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ORIGINAL RESEARCH

Carbapenem-Resistant *Enterobacter cloacae* Causing Nosocomial Infections in Southwestern China: Molecular Epidemiology, Risk Factors, and Predictors of Mortality

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Xiaolang Tian
Changwu Huang
Xiaoli Ye
Hongyan Jiang
Rufang Zhang
Xiaofang Hu
Dongshuang Xu

Department of Clinical Laboratory, University of Chinese Academy of Sciences Chongqing Renji Hospital (Fifth People's Hospital of Chongqing), Chongqing, People's Republic of China

Background: The emergence and spread of carbapenem-resistant *Enterobacter cloacae* (CR-ECL) have posed a serious threat to clinical management. This retrospective study assessed the epidemiological characteristics of CR-ECL to explore the risk factors and predictors of mortality in patients with CR-ECL infection.

Methods: We performed a retrospective 1:2 case-control study of hospitalized patients from January 2014 to December 2017. A total of 85 consecutive unique CR-ECL strains comprised the case group, and 170 matched patients with carbapenem-susceptible *Enterobacter cloacae* (CS-ECL) infection at the same period as the control group. Isolates were screened for potential resistance genes by polymerase chain reaction (PCR) and molecular typing was performed by multilocus sequence typing (MLST).

Results: The results of drug resistance gene detection showed that *blaNDM-1* was the most common carbapenem resistance gene. The MLST results showed that ST51 was the predominant epidemic type, followed by ST88. ICU admission ($P<0.001$), drainage tube ($P=0.002$), central venous catheter ($P=0.005$), and carbapenem exposure ($P=0.003$) were independent risk factors for CR-ECL infection. Significant predictors for 28-day mortality included solid tumours ($P=0.005$), septic shock ($P=0.019$), and mechanical ventilation ($P=0.027$).

Conclusion: Our study indicated that ST51 and ST88, which are closely related, were the predominant epidemic types of CR-ECL producing *blaNDM-1* in southwestern China. Strengthening the surveillance of patients with solid tumours, septic shock and mechanical ventilation is an urgent need.

Keywords: carbapenem-resistant *Enterobacter cloacae*, epidemiological characteristics, risk factors, predictors of mortality

Introduction

Bacteria belonging to *Enterobacterales*, such as *Escherichia coli, Klebsiella pneumoniae, Enterobacter, Citrobacter, Serratia, Proteus*, and *Morganella*, often cause nosocomial infections.[1,2] During the last decade, the emergence of carbapenem-resistant *Enterobacteriaceae* (CRE), which led to limited treatment options, has become the main cause of clinical anti-infective treatment failure.[3,4] It is worth noting that carbapenem-resistant *Enterobacter cloacae* (CR-ECL), which is one of the most common species that has been focused on in studies of a single species of *Enterobacterales*,[5] has been reported in many countries such as Korea, the United States, India, and China.[6–8]

Correspondence: Dongshuang Xu
Department of Clinical Laboratory, University of Chinese Academy of Sciences Chongqing Renji Hospital (Fifth People's Hospital of Chongqing), No. 24 Renji Road, Nanan District, Chongqing 400062, People's Republic of China
Tel +86 023-62895125
Fax +86 023-62881980
Email dongshuang_xu@163.com

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Resistance to carbapenems is associated with several mechanisms. Among them, carbapenemase production and loss of outer membrane proteins (ompC and ompF) are the main drug resistance mechanisms.[9] In China, carbapenemase production is attributed mainly to metallo-β-lactamases (MBLs) such as *blaVIM-1, blaIMP-4, blaIMP-8*, and *blaNDM-1*. Since the first case of carbapenem-resistant *E. cloacae* harbouring *blaNDM-1* was detected in Chongqing, *blaNDM-1*-producing *E. cloacae* strains have emerged in various regions across the country.[10] Notably, genes encoding MBLs are most commonly identified in *E. cloacae* and can be transmitted frequently through mobile genetic elements, leading to the prevalence of CR-ECL. Previous studies have certified that CR-ECL could increase the mortality rate, especially in vulnerable patients.[11,12] Further understanding of the molecular epidemiology of CR-ECL and investigation of carbapenemase gene-carrying plasmids, which are the most important mechanism of transmission of resistance, are needed to prevent the spread of CR-ECL. In addition, the identification of risk factors for CR-ECL infection would improve the choice and effcacy of empirical therapy.[5,7] Therefore, it is urgent to explore the epidemiological characteristics of CR-ECL infection.

