Emerging Microbes & Infections
2025, VOL. 14, 2479048 (13 pages)
https://doi.org/10.1080/22221751.2025.2479048

The emergence of highly resistant and hypervirulent *Escherichia coli* ST405 clone in a tertiary hospital over 8 years

Min Wang[a,b]*, Zhijun Zhang[a]*, Zhifei Sun[c]*, Xinying Wang[a], Jie Zhu[b], Meijie Jiang[a], Shuping Zhao[a], Liang Chen[d], Qiang Feng[a]† and Hong Du[b]†

[a]Center for Clinical Laboratory, Affiliated Taian City Central Hospital of Qingdao University, Taian, People's Republic of China; [b]Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, People's Republic of China; [c]Center for Cardiovascular Diseases, Affiliated Taian City Central Hospital of Qingdao University, Taian, People's Republic of China; [d]Department of Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, Buffalo, NY, USA

ABSTRACT

The emergence of carbapenem-resistant *Escherichia coli* (CREC) poses crucial challenges in clinical management, requiring continuous monitoring to inform control and treatment strategies. This study aimed to investigate the genomic and epidemiological characteristics of CREC isolates obtained from a tertiary hospital in China between 2015 and 2022. Next-generation sequencing was used for genomic profiling, and clinical data from patients were integrated into the analysis. ST405 (21.2%), ST167 (20.3%) and ST410 (15.9%) were the most prevalent of the 30 distinct sequence types (STs) identified among the 113 unique CREC isolates. Infections caused by the ST405 CREC clone and severe underlying diseases were associated with higher in-hospital mortality rates, particularly in patients aged ≥65 years. Furthermore, the ST405 clone exhibited a greater number of virulence and resistance genes than non-ST405 CREC clones. The virulence gene *eaeX* and resistance genes *mph(E)* and *msr(E)* were exclusively found in ST405 clones, while other virulence genes (*agn43*, *ipad* and *malX*) and resistance genes (*armA*, *catB3* and *arr-3*) were more prevalent in this clones. Additionally, ST405 showed higher minimum inhibitory concentrations for both meropenem and imipenem and showed superior growth under the meropenem challenge. *Galleria mellonella* virulence assays revealed that the ST405 CREC clone was more virulent than other predominant CREC STs. Our findings underscore the clinical threat posed by the ST405 CREC clone, which exhibits both enhanced virulence and extensive drug resistance. These results highlight the urgent need for stringent surveillance and targeted interventions to curb its further dissemination and prevent potential outbreaks.

ARTICLE HISTORY Received 5 December 2024; Revised 26 February 2025; Accepted 9 March 2025

KEYWORDS Multidrug resistance; restrospective study; *E.coli* high risk clones; whole-genome sequencing; ST405

Introduction

Antimicrobial resistance (AMR) has appeared as one of the most critical public health threats of the twenty-first century, affecting the effective prevention and treatment of infections in humans and animals [1–3]. *Escherichia coli* (*E. coli*) is a leading cause of urinary, respiratory and bloodstream infections [4–6]. Moreover, *E. coli* serves as a crucial reservoir for antimicrobial resistance genes within the "One Health" framework, which includes human, animal and environmental health [7,8]. Over the past two decades, the emergence and spread of carbapenem-resistant *E. coli* (CREC) have posed substantial challenges in clinical management [9–11]. The primary mechanism driving carbapenem resistance in CREC is the acquisition of carbapenemase genes, such as bla_{KPC}, bla_{NDM}, and $bla_{OXA\text{-}48\text{-}like}$ [9]. Among these, the bla_{NDM} gene has exhibited widespread global prevalence, with Asia – particularly China – serving as a significant reservoir [12,13]. A genomic analysis of 7,731 CREC isolates collected from various countries between 2005 and 2023 among humans determined ST167, ST410, ST131, ST38, ST405 and ST361 as dominant clones [9]. A genomic analysis of CREC isolates from Chinese hospitals between 2015 and 2017 identified ST131, ST167 and ST410 as the most prevalent CREC clones [10].

In China, *E. coli* has been the most frequently isolated clinical pathogen from 2005 to 2022, accounting for 16.0% to 20.4% of all isolates, with imipenem resistance rates ranging from 1.5% to 2.0% [14]. The prevalence of extended-spectrum beta-lactamases (ESBLs) in E. coli remains high, reaching approximately 50%, with $bla_{CTX\text{-}M}$ being the predominant

CONTACT Hong Du hong_du@126.com Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215004, People's Republic of China; Qiang Feng fengqiang915@163.com Center for Clinical Laboratory, The Affiliated Taian City Central Hospital of Qingdao University, Taian, Shandong 271000, People's Republic of China
*These authors contributed equally to this work.
†Hong Du and Qiang Feng contributed equally and share the role of corresponding authors.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/22221751.2025.2479048.

ESBL genotype [14]. A study from China reported that the carriage rate of CREC among ICU patients was 4.39% in 2020 and 5.79% in 2022. ST131 was identified as the most common bla_{KPC}-positive CREC isolate (30.09%), while bla_{NDM} was associated with ST617 and ST410 isolates, indicating the spread of multiple CREC clones among ICU patients in China [15].

In recent years, ST167 has emerged as one of the most common sequence types of extraintestinal pathogenic *E. coli* (ExPEC) globally, often linked to carbapenem resistance [9]. ST410 is another extraintestinal pathogen associated with multidrug resistance and has been recognized as a high-risk international clone [16,17]. ST131 has been extensively investigated for its association with ExPEC infections [18]. Its high virulence, broad metabolic capacity and multiple antibiotic resistance have contributed to its global prevalence and persistence [19,20]. ST405 is also a globally recognized clone, carrying virulence gene variants similar to those found in ST131 [21]. As part of phylogenetic group D, which is associated with increased virulence, ST405 is identified as a high-risk international clone with multidrug resistance (MDR) and is a potential reservoir of bla_{CTX-M} and bla_{NDM-5} [9,22,23]. ST405 CREC strains carrying both $bla_{CTX-M-3}$ and bla_{NDM-5} have been reported to transmit within communities [24]. In Pakistan, ST405 (44.4%) was the most frequently identified sequence type among 184 carbapenem-resistant clinical *E. coli* isolates recovered from clinical specimens in Lahore [22]. Similarly, in China, carbapenem-resistant *E. coli* isolates harbouring bla_{NDM-4} were identified in a tertiary hospital, all belonging to ST405 and inferred to have spread covertly [25]. Unlike ST131 and ST167, ST405 CREC exhibits higher median counts of resistance and virulence genes, posing a significant risk in clinical settings [26]. However, limited research has examined bla_{NDM-5}-carrying ST405 CREC, particularly studies integrating genomic features with clinical outcomes, leaving gaps in understanding the clinical significance of these isolates.

In this study, we conducted a comprehensive genomic epidemiological analysis of a large longitudinal cohort of CREC isolates collected over 8 years (2015–2022) from a tertiary teaching hospital in China. By combining genomic data with clinical information from patient electronic health records, we conducted a comparative analysis of the virulence and resistance genomic features of the predominant ST clonal lineages. Our results revealed the presence of a highly virulent and multidrug-resistant ST405 clone. Improved vigilance is required to monitor the spread of ST405 CREC, especially among critically ill patients in-hospital settings. Continuous surveillance of CREC will be important in controlling future outbreaks.

Materials and methods

Bacterial isolates and patient data collection

Carbapenem resistance was defined as a minimum inhibitory concentration (MIC) of ≥4 μg/mL for imipenem or meropenem and ≥2 μg/mL for ertapenem [27]. A total of 123 CREC isolates were collected from the patients admitted to Taian Central Hospital between January 2015 and December 2022. These CREC isolates were obtained as part of the routine clinical surveillance of carbapenem-resistant bacteria at Taian Central Hospital, during which a total of 123 CREC strains and 1,066 other carbapenem-resistant strains were collected. Species identification was performed using MALDI-TOF MS (Bruker Microflex LT, Bruker Daltonik GmbH, Germany). Infection and colonization were distinguished following the Infectious Diseases Society of America guidelines, with infections diagnosed based on clinical symptoms, local or systemic inflammation evidence, high drug resistance levels and strain isolation from a sterile site [28]. The clinical data collected included patient age, gender, length of hospital stay, admission and discharge dates, ward location during sample collection, sample collection date, sample type and diagnoses during the hospital stay. Community-acquired infections were those identified within 48 h of hospital admission. The clinical outcomes included 30-day mortality (within 30 days of the first positive culture) and in-hospital mortality (death during hospitalization after the first culture).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the VITEK-2 Compact system (bioMérieux, France) for ampicillin, cefuroxime, ceftazidime, ceftriaxone, cefepime, aztreonam, piperacillin–tazobactam, ertapenem, imipenem, meropenem, amikacin, gentamicin, trimethoprim–sulfamethoxazole, ciprofloxacin and levofloxacin. Colistin and tigecycline were tested using broth microdilution [29]. Results were interpreted based on the 2022 Clinical and Laboratory Standards Institute guidelines (CLSI M100, 2022) [29], except for tigecycline and colistin, which were interpreted according to the European Committee on Antimicrobial Susceptibility Testing criteria (https://www.eucast.org/). *E. coli* American Type Culture Collection (ATCC) 25922 was used as a quality control strain.

