Nasal carriage of CTX-M-55-producing *Escherichia coli* ST8369 in a healthy cohort in the city of Yangzhou, China

Zhen-Yu Wang[1,2†], Yue Jiang[1,2†], Yi-Qiao Shao[1],
Heng-Fan Lu[1], Meng-Jun Lu[1,2], Xinan Jiao[1,2],
Qiu-Chun Li[1,2*] and Jing Wang[1,2*]

[1]Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-Innovation Center for Prevention and Control of
Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China, [2]Key
Laboratory of Prevention and Control of Biological Hazard Factors (Animal Origin) for Agrifood
Safety and Quality, Ministry of Agriculture of China, Yangzhou University, Yangzhou, China

This study aimed to investigate the prevalence and diversity of extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli* isolates from healthy individuals in a community and to elucidate their dissemination mechanism. Cefotaxime-resistant *E. coli* were isolated from 95 samples of healthy persons from one community in Yangzhou, China, and were tested for minimal inhibitory concentrations of 14 antimicrobial agents. The isolates were subjected to whole genome sequencing by Illumina Hiseq or PacBio single-molecule real-time sequencing. A total of 30 cefotaxime-resistant *E. coli* isolates were obtained, carrying bla_{CTX-M} (n=29) or bla_{DHA} (n=1), of which the $bla_{CTX-M-55}$ (n=19) was the most predominant genotype. One novel bla_{CTX-M} variant $bla_{CTX-M-252}$ was identified. Thirteen CTX-M-55-producing *E. coli* isolates belonged to ST8369 from nasal (n=12) or faecal (n=1) samples shared the identical cgMLST type, resistance profiles, resistance genes, plasmid replicons, and a 5,053-bp $bla_{CTX-M-55}$ structure ΔIS*26*-ΔIS*Ecp1*-$bla_{CTX-M-55}$-Δ*orf477*-ΔTn*2*. The $bla_{CTX-M-55}$ gene was located on IncHI2/ST3 plasmid in *E. coli* ST8369. The lengths of bla_{CTX-M}/bla_{DHA}-carrying contigs in the remaining 17 *E. coli* strains ranged from 1,663 to 382,836 bp, located on chromosome (n=4) or plasmids (n=5); the location of the other eight contigs could not be determined due to incomplete assembly. The bla_{CTX-M} was associated with IS*Ecp1* as previously reported. Nasal colonization of CTX-M-55-producing ST8369 *E. coli* strains has occurred among healthy individuals in one community. There is a potential risk of antimicrobial resistance dissemination between humans within one community through close contact or environment *via* aerosols or dust. Therefore, surveillance of nasal carriage of bla_{CTX-M} in communities is warranted to further monitor the spread of the antimicrobial resistance genes in China.

KEYWORDS

bla_{CTX-M}, chromosome, *Escherichia coli*, IS*Ecp1*, plasmids, ST8369

Introduction

Extended-spectrum cephalosporins are widely used in human clinics and veterinary medicine to treat infections caused by multidrug-resistant Gram-negative bacteria; thus, extended-spectrum β-lactamases (ESBL), particularly CTX-M enzymes, have been increasingly reported in human clinical settings and animals worldwide (Bevan et al., 2017). Globally, incidence of CTX-M ESBLs is increasing, $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$ are the predominant genotypes detected in many parts of the world (Woerther et al., 2013; Bevan et al., 2017). The global dissemination of bla_{CTX-M} is mainly due to the rapid horizontal transfer mediated by conjugative plasmids; the epidemic plasmids such as IncF, IncI, and IncHI2 facilitate the global spread of bla_{CTX-M} in Enterobacteriaceae from humans, animals and the environment, particularly in *Escherichia coli* (Bevan et al., 2017; Rozwandowicz et al., 2018; Partridge et al., 2018). Mobile elements such as ISEcp1, IS26, and ISCR1 have also played an essential role in the blaCTX-M transmission (Bevan et al., 2017; Partridge et al., 2018). In addition, some successful *E. coli* clones, such as the *E. coli* clone ST131 lineage diffused worldwide, are also responsible for $bla_{CTX-M-15}$ global dissemination, mostly in human clinics (Bevan et al., 2017).

To date, many studies have focused on CTX-M-producing Enterobacteriaceae from clinical patients. However, the high prevalence of CTX-M-producing *E. coli* colonizing the intestinal tract of healthy persons in communities is of particular concern, since they could be a major reservoir of bla_{CTX-M} (Woerther et al., 2013; Bevan et al., 2017; Chen et al., 2021). In this study, we aimed to investigate the prevalence and characterization of extended-spectrum β-lactamases (ESBL)-producing *E. coli* isolates from healthy individuals in a community from Yangzhou, China, to elucidate their dissemination mechanism within this small-scale community.

Materials and methods

Sample collection and antimicrobial susceptibility testing

From April 9th to May 17th 2021, 58 fecal samples and 37 nasal swabs of 72 healthy volunteers (3 male and 69 female) with no obvious disease symptoms at the age of 15-46 were collected from 37 apartments located in three buildings in one community in Yangzhou, China. Participants had been exposed to antimicrobial agents in the three months prior to sample collection were excluded from this study. This small-scale community with approximately 2000 individuals located in the urban area of Yangzhou, and included three main areas, and one building was randomly selected to sample in each area (Supplementary Figure S1). Individual written informed consent for samples was obtained from all volunteers. Samples were incubated in LB broth (OXOID, Basingstoke, UK) for 18~24 h and then cultured on the MacConkey agar (Haibo, Qingdao, China) with 2 mg/L cefotaxime. One *E. coli* isolate per plate was selected and identified by 16S rRNA gene sequencing using PCR and Sanger sequencing (Kim et al., 2010). The cefotaxime-resistant *E. coli* isolates were tested susceptibility to 14 antimicrobial agents including ampicillin, cefotaxime, meropenem, gentamicin, amikacin, streptomycin, tetracycline, chloramphenicol, florfenicol, nalidixic acid, ciprofloxacin, colistin, fosfomycin, and sulfamethoxazole/trimethoprim by using the agar dilution or broth microdilution method (limited to colistin). The results were interpreted according to Clinical Laboratory Standards Institute (CLSI) M100, 30th edition (CLSI, 2020). *E. coli* ATCC 25922 was used as the quality control strain.

