Community Fecal Carriage and Molecular Epidemiology of Extended-Spectrum \(\beta\)-Lactamase- and Carbapenemase-Producing \textit{Escherichia coli} from Healthy Children in the Central South China

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Background: Fecal carriage of extended-spectrum \(\beta\)-lactamase-producing \textit{Escherichia coli} (ESBL-EC) and carbapenemase-producing \textit{E. coli} (CP-EC) is well reported among hospitalized adults and children. However, there are few studies on the carriage prevalence and ESBL-EC and CP-EC genotypes among healthy children in China.

Patients and Methods: Stool samples were collected from 330 students in 2021 from three randomly selected primary schools in Changsha, China. ESBL-EC and CP-EC were screened using CHROMagar\textsuperscript{TM} chromogenic plates. ESBL and carbapenemase production was confirmed using the double-disc synergy test and a modified carbapenem inactivation method, respectively. Antimicrobial susceptibility was tested using the broth microdilution method. Resistance determinants, virulence factors, and phylogenetic groups were determined by PCR and sequencing. Multi-locus sequence typing (MLST) was performed (seven housekeeping genes were amplified and sequenced) on the phylogenic group B2 \textit{E. coli} to detect high-risk clonal strains such as ST131 \textit{E. coli}. Then, ST131 \textit{E. coli} were characterized based on ST131 clades, O-type, and \textit{fimH} alleles.

Results: In total, 118 (35.8%) ESBL-EC and 3 (0.9%) CP-EC were isolated. \textit{bla}_{\text{CTX-M}} was the most common genotype (27.1%), identified in all ESBL-EC, except one, which carried \textit{bla}_{\text{SHV-12}}. One isolate with \textit{mcr-1} was found amongst ESBL-EC, whereas all three CP-EC carried \textit{bla}_{\text{NDM-1}}. The predominant sequence type (ST) clones in group B2 were ST131 and ST1193. The prevalence of ST131 \textit{E. coli} was 9.9%, displaying serotypes O16 and O25b, \textit{fimH} alleles 30, 41, and 89, and ST131 clades A and C1-M27.

Conclusion: In this study, high carriage rate of ESBL-EC was found among healthy children, and the dominant ESBL was CTX-M-14. In addition, high-risk clones (ST131 and ST1193) were also detected. This emphasizes the importance of monitoring ESBL-EC in community settings.

Keywords: community children, ESBL, CPE, ST131, \textit{mcr-1}

Introduction

The wide dissemination of extended-spectrum \(\beta\)-lactamase-producing \textit{Escherichia coli} (ESBL-EC) throughout the world is a major problem that has resulted in increased global mortality, morbidity, and health-care expenses.\(^1\) The emergence of ESBL-EC is now no longer limited to the clinical setting, and the 15-year combined prevalence of ESBL-EC carriage has reached 16.5% globally.\(^2\) Although individuals carrying ESBL-EC in the intestine are usually asymptomatic, long-term colonization and high carrying rates of the microbe increase the risk of infection with multidrug-resistant (MDR) pathogenic bacteria.\(^3\) Studies have shown that fecal colonization with ESBL-EC is associated with infection in healthy individuals.\(^4\)
Disconcertingly, there is a certain amount of ESBL-EC in the community. The distribution of sequence types (STs) and ESBL genes has been reported to be very similar between community fecal isolates and clinical isolates.\(^5\) In addition, with the increasing use of carbapenems and polymyxins, carbapenemase-producing E. coli (CP-EC) and colistin-resistant E. coli have also been isolated from feces samples procured from the community.\(^6,7\) This indicated that community may be a repository of ESBL-genes, carbapenemase-genes and colistin-resistant genes in the future. Plasmid-mediated horizontal transfer of resistance genes and the emergence of dominant clones of E. coli can be among the reasons for the increased global incidence of MDR E. coli, such as plasmids carrying \(\text{bla}_{\text{CTX-M}}\) and the E. coli ST131 clone.\(^3\) CTX-M is currently the most common ESBLs worldwide, and the spread of \(\text{bla}_{\text{CTX-M}}\) is mainly driven by IncF plasmids, which enable resistance genes to spread widely in communities and hospitals.\(^8-10\) E. coli ST131, a high-risk clone, is a type of extraintestinal pathogenic E. coli (ExPEC) with a multidrug-resistant spectrum that often causes community- and hospital-acquired infections.\(^11\) E. coli ST131 largely belongs to phylogenetic group B2 and can be divided into clade A, clade B, and clade C based on its \(\text{fimH}\) allele and O-type.\(^11\) Furthermore, E. coli ST131 is usually related to the production of ESBLs, especially CTX-M-15, CTX-M-14, and CTX-M-27.\(^12\)

