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

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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 structure *Δ*IS26-*Δ*ISep1-(*bla*CTX-M-55-Δorf477-ΔTn2. 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 ISep1 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*, ISep1, 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; Rozwandowicz et al., 2018; 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 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; Rozwandowicz et al., 2018; 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).

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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 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} -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...
ceftaxime 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 \( bla_{CTX-M-1} \) (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 (ISEcp1-\( bla_{CTX-M-55}/\text{\text{-}Dorf477} \)-\( \Delta \text{Tn} 2 \) was inserted into the chromosome and generated 5-bp direct repeats (DRs) (Figure 2A). A similar insertion (ISEcp1-\( bla_{CTX-M-55}/\text{\text{-}Dorf477} \)-\( \Delta \text{Tn} 2 \)) 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 F18A::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 \( \Delta \text{IS26-\text{ISEcp1-}\text{-blat}_{CTX-M-55}/\text{\text{-}Dorf477} \}-\Delta \text{Tn} 2 \), and three additional resistance genes \( \text{aph(3)-Ia, qnrS1} \) and \( \text{tet(A)} \) 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 (\( \Delta \text{ISEcp1-}\text{-blat}_{CTX-M-55}/\text{\text{-}Dorf477} \) and a truncated \( bla_{TEM-18} \)-downstream, which was commonly observed in many \( bla_{CTX-M-55} \)-carrying plasmids, e.g., pHNMC02 (MG179489). All ST8369 *E. coli* isolates (n=13) had identical 5,053-bp \( bla_{CTX-M-55} \)-positive contigs with structure \( \Delta \text{IS26-\text{ISEcp1-}\text{-blat}_{CTX-M-55}/\text{\text{-}Dorf477} \}-\Delta \text{Tn} 2 \), 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 IncHII/ST3 plasmid with a similar organization to other IncHI2 plasmids. In addition to \( bla_{CTX-M-55} \), pYUYZ18-1 contained numerous resistance genes, including \( \text{aac(3)-IId, aph(3)-Ia, strAB, tet(A), mph(A), qnrS1, floR, sul2, sul3, dfrA14, and arr-2} \). Similar pYUYZ18-1 plasmids were also present in other ST3869 *E. coli* strains in this study (Figure S3).
| Strains a | Source b | Sampling Date | MLST | Phylogenetic group | ESBL genotype | Antimicrobial susceptibility profile c | Plasmid | Mutations d | Length of blaCTX/DHA-1 contig (bp) |
|----------|----------|----------------|------|-------------------|--------------|---------------------------------------|---------|-------------|--------------------------|
| YZ21HCE7* | nasal swab, F | 2021/5/14 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE8* | nasal swab, F | 2021/5/14 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE9* | nasal swab, F | 2021/5/14 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE18* | nasal swab, F | 2021/5/14 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE19* | nasal swab, F | 2021/5/14 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE21* | nasal swab, F | 2021/5/16 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE22* | nasal swab, F | 2021/5/16 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE26* | feces, F | 2021/5/16 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE27* | nasal swab, F | 2021/5/17 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE28* | nasal swab, F | 2021/5/17 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE29* | nasal swab, M | 2021/5/17 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE30* | nasal swab, M | 2021/5/17 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE31* | nasal swab, F | 2021/5/17 | 8369 | B1 | blaCTX-M-55 | AMP/CTX/GEN/STR/TET/CHL/FFC/SXT | IncH12, F102: A17:B- | N | 5,053 |
| YZ21HCE1* | feces, F | 2021/4/9 | 773 | A | blaCTX-M-14 | AMP/CTX/NAL/CIP/FOS | F2:A-B10, IncX1, Col (BSS12) | gyrA (S83L) | 1,687 |
| YZ21HCE2* | feces, F | 2021/4/9 | 453 | B1 | blaCTX-M-14 | AMP/CTX/STR/TET/NAL | IncH12, IncFIB | gyrA (S83L), parC (S80I), parE (S458A) | 18,905 |
| YZ21HCE3* | feces, F | 2021/4/9 | 5614 | B1 | blaCTX-M-15 | AMP/CTX/STR/NAL | IncK | N | 21,609 |
| YZ21HCE4* | feces, F | 2021/4/10 | 1434 | A | blaCTX-M-14 | AMP/CTX/NAL | IncY, IncX1, IncFIB(9), Col156, IncFIB (pCRY) | N | 3,019 |
| YZ21HCE5 | feces, F | 2021/4/10 | 95 | B2 | blaCTX-M-43 | AMP/CTX/GEN/STR/TET/CHL/FFC/NAL/SXT | IncH12, F18:A-B1:C4 | gyrA (S83L) | 21,977 |
| YZ21HCE6* | feces, F | 2021/4/10 | 10 | C | blaCTX-M-51 | AMP/CTX/TET | IncI1, F18:A-B1:C4 | N | (Continued) |
TABLE 1 Continued