Whole genome sequencing and analysis

All cefotaxime-resistant *E. coli* isolates were sequenced by Illumina Hiseq. The library was constructed using NEB NEXT Ultra DNA Library Prep Kit for Illumina (New England Biolabs, USA) and 150 bp paired-end reads were obtained. For each *E. coli* isolate performed WGS, at least 100-fold coverage of raw reads was collected. The 150 bp pair-end raw reads were trimmed and filtered by the NGSQC toolkit 2.3.3, then were assembled into contigs using SPAdes 3.8.2 (Bankevich et al., 2012). One representative ST8369 *E. coli* isolate YZ21HCE18 was sequenced using PacBio single-molecule real-time sequencing. The phylogenetic groups of *E. coli* were confirmed according to previously described protocol by using assembled contigs (Clermont et al., 2000). The genomes were subjected to analysis of multilocus sequence typing (MLST), core genome multilocus sequencing typing (cgMLST), resistance genes, mutations and plasmids by using the Center for Genomic Epidemiology (CGE) pipelines (http://www.genomicepidemiology/org/). The phylogenetic tree of these isolates was constructed using Parsnp (https://harvest.readthedocs.io/en/latest/content/parsnp.html) and visualized by iTOL (Letunic and Bork, 2016). The bla_{CTX-M}/bla_{DHA}-carrying contigs were retrieved from the draft genomes and analyzed by ISfinder (https://www-is.biotoul.fr/) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The bla_{CTX-M}-bearing plasmid pYUYZ18-1 in strain YZ21HCE18 was compared with other ST8369 *E. coli* isolates using BRIG.

Conjugation assay

The transferability of cefotaxime resistance was determined using conjugation experiments as previously described (Chen et al., 2007) and streptomycin-resistant *E. coli* C600 as the recipient. Transconjugants were selected using 2 mg/L

cefotaxime and 3,000 mg/L streptomycin, and were confirmed by detecting bla_{CTX-M} or bla_{DHA-1} using PCR and sequencing (Chen et al., 2004; Liu et al., 2007).

Results

Prevalence and genotype distribution of cefotaxime-resistant *E. coli*

Thirty cefotaxime-resistant *E. coli* isolates were obtained from 12 nasal swabs and 18 fecal samples from 27 individuals (Table 1). The $bla_{CTX-M-55}$ (n=19) gene was identified as the most predominant genotype, followed by $bla_{CTX-M-14}$ (n=5), $bla_{CTX-M-65}$ (n=2), $bla_{CTX-M-15}$ (n=1), $bla_{CTX-M-64}$ (n=1), and bla_{DHA-1} (n=1) (Table 1). One novel bla_{CTX-M} variant $bla_{CTX-M-252}$ (GenBank accession no. OL884447) was identified, and differed from $bla_{CTX-M-65}$ by a single nucleotide resulting in one amino acid change (A32V).

Characterization of cefotaxime-resistant *E. coli* isolates

All ESBL-producing isolates exhibited an MIC of 8 to >128 mg/L to cefotaxime, and also showed resistance to multiple antibiotics, but were susceptible to meropenem and amikacin (Table 1). Twenty-two of them successfully transfer cefotaxime resistance to *E. coli* C600 by conjugation (Table 1). In addition to bla_{CTX-M}/bla_{DHA}, they carried one to 18 resistance genes, such as bla_{TEM}, *tet*(A), *floR*, *qnrS1*, *fosA3*, and *mcr-1* (Figure 1); mutations within *gyrA* (S83L, D87N/Y), *parC* (S80I), or *parE* (I355T, S458A) were observed in six of them (Table 1). Phylogenetic group analysis showed that group B1 was predominant (21; 70%), which was frequently associated with commensal or intestinal pathogenic strains (Clermont et al., 2000); followed by group A (3; 10%), group C (2; 6.67%), group F (2; 6.67%) and group E (1; 3.33%) (Table 1). Only one CTX-M-65-producing isolate belonged to extraintestinal virulent group B2.

Thirty ESBL-producing isolates were assigned to 14 known STs and three novel STs (ST12741, ST12743 and ST12744) (Figure 1). The most prevalent STs among them were ST8369 (n=13). So far, only nine ST8369 *E. coli* isolates were retrieved from EnteroBase (https://enterobase.warwick.ac.uk/) originating from humans, wild animals, and the environment (Table S1), and none of them carried resistance genes or mutations associated with quinolone resistance. To reveal the genetic differences between 22 *E. coli* ST8369 isolates, we analyzed their cgMLST profiles (cgSTs) based on 2513 alleles (Zhou et al., 2020). Among the identified seven cgSTs, cgST 75038 (n=14) was the dominant type shared by 13 strains in our study and one isolate PS00212 (Figure 1). Thirteen *E. coli* ST8369

strains from nasal (n=12) or fecal (n=1) samples of different individuals in this study shared the same cgST, resistance profiles, resistance genes, and plasmid replicons (Figure 1 and Table 1), indicating that there is a reservoir of this lineage in the community.

