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# Risk factors and molecular characterization of carbapenem resistant *Escherichia coli* recovered from a tertiary hospital in Fujian, China from 2021 to 2023

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## Abstract

**Background** There is a serious public health concern regarding the emergence of carbapenem-resistant *Escherichia coli* (CREC). The purpose of this study is to identify the molecular characterization and risk factors of CREC in Fujian province, China.

**Methods** A total of 48 CREC isolates were collected from various clinical samples. The strains were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS). Susceptibility to antibiotics was determined by the standard broth microdilution method. Polymerase chain reaction (PCR) was used to screen common drug resistance genes. Multilocus sequence typing (MLST) was used to type isolates. RT-qPCR was used to detect gene expression of *acrA*, *acrB*, and *tolC*. Conjugation assays were used to analyze the transferability of plasmids carrying *mcr-1* or $bla_{NDM}$. Risk factors for CREC infection were identified by logistic regression analysis.

**Results** 48 CREC strains were collected, with 81.25% producing carbapenemase (CP-CREC), and 18.75% were not producing carbapenemase (no-CP-CREC). They belonged to 21 sequence type (STs) and five unknown STs. Perianal swabs were the main sample type, with 25 patients found to have hematological malignancies. All isolates of CP-CREC were found to contain $bla_{NDM}$ ($bla_{NDM-5}$ ($n=32$), $bla_{NDM-1}$ ($n=5$), $bla_{NDM-4}$ ($n=1$), and $bla_{NDM-13}$ ($n=1$)), among which one isolate co-existence $bla_{NDM-5}$ and $bla_{OXA-48}$. Two $bla_{NDM}$-positive strains, specifically $bla_{NDM-5}$ and $bla_{NDM-4}$, were found to co-habor *mcr-1* with ST617. Conjugation assays confirmed that $bla_{NDM-1}$, $bla_{NDM-13}$, and most $bla_{NDM-5}$(68.75%, 22/32) could be transferred between *E. coli* strains. Four of the 9 non-CP-CREC isolates had deletions in *ompC* and *ompF* with $bla_{CTX-M}$ production, while the other five showed high expression of *acrA*, *acrB*, and *tolC*. Antibiotics usage, antifungal treatment, detection of other pathogens (prior to CREC infection), and respiratory disease were identified as independent risk factors for CREC infection. The area under the receiver operating

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characteristic curve for the scoring system was 0.937. Youden's index, with sensitivity and specificity of 0.96 and 0.78, was maximal when 2 points were scored.

**Conclusions** In CP-CREC, carbapenem resistance is caused primarily by multiple types of $bla_{NDM}$, while non-CP-CREC is caused by loss of porin protein or high expression of efflux pumps coupled with carrying $bla_{CTX-M}$. CREC isolates were highly diverse in terms of ST, with a total of 21 STs identified. Here, we first describe a clinical strain of CREC from China both *mcr-1* and $bla_{NDM-4}$ with ST617. An easy-to-use scoring system was developed to diagnose CREC infections.

**Keywords** CP-CREC, No-CP-CREC, $bla_{NMD}$, m *cr-1*, Efflux pumps, Risk factor

## Introduction

*Escherichia coli* (*E.coli*) is an opportunistic pathogen that can cause various infections, including UTIs, meningitis, bacteriemia, pneumonia, surgical site infections, and sepsis. Carbapenem antibiotics are highly effective against a variety of infections caused by extended-spectrum beta-lactamase (ESBL)-producing and multidrug-resistant (MDR) gram-negative bacteria. Carbapenem-resistant *E.coli* (CREC) is becoming more common due to the widespread use of carbapenem antibiotics. A survey conducted in Europe found that 19% of *E. coli* strains were classified as CREC from 2013 to 2014 [1]. The China Antimicrobial Surveillance Network (CHINET) reported that the isolation rates of CREC ranged from 0.7 to 1.9% from 2016 to 2023.

Antibiotic resistance is a global issue [2], with limited new antibiotics available to treat bacterial infections. According to the WHO, MDR pathogens, also known as 'superbugs', are a significant global threat, causing millions of deaths annually [3]. Carbapenems are crucial antibiotics used as a last resort for serious infections. Carbapenem resistance is mediated either by intrinsic mechanisms (such as coupled efflux pumps, AmpC overexpression, and porin loss) or by development of a carbapenemase [4]. First, transmissible carbapenemases are categorized into three classes: class A (serine carbapenemases, such as $bla_{KPC}$), class B (metallo-β-lactamases, such as $bla_{IMP}$, $bla_{VIM,}$ and $bla_{NDM}$) and class D ($bla_{OXA}$, such as $bla_{OXA-23}$ and $bla_{OXA-48}$) [5]. Second, in *E. coli*, AcrAB-TolC is the primary efflux pump. Research has found that AcrAB-TolC is linked to resistance against aminoglycosides, carbapenems, and other antibiotics [6]. Shiela Chetri et al., found a new link between ertapenem resistance and AcrA overexpression, as well as an increase in AcrB expression in *E. coli* under imipenem stress [7]. However, Howard T. H. Saw et al., found that efflux inhibitors may not enhance carbapenem effectiveness but instead could boost resistance in carbapenemase-producing organisms [8]. Lastly, a lack of certain outer membrane proteins (such as OmpF and OmpC) in *E. coli* can impact its response to carbapenem antibiotics. Yigit et al. [9]. found that changes in the outer membrane proteins OmpF and OmpC in *Enterobacter* strain 810 led to resistance to imipenem and decreased susceptibility to meropenem and cefepime.

CREC is a major clinical concern due to its resistance to all beta-lactam antibiotics and multiple resistance determinants, which limit treatment options. Polymyxin B is a last resort for treating multidrug resistant bacteria infections. A plasmid-mediated colistin *mcr-1* resistance gene was found by China in 2015 [10]. Since then nine derivatives (*mcr-2* to *mcr-10*) have been found in humans, animals, foods, and other sources [11]. While *mcr* is less common in clinical strains than in animal isolates, more reports of colistin-resistant carbapenemase-producing bacteria with combinations of $bla_{NDM}$ or $bla_{KPC}$ and *mcr* [12].

It has been shown that venous catheterization, exposure to penicillin and broad-spectrum cephalosporins, a longer hospital stay, presence of a urinary catheter, and intubation are independent risk factors for carbapenem-resistant *Enterobacter cloacae* infection [13, 14]. Limited data are available on the prevalence and mechanisms of CREC isolates from China. The risk factors for CRE infection have been examined in several studies [15], however, few studies have specifically assessed the risk factors for CREC acquisition.

Monitoring drug resistance in bacteria and understanding carbapenem resistance mechanisms can help improve antibiotic prescription guidelines and infection control strategies. This study analyzed the clinical and bacterial molecular characteristics of patients with CREC infections from 2021 to 2023 in order to identify risk factors. On the basis of the risk factors identified, we attempted to develop a scoring system to detect patients at-risk for CREC infection who have *E. oli*.

## Materials and methods

### Bacteria Isolates identification and clinical data collection

CREC isolates were obtained from Fujian Medical University Union Hospital (Fuzhou, China) between March 2021 and September 2023. The isolates were obtained from a variety of clinical specimens. We excluded duplicate isolates of the same species and specimen type from the same patient during the same year. For the defined CREC isolates, we evaluated susceptibility using broth

microdilution and disk diffusion, interpreting the results using the Clinical and Laboratory Standards Institute (CLSI) breakpoints. Resistances to imipenem (4 μg/ml or 19 mm), meropenem (4 μg/ml or 19 mm), or ertapenem (4 μg/ml or 18 mm) were defined as CRECs. All *E. coli* isolates from patients meeting the inclusion criteria of the study were stored in the laboratory at -80 °C in cryovials containing 20% glycerol and nutrient broth for further analysis. MALDI-TOF/MS was used to identify the bacterial species of the collected isolates using Bruker Biotyper™ system (Bruker Daltonics Inc., Billerica, Massachusetts). The clinical data used in this study were retrospectively analyzed.