| Strains\textsuperscript{a} | Source\textsuperscript{b} | Sampling Date | MLST Phylogenetic group | ESBL genotype | Antimicrobial susceptibility profile\textsuperscript{c} | Plasmid Mutations\textsuperscript{d} | Length of bla\textsubscript{CTX-DHA\textsuperscript{+}}-contig (bp) |
|---------------------------|-------------------------|---------------|--------------------------|---------------|---------------------------------------------------|----------------------------------|---------------------------------|
| YZ21HCE10\textsuperscript{a} | feces, F | 2021/5/14 | 12741 | B1 | bla\textsubscript{CTX-M-32} | AMP/CTX/GEN/CHL/FFC/NAL/CIP | IncI1, IncI2, IncI4, F84:A-B1:C4 | gyrA(S83L+D87N), parC (S80I), parE (S4S1A) | 1,663 |
| YZ21HCE12 | feces, F | 2021/5/14 | 4373 | F | bla\textsubscript{CTX-M-64} | AMP/CTX/STR/TET/CHL/FFC/FOC/SXT | F18:A-B1:C4 | N | 160,049 |
| YZ21HCE13 | feces, F | 2021/5/15 | 457 | F | bla\textsubscript{CTX-M-55} | AMP/CTX/GEN/NAL/CIP | F18:A-B1:C4 | gyrA(S83L+D87Y), parC (S80D+E43G), parE (I535T) | 382,836 |
| YZ21HCE14\textsuperscript{a} | feces, F | 2021/5/15 | 1049 | B1 | bla\textsubscript{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\textsubscript{CTX-M-55} | AMP/CTX/STR/TET/CHL/FFC/NAL/SXT | F24:A-B1, IncI1, IncX1, Col8282, Col1136 | gyrA (S83L), parE (I464F) | 2,763 |
| YZ21HCE16\textsuperscript{a} | feces, F | 2021/5/15 | 10 | C | bla\textsubscript{CTX-M-55} | AMP/CTX/TET/CHL/FFC | IncX1, F46:A-B24 | N | 46,033 |
| YZ21HCE17 | feces, F | 2021/5/14 | 2614 | B1 | bla\textsubscript{CTX-M-55} | AMP/CTX/STR/TET/CHL/FFC/NAL/SXT | IncY, IncQ1 | N | 3,895 |
| YZ21HCE20 | feces, F | 2021/5/16 | 12743 | A | bla\textsubscript{CTX-M-55} | AMP/CTX/GEN/TET/CHL/FFC/NAL/SXT | IncFIB, IncY | N | 38,904 |
| YZ21HCE23\textsuperscript{b} | feces, F | 2021/5/16 | 49 | B1 | bla\textsubscript{CTX-M-55} | AMP/CTX/GEN/STR/NAL/CIP/FOC/SXT | F18:A-B1:C4, IncK | N | 65,116 |
| YZ21HCE24\textsuperscript{a} | feces, F | 2021/5/16 | 442 | B1 | bla\textsubscript{CTX-M-55} | AMP/CTX/STR | F16:A-B6 | N | 2,763 |
| YZ21HCE25 | feces, F | 2021/5/16 | 12744 | B1 | bla\textsubscript{CTX-M-14} | AMP/CTX/GEN/STR/TET/CHL/FFC/NAL/CIP/FOC/SXT | F55:A-B6, pO111 | gyrA(S83L+D87N), parC (S80I) | 3,320 |

\textsuperscript{a} * indicates that strain could successfully transfer bla\textsubscript{CTX-M/DHA\textsuperscript{+}} to E. coli C600 by conjugation; Underline indicates nasal and fecal samples obtained from the same individual, YZ21HCE7 and YZ21HCE10, YZ21HCE15 and YZ21HCE18, YZ21HCE17 and YZ21HCE19; Boldface indicates individuals from the same apartment.