The genetic structures of bla_{CTX-M}/bla_{DHA} in 30 ESBL-producing *E. coli* isolates

The lengths of bla_{CTX-M}/bla_{DHA}-carrying contigs ranged from 1,663 to 382,836 bp, located on chromosome (n=4) or plasmids (n=5). Twenty-one contigs were short (1,663 to 5,053 bp) due to incomplete assembly and the high number of insertion elements; they did not have replicon genes or plasmid backbone; thus, it is difficult to determine their location (Table 1; Figure 2). In YZ21HCE13, the 3,241-bp transposition unit (IS*Ecp1*-$bla_{CTX-M-55}$-$\Delta orf477$-ΔTn*2*) was inserted into the chromosome and generated 5-bp direct repeats (DRs) (Figure 2A). A similar insertion (IS*Ecp1*-$bla_{CTX-M-55}$-$\Delta orf477$) with a different location had occurred in the chromosome of YZ21HCE20. Similarly, a 3,050-bp segment (IS*Ecp1*-$bla_{CTX-M-55}$-$\Delta orf477$-ΔTn*2*) with DRs (5'-TAACA-3') was inserted into the plasmid backbone in YZ21HCE6 (Figure 2A), this $bla_{CTX-M-55}$-bearing contig (21,977 bp) was identical to those of F18:A-:B1:C4 plasmids, such as pTREC1 (*E. coli*, MN158989) (Figure S2). In YZ21HCE16, the $bla_{CTX-M-55}$-carrying contig was 46,033 bp in size and similar to IncX1 plasmids such as p40EC-5 (CP070925) and pPK8277-49kb (CP080137) (Figure S2). $bla_{CTX-M-55}$ was associated with the commonly observed structure ΔIS*26*-ΔIS*Ecp1*-$bla_{CTX-M-55}$-$\Delta orf477$-ΔTn*2*, and three additional resistance genes *aph(3')-Ia*, *qnrS1* and *tet* (A) were co-located in this contig (Figure S2). The 2,763-bp $bla_{CTX-M-55}$ region in isolates YZ21HCE15 and YZ21HCE24 were identical, including the typical transposition unit (ΔIS*Ecp1*-$bla_{CTX-M-55}$-*orf477*) and a truncated bla_{TEM-1b} downstream, which was commonly observed in many $bla_{CTX-M-55}$-carrying plasmids, e.g., pHNMC02 (MG197489). All ST8369 *E. coli* isolates (n=13) had identical 5,053-bp $bla_{CTX-M-55}$-positive contigs with structure ΔIS*26*-ΔIS*Ecp1*-$bla_{CTX-M-55}$-$\Delta orf477$-ΔTn*2*, except strain YZ21HCE29 (one nucleotide change). The complete sequence of YZ21HCE18 as representative ST8369 *E. coli* was obtained (Table S2). The $bla_{CTX-M-55}$ gene was located on plasmid pYUYZ18-1 with a size of 248,665 bp, belonged to IncHI2/ST3 plasmid with a similar organization to other IncHI2 plasmids. In addition to $bla_{CTX-M-55}$, pYUYZ18-1 contained numerous resistance genes, including *aac(3)-IId*, *aph(3')-Ia*, *strAB*, *tet*(A), *mph* (A), *qnrS1*, *floR*, *sul2*, *sul3*, *dfrA14*, and *arr-2*. Similar pYUYZ18-1 plasmids were also present in other ST8369 *E. coli* strains in this study (Figure S3).

TABLE 1 Chracterization of cefotaxime-resistant *Escherichia coli* isolates in this study.

Strains[a]	Source[b]	Sampling Date	MLST	Phylogenetic group	ESBL genotype	Antimicrobial susceptibility profile[c]	Plasmid	Mutations[d]	Length of $bla_{CTX/DHA}$-contig (bp)
YZ21HCE7*	nasal swab, F	2021/5/14	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE8*	nasal swab, F	2021/5/14	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE9*	nasal swab, F	2021/5/14	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE18*	nasal swab, F	2021/5/14	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE19*	nasal swab, F	2021/5/14	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE21*	nasal swab, F	2021/5/16	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE22*	nasal swab, F	2021/5/16	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE26*	feces, F	2021/5/16	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE27*	nasal swab, F	2021/5/17	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE28*	nasal swab, F	2021/5/17	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE29*	nasal swab,M	2021/5/17	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE30*	nasal swab,M	2021/5/17	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE31*	nasal swab, F	2021/5/17	8369	B1	$bla_{CTX-M-55}$	AMP/CTX/GEN/STR/TET/CHL/FFC/SXT	IncHI2, F102:A17:B-	N	5,053
YZ21HCE1*	feces, F	2021/4/9	773	A	$bla_{CTX-M-14}$	AMP/CTX/NAL/CIP/FOS	F2:A-:B10, IncX1, Col (BS512)	*gyrA*(S83L+D87N), *parC* (S80I), *parE* (S458A)	1,687
YZ21HCE2*	feces, F	2021/4/9	453	B1	bla_{DHA-1}	AMP/CTX/STR/TET/NAL	IncHI2, IncFII, IncFIB	N	18,905
YZ21HCE3*	feces, F	2021/4/9	5614	B1	$bla_{CTX-M-15}$	AMP/CTX/STR/SXT	IncK	N	35,054
YZ21HCE4	feces, F	2021/4/10	1434	A	$bla_{CTX-M-14}$	AMP/CTX/NAL	IncY, IncX1, IncFIB(K), Col156, IncFII (pCRY)	N	21,609
YZ21HCE5	feces, F	2021/4/10	95	B2	$bla_{CTX-M-65}$	AMP/CTX/GEN/STR/TET/CHL/FFC/NAL/SXT	IncHI2, F18:A-:B1:C4	*gyrA* (S83L)	3,019
YZ21HCE6*	feces, F	2021/4/10	10	C	$bla_{CTX-M-55}$	AMP/CTX/TET	IncI1, F18:A-:B1:C4	N	21,977