Whole-genome sequencing, assembly and publicly available sequences

Genomic DNAs from the 123 CREC isolates were extracted using the Omega Bio-Tek Bacterial DNA

Kit (Doraville, GA, USA). The quality and concentration of the bacterial genomic DNA were evaluated via electrophoresis on a 1% agarose gel and analysis on a NanoDrop2000 system (Thermo Scientific, Waltham, MA, USA) and a Qubit 4 Fluorometer (Thermo Scientific, Waltham, USA). Libraries were constructed based on the qualified DNA by using a NEB Next Ultra TM II DNA Library Prep Kit (New England BioLabs, Ipswich, USA) and sequenced using paired-end libraries with an average insert size of 350 bp on the NovaSeq 6000 platform (Illumina, CA, USA). Raw reads with low quality were removed as described previously [30]. De novo assembly of the corrected reads was performed with SPAdes version 3.11 [31]. To further investigate the ST405 clone identified in this study, ST405 isolate genomes were retrieved from EnteroBase (accessed on 13 July, 2024), yielding 2,062 isolates with associated metadata (year of collection, country and source) and raw reads.

Genome annotation and characterization

Resistance genes, plasmid replicons, virulence factors and serotypes were determined using ABRicate v1.0.1 (https://github.com/tseemann/abricate) with the Resfinder, Plasmidfinder, Ecoli-vf and EcOH databases (updated 15 September 2022), using default parameters [32–34]. The Multilocus sequence typing (MLST) types were assigned using the Achtman scheme and performed using MLST v2.19.0 (https://github.com/tseemann/mlst) with the "ecoli" scheme. *fimH* typing was identified utilizing FimTyper version 1.0 [35].

Phylogenetic tree construction

Snippy version 4.6.0 (https://github.com/tseemann/snippy), with EB003 (GenBank accession no. CP086334) as the reference genome, was used to generate whole-genome alignment for the first CREC isolate (the initial CREC isolate detected from a patient's clinical specimen if more than one isolates were collected from the same patient) from each of the 113 patients. The full-length genome alignment was cleaned using the snippy-clean function and then processed using Gubbins version 2.4.1 to identify and remove homologous recombination regions [36]. SNP-sites version 2.5.1 was used to extract variant sites from the alignment, and FAST-tree was utilized to reconstruct the maximum likelihood phylogenetic tree [37]. The resulting tree was annotated using iTOL v7 [38].

Galleria mellonella larvae model

The virulence of the selected isolates was assessed using a *G. mellonella larvae* model, as previously described [16,39,40]. Supplementary Material presents the detailed methods for the *G. mellonella larvae* virulence assay.

In vitro competition assay

Competitive growth experiments were conducted to evaluate the interactions between ST405 and other STs following the protocol described by a previous study [27]. Supplementary Material shows the detailed methods for the in vitro competition assay.

Data statistical analysis

All statistical analyses were conducted using GraphPad Prism version 9.3.1 and Statistical Package for the Social Sciences (SPSS) version 29.0.1.0. Supplementary Material presents the detailed methods for statistical analysis.

Ethical approval

The study was reviewed and approved by the Ethical Review Committee of The Affiliated Taian City Central Hospital of Qingdao University. Isolates were collected from routine microbiological samples, and all samples were anonymised. Informed consent was waived as patients did not directly participate in the study.

Result

Clinical characteristics

We conducted whole-genome sequencing on 123 CREC isolates collected from clinical samples of 113 patients at Taian Central Hospital between 2015 and 2022. The isolates were recovered from urine (n = 50), sputum (n = 32), secretions (n = 12; including 9 skin wound exudates and 3 vaginal secretions), blood (n = 11), drainage fluid (n = 8), stool (n = 3) and other sources (n = 7) (Figure S1A). *In silico* MLST analysis revealed substantial diversity among the 123 CREC isolates, with 122 assigned to 30 known STs and one isolate possessing a novel ST type. Isolates of the same ST were recovered more than once in nine patients, with intervals ranging from 7 days to 30 months. In four of these patients, isolates of the same ST were recovered from different body sites, indicating potential intra-host colonization and secondary infection (Table S1). Among the first non-replicate isolates recovered from each patient, three STs were notably prevalent: ST405 (n = 24), ST167 (n = 23) and ST410 (n = 18) (Figure S1B), together comprising 57.5% of the total isolates. Serotyping revealed the dominant serotypes for these STs:

O102 for ST405, Onovel32 for ST167 and O8 for ST410, with frequencies of 100%, 69.6% and 94.4%, respectively (Table S1).

Phylogenetic analysis

To further investigate the evolutionary associations of the first non-replicated 113 CREC isolates, we constructed a phylogenetic tree based on core-genome single-nucleotide polymorphisms (SNPs). Phylogenetic analysis revealed five distinct clades. Clade 1 predominantly consisted of isolates from phylogroup D, along with others from phylogroups B2, F and G, including all ST405, ST38 and ST354 clones. 95.8% (23/24) of ST405 isolates carried *fimH29*. Clade 2 contained isolates from phylogroup A, including ST156, ST224, ST101 and several others. Clade 3 consisted of phylogroup C isolates, including all ST410 clones that carried *fimH24*. Clades 4 and 5 belonged to phylogroup A. Clade 4 included all ST361 clones, most of which carried *fimH54*, whereas clade 5 comprised ST167, ST617, ST10 and other clones, with 91.3% (21/23) of ST167 isolates lacking *fimH*. Notably, ST405 isolates clustered within the ICU in both 2019 and 2021, with other clades exhibiting clustering (Figure 1).

AMR and antibiotic resistance genes (ARG) diversity in CREC isolates

All CREC isolates exhibited resistance to at least one carbapenem. The resistance rates to meropenem and ertapenem were 99.1% (112/113) and that to imipenem was 97.4% (111/113). The resistance rates were 100% for ampicillin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, cefoperazone–sulbactam, piperacillin–tazobactam and cefoxitin. The resistance rate for aztreonam was 88.5% (100/113), whereas that for levofloxacin and ciprofloxacin were 93.8% (106/113) and 92.9% (105/113), respectively. Resistance to gentamicin, amikacin and trimethoprim–sulfamethoxazole was observed in 77.9% (88/113), 48.7% (55/113) and 92.9% (105/113) of the isolates. Colistin resistance was relatively rare, occurring in 5.3% (6/113) of the isolates (MIC: 4–8 μg/mL). All isolates were sensitive to tigecycline (Figure S2A). The resistance phenotype was largely attributable to the presence of the corresponding ARGs.