\textsuperscript{b} F, female; M, male.

\textsuperscript{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.

\textsuperscript{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.
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<sub>CTX-M-14</sub>-positive contigs from isolates YZ21HCE14 and YZ21HCE23 displayed >99.9% similarity to IncK1 plasmids pD16EC0206-1 (E. coli, CP088610) and pXi1-2 (Klebsiella pneumoniae, CP0664254) (Figure S2). The 3,060-bp bla<sub>CTX-M-14</sub> transposition unit (ISEcp1-bla<sub>CTX-M-14</sub>-ΔIS903) with 5-bp DRs (5'-GGCGGA-3') was inserted downstream of the plasmid conjugal transfer gene traK (Figure 2B). In isolates YZ21HCE1, YZ21HCE4, and YZ21HCE25, a similar bla<sub>CTX-M-14</sub> transposition unit was observed, differed by deletions involving ISEcp1 and/or IS903. In YZ21HCE4, the 2,996-bp fragment (ISEcp1-bla<sub>CTX-M-14</sub>-ΔIS903) was embedded in a 21,609-bp contig associated with the chromosome of E. coli (CP061185). Furthermore, a 3,045-bp region (ISYZ21HCE2. The core structure IS<sub>903</sub> (5')-GCGGA-3') was inserted downstream of the plasmid conjugal transfer gene traK (Figure 2B). In isolates YZ21HCE10, YZ21HCE17, (214 bp) and YZ21HCE10 (201 bp). The bla<sub>CTX-M-64</sub> was located on the chromosome of YZ21HCE5 (bla<sub>CTX-M-64</sub>-IS903-iroN) transposition unit was found in isolates YZ21HCE5 (bla<sub>CTX-M-64</sub>-IS903-iroN), YZ21HCE17 (bla<sub>CTX-M-64</sub>-IS903-iroN), and YZ21HCE10 (bla<sub>CTX-M-64</sub>-IS903-iroN), although ISEcp1 was incomplete. In YZ21HCE17, the bla<sub>CTX-M-64</sub> unit was inserted in an incomplete Tn7122. However, IS903 was truncated by IS26 at the 3' end in YZ21HCE5, resulting in the deletions of iroN and 701 bp of IS903. Similarly, an incomplete IS903 (80 bp) was also observed in YZ21HCE10. A 1,205-bp structure consisting of two hypothetical IS elements plus ΔIS26 (76-bp) was located upstream of bla<sub>CTX-M-64</sub> transposition unit with a 64-bp spacer in YZ21HCE5. Remnants of this structure were also identified upstream of the bla<sub>CTX-M-64</sub> unit in YZ21HCE17 (214 bp) and YZ21HCE10 (201 bp).

The bla<sub>CTX-M-64</sub> was located on the chromosome of YZ21HCE12. The 160,049-bp bla<sub>CTX-M-64</sub>-carrying contig showed highly (>98.0%) similarity to the corresponding region of E. coli chromosome such as Z30 (CP066844) and LD671 (CP061185). Furthermore, a 3,045-bp region (ISEcp1-bla<sub>CTX-M-64</sub>-orf477-ΔTn2) 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<sub>CTX-M-55</sub>-positive contig (35,054 bp) in YZ21HCE3 was similar to IncI1 plasmid pBH8STW-00321_3 (E. coli, CP056606) with 86% coverage and 98.36% identity (Figure S2). The bla<sub>CTX-M-55</sub> gene was associated with genetic content ΔTn2-ΔISEcp1-bla<sub>CTX-M-55</sub>-orf477-ΔTn2, seen in several plasmids, such as pS908-2 (Shigella flexneri, CP045523) and p92 (E. coli, CP041521). The bla<sub>CTX-M-55</sub> resistance module was followed by a 5,752-bp structure qnrS1-ISKpn19-ΔIS26 (Figure 2E).