Over the past decade, researches have reported the prevalence of ESBL-producing bacteria carried in the feces of healthy children.\(^3,7\) Among different geographic regions, the separation rate is significantly heterogeneous, ranging from 0.1% to 59%.\(^7,13\) Although there have been several reports in China regarding the prevalence of ESBL-carrying Enterobacteriaceae in healthy populations, most of these were restricted to examination of adults. Data regarding ESBL-EC and CP-EC carriage among community children in China are limited. Therefore, this study aimed to assess the current fecal carriage of ESBL-EC and CP-EC in healthy children in China and provide detailed molecular data.

**Materials and Methods**

**Participants**

A cross-sectional survey was conducted on healthy children in primary schools in Changsha, Hunan Province, China, in 2021. The resident population of Hunan province is 66,444,864, and Changsha, the capital city of Hunan province, has a resident population of 1,047,900 (Data updated to May 2021). Taking the geographical center of Changsha City as the center, the city was divided into four regions according to east, west, south and north directions. Large-scale primary schools (with more than 2000 students) were included in the selection range, and then schools that were relatively geographically scattered were considered. Three schools were finally selected in Kaifu District, Yuelu District, and Changsha County, and the geographic locations were shown in Figure S1. A total of 422 students from these schools participated in this study. After obtaining parental consent, fresh feces were collected from the children and immediately sent to the laboratory. In addition, a questionnaire was filled that requested information on the child’s gender, age, class, history of diarrhea, antibiotic treatments or hospitalization, whether they had pets, and whether they had been in contact with patients with diarrhea. Healthy children were defined as students who had no symptoms of diarrhea, no history of antibiotics use within one month, and who were younger than 14 years. Ultimately, 330 students were included in the study for subsequent analysis. This study was approved by the Ethics Committee of the Xiangya Hospital of Central South University (reference number 202110445).

**Screening of Fecal ESBL-EC and CP-EC Isolates, Species Identification, and Antimicrobial Susceptibility**

Within 4 h of feces sampling, an aliquot of fecal matter was smeared on two types of CHROMagar\(^\text{TM}\) chromogenic plates (CHROMagar, Paris, France) for the selection of samples containing ESBL-producing and carbapenemase-producing Enterobacteriaceae isolates. Subsequently, E. coli were identified by MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, German). The double-disc synergy test and a modified carbapenem inactivation method were used to confirm the production of ESBL and carbapenemase, respectively, according to Clinical and Laboratory Standards Institute (CLSI) guidelines.\(^14\) The antimicrobial susceptibility was then determined using the broth microdilution method, including cefazolin, cefuroxime, ceftriaxone, ceftazidime, cefepime, cefoxitin, amikacin, gentamicin, piperacillin/tazobactam, meropenem, tigecycline, aztreonam, levofloxacin, nitrofurantoin, cefoperazone/sulbactam, colistin, minocycline,
ampicillin, ampicillin-sulbactam, and trimethoprim/sulfamethoxazole. The susceptibility of the strains to other antibiotics was determined according to the CLSI guidelines, while that to tigecycline and colistin was investigated by the Food and Drug Administration and EUCAST criteria, respectively. *E. coli* ATCC25922 was used as the quality control strain.