Among the 113 CREC isolates, 107 carried carbapenemase genes, with bla_{NDM} being the most prevalent (94.7%, 107/113). The most predominant variant was $bla_{\text{NDM-5}}$, observed in 82.3% (93/113) of the isolates, followed by $bla_{\text{NDM-1}}$ (n = 10), $bla_{\text{NDM-6}}$ (n = 1), $bla_{\text{NDM-9}}$ (n = 2) and $bla_{\text{NDM-13}}$ (n = 1). Three isolates carried both $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-1}}$, whereas five

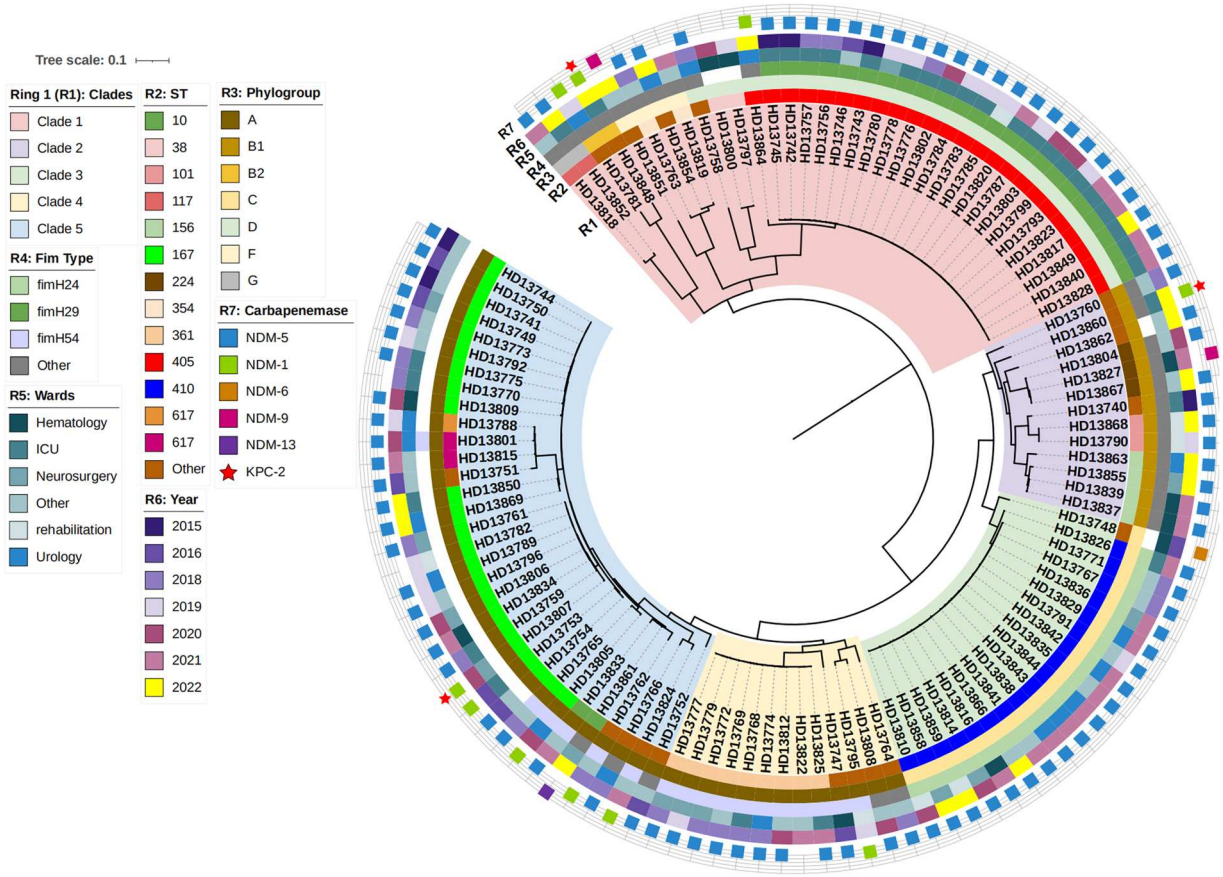

Figure 1. Phylogenetic tree of 113 clinical CREC isolates. The branch lengths are coloured according to the ST, phylogroup, Fim type, year, wards and carbapenemase gene. CREC: carbapenem-resistant *Escherichia coli*; ST: sequence type.

isolates harboured both bla_{NDM-5} and *mcr-1.1*. Additionally, one isolate carried both bla_{NDM-9} and *mcr-1.1* (Figure S2B).

In addition to carbapenemase genes, the CREC isolates contained various other resistance genes, including those conferring resistance to fosfomycin (*fosA3*, 54.9%), sulfonamides (*sul1*, 59.3%; *sul2*, 44.3%), aminoglycosides [*aadA2*, 39.8%; *aadA5*, 38.9%; *aac(3)-IId*, 45.1%], tetracyclines [*tet(A)*, 40.7%; *tet(B)*, 35.4%] and trimethoprim (*dfrA12*, 38.9%; *dfrA14*, 12.4%; *dfrA17*, 42.5%). A total of 104 CREC isolates (92%) tested positive for ESBL genes, with bla_{CTX-M} (84.1%, 95/113), bla_{SHV} (10.6%, 12/113), bla_{TEM} (46.9%, 53/113) and bla_{OXA-1} (37.2%, 42/113) being the most prevalent. The most predominant subtype was $bla_{CTX-M-55}$, observed in 53.1% (60/113) of the isolates. Additionally, 22 isolates carried AmpC β-lactamase genes, consisting of bla_{CMY-2} (n = 17), bla_{CMY-6} (n = 4) and bla_{CMY-42} (n = 1) (Table S2).

A heatmap of the resistance genes and plasmid replicons revealed distinct patterns among the ST405 and ST410 isolates. For example, *msr(E)* and *mph(E)* were exclusively observed in ST405 isolates, whereas bla_{CMY-2} was only present in the ST410 isolates (Figure S3). Among the 113 CREC isolates, the plasmid replicons IncX3 and IncFIB were the most prevalent, each detected in 66.4% (75/113) of the isolates (Table S3). The diversity of the resistance genes and plasmid replicons was higher in the ST167 and ST410 isolates, whereas the ST405 isolates exhibited minimal variation, with most harbouring identical resistance genes and plasmid replicons (Figure S3).

Relationships between genomic features and clinical data in ST405 and non-ST405 strains

The 113 isolates were categorized into four groups based on their STs: ST167, ST405, ST410 and others. We compared their clinical and resistance characteristics (Table 1). The median age of the patients was 65 years (range: 3–95), with significant differences in age distribution across the ST groups ($P = 0.01$). Patients infected with ST405 exhibited the highest median age (73.5 years), while those infected with ST410 demonstrated the lowest (59.5 years). The type of specimen did not significantly differ across STs ($P = 0.426$). However, significant differences were observed in the distribution of wards ($P <$

Table 1. Clinical characteristics and antimicrobial resistance of 113 CREC isolates.

Patient/bacterial characteristics	ST167 N = 23 n(%)	ST405 N = 24 n(%)	ST410 N = 18 n(%)	Other ST N = 48 n(%)	Total N = 113 n(%)	*P* Value[a]
Age, median, IQR	63 (6,88)	73.5 (36,94)	59.5 (38,75)	64 (3,95)	65 (3,95)	0.01
Sex						0.105
Male	11 (48)	17 (71)	11 (61)	20 (42)	59 (52)	
Female	12 (52)	7 (29)	7 (39)	28 (58)	54 (48)	
Samples Types						0.426
Urine	8 (35)	12 (50)	9 (50)	19 (40)	48 (42)	
Sputum	9 (39)	5 (21)	4 (22)	10 (21)	28 (25)	
Blood	1 (4)	4 (17)	2 (11)	3 (6)	10 (9)	
Other	5 (22)	3 (13)	3 (17)	16 (33)	27 (24)	
Year						0.016
2015–2016	6 (26)	4 (17)	0 (0)	5 (10)	15 (13)	
2017–2018	6 (26)	3 (13)	2 (11)	11 (23)	22 (20)	
2019–2020	8 (34)	10 (42)	3 (17)	11 (23)	32 (28)	
2021–2022	3 (13)	7 (29)	13 (72)	21 (44)	44 (39)	
Wards						<0.001
Intensive care unit	5 (22)	20 (83)	1 (6)	6 (13)	32 (28.3)	
Urology	3 (13)	1 (4)	5 (28)	8 (17)	17 (15.0)	
Neurosurgery	4 (17)	1 (4)	3 (17)	5 (10)	13 (11.5)	
Hematology	2 (9)	0 (0)	1 (6)	7 (15)	10 (8.8)	
Others	9 (39)	2 (8)	8 (44)	22 (46)	41 (36.2)	
Patient/bacterial characteristics	ST167 N = 23 n(%)	ST405 N = 24 n(%)	ST410 N = 18 n(%)	Other ST N = 48 n(%)	Total N = 113 n(%)	***P*** **Value[a]**
Antimicrobial resistance						
Trimethoprim–sulfamethoxazole	19 (83)	23 (96)	18 (100)	45 (94)	105 (93)	0.2
Amikacin	9 (39)	22 (92)	11 (61)	13 (27)	55 (49)	<0.0001
Gentamicin	15 (65)	23 (96)	17 (94)	33 (69)	88 (78)	0.005
Aztreonam	21 (91)	24(100)	18(100)	37 (77)	100(89)	0.007
Fluoroquinolones[b]	23 (100)	24 (100)	18 (100)	41 (85)	106 (94)	0.03
Cephalosporins[c]	23 (100)	24 (100)	18 (100)	48 (100)	113 (100)	–
Carbapenems[d]	23 (100)	24 (100)	18 (100)	48 (100)	113 (100)	–
Tigecycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–
Colistin	0 (0)	0 (0)	0 (0)	6 (13)	6 (5)	0.076
30 days morality	0 (0)	4 (17)	0 (0)	1 (2)	5 (4)	**0.029**
In-hospital mortality	2(9)	7(29)	0(0)	1(2)	10(9)	**0.001**

