in the mobilization of $bla_{\text{CTX-M}}$ genes and it is important for the dissemination of $bla_{\text{CTX-M}}$.[42] Recent study suggests that the molecular nature of IS*Ecp1* might be a factor contributing to the high detection rates of *E. coli* with $bla_{\text{CTX-M-14}}$ in both clinical and community settings.[53]

There are a few limitations to our study. Firstly, we faced difficulty retrieving clinical outcome data for some patients. Secondly, we did not investigate the underlying transmission mechanisms of $bla_{\text{CTX-M}}$-carrying plasmids in the isolates; however, this will be a focus of our subsequent research. Thirdly, as the study was conducted in a single district hospital, and the low number of strains included in our retrospective investigation may not be representative of the entire population. To address this issue, we plan to gather more strains from multiple district hospitals in the future to continue surveillance of *E. coli* infections using both conventional and WGS approaches, in order to better understand their development and transmission dynamics.

Conclusions

In conclusion, the prevalent type of ESBLs observed were CTX-M-27, CTX-M-55, CTX-M-14, and CTX-M-15, while the dominant lineage identified were ST131 and ST1193. Continuous monitoring of ESBL-producing *E. coli* infections using conventional and whole-genome sequencing approaches is crucial to better understand the mechanisms of resistance and transmission dynamics of this epidemic bacteria on a global scale.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and obtained approval from the Medical Ethics Committee at the Third People's Hospital of Xiaoshan, Hangzhou, China (Ethics 2022-1, Ethics 2022-2). All isolates were the result of regular laboratory procedures, and no patient-identifiable information was acquired. Therefore, informed consent was not needed.

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Disclosure

The authors report no conflicts of interest in this work.

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