**Detection of Antibiotic Resistance Genes**

ESBL-encoding genes *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub> were screened using multiplex polymerase chain reaction (PCR) as previously described. Specific primers were used to divide the *bla*<sub>CTX-M</sub> genes into five phylogenetic groups, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. Except for *bla*<sub>CTX-M-15</sub> that was detected by PCR, other *bla*<sub>CTX-M</sub>-positive isolates were determined by sequencing. The sequencing results were analyzed in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and ResFinder4.1 (https://cge.cbs.dtu.dk/services/ResFinder/). The presence of plasmid-mediated quinolone resistance determinants (*qnrA*, *qnrB*, *qnrC*, *qnrS*, *aac (6')-Ib-cr*, and *qepA*) and the colistin resistance gene (*mcr-1*) were detected in all the isolates by PCR. For three CP-EC, PCR was used to detect the carbapenemase genes (*bla*<sub>NDM-1</sub>, *bla*<sub>KPC-2</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>OXA-48</sub>).

**Virulence Genotyping and Phylogenetic Group Detection**

The presence of 26 virulence genes was assessed by multiplex PCR, and the virulence score was calculated as described previously. To investigate the phylogeny of ESBL-EC, multiplex PCR was performed according to the protocols provided by Clermont et al, in which *E. coli* was divided into seven groups (A, B1, B2, C, D, E, and F).

**Multi-Locus Sequence Typing (MLST)**

MLST analysis was performed on the phylogenic group B2 *E. coli* to screen the high-risk clone ST131. The seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) of B2 *E. coli* isolates were amplified and sequenced. The sequencing results were analyzed on PUBMLST (https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef), and the ST type of each strain was obtained. Furthermore, the carriage of resistance and virulence genes in ST131 and non-ST131 strains were compared.

**Molecular Characterization of ST131**

The ST131 clade of all 12 ST131 isolates was screened by multiplex PCR as described by Subramanya et al. O-type and *fimH* alleles were investigated according to a previous study.

**Statistical Analysis**

Categorical data were generally analyzed using the chi-square test or Fisher’s exact test. P < 0.05 was considered statistically significant. All data analysis was performed using the SPSS software (version 23.0).

**Results**

**Prevalence and Antimicrobial Susceptibility**

A total of 330 students (173 males and 157 females) participated in the study belonging to the age group of 6 to 12 years (average 8.8 years). Of the 330 feces samples, 118 were positive for ESBL production and 3 for carbapenemase production. For the ESBL-EC isolates, 100% of the isolates were resistant to ampicillin, 99.2% to ceftriaxone, 95% to cefuroxime, 85.6% to cefazolin, 59.3% to trimethoprim/sulfamethoxazole, 40.7% to levofloxacin, 28.8% to aztreonam, 25.4% to ceftazidime, 17.8% to gentamicin, and 13.6% to ampicillin-sulbactam, while none were resistant to meropenem, tigecycline, nitrofurantoin, pipercillin-tazobactam, or cefoperazone/sulbactam. For CP-EC, all of the isolates were susceptible to amikacin, tigecycline, colistin, and minocycline (Table 1).

**Molecular Analysis of the Antibiotic Resistance Genes**

The *bla*<sub>CTX-M</sub> gene was found in all of the ESBL-EC strains except for one isolate carrying *bla*<sub>SHV-12</sub>, while *bla*<sub>TEM</sub> was found in 45.8% of the strains. The PCR and sequencing analysis revealed that the most common *bla*<sub>CTX-M</sub> subtype was
blaCTX-M-14 (27.1%), followed by blaCTX-M-15 (24.6%), blaCTX-M-27 (24.6%), blaCTX-M-65 (8.5%), blaCTX-M-213 (4.2%), blaCTX-M-55 (4.2%), blaCTX-M-110 (1.7%), and blaCTX-M-24 (0.8%). Additionally, four strains co-harboring multiple blaCTX-M genes were found, two of which carried blaCTX-M-15 and blaCTX-M-27, one carried blaCTX-M-14 and blaCTX-M-15, and one carried blaCTX-M-15 and blaCTX-M-213. Three types of the plasmid-mediated quinolone resistance (PMQR) genes were found: qnrS (22.0%), aac (6')-Ib (3.4%), and qnrB (1.7%). Notably, one ESBL-EC isolate carried mcr-1 and all the three CP-EC isolates carried blaNDM-1. The detailed results are shown in Table 2.

