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Molecular epidemiology of carbapenem-resistant *Enterobacter cloacae* complex in a tertiary hospital in Shandong, China

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Abstract

Background The increasing incidence and prevalence of carbapenem-resistant *Enterobacter cloacae* complex (CREC) poses great challenges to infection prevention and disease treatment. However, much remains unknown about the clinical characteristics of CREC isolates. Our objective was to characterize antimicrobial resistance and carbapenemase production in CREC with 36 CREC isolates collected from a tertiary hospital in Shandong, China.

Results Three types of carbapenemases (NDM, IMP and VIM) were detected in these isolates. Among them, NDM carbapenemases were most prevalent, with a 61.2% (22/36) detection rate for NDM-1, 27.8% (10/36) for NDM-5 and 2.8% (1/36) for NDM-7. IMP-4 was found in two isolates and VIM-1 in only one isolate. The MLST analysis identified 12 different sequence types (STs), of which ST171 (27.8%) was the most prevalent, followed by ST418 (25.0%). ST171 isolates had significantly higher rates of resistance than other STs to gentamicin and tobramycin ($P_s < 0.05$), and lower rates of resistance to aztreonam than ST418 and other STs ($P_s < 0.05$). Among 17 carbapenemase-encoding genes, the *bla*_{NDM-5} gene was more frequently detected in ST171 than in ST418 and other isolates ($P_s < 0.05$). In contrast, the *bla*_{NDM-1} gene was more frequently seen in ST418 than in ST171 isolates. One novel ST (ST1965) was identified, which carried the *bla*_{NDM-1} gene.

Conclusion NDM-5 produced by ST171 and NDM-1 carbapenemase produced by ST418 were the leading cause of CREC in this hospital. This study enhances the understanding of CREC strains and helps improve infection control and treatment in hospitals.

Keywords *Enterobacter cloacae* complex, Carbapenemase, NDM-5, NDM-1, ST171, ST418

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Background

Enterobacter cloacae complex comprises several species, including *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii* and *E. nimipressuralis* [1, 2]. As Gram-negative opportunistic pathogens, they can cause several diseases such as minor infections of the skin, urinary tract infections, pneumonia and bloodstream infections (BSI) [2]. Due to the unreasonable use of antibiotics, multi-drug-resistant *E. cloacae* complex isolates have emerged and spread worldwide [3].

Carbapenems are regarded as the most effective antibiotics against many multidrug-resistant bacteria [4]. However, in recent years, carbapenem-resistant *E. cloacae* complex (CREC) isolates have been increasingly detected from clinical investigations, raising global public health concerns [5]. The production of carbapenemases was the most predominant mechanism associated with carbapenem resistance for the *E. cloacae* complex. Carbapenemases are members of class A, class B and class D β -lactamases [6, 7]. Among them, class A and class D carbapenemases are serine carbapenemases, including KPC, IMI, SME, GES, OXA-23, OXA-24, OXA-48 and OXA-58. Class B carbapenemases are metallo- β -lactamases which mainly include NDM, IMP, VIM, AIM, DIM, GIM and SPM [7]. In addition, some extended-spectrum β -lactamases (ESBLs), such as CTX-M, TEM and SHV, also lead to resistance of *E. cloacae* complex isolates to most β -lactam drugs, posing great challenges for clinical treatment [8].

The multilocus sequence typing (MLST) method has been widely utilized to trace CREC strains worldwide. Previous molecular epidemiological studies have found that the most abundant sequence types (STs) for CREC isolates were ST510 in Cali, Colombia [9], ST74 in Spain [10] and ST171 in the United States [11, 12]. In China, ST418 was previously reported as the predominant ST in Shenzhen [13], ST120 ST in Henan [14], ST190 in Wenzhou [15], ST93 in Liaoning [16] and ST544 in Ningxia [17]. However, until now, scarce data on CREC isolates have been available from Shandong, China.