However, the molecular epidemiological characteristics of CR-ECL infection are different from region to region.[13,14] In this study, we attempted to systematically analyse the epidemiological characteristics of CR-ECL infection to provide evidence for effective control of nosocomial infection with CR-ECL.

Materials and Methods

Study Design and Setting

We conducted a retrospective study to investigate the molecular epidemiological characteristics of CR-ECL in Chongqing Renji Affiliated Hospital of the Chinese Academy of Sciences University from January 2014 to December 2017. Carbapenem-resistant *E. cloacae* was defined as *E. cloacae* strains resistant to at least one of the carbapenem agents, with the criteria of MICs of ≥ 2 μg/mL for ertapenem, ≥ 4 μg/mL for imipenem, or ≥ 4 μg/mL for meropenem. To explore risk factors for CR-ECL infection, a retrospective 1:2 case-control study was performed. The case group consisted of patients with CR-ECL infection. Patients with CS-ECL infection were defined as the control group.

Identification and Drug Sensitivity of Bacteria

The VITEK 2 Compact system and the VITEK MS system (bioMérieux, Marcy l'Etoile, Lyon, France) were used for isolate identification, and the VITEK 2 Compact AST-GN13 card (bioMérieux) were used to test the antibiotic susceptibilities of all isolates. The MIC values for tested carbapenem agents, tigecycline (TGC) and polymyxin B (PB) were determined by the broth microdilution method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria.[15] Susceptibilities of tigecycline were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[16] In addition, the MIC ranges of carbapenem agents were also analyzed in our study.

The Detection of Resistance Gene

Polymerase chain reaction (PCR) testing was performed for detection of resistance genes using a previously described method.[17,18] Plasmid-mediated AmpC β-lactamase genes were detected using a multiplex PCR assay targeting MIR/ACT gene (closely related to chromosomal EBC family gene) and other plasmid-mediated genes, including *blaDHA, blaMOX, blaCMY, blaACC*, and *blaFOX*.[19] The PCR amplicons of carbapenemase and ESBLs resistance genes were sequenced.

Variables and Definitions

The data collected included information regarding demographics, baseline diseases, invasive procedures, and antibiotic exposure were also collected. All variables were analyzed to determine the risk factors for CR-ECL infection. Septic shock was defined as sepsis associated with organ dysfunction and accompanied by persistent hypotension after volume replacement,[20] and various infections are determined by the US Centers for Disease Control and Prevention (CDC).[21]

Multilocus Sequence Typing (MLST)

The *E. cloacae* MLST scheme used internal fragments of the following seven housekeeping genes: *dnaA, fusA, gyrB, leuS, pyrG, rplB, rpoB*. The PCR conditions were queried, and data were analysed using the *E. cloacae* MLST website (https://pubmlst.org/ecloacae/). Using its allelic profile, the sequence type (ST) was determined with the *E. cloacae* sequence definitions database.[22,23] In this study, if six of the seven alleles were homologous, strains would be grouped together.

Sample Size Calculations and Statistical Analysis

Based on the report of the CHINET Antimicrobial Resistance Surveillance Program in 2014,[24] we assumed that CR-ECL will comprise 1.5% of the cases and CS-ECL controls will comprise 10.9%. To determine a difference at the 0.05 significance level with 80% power, a 1:2 ratio between cases and controls. We estimated that we would need at least 83 CR-ECL vs 166 CS-ECL control cases (EpiInfo, version 3.3.2).

All statistical analyses were performed with SPSS 23.0 software. For univariate analysis, the results were presented as odds ratios (ORs), 95% confidence intervals (CIs) and *P* values. To identify the independent risk factors, significant variables with $P < 0.10$ in the univariate analysis were enrolled into the logistic regression model for multivariate analysis to evaluate risk factors for CR-ECL infection and mortality. For all statistical analyses, *P* value of < 0.05 was considered statistically significant.

Results

Bacterial Isolates

In this study, a total of 85 consecutive nonduplicate CR-ECL isolates were investigated during the study period. Isolates originated from different anatomical sites: urine (n=24, 28.2%), sputum (n=16, 18.8%), blood (n=13, 15.3%), secretions (n=10, 11.8%), puncture fluid (n=10, 11.8%), bile (n=6, 7.1%), pus (n=4, 4.7%), and cannula (n=2, 2.4%). The majority of patients were in the urinary surgery ward (n=17, 20.0%), followed by patients in the intensive care unit (n=14, 16.5%), hepatobiliary surgical ward (n=11, 12.9%), geriatric ward (n=10, 11.8%), neurology ward (n=10, 11.8%), respiratory ward (n=8, 9.4%), digestive medicine ward (n=7, 8.2%), orthopaedic ward (n=6, 7.1%), and endocrine ward (n=2, 2.4%).