**Virulence Genotyping and Phylogenetic Group**

For ESBL-EC (n=118), the most frequent virulence genes in their genome were fimH (88.1%), fyuA (72.9%), traT (65.3%), kpsMTII (61.0%), and intA (54.2%). Other virulence genes, such as papG allele I, gafD, cdtB, and cnf1, were not present. The median virulence score was 4.2 (ranging from 0 to 15) and approximately 52.5% of the isolates were ExPEC (Table 3). For CP-EC (n=3), the most frequent virulence genes observed were fimH (66.7%), kpsMTII (66.7%), and traT (66.7%), and two of the isolates were ExPEC (Table S1). All isolates were classified into seven phylogenetic groups (A, B1, B2, C, D, E, and F). The results revealed that the majority of isolates belonged to group B2 (33.1%), followed by groups D (18.1%), B1 (12.4%), F (9.9%), A (9.1%), E (6.6%), and C (2.5%). However, there were ten isolates that could not be classified into any group (Table S2).

**MLST**

The strains in the *E. coli* B2 group were classified into 13 different STs. ST131 (30.0%) was the most common ST, followed by ST1193 (27.5%), ST95 (10.0%), ST73 (7.5%), and ST2372 (5.0%). In addition, ST14, ST3483, ST4888, ST4508, ST1163, ST493, ST589, and ST92 each accounted for 2.5% (Table S3).
As indicated in Table S4, 12 (9.9%) of the 121 E. coli strains belonged to the ST131 lineage, including seven O16-ST131 and five O25b-ST131 isolates. The fimH alleles of all O16-ST131 isolates were fimH41, and the fimH alleles of O25b-ST131 isolates were fimH30 (n=4) and fimH89 (n=1). Among the 12 ST131 isolates, clade A (n=8) was the most common subclade, followed by clade C (n=4) which were C1-M27 subclades. All 12 ST131 isolates were found to harbor ESBL genes, including blaCTX-M-27, blaCTX-M-14, and blaCTX-M-15. The ST131 isolates had a significantly higher prevalence rate of blaCTX-M-27 compared with that of non-ST131 isolates (P < 0.05) (Table 2). In general, the virulence scores of the ST131 isolates were higher than those of the non-ST131 isolates (P < 0.05). In terms of the prevalence of virulence genes, kpsMII, traT, iutA, fyuA, and PAI appeared more frequently in the ST131 isolates (P < 0.05) (Table 3).

### Discussion

This study assessed the fecal carriage of ESBL-EC and CP-EC and their molecular characteristics in healthy children in China and showed a high rate of ESBL-EC carriage and predominance of the clone ST131 in the B2 group of E. coli. The correlation between infections caused by ESBL-producing bacteria and their colonization in feces has been