#### Resistance and virulence genes confirmation

Analyzing the gene sequence of carbapenemase confirmed the detection of carbapenemase genes ($bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{KPC}$, $bla_{SME}$, $bla_{GIM}$, $bla_{SIM}$, $bla_{IMI}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{GES}$, and $bla_{OXA\text{-}181}$). We conducted PCR assays to detect other antibiotic-resistant genes. The presence of resistance genes includes ESBLs ($bla_{CTX\text{-}M\text{-}1\text{-}like}$, $bla_{CTX\text{-}M\text{-}2\text{-}like}$, $bla_{CTX\text{-}M\text{-}8\text{-}like}$, $bla_{CTX\text{-}M\text{-}9\text{-}like}$, and $bla_{CTX\text{-}M\text{-}10\text{-}like}$ ), non-ESBL genes (*TEM* and *SHV*), fluoroquinolones (*qnrS*, *qnrA*, *qnrB*, *qepA*, and *gyrA*), sulfonamides (*sul1*), tetracyclines (*tetA*), *streptomycin* (*strA*), aminoglycosides (*ant3*, *aac6-IB*, *aac3-II*, *armA*, and *rmtB*), and porin-related genes (*OmpC* and *OmpF*). Genetic analysis of virulence genes: the following 9 virulence genes (i.e. *TraT*, *papC*, *afaC*, *iucD*, *hylA*, *ecpA*, *fimH*, *ompT*, and *iutA*) were examined by PCR in all strains. The sequence of primers is shown in Tables S1 and S2. Sequencing of positively amplified products was performed using the Sanger sequencing method, and a comparison was made with the NCBI BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/).

#### Antimicrobial susceptibility testing

We measured the minimum inhibitory concentration (MIC) of antibiotics as follows: cephalosporins (cefepime, ceftazidime), β-lactam/β-lactamase inhibitor combinations (cefoperazone-sulbactam, piperacillin-tazobactam), carbapenems (imipenem, meropenem), aminoglycosides (tobramycin, amikacin), tetracycline (minocycline, tigecycline), fluoroquinolones (ciprofloxacin, levofloxacin), trimethoprim–sulfamethoxazole, aztreonam, and polymyxin B using the standard broth microdilution method according to CLSI. In all cases except for tigecycline, antibiotic susceptibilities were based on the CLSI document standard. FDA standards were used to determine the breakpoint for tigecycline (susceptible: MIC≤2 mg/L; resistant: MIC≥8 mg/L). As controls, *E. coli* ATCC 25,922 and *P.aeruginosa* ATCC 27,853 were used.

#### Multilocus sequence typing

We performed MLST by PCR amplification and sequencing 7 housekeeping genes: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* for *E. coli*. The sequence of primers is shown in Table S3. The MLST database (www.mlst.net) assigned STs.

#### Transcriptional analysis real-time quantitative PCR

We extracted and transcribed RNA as described previously [16]. RT-qPCR was used to estimate relative gene expression of *acrA*, *acrB*, and *tolC* using the primer sets in Table S4. As a normalized standard, we used the 16 S rRNA gene as the normalized method. The obtained values were then normalized against those from carbapenem antibiotics susceptible strains.

#### Transferability of plasmids carrying *mcr-1* and $bla_{NDM}$

We studied the transferability of *mcr-1* or $bla_{NDM}$ using EC600 (which has rifampicin resistance) as the recipient bacteria. A total of 600μL of recipient bacteria (EC600) and 200μL of donor bacteria were mixed and cultured in an incubator at 37 °C for about 18 h. Double-resistant MH plates containing polymyxin B or meropenem and rifampicin were used to culture suspected donor, recipient, and mixed bacteria. As a determination method, only mixed bacteria grew colonies on the double-resistant plate, while neither the recipient bacteria nor the donor bacteria did. Transconjugants expressing *mcr-1* or $bla_{NDM}$ were confirmed by PCR.

#### Clinical data collection

For this study, 48 CREC isolates were collected from 48 patients. Due to missing data for 2 of the case patients, 46 cases were included in our study. The study defined a case as a patient from whom CREC was isolated in any clinical culture. At least 48 h after admission, patients with isolated CSEC from a clinical culture were considered controls. Controls were recruited in a 2:1 ratio to cases. The study matched cases and controls based on age, clinical manifestations, pathogens, hospital wards, date of admission, and other relevant factors. Cases and controls could only be included once in each study. The clinical medical record system was used to collect demographic and clinical data.

Several factors were examined as possible risk factors for the emergence of CREC, including antibiotic use (such as aminoglycosides, β-lactams, carbapenems, cephalosporins, quinolones, and polymyxin B), and invasive surgery. As well as the ECOG scores, the presence of multiple infections including in the lungs, urinary tract, blood, and abdominal cavity, was examined. We collected data on disease diagnoses, age, hospital ward, hospitalizations, outcome, invasive operation (such as PICC, catheter, and so on), co-morbidities (hypertension, diabetes,

solid tumor, surgical history, cardiac diseases, disease of the urinary system, liver disease, respiratory disease, and digestive system disease) and sample origin data for all CREC isolates at the same time.

### Statistical analysis

IBM SPSS ver. 21.0 statistical software (IBM Co., Armonk, NY) was used to analyze the data. In order to analyze categorical variables, frequency tables (n, %) and descriptive statistics (mean, median, standard deviation) were used. Logistic regression (Backward LR) was used for risk factor analysis (univariate and multivariate). Statistical significance was determined by the P value < 0.05.

## Results

### Clinical and microbiological characteristics

In this study, the detection rates of CREC from 2021 to 2023 were 3.0%, 2.8%, and 3.82%, respectively. 48 CREC isolates were collected from March 2021 to September 2023. With an age range of 3–98 years, the mean age of the patients was 46.14 years. 25 (52.08%) patients were diagnosed with hematological malignancies, 7 (18.6%) with urological malignancy, 4 (8.33%) with lung conditions, 3 (6.25%) with liver-related diseases, 3 (6.25%) with urinary-related diseases, 2 (4.16%) with acute pancreatitis, 2 (4.16%) with acute pancreatitis surgical wound infection, and 5 (10.42%) with other disease (cervical malignancy, osteoporosis and so on). Among the specimens, perianal swab ($n$=12,25%), urine ($n$=10, 20.83%), drainage fluid ($n$=7, 14.58%), stool ($n$=7, 14.558%), secretors ($n$=5, 10.42%), blood ($n$=4,8.33%), and sputum ($n$=3, 6.25%) were procured. Of the 48 carbapenem-resistant cases, 81.25% (39/48) produced carbapenemase (carbapenemase-producing CREC, CP-CREC), while 18.75% (9/48) did not produce carbapenemase (non carbapenemase-producing CREC, non-CP-CREC). Furthermore, ESBL production was observed in 87.5% (42/48) of the isolates.

### Resistance genes and virulence genes profile

A further test was performed to determine whether the CREC strains carried genes for carbapenemase. It was observed that 64.58% (31/48) of isolates carried $bla_{\text{NDM-5}}$ with the higher prevalence of $bla_{\text{NDM-1}}$ (10.42%, 5/48), while $bla_{\text{NDM-4}}$ and $bla_{\text{NDM-13}}$ were detected in 3.8% (1/48) of strains, respectively. One of 48 isolates had two carbapenem-resistance genes ($bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$) present simultaneously. We screened all the CREC strains for the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-8*, and *mcr-9* genes.Two strains carried *mcr-1* but not the other *mcr* genes, which coexist with $bla_{\text{NDM}}$.