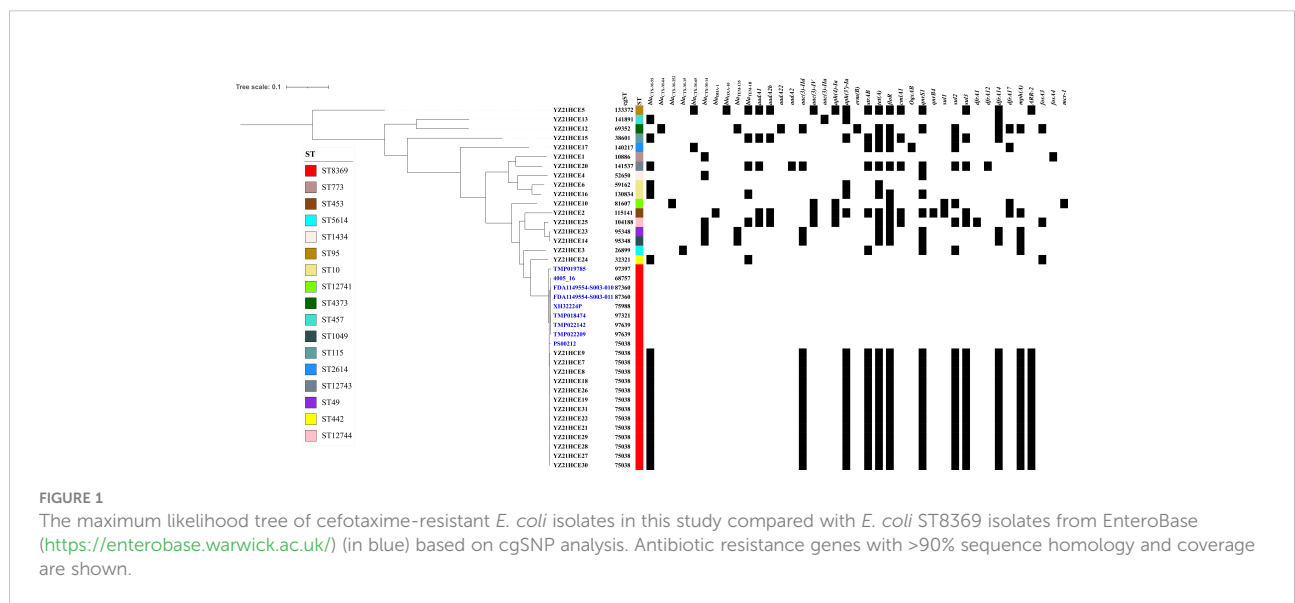*(Continued)*

TABLE 1 Continued

Strains[a]	Source[b]	Sampling Date	MLST	Phylogenetic group	ESBL genotype	Antimicrobial susceptibility profile[c]	Plasmid	Mutations[d]	Length of bla_{CTX/DHA^-} contig (bp)
YZ21HCE10*	feces, F	2021/5/14	12741	B1	$bla_{CTX-M-252}$	AMP/CTX/GEN/CHL/FFC/NAL/CIP/CL/SXT	IncI1, IncI2, IncY, F18:A-:B1:C4	$gyrA$(S83L+D87N), $parC$ (S80I), $parE$ (S458A)	1,663
YZ21HCE12	feces, F	2021/5/14	4373	F	$bla_{CTX-M-64}$	AMP/CTX/GEN/STR/TET/CHL/FFC/FOS/SXT	F18:A-:B1:C4, ColpVC	N	160,049
YZ21HCE13	feces, F	2021/5/15	457	F	$bla_{CTX-M-55}$	AMP/CTX/GEN/NAL/CIP	F18:A-:B1:C4	$gyrA$(S83L+D87Y), $parC$ (S80I+E84G), $parE$ (I355T)	382,836
YZ21HCE14*	feces, F	2021/5/15	1049	B1	$bla_{CTX-M-14}$	AMP/CTX/GEN/TET/CHL/FFC	IncK, F18:A-:B1:C4	N	96,577
YZ21HCE15	feces, F	2021/5/14	115	E	$bla_{CTX-M-55}$	AMP/CTX/STR/TET/CHL/FFC/NAL/SXT	F24:A-:B1, IncI1, IncX1, Col8282, Col156	$gyrA$ (S83L), $parE$ (I464F)	2,763
YZ21HCE16*	feces, F	2021/5/15	10	C	$bla_{CTX-M-55}$	AMP/CTX/TET/CHL/FFC	IncX1, F46:A-:B24	N	46,033
YZ21HCE17	feces, F	2021/5/14	2614	B1	$bla_{CTX-M-65}$	AMP/CTX/STR/TET/CHL/FFC/NAL/SXT	IncY, IncQ1	N	3,895
YZ21HCE20	feces, F	2021/5/16	12743	A	$bla_{CTX-M-55}$	AMP/CTX/GEN/TET/CHL/FFC/NAL/SXT	IncFIB, IncY	N	38,904
YZ21HCE23*	feces, F	2021/5/16	49	B1	$bla_{CTX-M-14}$	AMP/CTX/GEN/TET/CHL/FFC/SXT	F18:A-:B1:C4, IncK	N	65,116
YZ21HCE24*	feces, F	2021/5/16	442	B1	$bla_{CTX-M-55}$	AMP/CTX/FOS	F16:A-:B-	N	2,763
YZ21HCE25	feces, F	2021/5/16	12744	B1	$bla_{CTX-M-14}$	AMP/CTX/GEN/STR/TET/CHL/FFC/NAL/CIP/FOS/SXT	F55:A-:B6, p0111	$gyrA$(S83L+D87N), $parC$ (S80I)	3,320

[a], * indicates that strain could successfully transfer $bla_{CTX-M/DHA}$ to E. coli C600 by conjugation; Underline indicates nasal and fecal samples obtained from the same individual, YZ21HCE7 and YZ21HEC10, YZ21HCE15 and YZ21HCE18, YZ21HCE17 and YZ21HCE19; Boldface indicates individuals from the same apartment.
[b], F, female; M, male.
[c], AMP, ampicillin; CTX, cefotaxime; GEN, gentamicin; STR, streptomycin; TET, tetracycline; CHL, chloramphenicol; FFC, florfenicol; NAL, nalidixic acid; CIP, ciprofloxacin; CL, colistin; FOS, fosfomycin; SXT, sulfamethoxazole/trimethoprim; all strains were susceptible to meropenem and amikacin.
[d], N, not found.

