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# Characteristics and spatiotemporal changes in phenotypes and genotypes of extended-spectrum β-lactamases in *Escherichia coli* isolated from bloodstream infections in China from 2014 to 2021

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## Abstract

**Objective** To examine the characteristics and spatiotemporal changes in the phenotypes and genotypes of extended-spectrum β-lactamases (ESBLs) in *Escherichia coli* strains isolated from bloodstream infections (BSIs) across China between 2014 and 2021.

**Methods** 983 ESBL-positive *E. coli* strains were collected from BSIs in 66 hospitals across different geographic regions in China from 2014 to 2021. The phenotypic confirmation of ESBL was performed through the double-disc diffusion method. The genetic type was determined using polymerase chain reaction (PCR) followed by DNA sequencing.

**Results** Between 2014 and 2021, the prevalence of ESBL-positive *E. coli* steadily decreased from 61.2 to 49.6%. Among 983 phenotypically confirmed ESBL-positive *E. coli*, 763 (77.6%) were confirmed to carry ESBL genes, with the majority being of the CTX-M type, which is further divided into 23 subtypes and dominated by the CTX-M-9 and CTX-M-1 groups, with 457/763 and 333/763, respectively. Other ESBLs and *ampC* genes, such as $bla_{OXA-1}$, $bla_{CMY}$, and $bla_{DHA-1}$, often coexisted with either the CTX-M-9 or CTX-M-1 groups. $bla_{CTX-M-14}$ (34.3%, 157/457) and $bla_{CTX-M-55}$ (45.9%, 153/333) were the dominant subtypes in the CTX-M-9 and CTX-M-1 groups, respectively. A notable increase in $bla_{CTX-M-27}$ was observed, particularly from 2019 to 2021, with 26.4%, 23.1%, and 25.8% in all genotypes. Regarding the geographical distribution of the ESBLs, the highest rate of ESBL genetic positivity was observed in Southwest China, accounting for 84.9% (45/53), and the lowest was observed in Northeast China, with 73.2% (30/41). The abundance of the $bla_{CTX-M-27}$ genotype, in particular, exhibited a notable increase in Southwest China, with 31.4% (14/45) of the strains exhibiting this genotype, followed by the CTX-M-55 genotype, with 13.6% (6/45) of the strains exhibiting this genotype.

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**Conclusions** This study demonstrated a steadily decreasing trend in the incidence of ESBLs and predominant CTX-M type ESBLs, particularly the CTX-M-9 and CTX-M-1 groups, in *E. coli* strains across China, a notable increase in the $bla_{CTX-M-27}$ genotype and regional variations in the ESBL gene distribution were detected.

**Keywords** ESBLs, CTX-M, *E. coli*, Bloodstream infection, China

## Background

*Escherichia coli* is one of the most pathogenic bacteria that causes fatal infections [1]. *E. coli* is frequently identified as the primary causative agent of bloodstream infections (BSIs). It constitutes about 27% of the total cases of bacteremia in adult populations globally, with some studies reporting a prevalence reaching 57% [2]. Various primary infections caused by *E. coli* can lead to bacteremia or sepsis, such as surgical site infections, ventilator-associated pneumonia, abdominal and pelvic infections, and urinary tract infections [2, 3]. BSIs significantly contribute to illness and death on a global scale [4]. Around 28% of hospitalized patients in intensive care units get bacteremia or sepsis, while 18% develop bloodstream infections during their hospital stay [5]. BSIs are particularly exacerbated by the increasing prevalence of antibiotic resistance. BSI-attributable deaths could account for most of the AMR disease burden [6].

The clinically substantial prevalence of resistance to multiple antibiotic classes is one of the primary concerns regarding pathogenic *E. coli*. This includes the emergence and epidemiology of multidrug and extended-spectrum β-lactam-resistant *E. coli*, which poses a significant therapeutic challenge and can worsen the outcome of infected patients [7]. A decade ago, the percentage of ESBL-positive *E. coli* in mainland China was 68.9% in 2004–2005, 73.2% in 2007–2008, 67.9% in 2009–2010, 72.6% in 2011–2012, and 58.4% in 2013–2014 [8]. ESBL-producing *E. coli* was responsible for 38.0% of community-acquired urinary tract infections (CA-UTIs) in many regions of China in 2016–2017; this trend was also evident in 2015 in the southeastern region, where it caused 40.9% of BSIs of *E. coli* [9, 10].

This study examined the phenotypic and genotypic characteristics of ESBL-positive *E. coli* from BSIs across six geographic regions in China over eight years. By analyzing spatiotemporal patterns, we can identify regions where ESBL resistance is particularly prevalent or increasing, enabling the development of targeted and timely interventions. Such data provides actionable insights that guide the creation of tailored strategies, supporting efforts to curb the spread of resistant *E. coli* strains and reduce the AMR burden. The goal of this study was to analyze spatiotemporal changes in the phenotypic and genotypic profiles of ESBL-positive *E. coli* from BSIs across China, thereby providing insights to guide the development of effective strategies to address the growing challenge of ESBL resistance in *E. coli*-related BSIs.

## Methods

### Collection of clinical isolates

The Blood Bacterial Resistant Investigation Collaborative System (BRICS) is a national surveillance network in China that primarily identifies pathogens and monitors drug resistance in bloodstream infections (BSIs). Annually, BRICS collect bacterial isolates of BSIs from participating hospitals across various provinces, autonomous regions, and municipalities in China. The microbiologists of the participating hospitals conducted the isolation of bacterial pathogens from blood and cerebrospinal fluid (CSF), using standardized microbiological methods. These isolates were then shipped to the central BRICS laboratory, where the pathogens were reconfirmed using the Vitek 2 system (bioMérieux, Marcy-l'E' toile, France), and MALDI-TOF MS (Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany).