#### Table 2 Prevalence of Resistant Genes Among 118 ESBL-EC from Healthy Children

| Resistant Genes | No. (%) of Strains | P-value<sup>a</sup> |
|-----------------|--------------------|---------------------|
| **ESBL-encoding genes** |                   |                     |
| CTX-M-1 group   |                    |                     |
| bla<sub>CTX-M-15</sub> | 29 (24.6) | 1 (8.3) | 28 (26.4) | 0.289 |
| bla<sub>CTX-M-55</sub> | 5 (4.2) | 0 (0) | 5 (4.7) | 1.000 |
| CTX-M-9 group   |                    |                     |
| bla<sub>CTX-M-14</sub> | 32 (27.1) | 1 (8.3) | 31 (29.2) | 0.176 |
| bla<sub>CTX-M-27</sub> | 29 (24.6) | 10 (83.3) | 19 (17.9) | 0.001 |
| bla<sub>CTX-M-45</sub> | 10 (8.5) | 0 (0) | 10 (9.4) | 1.000 |
| bla<sub>CTX-M-110</sub> | 2 (1.7) | 0 (0) | 2 (1.9) | 1.000 |
| bla<sub>CTX-M-213</sub> | 5 (4.2) | 0 (0) | 5 (4.7) | 1.000 |
| bla<sub>CTX-M-24</sub> | 1 (0.8) | 0 (0) | 1 (0.9) | 1.000 |
| CTX-M-2 group   | 0 (0) | 0 (0) | 0 (0) | NA |
| CTX-M-8 group   | 0 (0) | 0 (0) | 0 (0) | NA |
| CTX-M-25 group  | 0 (0) | 0 (0) | 0 (0) | NA |
| CTX-M-11/CTX-M-9 group | 1 (0.8) | 0 (0) | 1 (0.9) | 1.000 |
| bla<sub>CTX-M-14</sub>+bla<sub>CTX-M-15</sub> | 1 (0.8) | 0 (0) | 1 (0.9) | 1.000 |
| bla<sub>CTX-M-15</sub>+bla<sub>CTX-M-213</sub> | 2 (1.7) | 0 (0) | 2 (1.9) | 1.000 |
| bla<sub>CTX-M-15</sub>+bla<sub>CTX-M-27</sub> | 5 (4.2) | 0 (0) | 5 (4.7) | 1.000 |
| SHV             | 1 (0.8) | 0 (0) | 1 (0.9) | 1.000 |
| TEM             | 54 (45.8) | 7 (58.3) | 47 (44.3) | 0.379 |
| **Fluoroquinolones-resistance genes** |                   |                     |
| qnrA            | 0 (0) | 0 (0) | 0 (0) | 1.000 |
| qnrB            | 2 (1.7) | 0 (0) | 2 (1.9) | 1.000 |
| qnrC            | 0 (0) | 0 (0) | 0 (0) | 1.000 |
| qnrS            | 26 (22.0) | 0 (0) | 26 (24.5) | 0.353 |
| aac (6')-Ib     | 4 (3.4) | 0 (0) | 4 (3.8) | 1.000 |
| qepA            | 0 (0) | 0 (0) | 0 (0) | 1.000 |
| **Colistin-resistance gene** |                   |                     |
| mcr-1           | 1 (0.8) | 0 (0) | 1 (0.9) | 1.000 |

<sup>a</sup>P-value < 0.05 are bolded.

**Abbreviations:** NA, not available; bla, beta-lactamase; ESBL, extended-spectrum beta-lactamase.
The emergence of resistant bacteria in intestinal commensal flora is a serious threat, as the intestine is an important site for the transmission of bacterial resistance, which may consequently lead to an increased risk of community- or hospital-acquired resistant infections.

Considering that the antimicrobial use for children differs from that for adults and that studies on fecal carriage of resistant bacteria in children have been mainly focused in a clinical setting, there is a need to monitor the prevalence of resistant bacteria in children in the community. To the best of our knowledge, this is the first systematic study focusing on the fecal carriage of ESBL-EC and CP-EC in healthy children from a community in China.

The carriage of ESBL-EC in feces was 35.8% in this study, which is higher than that in a study based in Taiwan (5.1%), in which the study population comprised of community children that were hospitalized within three days.