FIGURE 1
The maximum likelihood tree of cefotaxime-resistant E. coli isolates in this study compared with E. coli ST8369 isolates from EnteroBase (https://enterobase.warwick.ac.uk/) (in blue) based on cgSNP analysis. Antibiotic resistance genes with >90% sequence homology and coverage are shown.
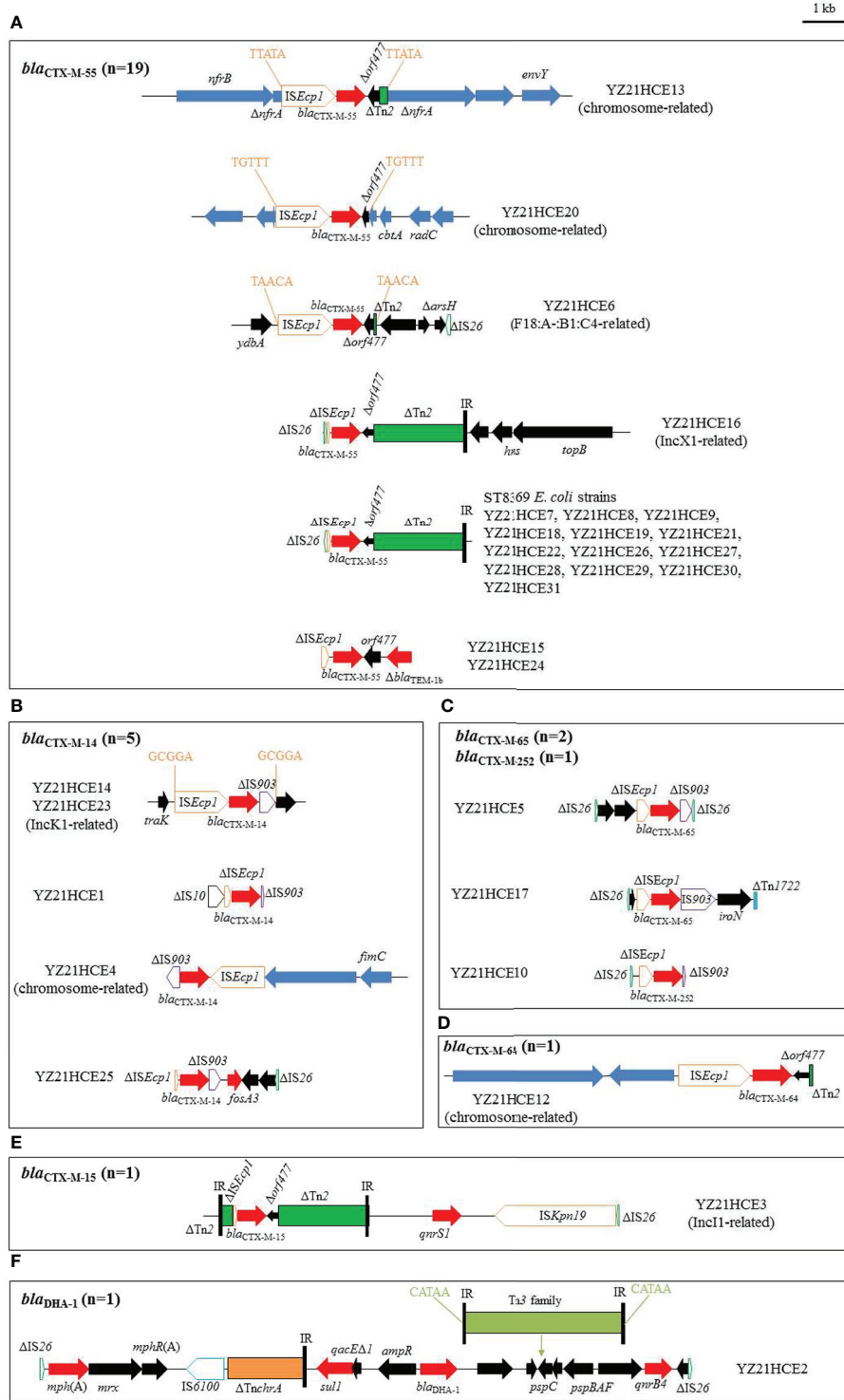
FIGURE 2

The genetic environments of blaCTX-M/blaDHA in 30 E. coli isolates in this study. **(A)** blaCTX-M-55; **(B)** blaCTX-M-14; **(C)** blaCTX-M-65/-252; **(D)** blaCTX-M-64; **(E)** blaCTX-M-15; **(F)** blaDHA. The extents and directions of antibiotic resistance (red arrows) and other genes (black arrows) are indicated. The blue arrows indicate chromosomal genes. ISs are shown as boxes labeled with their name. Tall bars represent the inverted repeats (IR) of transposon. Δ indicates a truncated gene or mobile element. Arrows and sequences indicate direct repeats.

Two $bla_{CTX-M-14}$-positive contigs from isolates YZ21HCE14 and YZ21HCE23 displayed >99.9% similarity to IncK1 plasmids pD16EC0206-1 (*E. coli*, CP088610) and pJX1-2 (*Klebsiella pneumoniae*, CP064254) (Figure S2). The 3,060-bp $bla_{CTX-M-14}$ transposition unit (IS*Ecp1*-$bla_{CTX-M-14}$-ΔIS*903*) with 5-bp DRs (5'-GCGGA-3') was inserted downstream of the plasmid conjugal transfer gene *traK* (Figure 2B). In isolates YZ21HCE1, YZ21HCE4, and YZ21HCE25, a similar $bla_{CTX-M-14}$ transposition unit was observed, differed by deletions involving IS*Ecp1* and/or IS*903*. In YZ21HCE4, the 2,996-bp fragment (IS*Ecp1*-$bla_{CTX-M-14}$-ΔIS*903*) was embedded in a 21,609-bp contig associated with the chromosome of *E. coli*. The 1,972-bp *fosA3* segment (*fosA3*-*orf1*-Δ*orf2*-ΔIS*26*) was present downstream of IS*903* in YZ21HCE25 (Figure 2B).