In this study, 100–150 phenotypically-confirmed ESBL-positive *E. coli* strains were randomly selected from the BRICS Central Laboratory Biobank yearly from 2014 to 2021; these strains were collected from all participant hospitals, and a total of 983 E. *coli* strains were selected for analysis of the genetic types of the ESBLs. As a part of the BRICS working group, our access to these strains does not require special permissions due to our central role in the network's operations.

### Antibacterial susceptibility test and ESBL phenotypic confirmation

The susceptibility of *E. coli* to various antimicrobial agents was determined using the methods recommended by the Clinical and Laboratory Standards Institute (CLSI) [11]. Antimicrobials tested included amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoperazone/sulbactam, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, moxalactam, aztreonam, ertapenem, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, fosfomycin, polymyxin B, and tigecycline. Broth microdilution was used for tigecycline and polymyxin B, while the agar dilution method was utilized to determine the minimum inhibitory concentration (MIC) of the remaining antibiotics. The confirmation of ESBL producers was carried out through a double disc diffusion method with cefotaxime and ceftazidime

(30 μg), both alone and in combination with clavulanic acid (10 μg) (Oxide Limited, UK), following the CLSI guidelines [11]. *E. coli* ATCC25922 and *Klebsiella pneumoniae* ATCC 700,603 were used as quality control organisms for antimicrobial susceptibility testing.

### Detection of ESBL genes by polymerase chain reaction and DNA sequencing

DNA was extracted from each isolate using a Steadypure universal genomic DNA extraction kit (AG21010) according to the manufacturer's protocol. PCR amplification was performed with a 50 μL reaction mixture under the following conditions: initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 55–60 °C for 30 s, and extension at 72 °C for 45 s. A final extension was conducted at 72 °C for 5 min. Polymerase chain reaction (PCR) was performed to detect ESBL genes ($bla_{CTX-M-1}$ group: $bla_{CTX-M-2}$ group, $bla_{CTX-M-8}$ group, $bla_{CTX-M-9}$ group, $bla_{TEM}$, $bla_{SHV}$, $bla_{KPC}$, $bla_{GES}$, $bla_{PER}$, $bla_{VEB,}$*ampC* ($bla_{ACC}$, $bla_{DHA}$, $bla_{FOX}$, $bla_{MOX}$, and $bla_{CIT,}$$bla_{CMY1}$, $bla_{CMY2}$, OXA: $bla_{OXA-1,}$$bla_{OXA-2}$$bla_{OXA-10}$, MOX: $bla_{MOX-1}$,$bla_{MOX-2}$, $bla_{CMY-}$1,$bla_{CMY-8}$ to $bla_{CMY-11}$, CIT: $bla_{LAT-1}$ to $bla_{LAT-4}$, $bla_{CMY-2}$ to $bla_{CMY-7}$, $bla_{BIL-1}$, FOX: $bla_{FOX-1}$ to $bla_{FOX-5B}$, EBC: $bla_{MIR-1T}$, $bla_{ACT-1}$, $bla_{BEL}$, $bla_{BES}$, $bla_{TLA}$, $bla_{SFO}$, $bla_{oxy}$) using ExTaq DNA polymerase (Takara, Dalian, China) and primers (Supplementary Table S1). PCR products were then visualized via electrophoresis, a 1% agarose gel to identify the amplified DNA fragments. Forward and reverse sequencing reactions were performed on an ABI 3730 automated sequencer using ABI Prism BigDye Terminator version 3.1 cycle sequencing (Applied Biosystems, Foster City, CA, USA). Positive PCR products were purified and directly sequenced from both ends or cloned in pMD18-T and then sequenced. The DNA sequences and deduced amino acid sequences were compared via BLAST, with sequences available at GenBank and with sequences at the Lahey Clinic (available at http://www.lahey.org/studies/webt.html) to identify the subtypes of β-lactamase genes.

### Statistical analysis

Statistical analysis was performed using IBM SPPS (version 27.0.1.0). The statistical significance was determined by the chi-square test. Statistical analyses were conducted to assess the occurrence and distribution of ESBL genes in *E. coli* strains over time and to compare the occurrence rates in various geographical regions. Significance was assumed at a $p$ value < 0.05 at the 95% confidence level.

## Results

### *E. coli* resistance surveillance results

From 2014 to 2021, BRICS collected 48,955 strains in total, including gram-positive (13690/48955, 28.0%) and gram-negative bacteria (35265/48955, 72.0%). *E. coli* accounted for 36.6% (17963) of the gram-negative isolates, whereas the percentage of ESBL-positive *E. coli* strains was 51.4% (9238/17963). All the *E. coli* strains exhibited a consistent pattern of high resistance to amoxicillin and cephalosporins but were susceptible to tigecycline, moxalactam, fosfomycin, imipenem, meropenem, amikacin, polymyxin B, and tigecycline. Significantly, a decreasing trend in resistance levels was observed over the study period for most antibiotics, including cefoxitin (from 19.5 to 9.2%), aztreonam (from 42.2 to 30.2%), amoxicillin/clavulanic acid (from 26.9 to 19.5%), piperacillin/tazobactam (from 12.3 to 7%), gentamicin (44–35.3%), ciprofloxacin (65.7–58.1%), trimethoprim/sulfamethoxazole (60.8–52.4%) and cephalosporins (Table 1).