In this study, we collected and characterized 36 CREC strains over a time span of five years (2018–2022) at a tertiary hospital in Shandong, China. Our work contributes to the understanding of the epidemiology and carbapenem resistance of *E. cloacae* complex strains.

Methods

Bacterial isolates

A total of 36 nonrepetitive CREC isolates were obtained from different departments (ICU, urinary surgery ward and other wards) at the First Affiliated Hospital of Shandong First Medical University (Shandong, China). These samples were obtained from 2018 to 2022. All isolates were identified using MALDI-TOF MS (Bruker) and

further verified by PCR targeting 16 S rRNA [18]. PCR products were sequenced by Tsingke BioTech Co., Ltd., followed by sequence alignment on the NCBI database.

Antimicrobial susceptibility test

To test susceptibility, all CREC isolates were exposed to 16 antibiotics, including piperacillin/tazobactam, cefazolin, cefotetan, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, imipenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole, by using a Vitek 2 compact system (bioMérieux, Marcy, France) with AST-GN-13 cards. The results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

mCIM test

To screen for suspected carbapenemase production in the 36 CREC strains, the modified carbapenem inactivation method (mCIM) was performed based on the CLSI guidelines.

Detection of resistance genes

The whole genomes of the 36 CREC strains were extracted using the DNA nucleic acid extraction kit (Tiangen, China). To detect resistance genes in CREC strains, PCR assays were carried out using conventional PCR amplification [9, 19–21]. The target resistance genes included the carbapenemase gene (bla_{NDM} , bla_{VIM} , bla_{IMP} , bla_{KPC} , bla_{SPM} , bla_{IMI} , bla_{OXA-23} , bla_{OXA-24} , bla_{OXA-48} , bla_{OXA-58} , bla_{SME} , bla_{DIM} , bla_{BIC} , bla_{GIM} , bla_{AIM} , and bla_{GES}) and the extended-spectrum β -lactamase genes (bla_{CTX-M} , bla_{SHV} , and bla_{TEM}). Positive amplicons were sequenced by Tsingke BioTech Co., Ltd. in both directions. The sequences were analyzed against the NCBI database by the Basic Local Alignment Search Tool (BLAST).

Multilocus sequence typing (MLST)

MLST analyses were performed for all CREC isolates as described previously (<https://pubmlst.org/organisms/enterobacter-cloacae/>). The amplified fragments of seven housekeeping genes (*dnaA*, *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*) were sequenced in both directions. The sequences were aligned with the reference sequence from the MLST database. Newly identified STs were submitted to the MLST database curator for approval, and new numbers were assigned. A minimum-spanning tree using the allelic difference between isolates of the seven housekeeping genes was constructed using BioNumerics software.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 21.0 for Windows. A two-sided p value of less than 0.05 was considered statistically significant.

Results

Clinical and demographic characteristics of CREC isolates

The clinical characteristics of the 36 CREC isolates are shown in Table 1. A total of 36 nonduplicate CREC isolates were collected from 2018 to 2022. Among them, one was collected in 2018, seven in 2019, three in 2020, 18 in 2021 and seven in 2022. The isolates were primarily from urine (n=10, 27.8%), sputum (n=7, 19.4%) and blood

(n=6, 16.7%) specimens. No more than three isolates were found in each of other specimens. The isolates were primarily collected from the ICU (n=16, 44.4%), followed by the urinary surgery ward (n=4, 11.1%). No more than three isolates were from each of the other hospital wards.

The demographic characteristics of the 36 CREC isolates are shown in Table 1 and summarized in Table 2. Briefly, 72.2% (26 of 36) of the infected patients were male and the rest were female. 58.3% (21 of 36) of them were older adults aged 60 years and over, 27.8% (10 of 36) middle-aged adults aged 41–60 and 2.8% (1 of 36) teenagers younger aged 12–20.