As observed in Figure 2C, the typical (IS*Ecp1*-$bla_{CTX-M-9G}$-IS*903*-*iroN*) transposition unit was found in isolates YZ21HCE5 ($bla_{CTX-M-65}$), YZ21HCE17 ($bla_{CTX-M-65}$), and YZ21HCE10 ($bla_{CTX-M-252}$), although IS*Ecp1* was incomplete. In YZ21HCE17, the $bla_{CTX-M-65}$ unit was inserted in an incomplete Tn*1722*. However, IS*903* was truncated by IS*26* at the 3' end in YZ21HCE5, resulting in the deletions of *iroN* and 701 bp of IS*903*. Similarly, an incomplete IS*903* (80-bp) was also observed in YZ21HCE10. A 1,205-bp structure consisting of two hypothetical proteins plus ΔIS*26* (76-bp) was located upstream of $bla_{CTX-M-65}$ transposition unit with a 64-bp spacer in YZ21HCE5. Remnants of this structure were also identified upstream of the $bla_{CTX-M-65}$ unit in YZ21HCE17 (214 bp) and YZ21HCE10 (201 bp).

The $bla_{CTX-M-64}$ was located on the chromosome of YZ21HCE12. The 160,049-bp $bla_{CTX-M-64}$-carrying contig showed highly (>98.0%) similarity to the corresponding region of *E. coli* chromosome such as Z30 (CP066844) and LD67-1 (CP061185). Furthermore, a 3,045-bp region (IS*Ecp1*-$bla_{CTX-M-64}$-Δ*orf477*-ΔTn*2*) in YZ21HCE12 was identical to those of chromosome of *E. coli* 3952 (MT773682) and plasmid pM-64-4467-1 (MT773679) from healthy humans in Hangzhou, China (Chen et al., 2021).

The $bla_{CTX-M-15}$-positive contig (35,054 bp) in YZ21HCE3 was similar to IncI1 plasmid pBHBSTW-00321_3 (*E. coli*, CP056606) with 86% coverage and 98.36% identity (Figure S2). The $bla_{CTX-M-15}$ gene was associated with genetic content ΔTn*2*-ΔIS*Ecp1*-$bla_{CTX-M-15}$-Δ*orf477*-ΔTn*2*, seen in several plasmids, such as p5908-2 (*Shigella flexneri*, CP045523) and p92 (*E. coli*, CP041521). The $bla_{CTX-M-15}$ resistance module was followed by a 5,752-bp structure *qnrS1*-IS*Kpn19*-ΔIS*26* (Figure 2E).

The bla_{DHA}-carrying contig (18,905 bp) of YZ21HCE2 was identical to the corresponding regions of multiple plasmids such as pM2901 (*Shigella sonnei*, CP061363), except for the insertion of one Tn*3* family transposon within *pspC* flanked by 5-bp DRs in YZ21HCE2. The core structure *sul1*-*qacEΔ1*-*ampR*-bla_{DHA-1}-*pspDCBAF*-*qnrB4* is commonly observed in numerous plasmids from various species (e.g., *Salmonella*, *Klebsiella pneumoniae*, *Citrobacter freundii*), highlighting the co-transfer ability of bla_{DHA-1} and *qnrB4*.

Discussion

The bla_{CTX-M} gene has been globally disseminated in different sources, with $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$ being dominant (Bevan et al., 2017). In this study, $bla_{CTX-M-55}$ is the most predominant genotype in healthy individuals, which agrees with the increasing prevalence of $bla_{CTX-M-55}$ in both animals and patients in China (Bevan et al., 2017). As a variant of $bla_{CTX-M-15}$, $bla_{CTX-M-55}$ was first reported in clinical *E. coli* and *K. pneumoniae* isolates in Thailand in 2007 (Kiratisin et al., 2007). Recently, $bla_{CTX-M-55}$ has become the predominant CTX-M genotype in *E. coli* and *Salmonella* from food animals, food products, and patients in China (Rao et al., 2014; Zhang et al., 2014; Fu et al., 2020; Huang et al., 2020; Liu et al., 2022; Zeng et al., 2022). One novel bla_{CTX-M} variant $bla_{CTX-M-252}$ was identified in this study. CTX-M-252 and CTX-M-65 differ by a single amino acid and share a similar genetic structure, suggesting a common lineage. Further investigation of $bla_{CTX-M-252}$ is needed.

In communities, faecal carriage rates of bla_{CTX-M} are increasing, particularly in developing countries (Woerther et al., 2013; Bevan et al., 2017). However, investigation of bla_{CTX-M} in nasal samples of healthy humans is rare. Previously, one (1/77, 1.30%) CTX-M-producing *E. coli* isolate was obtained from the nasal sample of a healthy human working at a pig abattoir in Cameroon (Founou et al., 2018). A high nasal carriage rate (32.43%) of bla_{CTX-M} was observed in this study, and nasal colonization of ST8369 *E. coli* producing CTX-M-55 among healthy persons occurred in one community in Yangzhou, China. It suggests that nasal carriage of bla_{CTX-M} is possibly common in humans. However, the small number of samples and communities is a limitation of this study. The acquisition of $bla_{CTX-M-55}$ and other resistance genes by ST8369 is mediated by the horizontal transfer of IncHI2 plasmid, followed by clonal dissemination. *E. coli* ST8369 is rarely described worldwide and may represent an emerging clone in humans, animals, and the environment. Nasal colonization of $bla_{CTX-M-55}$-carrying ST8369 *E. coli* suggests a potential risk of antimicrobial resistance dissemination between humans by the spread of clonal lineages in the small-scale community through close contact or environment *via* aerosols or dust. Therefore, the clinical importance of nasal carriage of CTX-M-producing *E. coli* might be underestimated. Although horizontal transfer is the main reason for bla_{CTX-M} dissemination, clonal spread of bla_{CTX-M}-harbouring strains, such as *E. coli* ST8369 in this study, CTX-M-15-producing *E. coli* ST949 in water surfaces, *E. coli* ST2179 encoding CTX-M-65 in retail meat, and $bla_{CTX-M-55}$-carrying *Salmonella* Typhimurium ST34 in patients (Bevan et al., 2017; Falgenhauer et al., 2021; Leão et al., 2021; Zeng et al., 2022) is another important route for bla_{CTX-M} transmission.