### Antimicrobial susceptibility of ESBL-positive *E. coli* strains

The trend of ESBL-positive *E. coli* decreased consistently from 2014 to 2021, ranging from 61.2% (412/673) in 2014 to 49.6% (2054/4141) in 2021. The susceptibility profiles of all the ESBL-positive *E. coli* strains demonstrated that imipenem, meropenem, and tigecycline were highly effective, exhibiting sensitivity rates above 90%. Amikacin, polymixin B, fosfomycin, and moxalactam were also active against ESBL-positive *E. coli*. Levofloxacin had low effectiveness, with a resistance rate of 70%, whereas resistance against trimethoprim/sulfamethoxazole was greater than 60%. Similarly, the susceptibility to ciprofloxacin declined from 17 to 15%, reaching a minimum rate of 8% in 2016. On the other hand, the sensitivity rates for gentamicin exhibited a constant increase from 46 to 57% throughout the study (Supplementary Table S2). The susceptibility data of the ESBL-positive *E. coli* strains that were selected for genotypic studies, specifically those carrying major ESBL genotypes, were also found to be susceptible to tigecycline, fosfomycin, ertapenem, meropenem and imipenem but resistant to gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole and, unexpectedly, moxalactam (Table 2). Surprisingly, the CTX-M-1 group carrying strains showed a high rate of resistance (80%) to aztreonam, whereas the CTX-M-9 group carrying strains showed varying resistance rates, with an average of 45%. Moreover, compared with the other genotype-carrying strains, the $bla_{CTX-M-27}$-carrying strains were more susceptible to cefoperazone/sulbactam and gentamicin.

**Table 1** Yearly susceptibility and resistance rates of ESBL-positive *E. coli* isolates to antimicrobial agents from 2014 to 2021. This table provides a trend analysis of how resistance levels among ESBL-positive *E. coli* isolates changed annually across different antimicrobial agents

| **Antibiotics** | ***E coli* (*N* = 17963)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2014 (*N* = 673)** | | **2015 (*N* = 947)** | | **2016 (*N* = 1490)** | | **2017 (*N* = 1339)** | | **2018 (*N* = 1812)** | | **2019 (*N* = 3683)** | | **2020 (*N* = 3878)** | | **2021 (*N* = 4141)** | |
| | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** | ***R*%** | **S%** |
| ESBL (+) rate | 61.2 - | | 55.6 - | | 55.9 - | | 55.4 - | | 50.4 - | | 51.0 - | | 48.4 - | | 49.6 - | |
| Amoxicillin | 93.2 | 5.1 | 83.4 | 15.8 | 84.9 | 14.4 | 85.6 | 13.9 | 84.6 | 14.5 | 83.4 | 15.0 | 83.3 | 14.9 | 83.1 | 15 1 |
| Amoxicillin/clavulanic acid | 26.9 | 40.6 | 14.6 | 60.7 | 18.0 | 56.4 | 19.7 | 51.5 | 24.2 | 48.7 | 44.0 | 38.1 | 19.5 | 44.5 | 19.5 | 49.2 |
| Piperacillin/tazobactam | 12.3 | 78.0 | 8.1 | 88.3 | 6.7 | 89.1 | 6.2 | 89.0 | 5.6 | 82.5 | 8.1 | 83.0 | 7.1 | 89.2 | 7 | 89.9 |
| Cefoperazone/sulbactam | 15.3 | 65.5 | 9.3 | 76.5 | 8.0 | 79.5 | 8.7 | 80.7 | 7.0 | 91.1 | 7.8 | 84.0 | 7.4 | 82.2 | 7.2 | 88.1 |
| Cefazolin | 69.1 | 26.7 | 62.6 | 30.7 | 63.5 | 29.8 | 61.8 | 31.2 | 57.1 | 37.0 | 56.1 | 39.6 | 58.2 | 34.8 | 56.3 | 38.2 |
| Cefuroxime | 64.8 | 31.8 | 58.7 | 40.1 | 59.9 | 37.4 | 50.6 | 45.4 | 53.6 | 43.0 | 52.7 | 43.1 | 52.8 | 43.9 | 53.8 | 43.5 |
| Ceftazidime | 33.9 | 58.7 | 30.6 | 62.3 | 30.9 | 61.9 | 28.9 | 62.6 | 25.6 | 65.8 | 28.2 | 63.7 | 27.4 | 66.3 | 29.8 | 618 |
| Ceftriaxone | 65.8 | 33.7 | 57.8 | 41.9 | 59.6 | 40.3 | 57.3 | 42.1 | 53.5 | 46.0 | 52.6 | 47.2 | 51.7 | 47.7 | 52.7 | 47.1 |
| Cefepime | 30.2 | 46.7 | 24.4 | 54.1 | 25.8 | 51.7 | 24.9 | 52.4 | 20.6 | 58.5 | 15.3 | 66.6 | 21.7 | 59 | 21.9 | 58.5 |
| Cefoxitin | 19.5 | 69.4 | 13.5 | 80.4 | 13.9 | 74.6 | 17.0 | 74.4 | 9.5 | 82.9 | 13.1 | 78.1 | 12.1 | 81 | 9.2 | 85.5 |
| Moxalactam | 3.0 | 93.9 | 2.3 | 94.4 | 2.6 | 94.9 | 2.5 | 95.5 | 2.3 | 96.8 | 2.5 | 95.9 | 2.6 | 96.3 | 1.7 | 97 |
| Aztreonam | 42.2 | 49.5 | 37.5 | 54.8 | 37.6 | 53.6 | 34.9 | 54.8 | 30.9 | 59.8 | 37.3 | 55.9 | 30.9 | 60.3 | 30.2 | 60.3 |
| Ertapenem | - | - | - | - | - | - | - | - | 1.3 | 98.2 | 1.7 | 97.7 | 2.3 | 96.8 | 2.1 | 97.3 |
| Imipenem | 1.0 | 97.8 | 1.4 | 98.1 | 1.1 | 98.7 | 1.2 | 98.4 | 1.3 | 98.5 | 1.6 | 98.2 | 1.5 | 97.7 | 1.3 | 98.4 |
| Meropenem | 1.2 | 98.7 | 1.5 | 98.2 | 1.1 | 98.7 | 1.0 | 98.6 | 1.3 | 98.6 | 1.6 | 98.4 | 1.6 | 98.2 | 1.3 | 98.4 |
| Amikacin | 4.9 | 93.8 | 3.3 | 96.3 | 3.6 | 95.5 | 3.1 | 96.3 | 2.5 | 97.2 | 2.3 | 97.7 | 2.2 | 97.2 | 2.6 | 96.8 |
| Gentamicin | 44.0 | 51.9 | 39.9 | 57.1 | 43.3 | 53.6 | 43.5 | 54.0 | 40.9 | 58.1 | 34.9 | 64.4 | 34.5 | 64.6 | 35.3 | 63.3 |
| Ciprofloxacin | 65.7 | 27.0 | 58.9 | 30.9 | 74.0 | 17.9 | 64.5 | 24.4 | 66.3 | 25.1 | 60.5 | 28.9 | 62.1 | 25.7 | 58.1 | 30.4 |
| Levofloxacin | 61.7 | 31.6 | 53.1 | 37.2 | 57.5 | 34.3 | 54.4 | 36.0 | 51.1 | 38.4 | 50.9 | 38.9 | 54.1 | 36.3 | 52 | 39.8 |
| Trimethoprim/sulfamethoxazole | 60.8 | 39.2 | 58.3 | 41.7 | 58.7 | 41.3 | 60.3 | 39.7 | 57.7 | 42.3 | 54.0 | 46.0 | 53.3 | 46.7 | 52.4 | 47.6 |
| Fosfomycin | 0.1 | 93.2 | 0.2 | 95.5 | 0.6 | 94.5 | 0.4 | 95.1 | 2.2 | 95.4 | 3.1 | 95.7 | 4 | 94.8 | 3.6 | 95.7 |
| Polymyxin B | 1.5 | 98.5 | 1.4 | 98.6 | 7.2 | 92.8 | 0.5 | 99.5 | 3.1 | 96.9 | 0.7 | 99.3 | 1 | 99 | 1.8 | 98.2 |
| Tigecycline | 0 | 99.6 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |

Note: S, susceptible; I, intermediate; R, resistant;

### ESBL genotypic characterization

PCR analyses were conducted on 983 phenotypically confirmed ESBL-positive *E. coli* strains. Of these, 763 (77.6%) were confirmed to carry both the ESBL and *ampC* genes. Furthermore, 75 strains harbored non-ESBL β-lactam genes, such as $bla_{TEM}$ variants, while 145 strains did not contain any β-lactamase genes. The study identified 23 CTX-M subtypes of the CTX-M-1 or CTX-M-9 group. The ESBL genes belong to the CTX-M type dominated by the CTX-M-9 group (457 strains; 223 strains have only the CTX-M-9 gene, while 234 strains have to coexist with other ESBL genes), followed by the CTX-M-1 group (333 strains, 132 single strains and 201 coexisting strains). In addition to these CTX-M genotypes, other ESBLs and *ampC* genes, such as $bla_{OXA-1}$, $bla_{CMY,}$ and $bla_{DHA-1,}$ were also identified and frequently coexisted with either the CTX-M-9 or CTX-M-1 group (Supplementary Table S3). In the CTX-M-9 group, $bla_{CTX-M-14}$ was the most common (157/457, 34.4%), followed by $bla_{CTX-M-27}$, $bla_{CTX-M-17,}$ and $bla_{CTX-M-65}$ (155/457, 33.9%; 89/457, 19.5%; 38/457, 8.3%). Similarly, $bla_{CTX-M-55}$ (153/333, 45.9%) was most frequent in the CTX-M-1 group, followed by the $bla_{CTX-M-15}$, $bla_{CTX-M-194}$ and $bla_{CTX-M-3}$ genotypes (100/333, 30%; 33/333, 9.9%; 16/333, 4.8%). Interestingly, 28 strains carrying $bla_{OXA-1}$ were exclusively found to coexist with the $bla_{CTX-M-15}$ gene. Other subtypes included $bla_{CTX-M-123}$ (3%), $bla_{CTX-M-137}$ (2.1%), $bla_{CTX-M-64}$ (2.1%), $bla_{CTX-M-101}$(0.9%), $bla_{CTX-M-199}$ (0.9%), and $bla_{CTX-M-163}$ (0.9%), belonging to the CTX-M-1 group; $bla_{CTX-M-24}$ (2.2%), $bla_{CTX-M-99}$ (0.7%), $bla_{CTX-M-18}$ (0.2%), and $bla_{CTX-M-125}$ (0.2%), along with less common genotypes such as $bla_{CTX-M-79}$, $bla_{CTX-M-82}$, $bla_{CTX-M-127}$, $bla_{CTX-M-190}$, and $bla_{CTX-M-294}$ from the CTX-M-9 group. These were present in strains carrying either single strains or coexisting with one or two ESBL or non-ESBL genotypes. Furthermore, the presence of the *ampC* gene was also identified alongside ESBL genotypes. Specifically, 12 strains harbored CMY ($bla_{CMY-2}$ and $bla_{CMY-42}$) genes, while two isolates harbored the $bla_{DHA-1}$ gene. Strains carrying the *ampC* gene coexisted with $bla_{CTX-M}$ and TEM variants; however, only three strains carried $bla_{CMY-2}$, one strain carried $bla_{CMY-42}$, and one carried $bla_{DHA-1}$ without coexisting with other genes.

### Yearly distribution of ESBLs

The yearly distribution of ESBL genes by *E. coli* strains showed variable trends. In 2014, these genes were present in 81.2% of the strains, which decreased to 66.4% in 2018, increased to 81.8% in 2020, and then slightly decreased to 78.1% in 2021. Statistically significant differences in gene distribution were observed only between 2018 and 2019 ($P<0.02$), while no significant variations were found in other years. The most frequently detected ESBL genotypes were $bla_{CTX-M-27,}bla_{CTX-M-14}$, and $bla_{CTX-M-55}$ (Fig. 1). Specifically, $bla_{CTX-M-27}$ was the most common gene from 2019 to 2021, with rates of 26.4%, 23.1% and 25.8%, respectively. In contrast, $bla_{CTX-M-14}$ was dominant in 2016 and 2018 (25.7% and 31.2%), while $bla_{CTX-M-55}$ was more common in 2014, 2015 and 2017, with rates of 23.4%, 20% and 19%. Other non-CTX-M genotypes, such as $bla_{CMY-2}$ (1.1% in 2018, 0.8% in 2019, 1.7% in 2020), $bla_{CMY-42}$ (1.7% in 2019), and $bla_{DHA-1}$ (0.8% in 2021), were also detected. Coexisting genotypes such as $bla_{OXA-1}$+$bla_{CTX-M-15}$ consistently appeared each year, ranging between 1% and 13% (Supplementary Table S4).