Moreover, bacteria also possess genes encoding resistance to antibiotics such as beta-lactams, sulfonamides, streptomycin, aminoglycosides, quinolones, and tetracycline. Among the ESBL-resistance genes positive strains (39/48,81.25%), 25 carried the $bla_{\text{CTXM-10}}$, 15 carried the $bla_{\text{CTXM-1}}$, 12 carried the $bla_{\text{CTXM-9}}$ gene, and 7 carried the $bla_{\text{CTXM-9}}$. 33.3% (16/48) of isolates had $bla_{\text{CTX-M}}$ and *TEM* genes simultaneously, along with 2.08% (1/48) of isolates that had both $bla_{\text{CTX-M}}$ and *SHV* genes. In total, 40 aminoglycoside resistance gene-positive CREC isolates were PCR positive for *ant3* (10 isolate), *ant3* plus *aac3-II* (10 isolates), *aac3-II* (8 isolates), *aac3-II* plus *aac6-IB* (3 isolates), *ant3* plus *rmtB* (2 isolates), *ant3*, *aac3-II* plus *rmtB* (2 isolates), *ant3* plus *aac6-IB* (2 isolates), *aac6-IB*, *aac3-II* plus *rmtB* (2 isolates), *ant3*, *aac3-II*, *rmtB* plus *aac6-IB* (1 isolates), *ant3*, *aac3-II*, *armA* plus *aac6-IB* (1 isolates), *aac6-IB* and *aac3-II* (1 isolates), *ant3*, *aac6-IB* and *aac3-II* (1 isolates), *aac6-IB* (1 isolates), *ant3* and *aac3-II* (1 isolates).

In total, 4 quinolone resistance genes were found, including 20 strains carrying *gyrA* (41.67%), 5 strains carrying *qnrS* (10.42%), 10 strains carrying both *gyrA* and *qnrS* (20.83%), 3 strains carrying both *gyrA* and *qepA* (6.25%), 1 strain carrying *qnrS*, *gyrA*, and *qepA* (2.08%), and 1 strain carrying *qnrS*, *qnrB*, and *gyrA* (2.08%). In addition, the *tetA*, *sul1* and *strA* genes were detected in 38, 20, and 13 isolates, respectively (Table 1; Fig. 1).

It was found that the most prevalent virulence-associated gene was *fimH* (93.75%, 45/48), followed by *ecpA* (89.6%, 43/48), *traT* (60.4%,29/48), *iucD* (45.8%, 22/48), *ompT* (35.4%,17/48), *afaC* (4.2%,2/48), papC (2.1%,1/48) (Fig. 2).

*CTX-M* type *ESBLs* were found in all non-CP-CREC. Of the 9 non-CP-CREC isolates, 44.44% ($n$=4) showed deletion in *ompC* and *ompF* porin-encoding genes (Table 2). The other five non-CP-CREC without pore-protein deletion had high expression of the efflux pumps genes *acrA*, *acrB*, and *tolC* (Fig. 3).

### Genetic profiling and antimicrobial susceptibility analysis

In order to understand the genetic variability of the CREC, MLST was conducted. In total, 48 CREC belonged to 21 STs and five unknown STs (untypable). As shown in Table 1, the most common ST was ST410 ($n$=9), followed by ST5229 ($n$=4), ST38 ($n$=3), ST405 ($n$=3), ST648 ($n$=3), ST617 ($n$=2), ST10 ($n$=2), ST155 ($n$=2), ST69($n$=2), and ST617 ($n$=2), and then by single ST isolates, including ST58, ST539, ST641, ST88, ST156, ST167, ST44, ST457, ST1730, ST297, ST361, and ST48 (Table 1).

All CREC isolates showed a high-level resistance to carbapenems, with 100% resistance to cephalosporins, including cefazolin, ceftazidime, ceftriaxone, and cefepime. Other antimicrobials showed irregular susceptibility and resistance, such as piperacillin-tazobactam (97.9%), ciprofloxacin (95.8%), levofloxacin (87.5%), sulfamethoxazole-trimethoprim (85.4%), gentamicin (76.2%),

**Table 1** Molecular characterization of 48 CREC strains

| Sample | Ward | Strain | Antimicrobial Resistance Genes | Virulence genes | MLST |
|---|---|---|---|---|---|
| Perianal swab | Hematology | CREC108 | *$bla_{CTX-M-10}$/sul1/aac3-II/TetA* | *ompC/ompF/afaC/ecpA/fimH* | ST38 |
| | Hematology | CREC054 | *$bla_{CTX-M-1}$/$bla_{CTX-M-10}$/sul1/aac3-II/TetA* | *ompC/ompF/afaC/ecpA/fimH* | ST38 |
| | Hematology | CREC821 | *SHV/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/ant3/aac6-Ib/aac3-II /armA/qnrS/qnrB/gyrA/TetA* | *ompC/ompF/TraT/ecpA/fimH* | |
| | Hematology | CREC761 | *TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-10}$/sul1/ant3/rmtB /gyrA/TetA* | *ompC/ompF/TraT/iucD/ecpA/fimH* | |
| | Hematology | CREC229 | *TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-10}$/strA/$aac3_{-II}$/qnrS /gyrA/TetA* | *ompC/ompF/TraT/ecpA/fimH* | ST405 |
| | Hematology | CREC640 | *$bla_{NDM-5}$/TEM/$bla_{CTX-M-10}$/sul1/ant3/qnrS /TetA* | *ompC/ompF/ecpA/fimH* | ST10 |
| | Hematology | CREC038 | *TEM/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/strA/aac6-Ib/aac3-II /gyrA/TetA* | *ompF/TraT/iucD/ecpA* | ST648 |
| | Hematology | CREC036 | *$bla_{NDM-5}$/TEM/$bla_{CTX-M-9}$/sul1/$aac3_{-II}$/TetA* | *TraT/iucD/ecpA/fimH/ompT* | ST88 |
| | Hematology | CREC435 | *TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/strA/sul1/ aac6-Ib/aac3-II/rmtB/qepA/gyrA/TetA* | *TraT/ecpA/fimH* | ST405 |
| | Hematology | CREC254 | *TEM/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/$bla_{strA}$/$aac6_{-Ib}$ /aac3_II /qepA/gyrA/TetA* | *TraT/iucD/ecpA* | ST648 |
| | Organ transplantation | CREC005 | *$bla_{NDM-5}$/TEM/ant3/qnrS/gyrA/TetA/* | *fimH/* | ST641 |
| | Hematology | CREC428 | *$bla_{NDM-5}$/strA/sul1/$aac3_{-II}$/qnrS/gyrA /TetA* | *TraT/iucD/ecpA/fimH/ompT* | ST69 |
| Stool | Hematology | CREC071 | *$bla_{NDM-1}$/sul1/ant3/$aac6_{-Ib}$/qnrS/TetA/* | *ecpA/fimH/* | |
| | Hematology | CREC301 | *$bla_{NDM-1}$/$bla_{CTX-M-10}$/sul1/ant3/aac6-Ib/aac3-II /qnrS/TetA* | *ecpA/fimH* | |
| | Hematology | CREC262 | *$bla_{NDM-5}$/sul1/ant3/aac6-Ib* | *ecpA/fimH* | |
| | ICU | CREC174 | *$bla_{NDM-5}$/$bla_{OXA-48}$/$bla_{OXA-181}$/TEM/sul1/ant3* | *ecpA/fimH* | ST410 |
| | Hematology | CREC937 | *$bla_{NDM-1}$/TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$ /strA/sul1/ant3/aac6-Ib/aac3-II/rmtB /qnrS/qepA/gyrA/TetA* | *TraT/iucD/ecpA/fimH/ompT* | ST155 |
| | Hematology | CREC109 | *$bla_{NDM-5}$/TEM/strA/ant3/aac3-II/qnrS/gyrA /TetA* | *TraT/iucD/ecpA/fimH/ompT* | ST155 |
| | Hematology | CREC009 | *TEM/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$/ant3/aac3-II/qnrS /gyrA /TetA* | *ompC/ompF/TraT/iucD/ecpA/fimH* | |
| Blood | Hematology | CREC084 | *$bla_{NDM-5}$/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$*/ant3/gyrA/TetA | *iucD/ecpA/fimH* | ST410 |
| | Hematology | CREC860 | *TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-10}$/ant3/aac3-II/rmtB /gyrA/TetA* | *ompC/TraT/iucD/ecpA/fimH/ompT* | ST410 |
| | Hematology | CREC102 | *$bla_{CTX-M-10}$/sul1* | *ompC/ompF/ecpA/fimH/ompT* | ST539 |
| | Hematology | CREC859 | *$bla_{NDM-5}$/TEM/ant3/aac3-II /qnrS /gyrA /TetA/* | *ompC/ompF/TraT/ecpA/fimH* | ST58 |
| Sputum | Hematology | CREC710 | *$bla_{NDM-5}$/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$ /ant3 /gyrA /TetA* | *TraT/iucD/ecpA/fimH/ompT* | ST410 |
| | ICU | CREC831 | *$bla_{NDM-5}$/TEM/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$ /ant3 /aac3-II/gyrA/TetA* | *ompC/ompF/TraT/iucD/ecpA/fimH/ompT* | ST48 |
| | Respiratory | CREC336 | *$bla_{NDM-5}$* | *ompC/ompF/TraT/papC/iucD/ecpA/fimH /ompT* | ST648 |
| Urine | Cadre | CREC916 | *$bla_{NDM-1}$/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$/sul1/aac3-II /gyrA* | *ompC/ompF/TraT/iucD/ecpA/fimH* | ST44 |
| | urology | CREC609 | *$bla_{NDM-5}$/sul1/ant3/TetA* | *ompC/ompF/TraT/ecpA/fimH* | ST361 |
| | urology | CREC225 | *$bla_{NDM-5}$/strA/gyrA/TetA* | *TraT/iucD/ecpA/fimH* | ST457 |
| | Rheumatology | CREC920 | *$bla_{NDM-5}$/TEM/ $bla_{CTX-M-1}$/$bla_{CTX-M-10}$ /strA /qepA/gyrA* | *TraT/ecpA/fimH/ompT* | ST410 |
| | Emergency | CREC981 | *$bla_{NDM-13}$/TEM/ant3/qnrS/gyrA/TetA* | *TraT/iucD/fimH/ompT* | ST5229 |
| | urology | CREC920 | *$bla_{NDM-5}$/$bla_{CTX-M-9}$/strA/ant3/aac3_II/gyrA /TetA* | *TraT/ecpA/fimH/ompT* | ST156 |
| | Medical oncology | CREC231 | *$bla_{NDM-5}$/TEM/ant3/aac3-II/gyrA/TetA* | *fimH* | ST5229 |
| | Gastroenterology | CREC346 | *$bla_{NDM-5}$/TEM/ant3/aac3_II/gyrA* | *ompT* | ST167 |
| | Hematology | CREC961 | *$bla_{NDM-5}$/TEM/strA/gyrA/TetA* | *ompC/ompF/iucD/ecpA/fimH/ompT* | ST410 |
| | Gastroenterology | CREC055 | *$bla_{NDM-5}$/mcr−1/TEM/$bla_{CTX-M-1}$/$bla_{CTX-M-10}$ /strA/ant 3/aac3_II/qnrS/gyrA/TetA/* | *ompC/ompF/TraT/ecpA/fimH/ompT* | ST617 |