Table 1 Microbiological and molecular characteristics of 36 CREC isolates

Isolate	Year of isolation	Gender/Age (Years)	Ward	Specimen	ST	Carbapenemase	ESBL
CREC01	2018	M/48	Hepatobiliary surgery	dr	1120	NDM-1	TEM
CREC02	2019	M/50	ICU	sp	171	NDM-5	TEM
CREC03	2019	M/51	Neurosurgery	ca	171	NDM-5	TEM
CREC04	2019	M/87	ICU	bl	418	NDM-1	TEM
CREC05	2019	F/27	Urinary surgery	ps	336	NDM-1	TEM, CTX-M
CREC06	2019	M/62	ICU	sp	418	NDM-1	TEM
CREC07	2019	F/81	ICU	sp	25	NDM-1	TEM, CTX-M
CREC08	2019	M/86	Respiratory medicine	sp	418	NDM-1	TEM
CREC09	2020	F/31	orthopedic	se	97	IMP-4	
CREC10	2020	M/36	Neurosurgery	sp	1965	NDM-1	TEM
CREC11	2020	F/67	Hepatobiliary surgery	dr	97	IMP-4	
CREC12	2021	F/81	ICU	bl	564	VIM-1	
CREC13	2021	M/65	Urinary surgery	ur	231	NDM-1	TEM, CTX-M
CREC14	2021	F/82	ICU	bl	171	NDM-5	TEM
CREC15	2021	F/61	Cardiovasology	bl	113	NDM-1	TEM, CTX-M
CREC16	2021	M/64	Cardiovasology	bl	113	NDM-1	TEM, CTX-M
CREC17	2021	M/58	ICU	sp	418	NDM-1	TEM
CREC18	2021	M/50	Neurosurgery	bl	171	NDM-5	TEM
CREC19	2021	M/76	Hepatobiliary surgery	pf	171	NDM-7	TEM
CREC20	2021	M/77	Urinary surgery	ur	231	NDM-1	
CREC21	2021	M/56	Gastroenterology	ur	127	NDM-1	
CREC22	2021	M/54	Gastroenterology	ca	171	NDM-5	TEM
CREC23	2021	M/60	ICU	ur	171	NDM-5	TEM
CREC24	2021	M/57	orthopedic	se	171	NDM-1	TEM, CTX-M
CREC25	2021	M/79	ICU	pf	418	NDM-1	TEM
CREC26	2021	M/77	Urinary surgery	ur	231	NDM-1	TEM, CTX-M
CREC27	2021	M/77	Nephrology	ur	127	NDM-1	TEM
CREC28	2021	M/61	ICU	se	418	NDM-1	TEM
CREC29	2021	M/56	ICU	ts	418	NDM-1	TEM
CREC30	2022	M/4	Hematology	sp	316	NDM-1	
CREC31	2022	F/83	ICU	ts	231	NDM-5	
CREC32	2022	F/38	General Practice	ur	171	NDM-5	TEM
CREC33	2022	M/81	ICU	ca	418	NDM-1	TEM
CREC34	2022	M/61	ICU	ur	418	NDM-1	
CREC35	2022	M/79	ICU	ur	231	NDM-5	
CREC36	2022	F/59	ICU	ur	171	NDM-5	TEM

CREC: carbapenem-resistant *Enterobacter cloacae* complex, M: Male; F: Female; ICU: Intensive Care Unit; bl: blood; ca.: catheter; dr: drainage; pf: peritoneal fluid; ps: preservation solution; se: secretion; sp: sputum; ts: throat swab; ur: urine

Table 2 Demographic characteristics corresponding of 36 CREC isolates

	Characteristic	n (%)
Age	Teenagers (12–20 years)	1 (2.8%)
	Young adult (21–40 years)	4 (11.1%)
	Middle adult (41–60 years)	10 (27.8%)
	Elderly (> 60 years)	21 (58.3%)
Gender	Male	26(72.2%)
	Female	10 (27.8%)