### Geographical distribution of ESBL

The detection rate of ESBL genes in *E. coli* strains was highest in Southwest China (84.9%, 44/53) and lowest in Northeast China (73.2%, 30/41). However, no statistically significant differences were observed between the different regions. $bla_{CTX-M-14}$, $bla_{CTX-M-27}$ (CTX-M-9 group), and $bla_{CTX-M-55}$ (CTX-M-1 group) were the most frequently encountered ESBL genotypes across all the regions (Fig. 2). Most geographic locations had high rates of $bla_{CTX-M-55}$, except for the southwest region (6/45, 13.6%), indicating its widespread occurrence. However, our data revealed a consistent increase in the $bla_{CTX-M-27}$ gene across China. Compared with that of $bla_{CTX-M-55}$ (6/45, 13.6%), the percentage of $bla_{CTX-M-27}$ appears to increase, particularly in Southwestern China, where it reached a rate of 31.8% (14/45). The frequency of this gene was 23.5% and 21.2% greater in the north and northwestern regions, respectively, than that of $bla_{CTX-M-14}$, which had frequencies of 17.6% and 16.9%, respectively. In addition to the CTX-M type, $bla_{OXA-1}$ was also the most common but was found only in coexisting with $bla_{CTX-M-15}$, such as in the northeast region, a rate of 10.0%, followed by the northwest and east regions 5.9%, and had the lowest rate of 4.0% in the south-central region (Supplementary Table S5).

### Characterization of non-ESBL β-lactamase genes

Genes encoding the broad-spectrum β-lactamase TEM were detected in 75 strains that did not coexist with other ESBL or non-ESBL genotypes. However, the SHV-1 (1 strain), OXA-10 (7 strains), ACT (1 strain), and NDM (5 strains) genes coexisted with other CTX-M genotypes.

## Discussion

Our study describes the antimicrobial susceptibility profiles and molecular epidemiological characteristics of ESBL-positive *E. coli* in China and can provide insight into treatment choices. For instance, we found that imipenem, meropenem, and tigecycline are highly effective against ESBL-positive *E. coli* in China, aligning

**Table 2** Susceptibility and resistance trends among major ESBL genotypes of *E. coli* from 2014 to 2021. This table highlights resistance profiles associated with specific ESBL genotypes, enabling comparison of resistance trends within each genotype over time

| Antibiotics | Total strains (*N* = 983) | | CTX-M-9 Group* (*N* = 223) | | | | | | CTX-M-1 Group (*N* = 132) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CTX-M-27* (*N* = 101) | | CTX-M-14*(*N* = 57) | | CTX-M-17* (*N* = 45) | | CTX-M-55*(*N* = 58) | | CTX-M-15*(*N* = 35) | |
| | %*R* | %S | %*R* | %S | %*R* | %S | %*R* | %S | %*R* | %S | %*R* | %S |
| Amoxicillin | 99.5 | 0.4 | 99 | 1 | 100 | 0 | 97.7 | 0 | 100 | 0 | 100 | 0 |
| Amoxicillin/clavulanic acid | 23.5 | 38 | 16.7 | 59.8 | 21.4 | 53.6 | 15.9 | 47.7 | 26.8 | 37.5 | 25.7 | 51.4 |
| Cefoperazone/sulbactam | 11.4 | 65 | 2 | 90.2 | 12.5 | 67.9 | 11.4 | 63.6 | 16.1 | 69.6 | 2.9 | 82.9 |
| Piperacillin/Tazobactam | 15.1 | 83 | 7.8 | 89.2 | 12.5 | 85.7 | 15.9 | 81.8 | 16.1 | 76.8 | 8.6 | 88.6 |
| Cefazolin | 97.0 | 11 | 99 | 0 | 98.2 | 1.8 | 97.7 | 2.3 | 96.4 | 0 | 97.1 | 2.9 |
| Cefuroxime | 93.7 | 5.8 | 96.1 | 2.9 | 98.2 | 1.8 | 93.2 | 6.8 | 100 | 0 | 94.3 | 5.7 |
| Ceftazidime | 48.3 | 35 | 48 | 27.5 | 28.6 | 62.5 | 29.5 | 59.1 | 69.6 | 21.4 | 65.7 | 22.9 |
| Ceftriaxone | 96.6 | 6.8 | 98 | 2 | 96.4 | 3.6 | 95.5 | 4.5 | 98.2 | 1.8 | 97.1 | 2.9 |
| Cefepime | 35.8 | 21 | 20.6 | 37.3 | 25 | 19.6 | 27.3 | 34.1 | 51.8 | 14.3 | 34.3 | 25.7 |
| Cefoxitin | 14.7 | 67 | 8.8 | 79.4 | 16.1 | 76.8 | 9.1 | 81.8 | 17.9 | 71.4 | 17.1 | 74.3 |
| Moxalactam | 0.9 | 94 | 98 | 2 | 98.2 | 1.8 | 95.5 | 4.5 | 98.2 | 1.8 | 97.1 | 2.9 |
| Aztreonam | 59.0 | 33 | 56.9 | 16.7 | 30.4 | 51.8 | 47.7 | 29.5 | 82.1 | 14.3 | 80 | 11.4 |
| Ertapenem | 0.0 | 86 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 97 | 0 | 100 |
| Imipenem | 0.0 | 98 | 0 | 99 | 0 | 100 | 0 | 100 | 0 | 98.2 | 0 | 100 |
| Meropenem | 0.0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| Amikacin | 3.5 | 96 | 7.8 | 69.6 | 5.4 | 71.4 | 6.8 | 63.6 | 10.7 | 67.9 | 5.7 | 71.4 |
| Gentamicin | 47.6 | 55 | 18.6 | 72.5 | 39.3 | 53.6 | 29.5 | 56.8 | 39.3 | 51.8 | 45.7 | 42.9 |
| Ciprofloxacin | 76.3 | 20 | 78.4 | 15.7 | 69.6 | 12.5 | 65.9 | 20.5 | 76.8 | 17.9 | 80 | 17.1 |
| Levofloxacin | 69.5 | 22 | 77.5 | 18.6 | 57.1 | 30.4 | 63.6 | 31.8 | 75 | 21.4 | 71.4 | 20 |
| Trimethoprim/Sulfamethoxazole | 65.3 | 33 | 68.6 | 31.4 | 48.2 | 51.8 | 52.3 | 47.7 | 53.6 | 46.4 | 71.4 | 28.6 |
| Fosfomycin | 2.8 | 86 | 1 | 98 | 3.6 | 91.1 | 6.8 | 90.9 | 7.1 | 87.5 | 0 | 97.1 |
| Polymyxin B | 2.1 | 97 | 0 | 100 | 1.8 | 82.1 | 2.3 | 90.9 | 3.6 | 89.3 | 0 | 94.3 |
| Tigecycline | 0.2 | 98 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |

Note: S, susceptible; I, intermediate; R, resistant; Only strains harboring the major genotypes from both groups (CTX-M-1 and CTX-M-9) MIC were included

* Only strains containing a single genotype were selected for inclusion

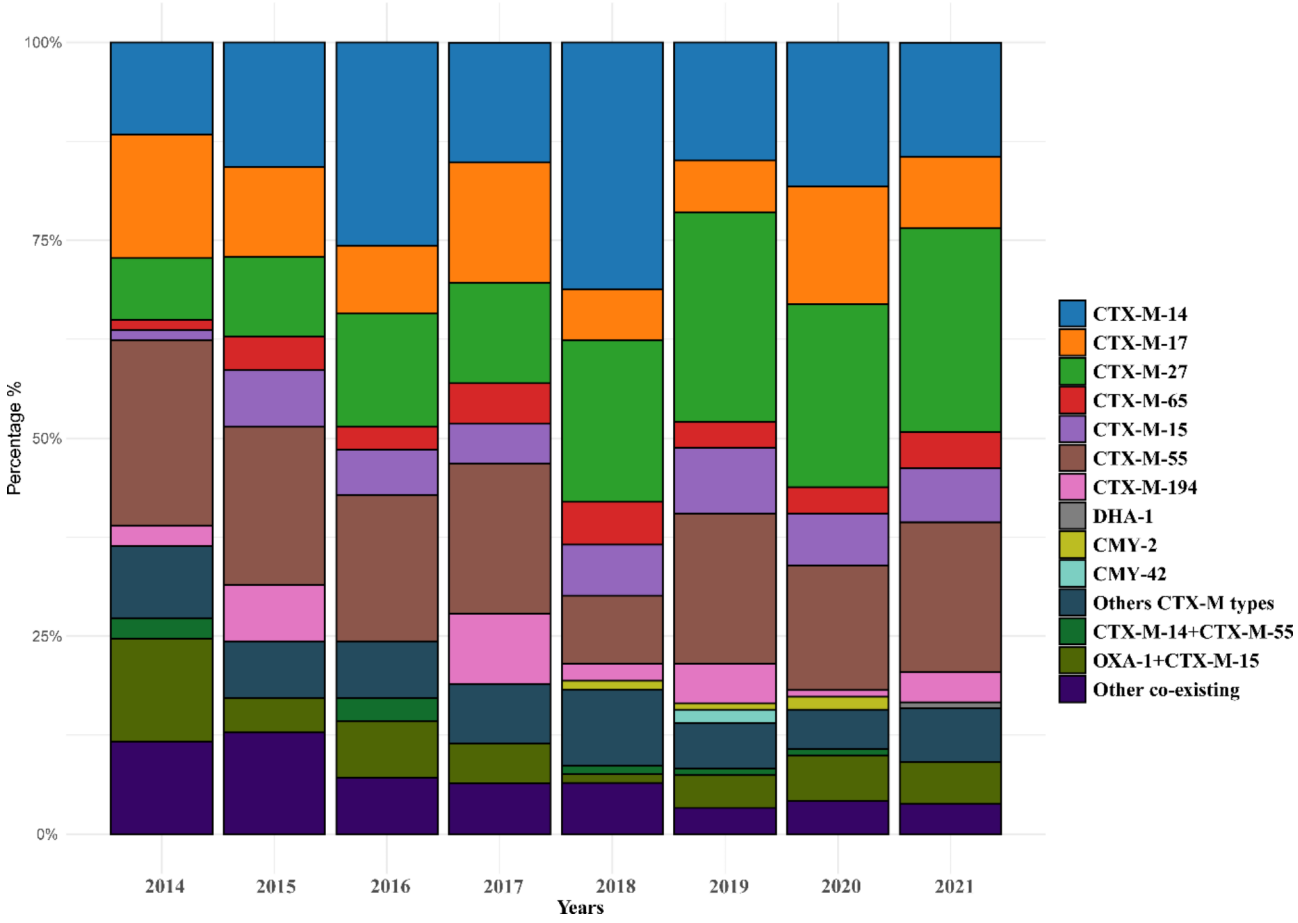


**Fig. 1** Yearly prevalence of ESBL and ampC genotypes among *E. coli* strains in China (2014–2021). This figure shows trends in the distribution of ESBL and ampC genotypes over time, depicting changes in genotype prevalence across the study period