### Table 3 Prevalence of Virulence Traits Among 118 ESBL-EC from Healthy Children

| Virulence Genes          | No. (%) of Strains | ST131 (n=12) | Non-ST131 (n=106) | P-value* |
|--------------------------|--------------------|--------------|-------------------|---------|
| Adhesin-associated       |                    |              |                   |         |
|  popAH                   | 14 (11.8)          | 1 (8.3)      | 13 (12.3)         | 1.000   |
|  popEF                   | 23 (19.5)          | 2 (16.6)     | 21 (19.8)         | 1.000   |
|  popC                    | 21 (17.8)          | 2 (16.6)     | 19 (17.9)         | 1.000   |
|  popG allele I           | 0 (0)              | 0 (0)        | 0 (0)             | NA      |
|  popGIII/III             | 5 (4.2)            | 1 (8.3)      | 4 (3.8)           | 0.421   |
|  popG allele II          | 14 (11.9)          | 2 (16.6)     | 12 (11.3)         | 0.634   |
|  sfa/focDE               | 7 (5.9)            | 0 (0)        | 7 (6.6)           | 1.000   |
|  afa/draBC               | 11 (9.3)           | 1 (8.3)      | 10 (9.4)          | 1.000   |
|  sfaS                    | 3 (2.5)            | 0 (0)        | 3 (2.8)           | 1.000   |
|  focG                    | 3 (2.5)            | 0 (0)        | 3 (2.8)           | 1.000   |
|  fimH                    | 104 (88.1)         | 12 (100.0)   | 92 (86.8)         | 0.356   |
|  nfaE                    | 2 (1.7)            | 0 (0)        | 2 (1.9)           | 1.000   |
|  gfD                     | 0 (0)              | 0 (0)        | 0 (0)             | NA      |
| Capsule-associated       |                    |              |                   |         |
|  kpsMTII                 | 72 (61.0)          | 11 (91.7)    | 61 (57.5)         | 0.027   |
|  kpsMTIII                | 2 (1.7)            | 0 (0)        | 2 (1.9)           | 1.000   |
|  kpsMT K5                | 33 (27.9)          | 6 (50.0)     | 27 (25.4)         | 0.092   |
|  kpsMT K1                | 34 (28.8)          | 3 (25.0)     | 31 (29.2)         | 1.000   |
| Toxin-associated         |                    |              |                   |         |
|  cdtB                    | 0 (0)              | 0 (0)        | 0 (0)             | NA      |
|  cnfI                    | 0 (0)              | 0 (0)        | 2 (1.9)           | NA      |
|  hlyA                    | 4 (3.4)            | 1 (8.3)      | 3 (2.8)           | 0.353   |
| Siderophore-associated   |                    |              |                   |         |
|  iutA                    | 64 (54.2)          | 10 (83.3)    | 54 (50.9)         | 0.037   |
|  fyuA                    | 86 (72.9)          | 12 (100.0)   | 74 (70.0)         | 0.035   |
| Others                   |                    |              |                   |         |
|  cvaC                    | 10 (8.5)           | 0 (0)        | 10 (9.4)          | 0.596   |
|  PAI                     | 45 (38.1)          | 11 (91.7)    | 34 (32.1)         | ≤0.001  |
|  traT                    | 77 (65.3)          | 12 (100.0)   | 65 (61.3)         | 0.008   |
|  rfc                     | 2 (1.7)            | 1 (8.3)      | 1 (0.9)           | 0.194   |
| Virulence score†         | 4.2 (0–15)         | 7 (5–11)     | 5 (0–15)          | 0.003   |
| ExPEC                    | 62 (52.5)          | 9 (75.0)     | 53 (50.0)         | 0.132   |

Notes: *P-value < 0.05 are bolded; †median (range); ExPEC, extraintestinal pathogenic E. coli; E. coli containing two of these five virulence genes (popAH/papC, sfa/focDC, afa/draBC, iutA, kpsMT II) is defined as ExPEC.

Abbreviations: NA, not available; ExPEC, extraintestinal pathogenic E. coli.
The prevalence of In our study, two ESBL-EC isolates showing colistin-resistance This **bla** Furthermore, in the present study, we observed a significantly higher prevalence rate in hospitalized patients was almost twice as high as in healthy individuals. A systematic review which may be partly due to the differences in sanitary conditions, climate, and eating habits in different regions of the world. Although the carriage rate of ESBL-EC in this study was relatively high compared to that observed in the above studies, it was still lower than that in pediatric patients in China, which has been reported to be 46.7%. Moreover, Babu et al reported that fecal carriage of ESBL-producing *Enterobacteriaceae* in hospitalized patients was almost twice as high as in healthy individuals. In addition, the carriage of CP-EC in the present study was 0.9%, which is lower than that of outpatient (1.4%) and inpatient children (1.9%) in Shanghai, China. Although few countries have reported CP-EC carriage among community healthy children to date, a report from Pakistan showed that a quarter of infants in their research harbored CP-EC, indicating that the intestine of healthy children may be a reservoir of CP-EC and should be considered as a serious health issue.