[a]χ^2 or Fisher exact test was used for categorical variables, and Kruskal-Wallis test was used for continuous variables; *P* value < 0.05 (bolded) was considered statistically significant.
[b]Fluoroquinolones included ciprofloxacin and levofloxacin.
[c]Cephalosporins included cefuroxime, ceftazidime, ceftriaxone and cefepime.
[d]Carbapenems included ertapenem, imipenem and meropenem.

0.001). CREC isolates were most frequently recovered from the ICU (32/113, 28.3%) (Figure S1C). ST405 was the dominant clone in the ICU, accounting for 62.5% of the isolates collected (Figure S1D).

ST frequencies changed over time, with a gradual increase in the number of CREC isolates. ST distribution by year significantly differed ($P = 0.016$). ST167 was the most prevalent ST between 2015 and 2016 and persisted through 2022. ST405 was consistently detected from 2015 to 2022, peaking at 31.3% (10/32) between 2019 and 2020. ST410 first emerged in 2018 and reached its highest proportion (29.5%, 13/44) between 2021 and 2022 (Figure S1E and F; Table 1).

Antibiotic resistance rates varied among different STs. The amikacin resistance rate was 92% for ST405 isolates and 39%, 67% and 27% for ST167, ST410 and other STs, respectively. Gentamicin resistance rates were >90% for ST167 and ST410 and 65% and 69% for ST167 and other STs, respectively. Aztreonam resistance rates were 100% for ST410 and ST405, 91% for ST167 and 77% for the other STs. Quinolone resistance was observed in 100% of ST167, ST405 and ST410 isolates and 85% of other STs. All colistin-resistant strains belonged to nondominant STs.

Significant differences in the mortality rates were observed among the different ST clones (30-day mortality, $P = 0.029$; in-hospital mortality, $P = 0.001$). Among the five patients who died within 30 days, four were isolated with ST405. The in-hospital mortality rate for patients with the ST405 clone was 29.1% (7/24, $P = 0.0007$), which was significantly higher than that of patients with the non-ST405 clone.

Clinical and genomic characterisation of the ST405 clone

Clinical and resistance characteristic analysis revealed that the ST405 clone was associated with a higher mortality rate and greater AMR compared with non-ST405 clones. An epidemiological investigation was conducted on 26 ST405 isolates from 24 patients in this study. Of the 26 isolates, 21 were recovered from patients in the ICU, whereas the remaining 5 were from 4 other wards. A phylogenetic tree based on the core-genome SNP analysis was developed and mapped with isolation year, wards and sample types (Figure 2(a)). Supplementary Table S4 shows the pairwise SNP differences between the isolates. The remaining 25 isolates carrying $bla_{\text{NDM-5}}$ clustered together, except for one isolate carrying $bla_{\text{NDM-1}}$ (HD13864), which formed a separate branch (Figure S3). This cluster exhibited SNP differences of 0–204

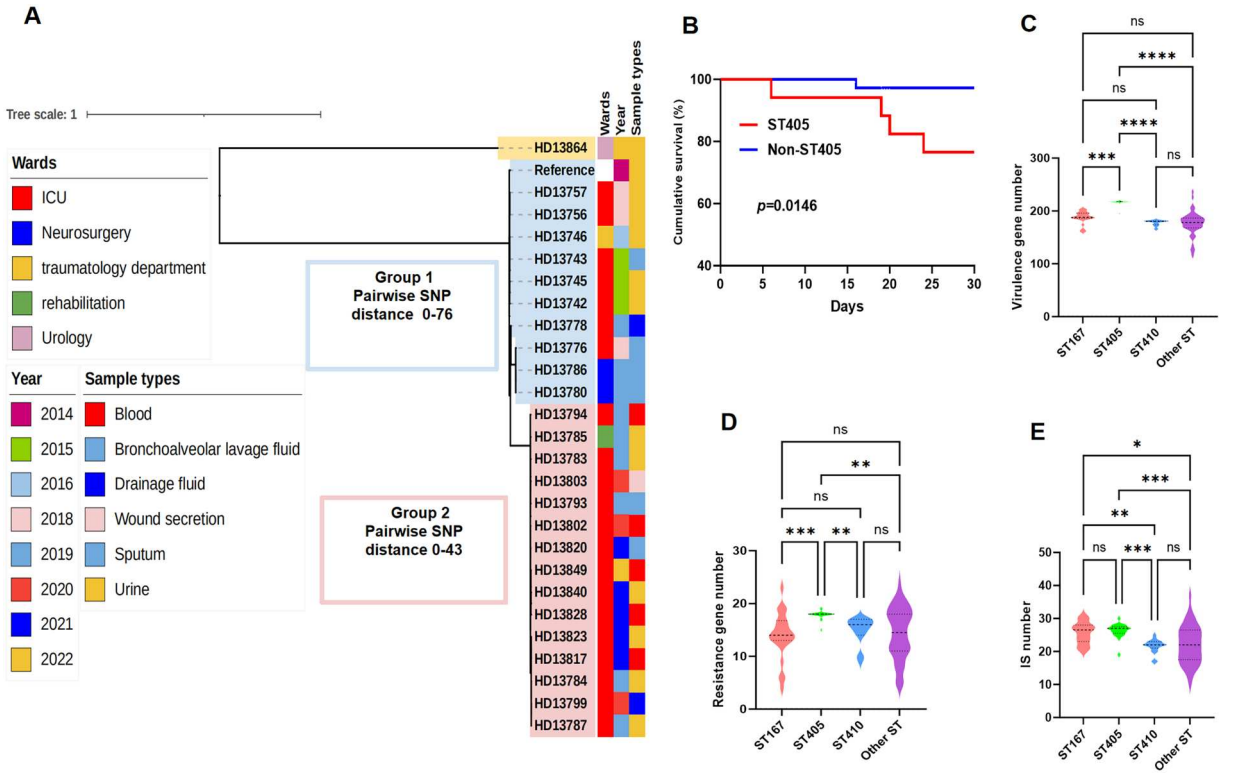

Figure 2. (A) Core-genome SNP phylogeny of the 26 ST405 CREC isolates in the hospital. Reference strains were selected based on the association of their core-genome multilocus sequence typing (cgMLST) with the earliest ST405 clone collected in this study. The branch lengths are coloured according to department, isolation time and sample types. (B) Thirty-day survival analysis curves for ST405 and non-ST405 clone-infected patients aged ≥65 years. (C–E) Comparison of resistance genes, virulence genes and insertion sequence number among ST167, ST405, ST410 and other STs. Statistical significance is indicated as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$. ns: not significant; CREC: carbapenem-resistant *Escherichia coli*; ST: sequence type; SNP: single-nucleotide polymorphism.

bp and was categorized into two main groups. Group 1 included isolates from patients hospitalized from July 2015 to May 2019, with SNP differences of 0–76. Group 2 consisted of isolates from patients hospitalized from May 2015 to 2022, with SNP differences of 0–43. Four isolates (HD13742, HD13743, HD13745, and HD13746) were isolated between 2015 and 2016, each exhibiting pairwise SNP distances of less than 10 bp. Additionally, eight isolates (HD13802 to HD13849) displayed pairwise SNP distances of less than 10 bp. These isolates were all recovered from ICU patients between 2019 and 2022. The close genetic relatedness and temporal clustering suggest a potential outbreak and transmission of ST405 clones within the ICU.