with surveillance data from the United States [12] and European countries [13]. Over 70% of the strains were susceptible to amikacin, polymixin B, fosfomycin, and moxalactam. These results provide crucial data for selecting specific drug and aminoglycoside combinations for the empiric therapy of infections caused by ESBL-positive *E. coli*. The study also demonstrated a continuous decline in the presence of ESBL-positive *E. coli* strains, which decreased from 61.2% in 2014 to 49.6% in 2021, with the lowest prevalence rate in 2020 at 48.4% across China. This decline can be attributed to the antimicrobial stewardship campaign launched by the Ministry of Health in 2011, which successfully reduced antibiotic consumption and irrational drug use in Chinese hospitals, which is associated with a decrease in the prevalence of antibiotic-resistant bacteria [14]. Our findings revealed that 77.6% of the phenotypically positive ESBL *E. coli* strains harbored ESBL genes; the ESBL genotypes of *E. coli* reported in this study were geographically distributed across the six regions with significantly high percentages, ranging from 73.3 to 84.9%. PCR amplification revealed a high diversity of CTX-M-type ESBLs, identifying 23 CTX-M subtypes, mainly from the CTX-M-9 and CTX-M-1 groups, aligning with global ESBL dissemination trends [15]. The dominance of the CTX-M-9 group in the study (457 strains) highlights the evolving nature of ESBL-positive *E. coli*. Additionally, the antibiotic susceptibility data indicated that all the *E. coli* strains that expressed CTX-M-1 and CTX-M-9 group genotypes exhibited significant resistance to moxalactam; moreover, $bla_{\text{CTX-M-27}}$-carrying strains also displayed resistance profiles distinct from those of strains with other ESBL genotypes. Specifically, gentamicin and cefoperazone/sulbactam exhibited higher levels of activity, whereas levofloxacin was ineffective. The observed increase in specific genotypes, such as $bla_{\text{CTX-M-27}}$, highlights the importance of integrating genotypic data into empiric therapy guidelines. It exhibits varying resistance profiles, suggesting that regional treatment protocols may benefit from genotype-based antibiotic selection to optimize efficacy. Tailoring empiric treatment strategies to account for prevalent genotypes could reduce the use of ineffective antibiotics, improve clinical outcomes, and support antibiotic stewardship efforts across China.

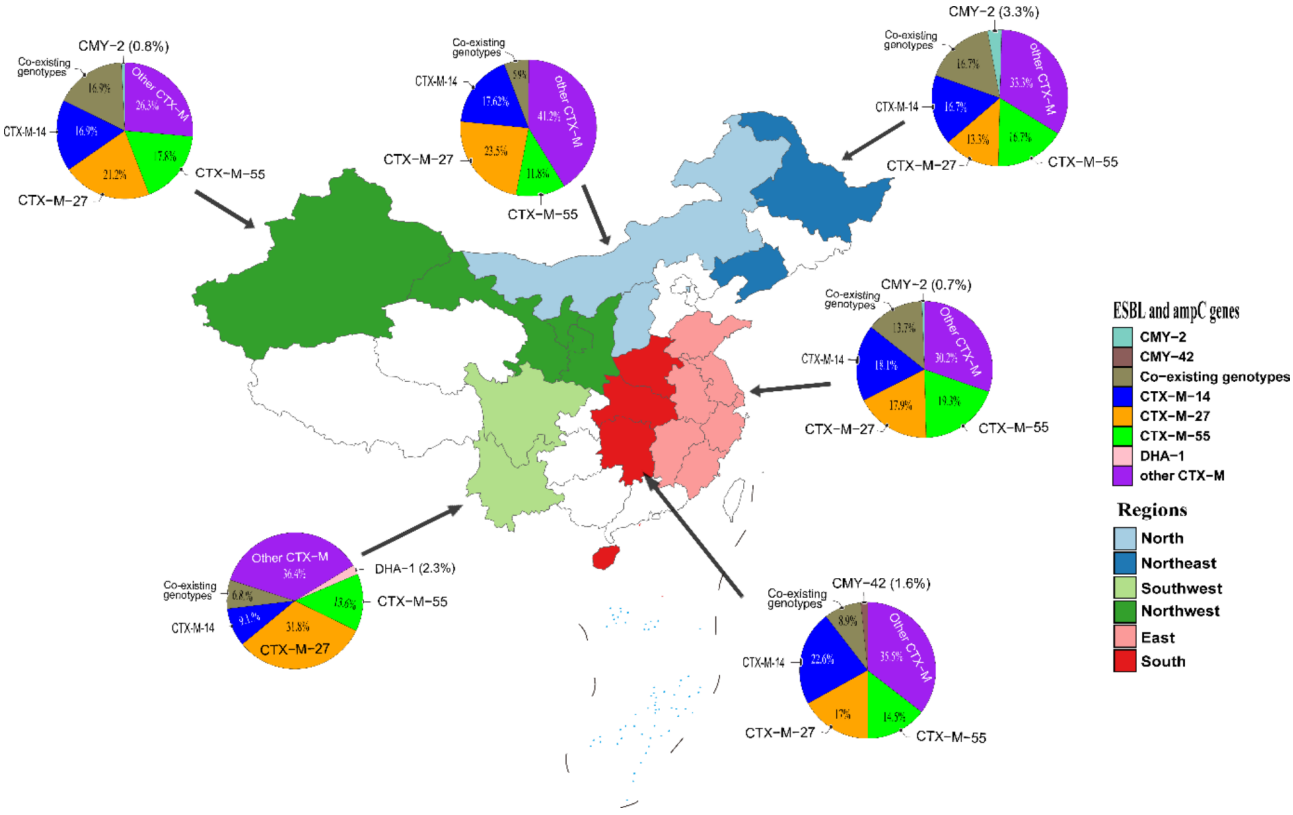


**Fig. 2** Geographical distribution of ampC and ESBL genotypes in *E. coli* strains across regions in China. This figure presents the regional prevalence of specific ESBL and ampC genotypes using pie charts, illustrating geographic patterns in genotype distribution across China

Incorporating this approach into local and national guidelines would provide a more targeted framework for managing ESBL-positive infections.