**Table 1** (continued)

| Sample | Ward | Strain | Antimicrobial Resistance Genes | Virulence genes | MLST |
|---|---|---|---|---|---|
| Drainage fluid | Colorectal | CREC743 | $bla_{CTX-M-1}$/$bla_{CTX-M-10}$/$aac6_{-Ib}$/*gyrA* | *ompF/TraT/iucD/ecpA/fimH* | ST405 |
| | Emergency | CREC765 | $bla_{NDM-5}$/$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/*sul1/ant3/rmtB/gyrA* | *ompC/ompF/ecpA/fimH* | ST410 |
| | Emergency | CREC648 | $bla_{CTX-M-9}$/$bla_{CTX-M-10}$/*gyrA* | *ompC/ompF/ecpA/fimH* | ST38 |
| | Colorectal | CREC801 | $bla_{NDM-5}$/*mcr−1/TEM/ant3/aac3-II /gyrA/TetA* | *ompC/ompF/fimH* | ST5229 |
| | General | CREC339 | $bla_{NDM-4}$/*mcr−1/TEM/*$bla_{CTX-M-1}$/$bla_{CTX-M-9}$ /$bla_{CTX-M-10}$/$_{strA}$/*sul1/ant3/gyrA/TetA* | *ompC/ompF/TraT/iucD/ecpA/fimH/ ompT* | ST617 |
| | Colorectal | CREC639 | *sul2/aac3-II/qnrS/TetA* | *ompC/ompF/TraT/iucD/ecpA/fimH/ ompT* | ST69 |
| Secretion | Gynecology | CREC701 | $bla_{NDM-5}$/*TEM/aac3-II/gyrA* | *ecpA/fimH* | ST297 |
| | Emergency | CREC542 | $bla_{NDM-5}$/*TEM/*$bla_{CTX-M-9}$/$bla_{CTX-M-10}$/*sul1/ant3/*$aac3_{-II}$ */rmtB/qnrS/gyrA/TetA* | *ompC/ecpA/fimH* | ST410 |
| | Colorectal | CREC155 | $bla_{NDM-5}$/*ant3/qnrS/gyrA/TetA* | *ompC/ompF/TraT/ecpA/fimH* | ST1730 |
| | Colorectal | CREC677 | $bla_{NDM-5}$/*TEM/ant3/*$aac3_{-II}$/*gyrA/TetA* | *TraT/iucD/ecpA/fimH* | ST5229 |
| | Hematology | CREC837 | $bla_{NDM-5}$/$bla_{CTX-M-9}$/*sul1/gyrA* | *ecpA/fimH* | ST410 |
| | Burn | CREC642 | $bla_{NDM-1}$/*qnrS/TetA* | *TraT/ecpA/fimH* | ST10 |

aztreonam (62.5%), and tobramycin (50%), whereas the highest susceptibility was recorded for polymyxin B (4.17%) (Fig. 4).

### Results of transferability of plasmids carrying *mcr-1* or $bla_{NDM}$

Conjugation assays confirmed that $bla_{NDM-1}$, $bla_{NDM-13}$, and most $bla_{NDM-5}$ (68.75%, 22/32) could be transferred between *E. coli* strains, with an observed transfer frequency ranging from $4.19\times10^{\wedge-1}$ to $1.80\times10^{\wedge-4}$. The antibiotic susceptibility testing results showed that the transconjugants, confirmed by PCR and sequencing, were resistant to imipenem (4 mg/L). It was found that the $bla_{NDM-4}$ gene carried on the plasmid in strain CREC339 could not be transferred to EC600. The plasmid carrying the *mcr-1* gene from strains CREC055 and CREC339, as well as the plasmid carrying the $bla_{NDM-5}$ gene from 10 CREC strains, were unsuccessfully transferred to the recipient.

### Risk factors for CREC infection

Due to incomplete case data, two patients detected by CREC infection were excluded. A comparison of the risk factors for acquiring CREC between CREC and CSEC groups is presented in Table 3, based on univariate and multivariate analyses. Using univariate conditional logistic regression analysis, it was demonstrated that hospital stay (>30days), hospitalizations (>3times), PICC, exposure to antibiotic agents (cephalosporin, aminoglycosides, fluoroquinolones, carbapenems, and antifungal agents), detection other pathogens (prior to CREC infection), surgical history, and respiratory disease were all risk factors for CREC infection. The multivariate conditional logistic regression analysis demonstrated that antibiotic usage ($P$=0.004), antifungal use ($P$=0.017), detection of other pathogens (prior to CREC infection) ($P$=0.000), and respiratory disease ($P$=0.016) were identified as independent risk factors for CREC infection (Table 3). After identifying these risk factors, we evaluated whether they could be used as scores to identify CREC infection. A point was assigned to each risk factor, resulting in a total score ranging from zero to four. For the purpose of determining the cutoff value for identifying cases with CREC infection, we performed receiver operating characteristic (ROC) curve analysis (Fig. 5). ROC analysis indicated high accuracy, with an area under the curve (AUC) of 0.937. Table 4 shows the sensitivity and specificity of each score. Youden's index, with sensitivity and specificity values of 0.96 and 0.78, was maximal when 2 points were scored.

## Discussion

It is important to know the key traits of CREC strains, such as antibiotic resistance and virulence, in order to effectively control their transmission. As far as we are aware, few studies have evaluated the risk factors for CREC infection. We aimed to investigate the molecular characterization of CREC and assess the potential risk factors for hospital-acquired CREC infection from matched case-control studies. In this study, the detection rate of CREC was generally stable at around 3.0% from 2021 to 2022, execpt for 2023. We suspect that the relative increase in the detection rate in 2023 may be related to infections co-occurring after the novel coronavirus infection.