The bla<sub>DHA-1</sub>-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 Tn3 family transposon within psr<sup>C</sup> flanked by 5-bp DRs in YZ21HCE2. The core structure sul1-quadA1+mprr-bla<sub>DHA-1</sub>-pspDCBA-qnrB4 is commonly observed in numerous plasmids from various species (e.g., Salmonella, Klebsiella pneumoniae, Citrobacter freundii), highlighting the co-transfer ability of bla<sub>DHA-1</sub> and qnrB4.

### Discussion

The bla<sub>CTX-M</sub> gene has been globally disseminated in different sources, with bla<sub>CTX-M-14</sub> and bla<sub>CTX-M-15</sub> being dominant (Bevan et al., 2017). In this study, bla<sub>CTX-M-55</sub> is the most predominant genotype in healthy individuals, which agrees with the increasing prevalence of bla<sub>CTX-M</sub> in both animals and patients in China (Bevan et al., 2017). As a variant of bla<sub>CTX-M-15</sub>, bla<sub>CTX-M-55</sub> was first reported in clinical E. coli and Salmonella isolates in Thailand in 2007 (Kiratisin et al., 2007). Recently, bla<sub>CTX-M-55</sub> 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<sub>CTX-M</sub> variant bla<sub>CTX-M-252</sub> 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<sub>CTX-M-252</sub> is needed.

In communities, faecal carriage rates of bla<sub>CTX-M</sub> are increasing, particularly in developing countries (Woerther et al., 2013; Bevan et al., 2017). However, investigation of bla<sub>CTX-M</sub> 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<sub>CTX-M</sub> 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<sub>CTX-M</sub> is possibly common in humans. However, the small number of samples and communities is a limitation of this study. The acquisition of bla<sub>CTX-M-55</sub> 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<sub>CTX-M-55</sub>-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<sub>CTX-M</sub> dissemination, clonal spread of bla<sub>CTX-M</sub>-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<sub>CTX-M-55</sub>-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<sub>CTX-M</sub> transmission.

Horizontal transfer mediated by plasmids and mobile elements is responsible for the global spread of bla<sub>CTX-M</sub> (Bevan et al., 2017; Partridge et al., 2018). For example, IncI, IncFII, and IncHI2 plasmids facilitate the horizontal
transmission of \( \text{bla}_{\text{CTX-M}} \) in \( E. \text{coli} \) and \( \text{Salmonella} \) 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 \( \text{IncHI2}, \text{IncK1}, \text{IncX1}, \) and \( \text{IncI1} \) were associated with \( \text{bla}_{\text{CTX-M}} \). Although we were not able to determine the location of \( \text{bla}_{\text{CTX-M}} \) in some \( E. \text{coli} \) isolates in this study due to incomplete assembly, sequence analysis indicates that \( \text{ISEcp1} \) plays an important role in \( \text{bla}_{\text{CTX-M}} \) dissemination among \( E. \text{coli} \) isolates and facilitates the horizontal transfer of \( \text{bla}_{\text{CTX-M}} \) from plasmids to chromosomes in distinct integration sites. The chromosomal integration of \( \text{bla}_{\text{CTX-M}} \) is increasingly reported in \( E. \text{coli} \), \( K. \text{pneumoniae} \), \( \text{Salmonella} \), \( \text{Proteus mirabilis} \) and some other species of \( \text{Enterobacteriaceae} \) 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 \( \text{bla}_{\text{CTX-M}} \) 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 \( E. \text{coli} \) \( \text{ST8369} \) associated with \( \text{IncHI2} \) plasmid in healthy individuals in one community from Yangzhou, China. Therefore, continued surveillance of nasal carriage of \( \text{bla}_{\text{CTX-M}} \) 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.

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**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.

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**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

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