Antibiotic Susceptibility Test

As shown in Table 1, of the 85 CR-ECL isolates, the ETP resistance rate was 100% (85/85), while 51.8% (44/85) and 42.4% (36/85) of the isolates were resistant to IMP and MEM, respectively. In addition, the rate of resistance to cephalosporins was relatively high. Specifically, 98.8%, 97.6%, and 65.9% of the isolates were resistant to CRO, CAZ, and FEP, respectively. However, the resistance rate to AK was relatively low (n=11, 12.9%). Notably, the drug resistance rate of CR-ECL infection group was significantly higher than that of the control group ($P < 0.05$). In addition, the distribution of carbapenem MIC ranges for CR-ECL with or without carbapenemase is listed in Table 2. Compared to carbapenemase-negative isolates, our results

Table 1 The Antimicrobial Susceptibility of CR-ECL and CS-ECL

Antibiotics	CR-ECL (n=85)			CS-ECL (n=170)			P-value
	S	I	R	S	I	R	
Ampicillin	0 (0)	2 (2.4)	83 (97.6)	37 (21.8)	6 (3.5)	127 (74.7)	**<0.001**
Ampicillin-sulbactam	5 (5.9)	1 (1.2)	79 (92.9)	72 (42.3)	4 (2.4)	94 (55.3)	**<0.001**
Piperacillin-tazobactam	46 (54.1)	3 (3.5)	36 (42.4)	143 (84.1)	7 (4.1)	20 (11.8)	**<0.001**
Ceftriaxone	1 (1.2)	0 (0)	84 (98.8)	106 (62.3)	3 (1.8)	61 (35.9)	**<0.001**
Ceftazidime	1 (1.2)	1 (1.2)	83 (97.6)	113 (66.4)	4 (2.4)	53 (31.2)	**<0.001**
Cefepime	23 (27)	6 (7.1)	56 (65.9)	141 (82.9)	8 (4.7)	21 (12.4)	**<0.001**
Cefoxitin	0 (0)	0 (0)	85 (100)	6 (3.5)	4 (2.4)	160 (94.1)	**0.023**
Aztreonam	6 (7)	2 (2.4)	77 (90.6)	78 (45.9)	11 (6.5)	81 (47.6)	**<0.001**
Ciprofloxacin	43 (50.6)	5 (5.9)	37 (43.5)	145 (85.3)	3 (1.8)	22 (12.9)	**<0.001**
Levofloxacin	54 (63.5)	8 (9.4)	23 (27.1)	156 (91.7)	2 (1.2)	12 (7.1)	**<0.001**
Gentamicin	27 (31.8)	7 (8.2)	51 (60.0)	146 (85.8)	5 (3.0)	19 (11.2)	**<0.001**
Tobramycin	22 (25.9)	9 (10.6)	54 (63.5)	146 (85.8)	4 (2.4)	20 (11.8)	**<0.001**
Amikacin	68 (80.0)	6 (7.1)	11 (12.9)	165 (97.0)	1 (0.6)	4 (2.4)	**<0.001**
Ertapenem	0 (0)	0 (0)	85 (100)	170 (100)	0 (0)	0 (0)	**<0.001**
Imipenem	26 (30.6)	15 (17.6)	44 (51.8)	170 (100)	0 (0)	0 (0)	**<0.001**
Meropenem	36 (42.4)	13 (15.2)	36 (42.4)	170 (100)	0 (0)	0 (0)	**<0.001**
Tigecycline	84 (98.8)	0 (0)	1 (1.2)	170 (100)	0 (0)	0 (0)	0.156
Polymyxin B	84 (98.8)	0 (0)	1 (1.2)	170 (100)	0 (0)	0 (0)	0.156

Note: Bold face indicates values that are significant (P<0.05).
Abbreviations: S, susceptible; I, intermediate-resistant; R, resistant.