The production of $bla_{NDM}$ is the major mechanism of CREC. Notable variations in carbapenemase production were observed among strains from different countries and regions. Greek and Israeli strains primarily produce carbapenemase class A *KPC* [17], while class D *OXA-48* is most common in Europe, particularly in Spain and France [18]. *NDM*-producing *enterobacteriaceae* are primarily found in the Iran [19], Indian subcontinent [20], and China [21]. However, in Anhui Province of China,

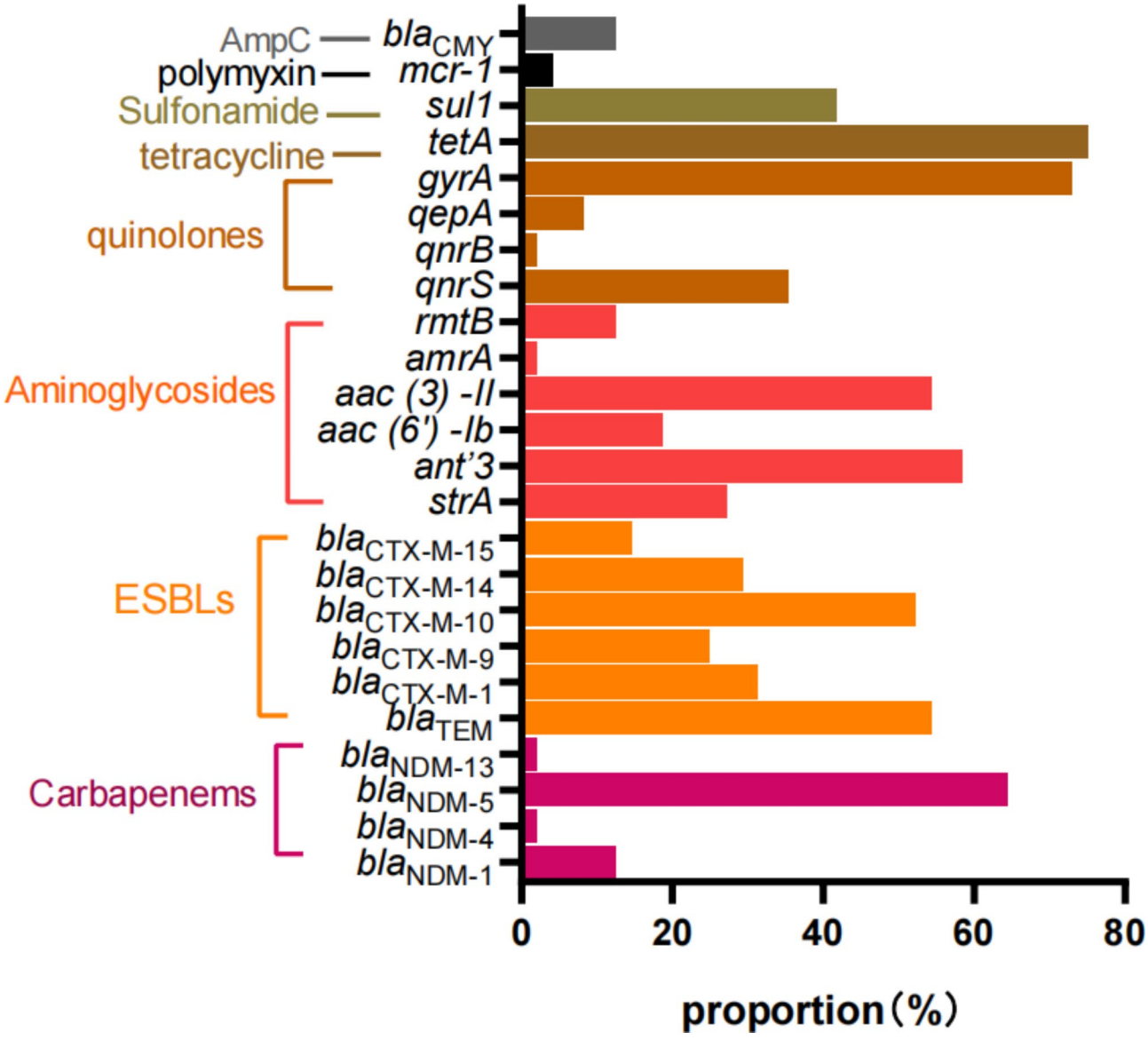


**Fig. 1** Molecular characterization of resistance genes in CREC isolates as detected by PCR

89.4% of *CREC* isolates had $bla_{\text{KPC-2}}$ [22]. $Bla_{\text{NDM}}$ was the main carbapenemase found in CREC, with $bla_{\text{NDM-5}}$ being the most common variant in China [23]. In this study, the resistance gene screening indicated that the $bla_{\text{NDM-5}}$ subtype was the most prevalent, followed by $bla_{\text{NDM-1}}$, $bla_{\text{NDM-4}}$, and $bla_{\text{NDM-13}}$. These findings suggest that the predominant mechanism of carbapenem resistance in *E. coli* is the production of *NDM*-type carbapenemases. This is consistent with the literature report. There have been reports that $bla_{\text{NDM}}$ is carried on plasmids with a variety of replicon types [24]. In addition, our study confirmed that the majority of $bla_{\text{NDM}}$ (71.80%, 28/39) could be transferred between *E. coli* strains. Therefore, monitoring these strains should be increased to prevent the spread of resistance between strains.

Moreover, our study identified one CREC strain with ST617 carrying $bla_{\text{NDM-4}}$ and *mcr-1*, as well as one CREC strain carrying $bla_{\text{NDM-5}}$ and *mcr-1*. In previous studies, $bla_{\text{NDM-5}}$ and *mcr-1*-producing *E. coli* were isolated from food animals in eastern China [24]. In spite of the fact that the two patients in this study were not engaged in animal husbandry, because they lived in a rural area, they might have been in contact with poultry. Additionally, this study had a few limitations. We did not obtain transconjugants that harbor *mcr-1* and either $bla_{\text{NDM-5}}$ or $bla_{\text{NDM-4}}$. Whether $bla_{\text{NDM}}$ and *mcr-1* can be transmitted from animals to the two patient is unclear. Further studies on the sequencing and transferability of *mcr-1* and $bla_{\text{NDM}}$ plasmids are needed. As far as we know, the presence of both *mcr-1* and $bla_{\text{NDM-4}}$ in CREC has not

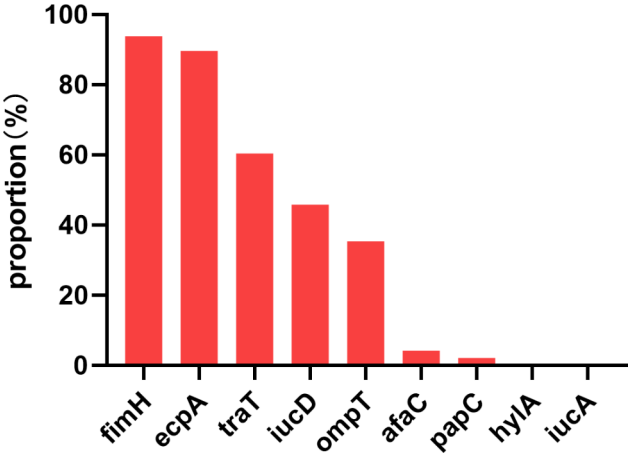


**Fig. 2** Molecular characterization of virulence genes in CREC isolates as detected by PCR

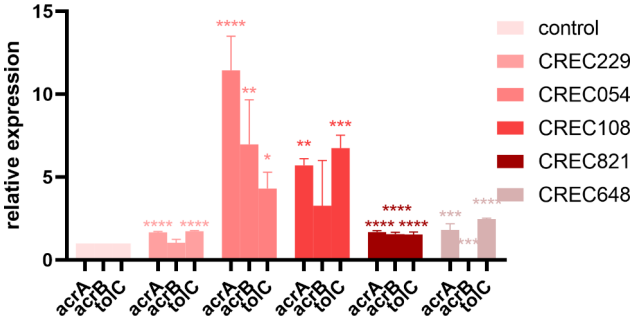


**Fig. 3** mRNA level of AcrA/B-TolC expressed in 5 non-CP-CREC strain Values represent the relative mRNA level of AcrA/B-TolC normalized to CSEC. Datas shown are the average values from three independent experiments, and bars represent standard deviations. ****$P < 0.001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

been reported in China from patients, but it was found in a study in North Lebanon [25]. Our study represents the first reported case of clinical CREC from China harboring both *mcr-1* and $bla_{\mathrm{NDM-4}}$, with ST617. Therefore, it is crucial to be aware of the potential spread of drug-resistant plasmids between animals and humans through the food chain.