Antibiotic susceptibility

The antimicrobial susceptibility profiles of 36 CREC strains are shown in Table 3 and Additional file 1. In general, all CREC isolates were resistant to ceftazidime, ceftazidime, ceftriaxone ertapenem and imipenem. Most isolates (over 86.1%) were also resistant to piperacillin/tazobactam, cefepime, ciprofloxacin and trimethoprim/sulfamethoxazole. In addition, 72.2% of the isolates were resistant to levofloxacin, 52.8% isolates resistant to aztreonam and gentamicin, and 41.7% to nitrofurantoin. In contrast, the resistance rates for tobramycin and amikacin were only 22.2% and 2.8%, respectively.

Distribution of carbapenemase- and ESBL-encoding genes

All 36 CREC strains showed positive phenotypes as detected by the modified carbapenem inactivation method (mCIM) indicating that they were

carbapenemase producers. All of them harbored carbapenemase-encoding genes. Three types of carbapenemases (NDM, IMP and VIM) were detected in these isolates. Among them, the *bla*_{NDM} gene was the most prevalent carbapenemase-encoding gene, with a 61.2% (22/36) detection rate for the *bla*_{NDM-1} gene, 27.8% (10/36) for the *bla*_{NDM-5} gene and 2.8% (1/36) for the *bla*_{NDM-7} gene. The *bla*_{IMP} gene was only found in only two isolates (5.6%), and both were *bla*_{IMP-4}. In addition, we found the *bla*_{VIM-1} gene in only one isolate (2.8%). None of these isolates had two or more carbapenemase-encoding genes, and none of them had other carbapenemase-encoding genes tested in this study.

Twenty of the 36 CREC isolates harbored the extended-spectrum β -lactamase (ESBL) genes. Among them, the *bla*_{TEM} gene was the most prevalent, with a 52.8% (19/36) detection rate, followed by the *bla*_{CTX-M} gene in seven isolates (19.4%). None of them harbored *bla*_{SHV} gene.

MLST profile

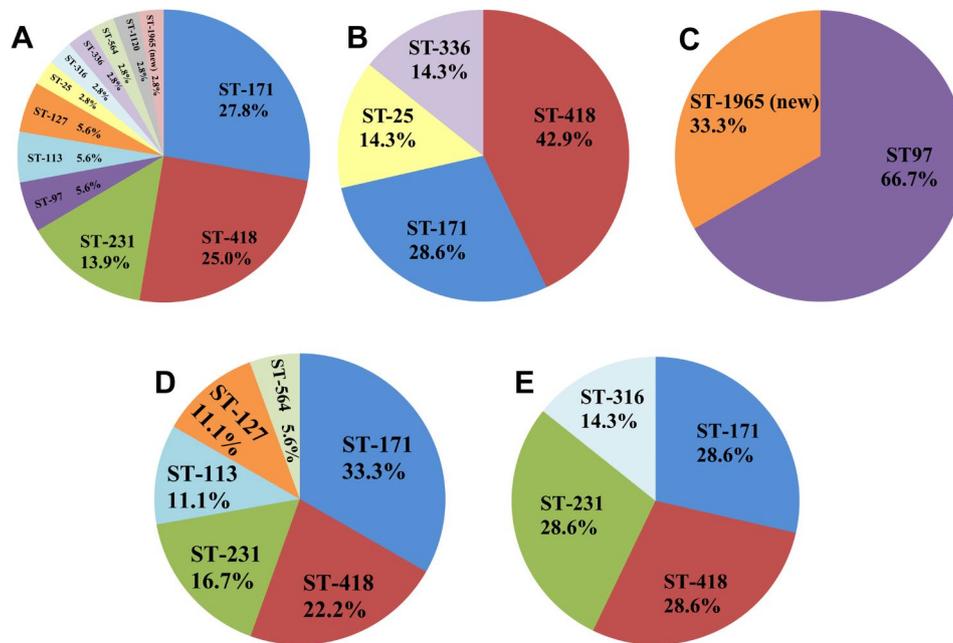
The MLST analysis revealed a total of 12 different STs, including 11 existing STs and one novel ST identified in this study. The new ST was submitted for ST assignment, which was ST1965. The profiles of the newly identified STs are listed in Table 4. In the alignment of the MLST sequence, a novel sequence was found in *pyrG*, which was designated as *pyrG*-461. Moreover, 83.3% (30/36) of the isolates were represented by six main STs (having ≥ 2 isolates). The most prevalent ST was ST171, which accounted for 27.8% (10/36) of the isolates, followed by