The global distribution of CTX-M variants showed that the CTX-M-1 group (especially CTX-M-15) was the dominant genotype in most regions, while the CTX-M-9 group (especially CTX-M-14) was dominant in China. In consistent with the above report, CTX-M-14 was the predominant CTX-M subtype in this study. The prevalence of CTX-M-27 was 24.6%, higher than the results of our previous study of healthy adults in 2015 (15.0%). Such an increase has also been seen in France, where the proportion of CTX-M-27 increased from 4.5% to 25% between 2010 and 2015 among the 1886 children screened. Furthermore, in the present study, we observed a significantly higher prevalence rate of *bla*CTX-M-27 among ST131 isolates than that in non-ST131 samples. In line with this, Birgy et al also reported a high proportion of CTX-M-27 in ST131 isolates. Therefore, ST131 isolates may play a role in the transmission of *bla*CTX-M-27.

Like the resistance pattern of ESBL-EC from pediatric patients, ESBL-EC isolates from healthy children were highly resistant to ampicillin, ceftriaxone, and cefazolin, and were susceptible to meropenem, colistin, and tigecycline. This underscores the importance of investigating resistant strains in human fecal matter, as clinical strains may originate from the intestinal tract. Fluoroquinolones are first-line antimicrobial agents for *E. coli* infections but they are not recommended for children. Surprisingly, the isolates in this study showed up to 40.7% resistance to levofloxacin, and the resistance to ciprofloxacin reported in pediatrics was also as high as 60%. The reason for this high resistance rate may be related to the PMQR genes, which can undergo horizontal transfer between strains. PMQR genes were also detected in 27.1% of the ESCL-EC strains in this study. However, the mechanisms of the high resistance to fluoroquinolones in children remain to be explored further.

Infections due to carbapenem-resistant *Enterobacteriaceae* (CRE) in children are often associated with adverse clinical outcomes. Disconcertingly, we found three *E. coli* strains carrying *bla*NDM-1 gene. In 2016, a study conducted in eastern China also reported that NDM-1 was the major carbapenemase produced by ESBL-EC isolated from the feces of outpatient children. NDM-1 is a metal-β-lactamase (MBL) and cannot be inhibited by β-lactamase inhibitors, which significantly limits the utility of β-lactam-β-lactamase inhibitors, such as ceftazidime-avibactam. A systematic review showed that *bla*NDM-1 was the most common carbapenem-resistant genotype causing neonatal sepsis in China. Therefore, given the complexity of treating CRE (especially MBL-induced CRE) infections, it is necessary to monitor the prevalence of such resistant bacteria among commensal bacteria.

In addition, a lot of focus has recently been drawn to the emergence of *mcr-1*. A high prevalence of *mcr-1* in healthy children has been reported in Bolivia (38.8%) and Lebanon (33.3%). However, the carriage of *mcr-1* in the intestine of healthy children remains understudied in China. Only Hu et al reported a carriage rate of 9.6% for *mcr-1* among hospitalized children who did not suffer from diarrhea. In our study, two ESBL-EC isolates showing colistin-resistance were identified, one of which co-harborred both *mcr-1* and *bla*CTX-M-14 and was isolated from a child with pets, including dogs and pet mice. This was consistent with a report from Guangzhou, China, suggesting that animals may be the source of presence of *mcr-1* in humans. Colistin resistance in another isolate may be mediated by other subtypes of the *mcr* gene or other resistance mechanisms, which needs further investigation.
Pathogenic *E. coli* strains are usually assigned to the B2 phylogenetic group, which accounted for the main proportion (33.1%) in our research, in which ST131 *E. coli* was the predominant clone. ST131 ESBL-EC is commonly found in community-acquired infections, and ST131 carriers can transmit it within the families, highlighting the potential health threat from ST131 carriers. Even though in this research, it was found that the detected ST131 ESBL-EC carried several virulence genes (*kpsMTII*, *traT*, *iutA*, *fyuA*, and *PAI*), the carrier did not show infection symptoms. ESBL and virulence genes (*fyuA*) were the risk factors of *E. coli* infection. However, the occurrence of infection is also host-dependent. Therefore, infection by the virulent ST131 *E. coli* does not necessarily occur when the body is immune-competent and the intestinal barrier is intact. Ferjani et al reported that *E. coli* carried virulence genes and resistance genes in the feces of healthy children. 