According to previous studies, *NDM*-producing *E. coli* cover a wide range of sequence types, with ST167, ST410, and ST617 being found in several countries (Korea, India, South Africa, Japan, the USA, and Switzerland) [26]. A multicentre study in China found that ST167 was the most common clonal lineage of *NDM*-producing *E. coli*, followed by ST410 [27]. The most frequent ST was ST405 in CREC in Lebanon [28]. Out of 214 *E. coli* isolates, 16 were carbapenem resistant, with the $bla_{\mathrm{NDM-5}}$ gene as the main carbapenemase-encoding gene and ST1656 as the main ST type [29]. While our findings revealed that among the 39 strains of *NDM*-producing *E. coli*, there were 21 different sequence types, with ST410 being the most abundant, followed by ST5229. This is inconsistent with previous reports. According to our knowledge, *E. coli* ST5229 has been reported less frequently in China; however, in integrated and conventional farms in Korea, ST5229 was the most common ST, followed by ST101 and then ST10 [30]. Therefore, vigilant monitoring of clinical CREC transmission, along with other resistance genes is imperative.

In addition, we analyzed the remaining 9 non-CP-CREC strains and found that 7 of these strains were isolated from perianal swabs. The non-CP-CREC strains in this study included ST405 and ST38, which were exclusive to these strains. Non-CP-CREC exhibited high levels of resistance to ETP but demonstrated lower levels of resistance to MEM and IMP. Non-CP-CREC strains showed a 44.4% deletion of the ompC or ompF genes, and 100% produced $bla_{\mathrm{CTX-M-10}}$, suggesting that $bla_{\mathrm{CTX-M-10}}$ and membrane porin may be involved in carbapenem resistance. Research has found that the loss of OmpC is critically important for the phenotypic development of ertapenem-resistant and meropenem-susceptible strains, along with the expression of $bla_{\mathrm{CTX-M}}$ [31]. In this study, the other 5 non-CP-CREC strains with ompC and ompF expression had high expressions of efflux pump genes *acrA*, *acrB*, and *tolC*. The study shows that there is a strong correlation between ertapenem resistance and AcrA over-expression [32]. $Bla_{\mathrm{CTX-M-10}}$ and the high expression of efflux pumps may contribute to the drug resistance mechanisms of these 5 strains, but further

**Table 2** Molecular characterization of 9 non-carbapenemase-producin CREC

| Strain | ST | sample type | MICs (μg/mL) | | | ESBLs/AmpC | OMP |
|---|---|---|---|---|---|---|---|
| | | | ETP | MEM | IMP | | |
| CREC038 | ST648 | Perianal swab | 64 | 8 | 8 | *TEM*, $bla_{\mathrm{CTX-M-9}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-14}}$ | *ompF* |
| CREC743 | ST405 | Drainage fluid | 128 | 8 | 4 | $bla_{\mathrm{CTX-M-1}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-15}}$ | *ompF* |
| CREC229 | ST405 | Perianal swab | 16 | 2 | 1 | *TEM*, $bla_{\mathrm{CTX-M-1}}$, $bla_{\mathrm{CTX-M-10}}$ | *OmpC,ompF* |
| CREC435 | ST405 | Perianal swab | 64 | 8 | 8 | *TEM*, $bla_{\mathrm{CTX-M-1}}$, $bla_{\mathrm{CTX-M-9}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-14}}$, $bla_{\mathrm{CTX-M-15}}$ | |
| CREC254 | ST648 | Perianal swab | 64 | 16 | 8 | $bla_{\mathrm{CTX-M-9}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-14}}$ | |
| CREC648 | ST38 | Drainage fluid | 32 | 8 | 1 | $bla_{\mathrm{CTX-M-9}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-14}}$ | *OmpC, ompF* |
| CREC108 | ST38 | Perianal swab | 128 | 16 | 8 | $bla_{\mathrm{CTX-M-10}}$ | *OmpC, ompF* |
| CREC054 | ST38 | Perianal swab | 128 | 16 | 4 | $bla_{\mathrm{CTX-M-1}}$, $bla_{\mathrm{CTX-M-10}}$ | *ompC*、*ompF* |
| CREC821 | unknow | Perianal swab | 16 | 2 | 4 | $bla_{\mathrm{CTX-M-9}}$, $bla_{\mathrm{CTX-M-10}}$, $bla_{\mathrm{CTX-M-14}}$, $bla_{\mathrm{CMY}}$ | *ompC*、*ompF* |

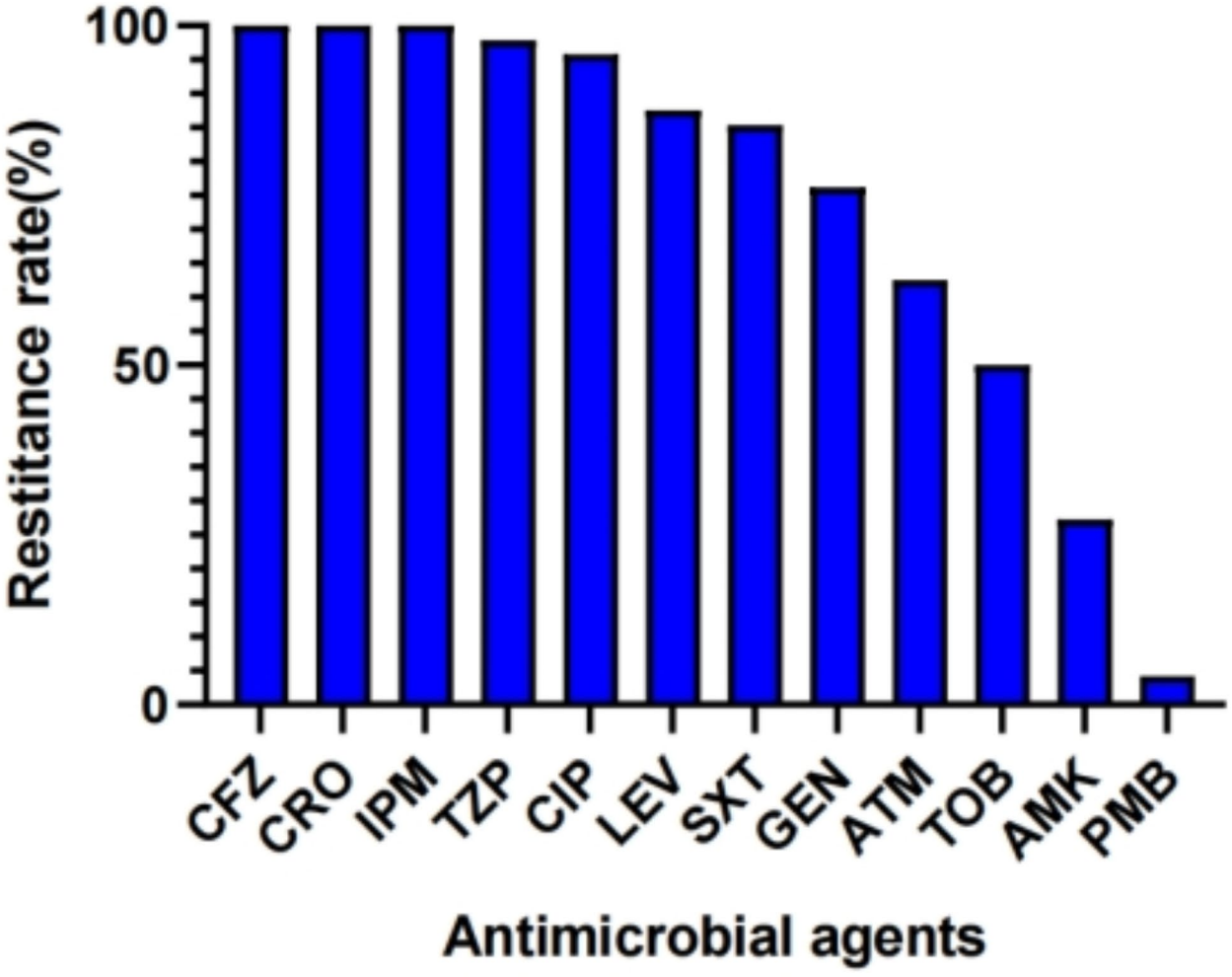


**Fig. 4** Susceptibility of CREC isolates to different antimicrobial agents. Note: CFZ: cefazolin; PMB, polymyxin B; SXT, Trimethoprim-Sulfamethoxazole; CRO, Ceftriaxone; IPM, imipenem; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; LEV, levofloxacin; GEN, gentamicin; ATM, aztreonam; TOB, tobramycin; AMK, amikacin

research is needed to determine the specific mechanism involved.