Table 2 Distribution of Carbapenem MIC Ranges for CR-ECL with or Without Carbapenemase

Carbapenem Agents	Total (N = 85)	Carbapenemase Positive (N = 43)				Carbapenemase Negative (N = 42)				*P*-value
	R (%)	R	MIC_{50}	MIC_{90}	Range	R	MIC_{50}	MIC_{90}	Range	
Ertapenem	85 (100)	43 (100)	32	128	4–256	42 (100)	8	32	4–64	-
Imipenem	44 (51.8)	36 (83.7)	8	32	0.25–128	8 (19.1)	1	4	0.25–16	**<0.001**
Meropenem	36 (42.4)	30 (69.8)	8	64	0.5–128	6 (14.3)	1	4	0.25–32	**<0.001**

Notes: Data are number resistant (% of resistance rates). *P*-value for comparisons of the resistance rates of carbapenemase-positive and carbapenemase-negative groups. Bold face indicates values that are significant (P<0.05).
Abbreviation: R, resistance.

showed that resistance was a significantly greater proportion of CR-ECL isolates that were carbapenemase-positive for carbapenem agents (P < 0.05).

Detection of Drug Resistance Genes and ST Types

Overall, 50.6% (43/85) of the CR-ECL isolates harboured carbapenemase-encoding genes. Among them, 24 isolates possessed *blaNDM-1*, three isolates had *blaNDM-5*, eight isolates contained *blaKPC-2*, and eight isolates contained *blaIMP-8*. The *blaOXA-48* gene was also detected in two CR-ECL strains in our study, and two CR-ECL isolates were determined to carry the *blaNDM-1* and *blaIMP-8* genes at the same time. In addition to the production of carbapenemase, the deletion of outer membrane porins such as ompC and/or ompF was been detected in 44 CR-ECL strains. Notably, 83.3% (35/42) of non-carbapenemase-producing isolates had lost at least one porin, which indicated that the deletion of outer membrane porin genes played an important role in carbapenem resistance.The MLST results showed that ST51 was the predominant epidemic type (n=13, 30.2%), followed by ST88 (n=11, 25.6%) and the other types were relatively disperse. In addition, there was no outbreak of carbapenemase-positive CR-ECL isolates in our hospital, but some strains had the same ST type, which indicated that CR-ECL strains were dispersively transmitted in clinical departments (Table 3).

Risk Factor Analysis of CR-ECL Infection

The comparison of the CR-ECL infection group to controls according to 36 clinical variables is listed in Table 4. The following factors were most frequently associated with the development of CR-ECL infection: ICU admission, mechanical ventilation, drainage tube, central venous catheter, and exposure to carbapenem antibiotics. As shown in Table 5. ICU admission (OR: 3.62, 95% CI: 1.93–6.80, *P*<0.001), drainage tube (OR: 3.06, 95% CI: 1.50–6.22, *P*=0.002), central venous catheter (OR: 3.21, 95% CI: 1.43–7.21, *P*=0.005), and carbapenem exposure (OR: 3.43, 95% CI: 1.53–7.70, *P*=0.003) were identified as independent risk factors for infection with CR-ECL.

Clinical Outcome of CR-ECL Infection

At 28 days after infection onset, mortality was significantly higher in patients with CR-ECL infection than in those with CS-ECL infection (23/85 vs.5/170, *P*<0.001). In the CR-ECL group, 28-day mortality of patients who

Table 3 Distribution of Resistance Genes and MLST Types of CR-ECL

Microorganism (No. of Strains)	Resistance Genes and MLST Types (no. of Strains)						
	Carbapenemase Resistance Genes	ESBL Resistance Genes	AmpC Resistance Genes	Quinolone Resistance Genes	Aminoglycoside Resistance Genes	Outer Membrane Porin genes	ST Types
CR-ECL (85)	*KPC-2* (8) *NDM-1* (24) *NDM-5* (3) *IMP-8* (8) *OXA-48* (2)	*CTX-M-1* (21) *CTX-M-9* (19) *CTX-M-14* (5) *SHV-2* (36)	*DHA* (43) *CMY* (20) *MOX* (13)	*qnrA* (12) *qnrB* (46) *qnrS* (14) *acc(6')-Ib-cr* (40)	*armA* (16) *rmtB* (7) *acc(6')-Ib* (42)	*ompC* (28) *ompF* (13)	ST32 (3) ST51 (13) ST88 (11) ST89 (3) ST97 (5) ST177 (6) ST591 (2)

Abbreviations: MLST, multilocus sequence typing; ESBL, extended-spectrum β-lactamase.