O25b has traditionally been considered the predominant serotype of ST131, but the prevalence of *E. coli* O16-ST131 has increased significantly in recent years. In our study, the detection rate of O16-ST131 was higher than that of O25b-ST131, which was consistent with our past results of a study on healthy adults in Changsha. The rise of O16-ST131 may be related to the improvement of novel PCR detection methods. Clade A ST131 *E. coli* predominated (66.6%) in our study and the others belonged to clade C1-M27 (33.3%); moreover, in another multi-center study, it was observed that all clade A ST131 *E. coli* were associated with the community infections. This finding implies clade A may become a major clade of ST131 in the community in China. However, a similar study in France reported that the predominant ST131 clade was clade C (74%), followed by clade A (26%). This may be due to the geographical differences between the two regions. Additionally, C1-M27 is a novel subclade of clade C carrying CTX-M-27, which has a higher degree of dissemination in the hospital setting. Currently, clade C1-M27 has emerged and become prominent in many countries, such as Japan and Canada, and the present study is the first to show the presence of clade C1-M27 in China. More studies are needed to further monitor the prevalence of this clade.

Notably, ST1193 accounted for a high proportion of the B2 group. *E. coli* ST1193 was first described in 2012, and its incidence has risen dramatically in recent years. Birgy et al reported a significant increase (from zero to 9.3%) in the detection of *E. coli* ST1193 among children with febrile urinary tract infections in France from 2014 to 2017. Ding et al found that *E. coli* ST1193 accounted for 21.4% of *E. coli* clinical isolates that cause neonatal invasive infections in the population of China. Meanwhile, clones related to urinary tract infection were also detected, such as ST95 and ST73. The current findings suggest that the specific global risk clones are lurking in humans, even in non-clinical settings, which also poses a potential but not negligible risk to public health.

The study has several limitations. First, although we chose three schools from different urban areas to represent the characteristics of the Changsha region, inevitably, the representativeness of our study population remains limited. In addition, there was one colistin-resistant isolate in need of further research to explore its resistance mechanism.

**Conclusion**

In conclusion, high fecal carriage and significant CTX-M genetic diversity of ESBL-EC were detected in children from Changsha, China. The emergence of the *mcr-1* gene, *bla*NDM-1* gene, and ST131 *E. coli* (especially C1-M27) among community children should not be taken lightly.

**Abbreviations**

ESBL-EC, extended-spectrum β-lactamase-producing *Escherichia coli*; CP-EC, carbapenemase-producing *E. coli*; MLST, multi-locus sequence typing; ST, sequence type; *bla*, beta-lactamase; MDR, multidrug-resistant; ExPEC, extraintestinal pathogenic *E. coli*; PCR, polymerase chain reaction; CRE, carbapenem-resistant Enterobacteriaceae; MBL, metal-β-lactamase; R, resistant; I, intermediate; S, susceptible; CZO, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; AMK, amikacin; GEN, gentamicin; TZP, piperacillin/tazobactam; MEM, meropenem; TGC, tigecycline; ATM, aztreonam; AMP, ampicillin; SAM, ampicillin-sulbactam; COL, colistin; MNO, minocycline; SXT, trimethoprim/sulfamethoxazole; LVX, levofloxacin; NIT, nitrofurantoin; CSL, cefoperazone/sulbactam.
**Ethics Statement**

All procedures of this study involving humans (individuals, human samples, isolates) were reviewed and approved by Ethics Committee of the Xiangya Hospital of Central South University (reference number 202110445). This study was conducted in accordance with the Declaration of Helsinki. The parents or legal guardians of the children have been informed of the purpose of this study.

**Consent for Publication**

All authors confirm that the details of any images/recordings can be published.

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**Disclosure**

The authors report no conflicts of interest in this work.

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