In addition to analyzing the molecular characteristics of CREC, we further analyzed the risk factors of CREC infection. A previous report found that CREC were mainly isolated from urine samples of *NDM*-producing *E.coli* around the world [33], rather than from perianal swabs in this study. Perhaps this is due to the fact that most of the patients in this study were hematology patients. The univariate analyses revealed 13 variables that were significantly different between the case (CREC) and control (CSEC) groups, all of which were associated with the CREC infection. PICC seems to be specific to this population since other risk factors are consistent with other reports [34]. This may be related to the high proportion of patients with blood diseases in this study.

Clinical case data suggest that most patients in the CREC group had received antibiotics before the CREC infection was detected, while only a minority had received antibiotics in the CSEC group. It may be because of this that CREC strains frequently harbor resistance genes to various classes of antibiotics. There were 26 strains with resistance genes for carbapenemase, aminoglycoside, quinolone, sulfonamide, and tetracycline; and six strains with resistance genes for carbapenems, aminoglycosides, quinolones, sulfonamides, and tetracycline. As a result, most CREC isolates showed high levels of resistance to carbapenems, cephalosporins, β-lactam/β-lactamase inhibitors, aminoglycosides, and fluoroquinolones, except for amikacin and polymyxin B. It poses a major challenge for clinical treatments of CREC infection. To our knowledge, in addition to inhibiting bacterial growth, the combined use of multiple antimicrobial regimens could also lead to mutations and drug resistance because selective antibiotics can exert pressure on the microbiome [35, 36], especially with the exposure to

**Table 3** Univariate and multivariate analyses of risk factors for CREC infection

| Variable | CREC infection group | CSEC infection group | Univariate analyses | | Multivariate analyses | |
|---|---|---|---|---|---|---|
| | *n* = 46 | *n* = 92 | OR (95% CL) | P | OR (95% CL) | P |
| sex | | | | | | |
| Male | 26(56.5%) | 52(56.5%) | 1.000(0.490–2.042) | 1.000 | | |
| female | 20(43.5%) | 40(43.5%) | | | | |
| Age (year) | 47.0 ± 22.6 | 48.9 ± 18.0 | 0.995(0.977–1.013) | 0.582 | | |
| Hospital stay (> 30 day) | 23(50.0%) | 16(17.4%) | 4.750(2.155–10.470) | 0.000 | 2.030(0.698–5.904) | 0.193 |
| Hospitalizations (> 3 times) | 22(47.8%) | 22(23.9%) | 2.917(1.376–6.181) | 0.005 | 1.861(0.681–5.084) | 0.226 |
| ECOG (3–5 score) | 12(26.1%) | 9(9.8%) | 3.255(1.256–8.433) | 0.015 | 2.347(0.611–9.011) | 0.214 |
| Outcome (drug survival) | 45(97.8%) | 92(100%) | q | 1.000 | | |
| Invasive operation | 35(76.1%) | 47(51.1%) | 3.046(1.381–6.721) | 0.006 | | |
| PICC | 26(56.5%) | 29(31.5%) | 2.824(1.361–5.862) | 0.005 | 0.301(0.049–1.859) | 0.196 |
| catheter | 6(13.0%) | 21(22.8%) | 0.507(0.189–1.360) | 0.177 | | |
| antibiotic use | 44(95.7%) | 30(32.6%) | 45.467(10.323-200.249) | 0.000 | 25.201(5.156-123.164) | 0.000 |
| β-lactam/β-lactamase inhibitors | 22(47.8%) | 4(4.3%) | 20.167(6.341–64.139) | 0.000 | | |
| cephalosporins | 11(23.9%) | 8(8.7%) | 3.300(1.223–8.903) | 0.018 | | |
| Aminoglycosides | 21(45.7%) | 10(10.9%) | 6.888(2.868–16.542) | 0.000 | | |
| carbapenems | 21(45.7%) | 6(6.5%) | 12.040(4.382–33.080) | 0.000 | | |
| Fluoroquinolones | 14(30.4%) | 2(2.2%) | 19.687(4.240-91.424) | 0.000 | | |
| Antifungal | 24(52.2%) | 9(9.8%) | 10.061(4.095–24.715) | 0.000 | 2.167(0.666–7.050) | 0.199 |
| Detected other pathogens (prior to CREC infection) | 30(65.2%) | 8 (8.7%) | 19.687(7.648–50.680) | 0.000 | 27.928(6.041-129.108) | 0.000 |
| Co-morbidities | | | | | | |
| Hypertension | 10(21.7%) | 21(22.8%) | 0.939(0.400-2.204) | 0.885 | | |
| Diabetes | 7(15.2%) | 7 (7.6%) | 2.179(0.715–6.641) | 0.171 | | |
| Solid tumor | 7(15.2%) | 17(18.5%) | 0.792(0.303–2.071) | 0.634 | | |
| Surgical history | 13(28.3%) | 7 (7.6%) | 4.784(1.755–13.041) | 0.002 | 0.914(0.170–4.917) | 0.917 |
| Cardiac diseases | 8(17.4%) | 11(12.0%) | 1.550(0.577–4.167) | 0.385 | | |
| Disease of the urinary System | 15(32.6%) | 34(37.0%) | 0.825(0.391–1.744) | 0.615 | | |
| Liver disease | 16(34.8%) | 29(31.5%) | 1.159(0.548–2.451) | 0.700 | | |
| Respiratory disease | 20(43.5%) | 21(22.8%) | 2.601(1.217–5.558) | 0.014 | 5.845(1.387–24.641) | 0.016 |
| Digestive system disease | 15(32.6%) | 25(27.2%) | 1.297(0.601–2.797) | 0.508 | | |
| hospital stay (day) | 36.6 ± 22.3 | 18.5 ± 18.6 | | | | |
| hospitalizations(times) | 4.9 ± 5.3 | 2.9 ± 4.9 | | | | |
| ECOG scores | | | | | | |
| 0 | 0 | 14 | | | | |
| 1 | 23 | 42 | | | | |
| 2 | 11 | 27 | | | | |
| 3 | 7 | 8 | | | | |
| 4 | 4 | 1 | | | | |
| 5分 | 1 | 0 | | | | |

PICC:Peripherally inserted central catheter ; ECOG: Eastern Cooperative Oncology Group

broad-spectrum antibiotics, such as aminoglycosides and carbapenems, which have been attributed to the emergence of CREC. Due to this, instead of using broad spectrum antibiotics to prevent in-hospital mortality from rising CREC infections, we applied antibiotics according to the results of antimicrobial susceptibility testing.