Table 4 Univariate Analysis of Risk Factors for Infection with CR-ECL and CS-ECL

Variables	CR-ECL Infection (n=85)	Control Group (n=170)	OR (95% CI)	*P*-value
Demographics				
Male gender	45 (52.9)	87 (51.2)	1.07 (0.64–1.81)	0.790
Elderly	37 (43.5)	72 (42.4)	1.05 (0.62–1.78)	0.858
ICU admission	47 (55.3)	44 (25.9)	3.54 (2.05–6.13)	**<0.001**
Transferring from another hospital	26 (30.6)	38 (22.4)	1.53 (0.85–2.75)	0.153
Baseline diseases and acquired infection				
Hypertension	25 (29.4)	42 (24.7)	1.27 (0.71–2.27)	0.421
Diabetes	22 (25.9)	34 (20.0)	1.40 (0.76–2.58)	0.285
Hypoproteinemia	24 (28.2)	35 (20.6)	1.52 (0.83–2.77)	0.172
Hypokalemia	16 (18.8)	21 (12.4)	1.65 (0.81–3.35)	0.167
Severe anemia	6 (7.1)	14 (8.2)	0.85 (0.31–2.29)	0.742
Septic shock	24 (28.2)	33 (19.4)	1.63 (0.89–3.00)	0.111
Solid tumor	28 (32.9)	47 (27.6)	1.29 (0.73–2.26)	0.382
Respiratory diseases	29 (34.1)	43 (25.3)	1.53 (0.87–2.69)	0.140
Liver diseases	20 (23.5)	34 (20.0)	1.23 (0.66–2.30)	0.516
Gastrointestinal diseases	17 (20.0)	35 (20.6)	0.96 (0.50–1.85)	0.912
Cardiovascular diseases	13 (15.3)	14 (8.2)	2.01 (0.90–4.50)	0.084
Renal diseases	18 (21.2)	43 (25.3)	0.79 (0.43–1.48)	0.467
Endocrine system diseases	19 (22.4)	40 (23.5)	0.94 (0.50–1.74)	0.834
Pulmonary infection	19 (22.4)	25 (14.7)	1.67 (0.86–3.24)	0.128
Intra-abdominal infection	10 (11.8)	22 (12.9)	0.89 (0.40–1.99)	0.789
Urinary tract infection	11 (12.9)	28 (16.5)	0.75 (0.36–1.60)	0.460
Invasive procedures during hospital stay				
Parenteral nutrition	16 (18.8)	23 (13.5)	1.48 (0.74–2.98)	0.268
Mechanical ventilation	18 (21.2)	20 (11.8)	2.02 (1.01–4.05)	**0.047**
Urinary catheter	35 (41.2)	66 (38.8)	1.10 (0.65–1.88)	0.717
Drainage tube	33 (38.8)	23 (13.5)	4.06 (2.18–7.53)	**<0.001**
Tracheal cannula	9 (10.6)	28 (16.5)	0.60 (0.27–1.34)	0.209
Previous surgery in the past 6 months	31 (36.5)	44 (25.9)	1.64 (0.94–2.88)	0.080
Central venous catheter	25 (29.4)	18 (10.6)	3.52 (1.79–6.91)	**<0.001**
Arterial catheter	13 (15.3)	30 (17.6)	0.84 (0.41–1.71)	0.636
Antibiotic exposure within 3 months				
Cephalosporins	32 (37.6)	56 (32.9)	1.23 (0.71–2.12)	0.456
Carbapenem	26 (30.6)	15 (8.8)	4.56 (2.26–9.19)	**<0.001**
Aminoglycosides	7 (8.2)	26 (15.3)	0.50 (0.21–1.20)	0.113
Quinolones	17 (20.0)	36 (21.2)	0.93 (0.49–1.78)	0.827
Tetracyclines	6 (7.1)	7 (4.1)	1.77 (0.58–5.44)	0.314
Macrolides	7 (8.2)	9 (5.3)	1.61 (0.58–4.47)	0.361
Metronidazole	22 (25.9)	31 (18.2)	1.57 (0.84–2.92)	0.156
Glycopeptides	12 (14.1)	18 (10.6)	1.39 (0.64–3.03)	0.410

Notes: Values are presented as n (%), unless otherwise noted. Bold face indicates values that are significant (P<0.05).
Abbreviation: ICU, intensive care unit.

received carbapenem-including treatment, which was administered to 26 patients, was not significantly different from the mortality of patients without carbapenem treatment (8/26 vs.15/59, *P*=0.609), which indicated that the use of carbapenem is not associated with mortality in patients with CR-ECL infection. As shown in Table 6, the results showed that solid tumours (OR: 6.06, 95% CI: 1.73–21.21, *P*=0.005), septic shock (OR: 4.81, 95%

Table 5 Multivariate Analysis of Risk Factors for Infection with CR-ECL and CS-ECL

Variables	B	S.E,	Wals	OR	95% CI	*P*-value
ICU admission	1.29	0.32	16.03	3.62	1.93–6.80	**<0.001**
Drainage tube	1.12	0.36	9.51	3.06	1.50–6.22	**0.002**
Central venous catheter	1.17	0.41	7.98	3.21	1.43–7.21	**0.005**
Carbapenem exposure	1.23	0.41	8.94	3.43	1.53–7.70	**0.003**

Note: Bold face indicates values that are significant (P<0.05).
Abbreviation: ICU, intensive care unit.