Horizontal transfer mediated by plasmids and mobile elements is responsible for the global spread of bla_{CTX-M} (Bevan et al., 2017; Partridge et al., 2018). For example, IncI, IncFII, and IncHI2 plasmids facilitate the horizontal

transmission of bla_{CTX-M} in *E. coli* and *Salmonella* from various sources (Chen et al., 2021; Guo and Zhao, 2021; Yang et al., 2014; Nadimpalli et al., 2019; Zhang et al., 2021; Zeng et al., 2021). In this study, various plasmids such as IncHI2, IncK1, IncX1, and IncI1 were associated with bla_{CTX-M}. Although we were not able to determine the location of bla_{CTX-M} in some *E. coli* isolates in this study due to incomplete assembly, sequence analysis indicates that IS*Ecp1* plays an important role in bla_{CTX-M} dissemination among *E. coli* isolates and facilitates the horizontal transfer of bla_{CTX-M} from plasmids to chromosomes in distinct integration sites. The chromosomal integration of bla_{CTX-M} is increasingly reported in *E. coli*, *K. pneumoniae*, *Salmonella*, *Proteus mirabilis* and some other species of Enterobacteriaceae with the help of mobile elements (Huang et al., 2017; He et al., 2017; Zeng et al., 2022; Yoon et al., 2022). Chromosomal integration of bla_{CTX-M} seems to be an adaptive evolution in response to antimicrobial pressure (Yoon et al., 2022).

In conclusion, we report nasal colonization of CTX-M-55-producing *E. coli* ST8369 associated with IncHI2 plasmid in healthy individuals in one community from Yangzhou, China. Therefore, continued surveillance of nasal carriage of bla_{CTX-M} in communities is warranted.

Accession Numbers

The sequences have been deposited in the GenBank under accession number: PRJNA819533.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving human participants were reviewed and approved by Yangzhou University. The participants provided their written informed consent to participate in this study.

Author contributions

JW and Q-CL conceived and designed the experiments, Z-YW, YJ, Y-QS, H-FL, and M-JL carried out the experiments. Z-YW and JW analyzed the data and wrote the manuscript. Q-CL and XJ revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.970940/full#supplementary-material

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021

Bevan, E. R., Jones, A. M., and Hawkey, P. M. (2017). Global epidemiology of CTX-m β-lactamases: temporal and geographical shifts in genotype. *J. Antimicrob. Chemother.* 72, 2145–2155. doi: 10.1093/jac/dkx146

Chen, J., Chen, S., Jiang, Y., Zhang, R., and Cai, J. (2021). Fecal carriage and genetic characterization of CTX-M-1/9/1-producing escherichia coli from healthy humans in hangzhou, China. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.616687

Chen, L., Chen, Z., Liu, J., Zeng, Z., Ma, J., and Jiang, H. (2007). Emergence of RmtB methylase-producing escherichia coli and enterobacter cloacae isolates from pigs in China. *J. Antimicrob. Chemother.* 59, 880–885. doi: 10.1093/jac/dkm065

Chen, S., Zhao, S., White, D. G., Schroeder, C. M., Lu, R., Yang, H., et al. (2004). Characterization of multiple-antimicrobial-resistant salmonella serovars isolated from retail meats. *Appl. Environ. Microbiol.* 70 (1), 1–7. doi: 10.1128/AEM.70.1.1-7.2004

Clermont, O., Bonacorsi, S., and Bingen, E. (2000). Rapid and simple determination of the escherichia coli phylogenetic group. *Appl. Environ. Microbiol.* 66, 4555–4558. doi: 10.1128/AEM.66.10.4555-4558.2000

CLSI (2020). *Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 30th ed* (Wayne, PA: Clinical and Laboratory Standards Institute).

Falgenhauer, L., Zur Nieden, A., Harpel, S., Falgenhauer, J., and Domann, E. (2021). Clonal CTX-M-15-producing escherichia coli ST-949 are present in German surface water. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.617349

Founou, L. L., Founou, R. C., Allam, M., Ismail, A., and Essack, S. Y. (2018). Draft genome sequence of an extended-spectrum β-lactamase (CTX-M-15)-producing escherichia coli ST10 isolated from a nasal sample of an abattoir worker in Cameroon. *J. Glob. Antimicrob. Resist.* 14, 68–69. doi: 10.1016/j.jgar.2018.06.004

Fu, Y., Xu, X., Zhang, L., Xiong, Z., Ma, Y., Wei, Y., et al. (2020). Fourth generation cephalosporin resistance among salmonella enterica serovar enteritidis isolates in shanghai, China conferred by blaCTX-M-55 harboring plasmids. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.00910

Guo, L., and Zhao, Y. (2021). Global spread and molecular characterization of CTX-m-producing salmonella typhimurium isolates. *Antibiotics.* 10 (11), 1417. doi: 10.3390/antibiotics10111417

He, D., Liu, L., Guo, B., Wu, S., Chen, X., Wang, J., et al. (2017). Chromosomal location of the fosA3 and blaCTX-m genes in Proteus mirabilis and clonal spread of escherichia coli ST117 carrying fosA3-positive IncHI2/ST3 or F2:A-:B- plasmids in a chicken farm. *Int. J. Antimicrob. Agents.* 49 (4), 443–448. doi: 10.1016/j.ijantimicag.2016.12.009

Huang, W., Wang, G., Sebra, R., Zhuge, J., Yin, C., Aguero-Rosenfeld, M. E., et al. (2017). Emergence and evolution of multidrug-resistant klebsiella pneumoniae with both blaKPC and blaCTX-m integrated in the chromosome. *Antimicrob. Agents Chemother.* 61 (7), e00076–e00017. doi: 10.1128/AAC.00076-17