Our study shows that antibiotics usage, antifungal treatment, detection of other pathogens (prior to CREC infection), and respiratory disease were identified as independent risk factors for CREC infection. Unlike other studies, respiratory disease was shown to be an independent risk factor in this study. Studies have found that if respiratory disease is the underlying illness, it may protect against CRKP infection [37], whereas if it is a comorbidity, it may have the opposite effect [38]. The different genotypes carried by strains and the population differences may be important factors in determining whether respiratory diseases are independent risk factors. In addition, our results show that patients with CREC infection have a higher ECOG score and a worse

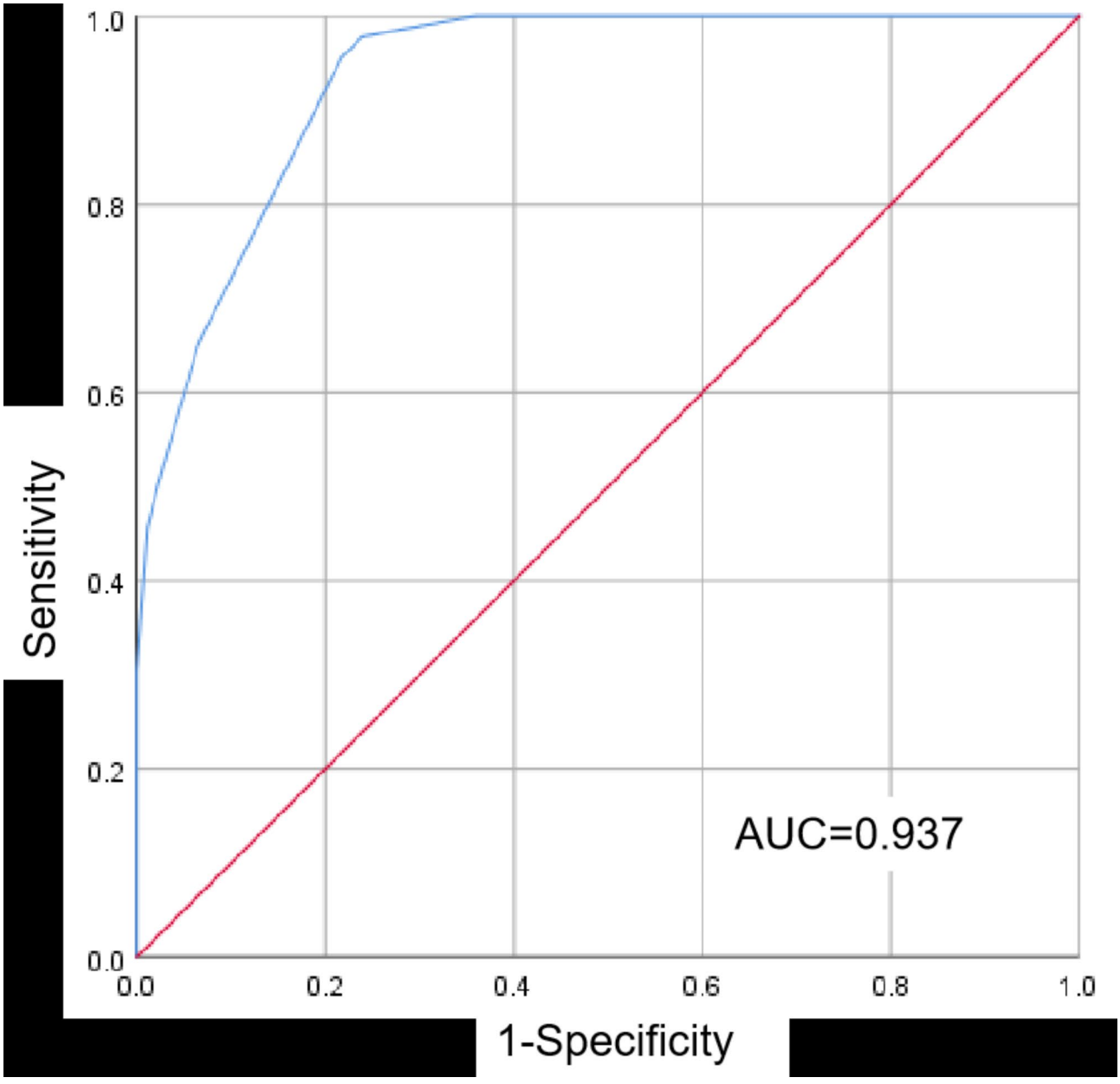


**Fig. 5** Receiver operator characteristic curve analysis for the scoring system

**Table 4** Accuracy of the proposed scoring system for the diagnosis of CREC infection

| Score | CREC infection group (*n* = 46) | CSEC infection group (*n* = 92) | Sensitivity | Specifcity | Youden's index (J) |
|---|---|---|---|---|---|
| ≥0 | 46 | 92 | 1.00 | 0 | 0 |
| ≥1 | 46 | 46 | 1.00 | 0.50 | 0.50 |
| ≥2 | 44 | 20 | 0.96 | 0.78 | 0.74 |
| ≥3 | 23 | 2 | 0.50 | 0.98 | 0.48 |
| ≥4 | 5 | 0 | 0.11 | 1.00 | 0.11 |

prognosis than those with CSEC infection. Therefore, it is necessary to build predictive models to quickly identify CREC infections. In this study, a scoring system was developed that assigns points based on four independent risk factors. It seems that the system is accurate, with an AUROC of 0.937, and a maximal Youden's index of 2 points was obtained. A score system like this may be useful for identifying patients at risk of infection with CREC. To prevent the spread of CREC, more rigorous infection control measures must be implemented, such as antibiotic stewardship and timely investigation of epidemiology data.

The present study is subject to certain limitations. Firstly, although we tested numerous drug resistance genes in the strains, we did not conduct whole-genome sequencing. This omission resulted in a lack of plasmid data and hindered our ability to identify other uncommon resistance mechanisms. Future research will address this limitation by exploring the genetic characteristics of resistance genes, including their chromosomal or plasmid locations, homology characteristics, and insights into the evolutionary and developmental patterns of the strains over the three-year period. In addition, this study has a small sample size and is conducted at a single center. There is still a need to confirm these findings in multicenter, large-sample, randomized controlled trials.

## Conclusion

Our study found that CREC isolates were resistant to most antibiotics, primarily due to *NDM*-related mechanisms. The isolates exhibited high sequence type diversity, with ST410 being the most common, followed by ST5229. Notably, we first describe a clinical CREC strain carrying both *mcr-1* and $bla_{NDM-4}$ from China. Vigilance is crucial to prevent the spread of drug-resistant plasmids from animals to humans through the food chain. Antibiotic usage, antifungal treatment, detection of other pathogens (prior to CREC infection), and respiratory disease were identified as independent risk factors for CREC infection. Using the results, we developed a simple scoring system to identify CREC infections with the sensitivity of 96% and specificity of 78% when scores are ≥2 points.This indicated the monitoring of this isolate should be enhanced.

### Abbreviations

| | |
|---|---|
| *E.coli* | *Escherichia coli* |
| CREC | Carbapenem-resistant *E.coli* |
| non-CP-CREC | Non carbapenemase-producing CREC |
| CP-CREC | Carbapenemase-producing CREC |
| CSEC | Carbapenem sensitive *Escherichia coli* |
| ESBLs | Extended-spectrumβ-lactamases |
| PMQR | Plasmid-mediated quinolone resistance |
| MLST | Multilocus sequence typing |
| STs | Sequence type |
| CHINET | China Antimicrobial Surveillance Network |
| PICC | Peripherally inserted central catheter |
| MIC | Minimum inhibitory concentration |
| PCR | Polymerase chain reaction |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03525-9.

Supplementary Material 1

### Acknowledgements

Not applicable.

### Author contributions

Study design: Yingping Cao and Xiaohong Xu. Study conduct: Siyan Lian, Chang Liu and Meili Cai. Data collection: Siyan Lian and Chang Liu. Data analysis: Meili Cai and Xiaohong Xu. Data interpretation: Chang Liu and Meili Cai. Drafting manuscript: Xiaohong Xu. Revising manuscript content: Yingping Cao and Xiaohong Xu. Approving the final version of the manuscript: Yingping Cao and Xiaohong Xu. All authors read and approved the final manuscript.

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### Data availability

Data is provided within the manuscript or supplementary information files.The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The Medical Ethics Committee of Fujian Medical University Union Hospital (2024KY077) thoroughly reviewed and granted approval for all procedures pertaining to human subjects, including individuals, medical records, human samples, and clinical isolates, in this study. We affirm that the execution of this study adhered to the principles outlined in the Declaration of Helsinki.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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