Table 6 Risk Factors Associated with 28-Day Mortality

Variables	Nonsurvivors (n=23)	Survivors (n=62)	OR (95% CI)	P-value	OR (95% CI)	P-value
Elderly	11 (47.8)	26 (41.9)	1.27 (0.49–3.32)	0.627	–	–
ICU admission	13 (56.5)	34 (54.8)	1.07 (0.41–2.81)	0.890	–	–
Solid tumor	15 (65.2)	13 (21.0)	7.07 (2.46–20.27)	**<0.001**	6.06 (1.73–21.21)	**0.005**
Respiratory system disease	9 (39.1)	20 (32.3)	1.35 (0.50–3.64)	0.553	–	–
Digestive system disease	11 (47.8)	26 (41.9)	1.27 (0.49–3.32)	0.627	–	–
Urinary system disease	5 (21.7)	13 (21.0)	1.05 (0.33–3.35)	0.938	–	–
Cardiovascular diseases	3 (13.0)	10 (16.1)	0.78 (0.19–3.13)	0.725	–	–
Hypoproteinemia	7 (30.4)	17 (27.4)	1.16 (0.41–3.31)	0.784	–	–
Hypokalemia	6 (26.1)	10 (16.1)	1.84 (0.58–5.80)	0.297	–	–
Septic shock	13 (56.5)	11 (17.7)	6.03 (2.11–17.24)	**<0.001**	4.81 (1.30–17.79)	**0.019**
Parenteral nutrition	4 (17.4)	12 (19.4)	0.88 (0.25–3.06)	0.837	–	–
Tracheal cannula	2 (8.7)	7 (11.3)	0.75 (0.14–3.90)	0.730	–	–
Drainage tube	13 (56.5)	20 (32.3)	2.73 (1.02–7.28)	**0.041**	–	–
Previous surgery in the past 6 months	9 (39.1)	22 (35.5)	1.17 (0.44–3.13)	0.756	–	–
Central venous catheter	7 (30.4)	18 (29.0)	1.07 (0.38–3.04)	0.900	–	–
Arterial catheter	4 (17.4)	9 (14.5)	1.24 (0.34–4.50)	0.744	–	–
Mechanical ventilation	12 (52.2)	6 (9.7)	10.18 (3.15–22.94)	**<0.001**	4.70 (1.20–18.45)	**0.027**
Cephalosporins	10 (43.5)	22 (35.5)	1.40 (0.53–3.71)	0.499	–	–
Carbapenem	8 (34.8)	18 (29.0)	1.30 (0.47–3.61)	0.609	–	–
Aminoglycosides	2 (8.7)	5 (8.1)	1.09 (0.20–6.03)	0.925	–	–
Quinolones	5 (21.7)	12 (19.4)	1.16 (0.36–3.75)	0.807	–	–
Macrolides	3 (13.0)	4 (6.5)	2.18 (0.45–10.57)	0.326	–	–

Notes: Values are presented as n (%), unless otherwise noted. Bold face indicates values that are significant (P<0.05). "–" indicates data not available.
Abbreviation: ICU, intensive care unit.

CI: 1.30–17.79, *P*=0.019), and mechanical ventilation (OR: 4.70, 95% CI: 1.20–18.45, *P*=0.027) were the predictors independently associated with 28-day mortality.

Discussion

At present, the global spread of CR-ECL strains gravely threatens public health because of the limited treatment options and unfavourable impact on prognosis.[25,26] Previous studies showed that the detection rate of CR-ECL strains in North America, Europe, the Middle East and Southeast Asia was relatively high,[7] and even outbreaks occurred in some areas.[27] In China, the molecular epidemiology of CR-ECL has been reported in Henan, Guangdong and northwestern China, and ST418 and ST78 were the predominant epidemic types of CR-ECL producing *blaNDM-1* in Guangdong and northwestern China, respectively.[28,29] However, there have been no reports on the clinical molecular epidemiology of CR-ECL infection in southwestern China, and there is an urgent need to systematically analyse the epidemiological characteristics of CR-ECL infection.