Within Group 2, two patients had two CREC isolates each. For one patient, the first CREC isolate was obtained from the respiratory tract, and 31 days later, another CREC isolate was obtained from the bloodstream, with a 2-SNP difference between the two isolates. In another patient, the first CREC isolate was recovered from sputum, and 7 months later, a second CREC isolate was obtained from sputum, showing an 8-SNP difference. The results suggest that in the first patient, the CREC strain may have disseminated from the respiratory tract to the bloodstream, while in the second patient, the persistence of the strain over several months indicates prolonged colonization or recurrent infection. The low SNP differences between isolates further support in-host evolution rather than reinfection with a distinct strain.

Considering the potential effect of age on patient outcomes, we categorized the patients into two groups: <65 years and ≥65 years of age. The univariable analysis identified ICU location on the day of CREC isolation, Charlson Comorbidity Index (CCI), polymicrobial infections and ST405 clone infection as risk factors for CREC-infected patients aged ≥65 years (Table 2 and Table S5). In the final multivariable model, CCI [$P = 0.033$, odds ratio (OR): 2.96 (1.089–8.045]) and infection with the ST405 clone ($P = 0.038$, OR: 33.054 (1.218–897.008)] were independently associated with death in patients aged ≥65 years after adjusting for ICU location on the day of CREC isolation and polymicrobial infections (Table 3). Survival curve analysis revealed that among CREC-infected patients aged ≥65 years, those infected with ST405 isolates demonstrated a significantly higher risk of mortality than those infected with non-ST405 isolates ($P = 0.0146$) (Figure 2(b)).

A comparison of the resistance genes, virulence genes and insertion sequence counts between the three dominant ST clones and other ST clones revealed that ST405 harboured a greater number of resistance genes, virulence genes and insertion sequences (Figure 2(c–e)). Several of these genes were unique or more prevalent in ST405. Notably, the resistance genes *mph(E)* and *msr(E)* were found exclusively in ST405 clones. while the invasiveness-associated gene *eaeX* was present in 79.2% of ST405 isolates but absent in non-ST405 isolates. Compared with non-ST405 clones, certain virulence genes, such as those encoding type III secretion system proteins (*espX3*, *espY3*, *espY4*, *eivACEFGIJ*), iron acquisition-related proteins (*fyuA*, *chuASTVWXY*, *irp1*, *irp2*, *ybtAEPQSTUX*), autotransporter-related proteins (*agn43*), protectins (*kpsM*), miscellaneous proteins (*malX*, *ipad*, *ECs3728*, *EcE24377A_0237*), and resistance genes (*armA*, *catB3*, *ARR-3*), were more prevalent in ST405 clones (Tables S6 and S7) [41,42].

Enhanced carbapenem resistance and virulence in ST405

The median MICs for imipenem and meropenem were 64 μg/mL in the ST405 clone and 32 μg/mL in both ST167 and ST410 clones (Figure 3(b)). A correlation between the copy number and expression levels of carbapenemase genes and the MIC values of carbapenem antibiotics in the strains was observed in *E. coli* [43]. Using quantitative reverse transcription polymerase chain reaction, we confirmed that the relative

Table 2. Univariate analysis of factors influencing in-hospital mortality in patients aged 65 and older with CREC infection.

Patient characteristics	Survival N = 45 n (%)	Non-survival N = 9 n (%)	*P* Value[a]	Odds ratio (95%CI)
Sex(male vs female)			0.709	1.333 (0.295–6.028)
Male	27 (60)	6 (67)		
Female	18 (40)	3 (33)		
Age(median)	75	76	0.168	1.065 (0.974–1.164)
ICU location on the day of CREC isolation	18 (40)	8 (89)	**0.024**	12.000 (1.380–104.336)
Comorbidities				
CCI (Charlson Comorbidity Index) (median)	2	4	**0.031**	1.522 (1.040–2.227)
Urinary system disease	27 (60)	6 (67)	0.709	0.75 (0.166–3.391)
Cerebrovascular disease	27 (60)	8 (89)	0.129	0.188 (0.022–1.630)
Chronic kidney disease	17 (38)	4 (44)	0.709	0.759 (0.179–3.223)
History of malignancy	12 (27)	3 (33)	0.684	0.727 (0.157–3.377)
Diablte disease	16 (36)	3 (33)	0.899	1.103 (0.243–5.017)
Polymicrobioal	21 (47)	8 (89)	**0.045**	9.143 (1.055–79.261)
ST(ST405 vs Non-ST405)			**0.004**	12.250 (2.190–68.510)
ST405	10 (22)	7 (78)		
Non-ST405	35 (78)	2 (22)		

[a]Univariate logistic regression as indicated; *P* value < 0.05 (bolded) was considered statistically significant.

Table 3. Multivariate analysis of factors influencing in-hospital mortality in patients aged 65 and older with CREC infection.

Variable	Odds ratio	95% CI	*P* value[a]
ICU location on the day of CREC isolation	12.497	0.198–787.855	0.232
CCI	2.960	1.089–8.045	**0.033**
Polymicrobioal	1.824	0.113–29.373	0.672
ST405	33.054	1.218–897.008	**0.038**

[a]Multivariate logistic regression as indicated; *P* value < 0.05 (bolded) was considered statistically significant.

expression and copy number of the bla_{NDM-5} genes in ST405 clones were higher than those in ST167 and ST410 clones (Figure S5). This may partially explain the higher MIC values for carbapenem antibiotics observed in ST405 clones compared with other dominant clones.

To assess the growth fitness of these clones, we conducted *in vitro* competition assays. We selected three isolates from ST167 and ST410 clones that exhibited comparable individual growth rates to the ST405 clone (Figure S4). These isolates were utilized in competition assays to assess the fitness of ST405 compared to ST167 and ST410 under both meropenem and non-meropenem conditions. No significant growth differences were observed in the absence of meropenem; however, ST405 exhibited a significant competitive advantage under meropenem pressure (1 μg/mL) (Figure 3(c and d)).

The survival rate of larvae infected with ST405 isolates in the *G. mellonella* infection model was significantly lower than those infected with ST167, ST410 and non-virulent isolates after 72 h ($P < 0.05$). However, the survival rate of larvae infected with ST405 was higher than that of larvae infected with the hypervirulent *Klebsiella pneumoniae* strain ATCC43816 ($P < 0.05$) (Figure 3(a)). Based on these data and comparisons with highly virulent isolates, we consider ST405 to be a relatively high virulence *E. coli* clone, demonstrating greater virulence than other major MDR CREC clones such as ST167 and ST410.

Global dissemination of ST405

We analysed metadata obtained from 2,062 ST405 *E. coli* isolates in the EnteroBase database. As of 13 July, 2024, the EnteroBase database consisted of 2,062 ST405 *E. coli*