Table 3 Resistance rates for ST171 and ST418 isolates

Antimicrobial	Resistance rate (%)				Resistance rate (%)		
	Overall	ST171	Other STs	P-value	ST171	ST418	P-value
	n = 36	n = 10	n = 26		n = 10	n = 9	
Piperacillin/tazobactam	94.4%	100.0%	92.3%	1.000	100.0%	100.0%	-
Cefazolin	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Cefotetan	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Ceftazidime	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Ceftriaxone	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Ertapenem	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Imipenem	100.0%	100.0%	100.0%	-	100.0%	100.0%	-
Cefepime	88.9%	100.0%	84.6%	0.559	100.0%	100.0%	-
Ciprofloxacin	88.9%	100.0%	84.6%	0.559	100.0%	100.0%	-
Trimethoprim/sulfamethoxazole	86.1%	100.0%	80.8%	0.293	100.0%	88.9%	0.474
Levofloxacin	72.2%	80.0%	69.2%	0.689	80.0%	100.0%	0.474
Aztreonam	52.8%	20.0%	65.4%	0.025	20.0%	100.0%	0.001
Gentamicin	52.8%	90.0%	38.5%	0.008	90.0%	77.8%	0.582
Nitrofurantoin	41.7%	30.0%	46.2%	0.468	30.0%	55.6%	0.370
Tobramycin	22.2%	60.0%	7.7%	0.002	60.0%	22.2%	0.170
Amikacin	2.8%	10.0%	0.0%	0.278	10.0%	0.0%	1.000

Table 4 Allelic profiles of the new ST found in this study

ST	<i>dnaA</i>	<i>fusA</i>	<i>gyrB</i>	<i>leuS</i>	<i>pyrG</i>	<i>rplB</i>	<i>rpoB</i>
ST-1965	46	20	20	96	461	29	54

**Fig. 1** MLST population analysis over the different years. (A) STs in all 36 isolates. (B) STs distribution in 2019. (C) STs distribution in 2020. (D) STs distribution in 2021. (E) STs distribution in 2022**Table 5** Prevalence of carbapenem resistance genes among ST171 and ST418 isolates

gene	distributing in		P-value	distributing in		P-value
	ST171(n=10) n (%)	ST418(n=9) n (%)		ST171(n=10) n (%)	Other STs(n=26) n (%)	
<i>bla</i> _{NDM-1}	1(10.0%)	9(100.0%)	0.000	1(10.0%)	21(80.8%)	0.000
<i>bla</i> _{NDM-5}	8(80.0%)	0(0.0%)	0.001	8(80.0%)	2(7.7%)	0.000
<i>bla</i> _{NDM-7}	1(10.0%)	0(0.0%)	1.000	1(10.0%)	0(0.0%)	0.278
<i>bla</i> _{IMP-4}	0(0.0%)	0(0.0%)	-	0(0.0%)	2(7.7%)	1.000
<i>bla</i> _{VIM-1}	0(0.0%)	0(0.0%)	-	0(0.0%)	1(3.8%)	1.000

ST418 accounting for 25.0% (9/36) (Fig. 1 and Additional file 2).