Patients with CR-ECL infection often received more effective empirical treatment due to high clinical suspicion for multidrug-resistant gram-negative bacteria. Previous studies have shown that appropriate antimicrobial treatment can help to improve survival rate.[30] Regarding the drug sensitivity of CR-ECL strains, most CR-ECL strains have been

shown to be highly multidrug resistant. Actually, tigecycline and polymyxin B are the most effective antibiotics in the treatment of CR-ECL infection.[31] However, the use of polymyxin B as a monotherapy against CR-ECL infection is debatable. Increasing reports about heterologous resistance to polymyxin B of several gram-negative bacteria indicate that rapid resistance to polymyxins can develop as a result of polymyxin B monotherapy.[32] Notably, with the emergence of *mcr-1*-positive *E. cloacae*, which may lead to resistance to colistin, the treatment of CR-ECL infection is increasingly diffcult. Therefore, combined antibiotic therapy should be considered as the optimal treatment option for severe infections caused by CR-ECL.[33–35]

In this study, we found that most CR-ECL isolates contained multiple drug resistance genes at the same time. The production of ESBLs and AmpC β-lactamases has been demonstrated to play a role in the resistance of *E. cloacae* to broad-spectrum beta-lactam antibiotics. In addition, *blaDHA* was reported to be the most common gene in *E. cloacae* producing AmpC β-lactamases in many studies, which is consistent with our study.[36,37] However, it is worth noting that carbapenemase acquisition and loss of outer membrane proteins are the main causes of bacterial resistance to carbapenem, rather than ESBLs and AmpC β-lactamases.[38] Notably, under antibiotic selective pressure, resistance genes can be transmitted frequently through mobile genetic elements, which will ultimately result in the development of extensively drug-resistant (XDR) and even pandrug-resistant (PDR) *E. cloacae*.[39,40]

Most previous studies appeared to be restricted to reports of sporadic cases of *E. cloacae* isolates harbouring *blaNDM-1*, with diverse clones from geographic regions such as ST92 in Croatia, ST265 in Australia, and ST78, ST93, ST120, and ST418 in China.[41,42] In the current study, ST51 and ST88 were the predominant epidemic types among the seven different ST types identified, which provides a new model for the spread of *blaNDM-1*-carrying *E. cloacae* in China and should be of great-concern. Interestingly, only two of the seven alleles were different between ST51 (*dnaA, fusA, gyrB, leuS, pyrG, rplB*, and *rpoB*: 4, 4, 4, 6, 37, 4, and 6, respectively) and ST88 (*dnaA, fusA, gyrB, leuS, pyrG, rplB*, and *rpoB*: 4, 4, 4, 62, 59, 4, and 6, respectively), suggesting that they are closely related to each other. Notably, in contrast to the close connection between ST258/ST11 and *blaKPC-2* in *K. pneumoniae*,[43] the *blaNDM-1* gene has a high detection rate in the above two ST types (n=20, 83.3%), suggesting that the *blaNDM-1* gene was involved in the epidemic dissemination of CR-ECL strains. Diverse clones of *blaNDM-1*-carrying *E. cloacae* have been widely geographically distributed.[44]

We also performed a retrospective analysis to assess clinical predictors of CR-ECL infection. Compared to previous studies,[45,46] ICU admission, drainage tube, central venous catheter, and carbapenem exposure were identified as independent risk factors for infection with CR-ECL in our study. As we all know, the immunity of patients in ICU is relatively low, and the possibility of infection of CR-ECL strain will be increased during invasive operations such as drainage tube and central venous catheter. In addition, when the *E. cloacae* are exposed to carbapenem antibiotics, the sensitive strains are inhibited or killed, and the resistant strains survive and become the dominant strains, even lead to the spread of CR-ECL strains.[47] Therefore, we should reasonably use carbapenem antibiotics to reduce the production of CR-ECL strain.

Consistent with previous studies,[48,49] our study highlighted the high mortality associated with CR-ECL infection. Although our study showed that exposure to carbapenem antibiotics was strongly correlated with CR-ECL infection, we did not identify an association between the use of carbapenems and mortality in patients with CR-ECL infection, we found that solid tumours, septic shock, and mechanical ventilation were independent risk factors for death caused by CR-ECL infection. Most cancer patients usually have low immunity after radiotherapy or chemotherapy. In addition, the basic condition of these patients is very poor, which increases the risk of infection and even death. Moreover, CR-ECL strains often cause septic shock, which leads to multiple organ failure and death.[50] Therefore, We should strengthen the monitoring of these patients with a high risk of death to reduce their mortality.