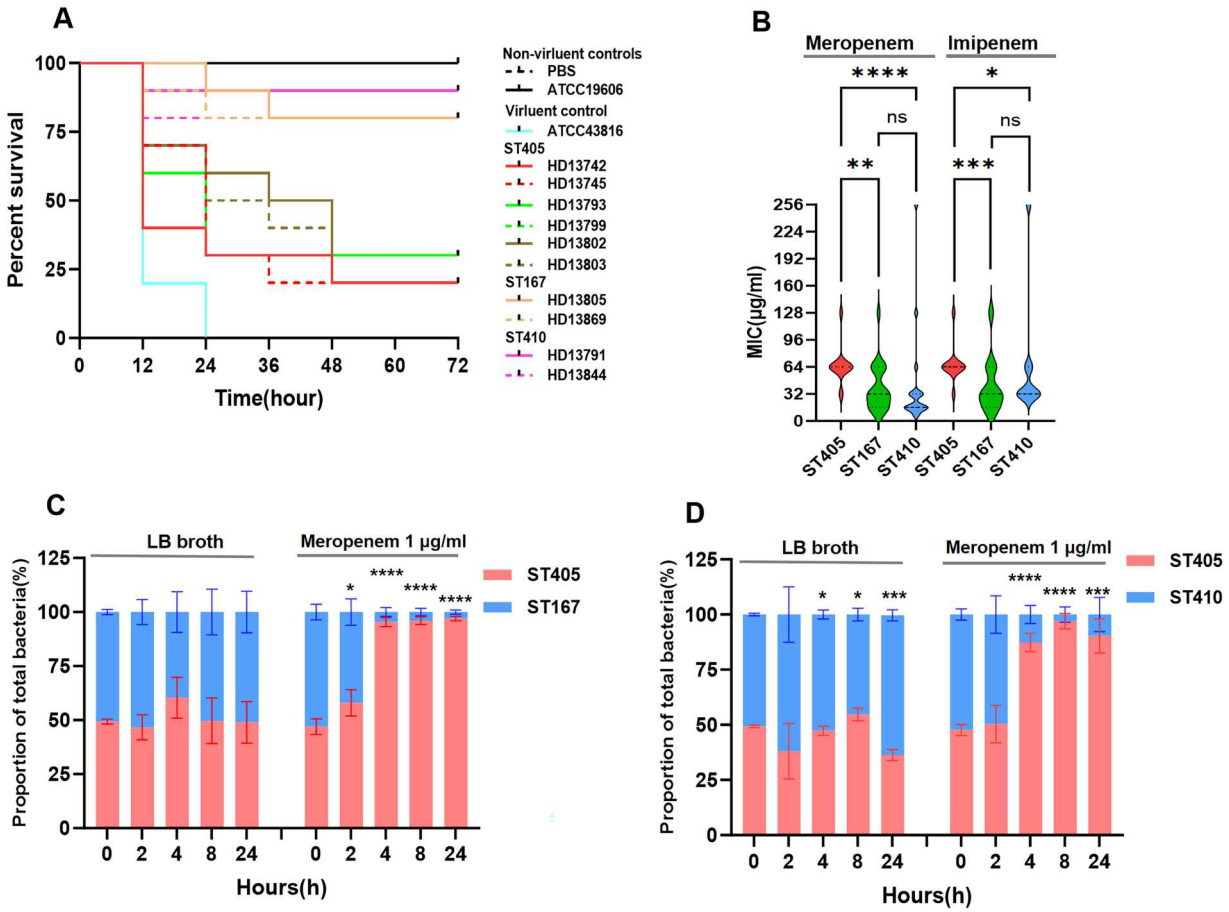

Figure 3. Clinical hazards of the CREC high-risk clone. (A) Survival curves for wax moth larvae (*G. mellonella*) infected with 2×10^5 CFU of different isolates from ST405, ST167 and ST410. (B) Comparison MIC of meropenem and imipenem for isolates from ST405, ST167 and ST410. (C) Comparison of the *in vitro* competitive growth ability between ST405 and ST167 in the presence or absence of meropenem. (D) Comparison of the i*n vitro* competitive growth ability between ST405 and ST410 in the presence or absence of meropenem. Statistical significance is indicated as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$. ns: not significant; CREC: carbapenem-resistant *Escherichia coli*; ST: sequence type; MIC: minimum inhibitory concentration.

isolates, covering 5 continents and 64 countries (Figure S6A and B). These isolates were primarily collected from humans, food, companion animals, poultry, wildlife and environmental samples (Figure S6C). The first carbapenemase-producing ST405 isolate was detected in 2013, demonstrating an increasing prevalence since then, surpassing 50% after 2018 (Figure S6D). Among the human-derived ST405 isolates, urine samples were the most predominant, followed by rectal swabs, blood and respiratory specimens (Figure S6E). Among these, the *fimH27* type was the most prevalent (70.6%, 1,456/2,062), followed by *fimH29* (19.2%, 396/2,062) (Figure S6G). Analysis of the two major *fimH* types revealed that ST405 *fimH29* isolates were predominantly derived from urine samples (49.74%), which was significantly higher than the 24.72% observed for *the fimH27* isolates ($P = 0.0284$) (Figure S6H). Furthermore, the proportion of *fimH29* isolates carrying carbapenemase genes was 64.78%, which was much higher than the 35.51% observed in *fimH27* isolates ($P < 0.0001$) (Figure S6I).

Phylogenetic analysis of 1,508 isolates with available genomic sequences indicated that ST405 primarily formed three distinct clades, corresponding to *fimH27*, *fimH29* and other *fimH* types (Figure S7). The isolates from this study primarily clustered with those from Asia, with a smaller number from North America and Oceania. Interestingly, one ST405 isolate from a companion animal also clustered with the isolates from this study (Figure S7). Among the *bla*ESBL genes, $bla_{\text{CTX-M-15}}$ was the most prevalent in the *fimH*29 ST405 isolates. In contrast, the ST405 CREC isolates in this study primarily carried $bla_{\text{CTX-M-55}}$ (Figure S7).

Discussion

We conducted *in silico* MLST typing and determined ST405, ST167 and ST410 as the dominant CREC clones in our hospital. Our integration of clinical data with *in vitro* experiments revealed that the ST405 CREC clone is particularly concerning because of its high virulence and resistance profile.

Previous studies have highlighted ST405 as a successful MDR ExPEC lineage, with rising infection rates worldwide and a strong association with bla_{NDM} and $bla_{\text{CTX-M}}$ variants [9,12,44–47]. While ST405 has been previously noted for its high virulence, similar to ST131, in terms of epithelial adherence, invasion, and resistance to serum bactericidal activity [48]. Both ST405 and ST131 are notable for their high virulence, as demonstrated by virulence gene profiling and animal models [24]. However, the clinical impact of ST405 CREC remains poorly understood.

Our study demonstrates that the ST405 CREC clone exhibits a significantly higher 30-day mortality rate (16.7%) compared to non-ST405 CREC infections, as well as greater virulence in *G. mellonella* assays than ST167 and ST410. In the multivariate analysis, ST405 CREC infection and the Charlson Comorbidity Index (CCI) were identified as independent risk factors for in-hospital mortality among patients aged 65 years and older. Notably, 83.3% of patients with ST405 infection originated from the intensive care unit (ICU), accounting for 62.5% of all ICU CREC infections. The presence of the ST405 CREC clone poses a significant threat to elderly and frail patients, particularly those admitted to the ICU.

Genomic studies have demonstrated that certain AMR determinants, virulence factors, and adaptive mechanisms may be critical for the persistence of high-risk clones [27,49]. Comparative genomics in our study reveals that ST405 harbours a greater number of resistance genes, virulence genes, and insertion sequences than non-ST405 clones. Without incurring substantial fitness costs, this genomic plasticity likely explains its persistence over a 7-year period in our hospital. Further comparative genomics analysis revealed that the macrolide resistance-associated genes *msr(E)* and *mph(E)*, as well as the invasiveness-associated gene *eaeX*, are uniquely present in the ST405 clone. This finding suggests that the enhanced dissemination and virulence of the ST405 clone may be driven by these specific genetic elements. We hypothesize that these virulence factors (Table S7), which are involved in iron acquisition, T3SS, invasiveness, and adhesion, play a crucial role in the increased pathogenicity and adaptability of the ST405 clone, potentially contributing to its higher virulence compared to ST167 and ST410 [50,51]. The unique combination of antimicrobial resistance and virulence in ST405 may account for its epidemiological success and severe clinical impact. Given its persistence and pathogenic potential, future studies should investigate the functional roles of these resistance and virulence genes in antimicrobial resistance, bacterial fitness, and disease severity.

A high-risk clone possesses the ability to effectively colonize hosts, adapt to diverse environments, spread efficiently, and cause severe or recurrent infections [19,49]. In our study, ST405 exhibited a strong potential for long-term colonization and recurrence. Previous reports have indicated that the ST405 CREC clone has been implicated in community transmission and is capable of colonizing the human gut [24,52]. Our findings strongly suggest that persistent colonization by these strains may lead to higher transmission rates, ultimately contributing to increased hospital mortality. Although the exact mechanisms of indirect ST405 CREC transmission remain unclear, potential sources may include colonization, medical equipment, or healthcare workers [53].

In vitro competitive growth assays revealed that ST405 exhibits a significant growth advantage under

meropenem pressure. We observed that the relative copy number and mRNA expression levels of the bla_{NDM-5} in ST405 clones were higher than those in ST167 and ST410 clones. Furthermore, in vitro experimental evolution under continuous meropenem pressure demonstrated an increase in bla_{NDM-5} copy number in CREC, and this phenotype persisted even after the removal of antibiotic pressure [43]. Other factors may also contribute to the elevated MIC, which warrant further investigation in future studies. All 24 patients infected with ST405 reported ICU admission history, polymicrobial infections and extensive antibiotic use, which may have caused ST405's increased resistance to carbapenems and other antibiotics [54]. Broad-spectrum antibiotic administration has been identified as a key driver of AMR development, particularly for resistant Gram-negative pathogens [55]. Antibiotics act as "colonisation assistants" by reducing the fitness of competitors, thereby improving the spread of resistant cells between hosts [56].