Huang, Y., Zeng, L., Doi, Y., Lv, L., and Liu, J. H. (2020). Extended-spectrum β-lactamase-producing escherichia coli. *Lancet Infect. Dis.* 20 (4), 404–405. doi: 10.1016/S1473-3099(20)30115-8

Kim, T. W., Kim, Y. H., Kim, S. E., Lee, J. H., Park, C. S., and Kim, H. Y. (2010). Identification and distribution of bacillus species in doenjang by whole-cell protein patterns and 16S rRNA gene sequence analysis. *J. Microbiol. Biotechnol.* 20, 1210–1214. doi: 10.4014/jmb.1002.02008

Kiratisin, P., Apisarnthanarak, A., Saifon, P., Laesripa, C., Kitphati, R., and Mundy, L. M. (2007). The emergence of a novel ceftazidime-resistant CTX-m extended-spectrum β-lactamase, CTX-M-55, in both community-onset and hospital-acquired infections in Thailand. *Diagn. Microbiol. Infect. Dis.* 58 (3), 349–355. doi: 10.1016/j.diagmicrobio.2007.02.005

Leão, C., Clemente, L., Moura, L., Seyfarth, A. M., Hansen, I. M., Hendriksen, R. S., et al. (2021). Emergence and clonal spread of CTX-M-65-producing escherichia coli from retail meat in Portugal. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.653595

Letunic, I., and Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 44, W242–W245. doi: 10.1093/nar/gkw290

Liu, Z., Wang, K., Zhang, Y., Xia, L., Zhao, L., Guo, C., et al. (2022). High prevalence and diversity characteristics of blaNDM, mcr, and blaESBLs harboring multidrug-resistant escherichia coli from chicken, pig, and cattle in China. *Front. Cell Infect. Microbiol.* 11. doi: 10.3389/fcimb.2021.755545

Liu, J., Wei, S., Ma, J., Zeng, Z., Lü, D., Yang, G., et al. (2007). Detection and characterization of CTX-m and CMY-2 β-lactamases among escherichia coli isolates from farm animals in guangdong province of China. *Int. J. Antimicrob. Agents.* 29, 576–581. doi: 10.1016/j.ijantimicag.2006.12.015

Nadimpalli, M., Fabre, L., Yith, V., Sem, N., Gouali, M., Delarocque-Astagneau, E., et al. (2019). CTX-M-55-type ESBL-producing salmonella enterica are emerging among retail meats in Phnom Penh, Cambodia. *J. Antimicrob. Chemother.* 74 (2), 342–348. doi: 10.1093/jac/dky451

Partridge, S. R., Kwong, S. M., Firth, N., and Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* 31 (4), e00088–e00017. doi: 10.1128/CMR.00088-17

Rao, L., Lv, L., Zeng, Z., Chen, S., He, D., Chen, X., et al. (2014). Increasing prevalence of extended-spectrum cephalosporin-resistant escherichia coli in food animals and the diversity of CTX-m genotypes during 2003-2012. *Vet. Microbiol.* 172 (3-4), 534–541. doi: 10.1016/j.vetmic.2014.06.013

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., et al. (2018). Plasmids carrying antimicrobial resistance genes in enterobacteriaceae. *J. Antimicrob. Chemother.* 73 (5), 1121–1137. doi: 10.1093/jac/dkx488

Woerther, P. L., Burdet, C., Chachaty, E., and Andremont, A. (2013). Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-m. *Clin. Microbiol. Rev.* 26, 744–758. doi: 10.1128/CMR.00023-13

Yang, X., Liu, W., Liu, Y., Wang, J., Lv, L., Chen, X., et al. (2014). F33: A-: B-, IncHI2/ST3, and IncI1/ST71 plasmids drive the dissemination of fosA3 and blaCTX-M-55/-14/-65 in escherichia coli from chickens in China. *Front. Microbiol.* 5. doi: 10.3389/fmicb.2014.00688

Yoon, E. J., Choi, Y. J., Kim, D., Won, D., Choi, J. R., and Jeong, S. H. (2022). Amplification of the chromosomal blaCTX-M-14 gene in escherichia coli expanding the spectrum of resistance under antimicrobial pressure. *Microbiol. Spectr.*, 10(3):e0031933. doi: 10.1128/spectrum.00319-22

Zeng, S., Luo, J., Li, X., Zhuo, C., Wu, A., Chen, X., et al. (2021). Molecular epidemiology and characteristics of CTX-M-55 extended-spectrum β-lactamase-producing escherichia coli from guangzhou, China. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.730012

Zeng, S., Zhuo, Z., Huang, Y., Luo, J., Feng, Y., Gong, B., et al. (2022). Prevalence of chromosomally located blaCTX-M-55 in salmonella typhimurium ST34 isolates recovered from a tertiary hospital in guangzhou, China. *Microbiol. Spectr.*, 10(3): e0277121. doi: 10.1128/spectrum.02771-21

Zhang, Z., Chang, J., Xu, X., Zhou, M., Shi, C., Liu, Y., et al. (2021). Dissemination of IncFII plasmids carrying fosA3 and blaCTX-M-55 in clinical isolates of salmonella enteritidis. *Zoonoses. Public Health* 68 (7), 760–768. doi: 10.1111/zph.12825

Zhang, J., Zheng, B., Zhao, L., Wei, Z., Ji, J., Li, L., et al. (2014). Nationwide high prevalence of CTX-m and an increase of CTX-M-55 in escherichia coli isolated from patients with community-onset infections in Chinese county hospitals. *BMC Infect. Dis.* 14, 649. doi: 10.1186/s12879-014-0659-0

Zhou, Z., Alikhan, N. F., Mohamed, K., Fan, Y.Agama Study Group, , Achtman, M. (2020). The EnteroBase user's guide, with case studies on salmonella transmissions, yersinia pestis phylogeny, and escherichia core genomic diversity. *Genome Res.* 30, 138–152. doi: 10.1101/gr.251678.119