Our data further confirmed that $bla_{\text{CTX-M-55}}$ remained the most abundant CTX-M gene, but $bla_{\text{CTX-M-27}}$ showed an increasing pattern, especially in the southwest (36.4%), northern (23.5%) and northwestern (21.2%) regions of China. These findings reflect the patterns reported in the literature, where the emergence of $bla_{\text{CTX-M-27}}$ has been reported in some regions of China [16]. Research by Jia et al. in 2021 revealed $bla_{\text{CTX-M-27}}$ in several regions in China, with a 25% prevalence rate in *E. coli* strains. On the other hand, in a 2022 study, Zhao et al. noted a 9% lower occurrence of $bla_{\text{CTX-M-27}}$ in BSIs in Southeast China. Additionally, in Hangzhou, Zhejiang Province, Shao et al. identified $bla_{\text{CTX-M-27}}$ in a mere 9 isolates from hospital patients in 2023 despite a limited sample size [9, 10, 17]. Our study, which involved a larger sample size and comprehensive coverage of $bla_{\text{CTX-M-27}}$ across China, is separate from earlier research. Besides that, the spread of $bla_{\text{CTX-M-27}}$ in animals and the environment [18, 19] may also contribute to its emergence, suggesting its mobilization across different domains. The emergence of the $bla_{\text{CTX-M-27}}$ gene in *E. coli* strains in humans, the environment, and food animals across China [9, 19, 20] suggests that $bla_{\text{CTX-M-27}}$ has already been disseminated in humans, animals and the environment. Supporting this conclusion, a study by Zhao et al. provided evidence for the transmission mechanisms of $bla_{\text{CTX-M-27}}$. They investigated Tn*1721*-like transposons that carry the $bla_{\text{CTX-M-27}}$ gene and found that they can transferred between different plasmids in *E. coli*. Furthermore, they revealed that the P1-like bacteriophage facilitates the dissemination of $bla_{\text{CTX-M-27}}$ among Salmonella spp [21]. It has also been observed that the IncF group F18:A-:B10 plasmid families also play a role in promoting the transfer of $bla_{\text{CTX-M-27}}$ in *E. coli*. A novel structural arrangement has been identified for the $bla_{\text{CTX-M-27}}$ gene within the F18:A-:B10 plasmids. Additionally, the F24:A-:B1 plasmids were probably transferred before obtaining the $bla_{\text{CTX-M-27}}$ gene. It is also hypothesized that Tn*2*-related events may have had a role in facilitating the dissemination of the $bla_{\text{CTX-M-27}}$ gene within the F24:A-:B1 plasmids. This means that the number of prevalent vehicles for $bla_{\text{CTX-M-27}}$ transmission has diversified over time [18]. The results from the current study, along with the literature, indicate that $bla_{\text{CTX-M-27}}$ is capable of mobilization across these domains.

Also, the coexistence of ESBLs with *ampC* genes, such as $bla_{\text{OXA-1}}$, $bla_{\text{CMY}}$, $bla_{\text{DHA-1}}$, and specifically $bla_{\text{OXA-1}}$ in 28 strains with the $bla_{\text{CTX-M-15}}$ genotype highlights a complex pattern of gene coexistence that may have

significant implications for antibiotic resistance profiles. The presence of *ampC* genes, particularly $bla_{CMY}$ and $bla_{DHA-1}$, alongside ESBL genotypes and the coexistence of *ampC* genes, present significant challenges to the efficacy of the national action plan for antibiotic stewardship. These findings point to an urgent need for targeted therapeutic strategies, including the selection of specific antimicrobial agents based on genetic profiling of ESBL-positive strains. Furthermore, in our study, we also found that 145 (14.7%) strains lacked ESBL genes, but they all phenotypically conformed to ESBL. The *E. coli* strains that exhibit a phenotypic ESBL-positive profile but lack the corresponding ESBL genes may be due to nonenzymatic mechanisms to resist antibiotics, such as modifications in the bacterial cell wall or outer membrane, efflux pumps that expel antibiotics from the bacterial cell or alterations in target sites.

However, our study has some limitations. The study focuses solely on BSIs, which may not fully represent ESBL resistance patterns in other infection types, such as urinary or respiratory tract infections. Additionally, while we collected data from multiple regions, the lack of uniform coverage across all regions of China could limit the generalizability of our results. These factors may affect the overall representation of resistance patterns nationwide. Future research that includes various infection sites and more comprehensive regional sampling would help provide a fuller picture of resistance trends and strengthen the applicability of these findings.

## Conclusions

This study contributes significantly to the evolving backdrop of the ESBL epidemic in BSI *E. coli* across China. We observed a notable decline in ESBL-positive *E. coli*, from 61.2% in 2014 to 49.6% in 2021. Although the CTX-M-9 and CTX-M-1 groups maintained predominant roles in the genotypes of ESBLs, $bla_{CTX-M-27}$ had a predominant increasing trend, especially in the southwest region. Overall, our findings underscore the importance of continued surveillance and research to guide effective antibiotic strategies and public health policies. Future research should focus on exploring the underlying mechanisms driving the regional prevalence of specific genotypes, such as $bla_{CTX-M-27}$, and on evaluating the effectiveness of tailored antibiotic stewardship programs in reducing ESBL prevalence in diverse healthcare settings.

## Supplementary Information

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Supplementary Material 1

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NA.

**Author contributions**
YHX designed the study. YC carried out data processing. JJ, and ZL conducted laboratory assays. SS performed the genotypic analysis and analyzed data with assistance from HX. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. SS wrote the first draft of the manuscript. All the authors contributed to and reviewed the final manuscript.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's note