Despite a sampling bias that skewed the majority of ST405 isolates toward human sources, the detection of a minority from food, companion animals, poultry, wildlife, and environmental samples suggest that multiple transmission pathways may have facilitated its dissemination (Figure S6C). Global genomic data on ST405 strains indicate that *fimH29* is the second most common *fim* type of ST405 *E. coli*, predominantly isolated from urine, with a significant higher probability of carrying carbapenemases compared to *fimH27* isolates. The ST405 strains in our study belonged to the *fimH29* type, highlighting the need for vigilance against the spread of this high-risk clone. ST405 is a reservoir of CTX-M, with CTX-M-15 being the predominant variant globally. However, in our study, the ST405 clone primarily harboured $bla_{CTX-M-55}$(Figure S7), a major ESBL gene commonly identified in animal-derived *E. coli* [57].

MDR high-risk clones, such as ST405, are key drivers of the global increase in AMR. Their successful dissemination is due to their adaptability, enabling them to dominate within populations [58].

Previous studies have demonstrated that colistin resistance in *E. coli* is primarily mediated by *mcr-1* gene. Similarly, our study identified six colistin-resistant CREC isolates, all of which harboured *mcr-1* and belonged to five distinct sequence types (STs), none of which representing dominant clones in our study setting. The prevalence of *mcr-1*-carrying CREC strains in our work was 5.3% (6/113), exceeding the global average of 2.3% and the previously reported rate of 1.48% in China [9,59]. Consistent with other findings, we speculate that the spread of *mcr-1* in our setting is driven more by horizontal gene transfer and plasmid-mediated resistance than by clonal expansion [60]. Therefore, stringent surveillance measures are warranted to curb the horizontal dissemination of *mcr-1* among clinical isolates.

Our study was limited to a single region and did not include isolates from across China, it provides valuable information into the molecular and clinical characteristics of CREC within this specific locale. Considering the localized nature of CREC dissemination, understanding the molecular and clinical features of CREC in individual regions is crucial. This study was retrospective; thus, future prospective studies are warranted to monitor the spread of carbapenem-resistant isolates and address emerging trends.

Conclusion

In this study, we investigated the clinical and genomic characteristics of CREC isolates from patients between 2015 and 2022 at a tertiary hospital in China. We revealed that CREC ST405 clones are associated with increased virulence in patients with critical illness and exhibit high resistance to antibiotics, including carbapenems, posing a significant clinical treatment challenge. Increased vigilance is crucial to monitor the emergence and spread of these MDR and potentially highly virulent *E. coli* isolates in clinical settings.

Author contributions

Min Wang, Hong Du, and Qiang Feng were responsible for overall analysis development, supervision of the project, and review of the final manuscript; Hong Du acquired funding for the study; Zhifei Sun and Xinying Wang collected clinical data; Min Wang, Zhijun Zhang, and Jie Zhu performed laboratory work; Min Wang and Zhijun Zhang performed the statistical analysis; Min Wang and Zhifei Sun performed the bioinformatic analysis; Meijie Jiang and Shuping Zhao accessed the data in the study; Min Wang, Liang Chen, and Hong Du prepared the manuscript. All authors critically reviewed the manuscript and approved the final report before submission.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the Key Research and Development Project of Jiangsu Provincial Science and Technology Department (BE2023704).

Data availability statement

Data pertaining to this study are available from the corresponding authors upon request.

References

[1] Laxminarayan R, Sridhar D, Blaser M, et al. Achieving global targets for antimicrobial resistance. Science. 2016;353(6302):874–875. doi:10.1126/science.aaf9286

[2] GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet. 2024;404(10459):1199–1226. doi:10.1016/S0140-6736(24)01867-1

[3] Li X, Hu H, Zhu Y, et al. Population structure and antibiotic resistance of swine extraintestinal pathogenic *Escherichia coli* from China. Nat Commun. 2024;15:5811. doi:10.1038/s41467-024-50268-2

[4] Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2004;2:123–140. doi:10.1038/nrmicro818

[5] Denamur E, Clermont O, Bonacorsi S, et al. The population genetics of pathogenic *Escherichia coli*. Nat Rev Microbiol. 2021;19:37–54. doi:10.1038/s41579-020-0416-x

[6] Tenaillon O, Skurnik D, Picard B, et al. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol. 2010;8:207–217. doi:10.1038/nrmicro2298

[7] Léger A, Lambraki I, Graells T, et al. Characterizing social-ecological context and success factors of antimicrobial resistance interventions across the one health spectrum: analysis of 42 interventions targeting *E. coli*. BMC Infect Dis. 2021;21:873. doi:10.1186/s12879-021-06483-z

[8] Smit C, Lambert M, Rogers K, et al. One health determinants of *Escherichia coli* antimicrobial resistance in humans in the community: an umbrella review. Int J Mol Sci. 2023;24:17204. doi:10.3390/ijms242417204

[9] Li Y, Sun X, Dong N, et al. Global distribution and genomic characteristics of carbapenemase-producing *Escherichia coli* among humans, 2005-2023. Drug Resist Updat. 2024;72:101031. doi:10.1016/j.drup.2023.101031

[10] Li F, Ye K, Li X, et al. Genetic characterization of carbapenem-resistant *Escherichia coli* from China, 2015-2017. BMC Microbiol. 2021;21:248. doi:10.1186/s12866-021-02307-x

[11] Grundmann H, Glasner C, Albiger B, et al. Occurrence of carbapenemase-producing Klebsiella pneumoniae and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis. 2017;17:153–163. doi:10.1016/S1473-3099(16)30257-2

[12] Macesic N, Hawkey J, Vezina B, et al. Genomic dissection of endemic carbapenem resistance reveals metallo-beta-lactamase dissemination through clonal, plasmid and integron transfer. Nat Commun. 2023;14:4764. doi:10.1038/s41467-023-39915-2

[13] Ma J, Song X, Li M, et al. Global spread of carbapenem-resistant enterobacteriaceae: epidemiological features, resistance mechanisms, detection and therapy. Microbiol Res. 2023;266:127249. doi:10.1016/j.micres.2022.127249

[14] Qin X, Ding L, Hao M, et al. Antimicrobial resistance of clinical bacterial isolates in China: current status and trends. JAC Antimicrob Resist. 2024;6:dlae052. doi:10.1093/jacamr/dlae052

[15] Zhang R, Li Y, Chen J, et al. Population genomic analysis reveals the emergence of high-risk carbapenem-resistant *Escherichia coli* among ICU patients in China. J Infect. 2023;86:316–328. doi:10.1016/j.jinf.2023.02.004

[16] Ba X, Guo Y, Moran RA, et al. Global emergence of a hypervirulent carbapenem-resistant *Escherichia coli* ST410 clone. Nat Commun. 2024;15:494. doi:10.1038/s41467-023-43854-3

[17] Roer L, Overballe-Petersen S, Hansen F, et al. *Escherichia coli* sequence type 410 is causing new international high-risk clones. mSphere. 2018;3:e00337-18. doi:10.1128/mSphere.00337-18

[18] Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev. 2014;27:543–574. doi:10.1128/CMR.00125-13

[19] Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev. 2015;28:565–591. doi:10.1128/CMR.00116-14

[20] Taati Moghadam M, Mirzaei M, Fazel Tehrani Moghaddam M, et al. The challenge of global emergence of novel colistin-resistant *Escherichia coli* ST131. Microb Drug Resist. 2021;27:1513–1524. doi:10.1089/mdr.2020.0505

[21] Matsumura Y, Yamamoto M, Nagao M, et al. Association of fluoroquinolone resistance, virulence genes, and IncF plasmids with extended-spectrum-β-lactamase-producing *Escherichia coli* sequence type 131 (ST131) and ST405 clonal groups. Antimicrob Agents Chemother. 2013;57:4736–4742. doi:10.1128/AAC.00641-13

[22] Mujahid F, Rasool MH, Shafiq M, et al. Emergence of carbapenem-resistant uropathogenic *Escherichia coli* (ST405 and ST167) strains carrying bla(CTX-M-15), bla(NDM-5) and diverse virulence factors in hospitalized patients. Pathogens. 2024;13:964. doi:10.3390/pathogens13110964