ST171 and ST418 were the most predominant STs found in 2019, 2021 and 2022; however, in 2020 these two STs were not detected and the dominant ST was ST97. In addition, both ST97 isolates harbored the *bla*_{IMP-4} gene, with the detection rate higher than that of the other STs ($P < 0.05$) (Table 5). For other minor STs, the distributions varied across years, with some STs diminishing or switching to another minor ST (Fig. 1). For instance, ST231 isolates were not observed in 2019 and 2020. However, in 2021, the proportion of this ST increased to 22.2% in 2021 and 28.6% in 2022.

One clonal complex (CC) and 10 singletons were identified, which suggested high genetic diversity. CC231 (accounting for six isolates) was the only CC that contained ST231 and the new ST1965 (Table 4). In addition,

the other 10 individual STs were all singletons which accounted for 30 isolates. The detailed MLST profiles are presented in Additional file 2.

Comparisons of resistance and carbapenemase-encoding genes between ST171 and ST418

As described in Tables 3 and 90% and 60% of ST171 isolates were resistant to gentamicin and tobramycin, respectively, which was significantly higher than other STs isolates ($P < 0.05$). In contrast, only 20% of ST171 isolates were resistant to aztreonam which was significantly lower than ST418 and other STs isolates (both $P < 0.05$). No significant differences were found in the resistance to other antibiotics between ST171 and ST418 isolates or between ST171 and other STs. Among the 17 carbapenemase-encoding genes tested in this study, 80% ST171 (8 of 10) isolates harbored *bla*_{NDM-5} gene which was more

results were consistent with previous studies of these cities in China [13].

We also observed one new ST (ST1965) in this study, and it was classified into CC231, which suggested that *E. cloacae* complex isolates were diverse and still in clonal expansion. Besides, we found that this new ST isolate harbored the *bla*_{NDM-1} gene. To our knowledge, this is the first report in the world of ST1965 carbapenem-resistant *E. cloacae* complex isolate carrying the *bla*_{NDM-1} gene. Although this new ST was in the minority, the isolate within it may give rise to future disease outbreaks; therefore, close attention should be paid to this new ST to identify and further limit both transmission and outbreaks.

Conclusions

In our study, we characterized the molecular epidemiology and carbapenem-resistance mechanisms of *E. cloacae* complex strains in a tertiary hospital in Shandong, China. NDM-5 carbapenemase produced by ST171 and NDM-1 carbapenemase produced by ST418 were the leading cause for the carbapenem resistance of *E. cloacae* complex strains in this hospital. One novel ST (ST1965) was detected, and this new ST isolate carried the *bla*_{NDM-1} gene. This study contributes to a better understanding of CREC strains and improves infection control and treatment in hospitals.

Abbreviations

CREC	carbapenem-resistant <i>Enterobacter cloacae</i> complex
PCR	polymerase chain reaction
MLST	Multilocus sequence typing
ST	sequence type
BSI	Bloodstream infection
ESBLs	extended-spectrum β -lactamases
MBLs	metallo β -lactamases
NDM	New Delhi metallo- β -lactamase
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
BLAST	Basic Local Alignment Search Tool
CC	Clonal complex
CLSI	Clinical and Laboratory Standards Institute
MIC	minimum inhibitory concentration
MALDI-TOF MS	matrix assisted laser desorption ionization time of flight mass spectrometry

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-023-02913-x>.

Supplementary Material 1

Supplementary Material 2

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Authors' contributions

JZW conceived the idea and designed the experiment. JZW, SNH and QC analyzed the results. JZW, SNH and WYX drafted the manuscript. WYX, XNZ and XTD performed the experiment. JZW and HQJ participated in manuscript

revision. All authors read and approved the final manuscript. SNH and WYX contributed equally to this work.

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Data Availability

The datasets used and/or analyzed during the current study are within the manuscript and the Additional files. The sequences analysed during the current study were deposited in the GenBank database (accession numbers: OP806578-OP806829 and OP806908-OP806970).

Declarations

Competing interests

Authors declare that they have no competing interests.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University. As this study only focused on bacteria alone and did not use any human material or patient information, the Review Board of the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University exempted this study from review and waived the need for informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

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