[23] Naseer U, Sundsfjord A. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. Microb Drug Resist. 2011;17:83–97. doi:10.1089/mdr.2010.0132

[24] Mihaila L, Wyplosz B, Clermont O, et al. Probable intrafamily transmission of a highly virulent CTX-M-3-producing *Escherichia coli* belonging to the emerging phylogenetic subgroup D2 O102-ST405 clone. J Antimicrob Chemother. 2010;65:1537–1539. doi:10.1093/jac/dkq155

[25] Zhang X, Feng Y, Zhou W, *et al*. Cryptic transmission of ST405 Escherichia coli carrying bla (NDM–4) in hospital. Sci Rep. 2018;8(1):390. doi:10.1038/s41598–017–18910–w

[26] Huang J, Lv C, Li M, et al. Carbapenem-resistant *Escherichia coli* exhibit diverse spatiotemporal epidemiological characteristics across the globe. Commun Biol. 2024;7:51. doi:10.1038/s42003-023-05745-7

[27] Wang Q, Wang R, Wang S, et al. Expansion and transmission dynamics of high risk carbapenem-resistant Klebsiella pneumoniae subclones in China: an epidemiological, spatial, genomic analysis. Drug Resist Updat. 2024;74:101083. doi:10.1016/j.drup.2024.101083

[28] Horan TC, Andrus M, Dudeck MA. Cdc/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309–332. doi:10.1016/j.ajic.2008.03.002

[29] CLSI. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
[30] Peng Z, Liang W, Wang F, et al. Genetic and phylogenetic characteristics of Pasteurella multocida isolates from different host species. Front Microbiol. 2018;9:1408. doi:10.3389/fmicb.2018.01408
[31] Bankevich A, Nurk S, Antipov D, et al. Spades: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–477. doi:10.1089/cmb.2012.0021
[32] Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640–2644. doi:10.1093/jac/dks261
[33] Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–3903. doi:10.1128/AAC.02412-14
[34] Ingle DJ, Valcanis M, Kuzevski A, et al. In silico serotyping of *E. coli* from short read data identifies limited novel O-loci but extensive diversity of O:H serotype combinations within and between pathogenic lineages. Microb Genom. 2016;2:e000064. doi:10.1099/mgen.0.000064
[35] Roer L, Tchesnokova V, Allesøe R, et al. Development of a web tool for *Escherichia coli* subtyping based on fimH alleles. J Clin Microbiol. 2017;55:2538–2543. doi:10.1128/JCM.00737-17
[36] Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015;43:e15. doi:10.1093/nar/gku1196
[37] Page AJ, Taylor B, Delaney AJ, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom. 2016;2:e000056. doi:10.1099/mgen.0.000056
[38] Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 2021;49:W293–W296. doi:10.1093/nar/gkab301
[39] Feng Y, Liu L, Lin J, et al. Key evolutionary events in the emergence of a globally disseminated, carbapenem resistant clone in the *Escherichia coli* ST410 lineage. Commun Biol. 2019;2:322. doi:10.1038/s42003-019-0569-1
[40] Jønsson R, Struve C, Jenssen H, et al. The wax moth Galleria mellonella as a novel model system to study Enteroaggregative *Escherichia coli* pathogenesis. Virulence. 2017;8:1894–1899. doi:10.1080/21505594.2016.1256537
[41] Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*. Int J Med Microbiol. 2011;301:642–647. doi:10.1016/j.ijmm.2011.09.006
[42] Azam MW, Zarrilli R, Khan AU. Updates on the virulence factors produced by multidrug-resistant Enterobacterales and strategies to control their infections. Microorganisms. 2023;11:1901. doi:10.3390/microorganisms11081901
[43] Xiang G, Zhao Z, Zhang S, et al. Porin deficiency or plasmid copy number increase mediated carbapenem-resistant *Escherichia coli* resistance evolution. Emerg Microbes Infect. 2024;13:2352432. doi:10.1080/22221751.2024.2352432
[44] Ko W, Tseng S, Chou C, et al. Molecular epidemiology and comparative genomics of carbapenemase-producing *Escherichia coli* isolates from 19 tertiary hospitals in China from 2019 to 2020. Front Microbiol. 2023;14:1056399. doi:10.3389/fmicb.2023.1056399
[45] Takayama Y, Sekizuka T, Matsui H, et al. Characterization of the IncFII-IncFIB(pB171) plasmid carrying bla (NDM-5) in *Escherichia coli* ST405 clinical isolate in Japan. Infect Drug Resist. 2020;13:561–566. doi:10.2147/IDR.S232943
[46] Sumbana JJ, Santona A, Fiamma M, et al. Extraintestinal pathogenic *Escherichia coli* ST405 isolate coharboring blaNDM-5 and blaCTXM-15: a new threat in Mozambique. Microb Drug Resist. 2021;27:1633–1640. doi:10.1089/mdr.2020.0334
[47] Roy Chowdhury P, McKinnon J, Liu M, et al. Multidrug resistant uropathogenic *Escherichia coli* ST405 with a novel, composite IS26 transposon in a unique chromosomal location. Front Microbiol. 2018;9:3212. doi:10.3389/fmicb.2018.03212
[48] Shaik S, Ranjan A, Tiwari SK, et al. Comparative genomic analysis of globally dominant ST131 clone with other epidemiologically successful extraintestinal pathogenic *Escherichia coli* (ExPEC) Lineages. mBio. 2017;8:e01596-17. doi:10.1128/mBio.01596-17
[49] Kocsis B, Gulyás D, Szabó D. Emergence and dissemination of extraintestinal pathogenic high-risk international clones of *Escherichia coli*. Life (Basel). 2022;12:2077. doi:10.3390/life12122077
[50] Yao Y, Xie Y, Perace D, et al. The type III secretion system is involved in the invasion and intracellular survival of *Escherichia coli* K1 in human brain microvascular endothelial cells. FEMS Microbiol Lett. 2009;300:18–24. doi:10.1111/j.1574-6968.2009.01763.x
[51] Larzábal M, Marques Da Silva W, Riviere NA, et al. Novel effector protein EspY3 of type III secretion system from enterohemorrhagic *Escherichia coli* is localized in actin pedestals. Microorganisms. 2018;6:112. doi:10.3390/microorganisms6040112
[52] Bitar I, Piazza A, Gaiarsa S, et al. St405 NDM-5 producing *Escherichia coli* in Northern Italy: the first two clinical cases. Clin Microbiol Infect. 2017;23:489–490. doi:10.1016/j.cmi.2017.01.020
[53] Ding Y, Zhuang H, Zhou J, et al. Epidemiology and genetic characteristics of Carbapenem-resistant *Escherichia coli* in Chinese intensive care unit analyzed by whole-genome sequencing: a prospective observational study. Microbiol Spectr. 2023;11:e0401022. doi:10.1128/spectrum.04010-22
[54] Taur Y, Xavier JB, Lipuma L, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012;55:905–914. doi:10.1093/cid/cis580
[55] Boutzoukas AE, Komarow L, Chen L, et al. International epidemiology of carbapenemase-producing *Escherichia coli*. Clin Infect Dis. 2023;77:499–509. doi:10.1093/cid/ciad288
[56] Morjaria S, Schluter J, Taylor BP, et al. Antibiotic-induced shifts in fecal microbiota density and composition during hematopoietic stem cell transplantation. Infect Immun. 2019;87:e00206-19. doi:10.1128/IAI.00206-19
[57] Yang JT, Zhang LJ, Lu Y, et al. Genomic insights into global bla(CTX-M-55)-positive *Escherichia coli* epidemiology and transmission characteristics. Microbiol Spectr. 2023;11:e0108923. doi:10.1128/spectrum.01089-23

[58] Baker S, Thomson N, Weill FX, et al. Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. Science. 2018;360:733–738. doi:10.1126/science.aar3777

[59] Huang H, Dong N, Shu L, et al. Colistin-resistance gene mcr in clinical carbapenem-resistant Enterobacteriaceae strains in China, 2014–2019. Emerg Microbes Infect. 2020;9:237–245. doi:10.1080/22221751.2020.1717380

[60] Liu JH, Liu YY, Shen YB, et al. Plasmid-mediated colistin-resistance genes: mcr. Trends Microbiol. 2024;32:365–378. doi:10.1016/j.tim.2023.10.006