Limitations

This study has several limitations. First, it was a retrospective case-control study and was conducted in a single centre, our sample size was relatively small, which might have led to errors in statistical analysis and the omission of some other risk factors. Second, we were unable to determine all the resistance mechanisms of CR-ECL to carbapenems, such as the mutation of outer membrane porins, overexpression of efflux pumps and so on, owing to the limitations of the research conditions. Third, due to methodological limitations, this retrospective study was not able to evaluate the expected efficacy and outcome of the treatment, and further prospective and multicentre clinical trials are expected to be performed.

Conclusions

Our findings showed that ST51 and ST88, which are closely related, were the predominant epidemic types of CR-ECL producing *blaNDM-1* in southwestern China. In addition, we identified important risk factors and predictors of mortality in patients with CR-ECL infection, and provided some recommendations for the diagnosis and treatment of patients infected with CR-ECL strains in southwestern China.

Ethics Approval

The study was approved by the Ethics Committee of Renji Hospital, Chinese Academy of Sciences University, Chongqing, China. The ethics committee waived the need for written informed consent provided by participants due to the retrospective nature of the study. Because all patient data were analyzed in anonymity, no additional informed consent was required.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Alraddadi BM, Saeedi M, Qutub M, et al. Efficacy of ceftazidime-avibactam in the treatment of infections due to carbapenem-resistant *Enterobacteriaceae*. *BMC Infect Dis*. 2019;19(1):772. doi:10.1186/s12879-019-4409-1
2. Ballot DE, Bandini R, Nana T, et al. A review of -multidrug-resistant *Enterobacteriaceae* in a neonatal unit in Johannesburg, South Africa. *BMC Pediatr*. 2019;19(1):320. doi:10.1186/s12887-019-1709-y
3. Saeed NK, Alkhawaja S, Azam N, Alaradi K, Al-Biltagi M. Epidemiology of carbapenem-resistant *Enterobacteriaceae* in a tertiary care center in the Kingdom of Bahrain. *J Lab Physicians*. 2019;11(2):111–117. doi:10.4103/JLP.JLP_101_18
4. Perez F, Bonomo RA. Carbapenem-resistant *Enterobacteriaceae*: global action required. *Lancet Infect Dis*. 2019;19(6):561–562. doi:10.1016/S1473-3099(19)30210-5
5. Tetsuka N, Hirabayashi A, Matsumoto A, et al. Molecular epidemiological analysis and risk factors for acquisition of carbapenemase-producing *enterobacter cloacae* complex in a Japanese University Hospital. *Antimicrob Resist Infect Control*. 2019;8:126. doi:10.1186/s13756-019-0578-3
6. Lee JH, Bae IK, Lee CH, Jeong S. Molecular characteristics of first *IMP-4*-producing enterobacter cloacae sequence type 74 and 194 in Korea. *Front Microbiol*. 2017;8:2343. doi:10.3389/fmicb.2017.02343
7. Annavajhala MK, Gomez-Simmonds A, Uhlemann AC. Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. *Front Microbiol*. 2019;10:44. doi:10.3389/fmicb.2019.00044
8. Ho PL, Li Z, Lo WU, et al. Identification and characterization of a novel incompatibility group X3 plasmid carrying *bla NDM-1* in Enterobacteriaceae isolates with epidemiological links to multiple geographical areas in China. *Emerg Microbes Infect*. 2012;1(11):e39. doi:10.1038/emi.2012.37
9. Majewski P, Wieczorek P, Ojdana D, et al. Altered outer membrane transcriptome balance with AmpC overexpression in carbapenem-resistant *enterobacter cloacae*. *Front Microbiol*. 2016;7:2054. doi:10.3389/fmicb.2016.02054
10. Dai W, Sun S, Yang P, et al. Characterization of carbapenemases, extended spectrum Beta-Lactamases and molecular epidemiology of carbapenem-non-susceptible *Enterobacter Cloacae* in a Chinese hospital in Chongqing. *Infect Genet Evol*. 2013;14:1–7. doi:10.1016/j.meegid.2012.10.010
11. Pecora N, Zhao X, Nudel K, et al. Diverse vectors and mechanisms spread New Delhi metallo-beta-lactamases among carbapenem-resistant *Enterobacteriaceae* in the greater Boston Area. *Antimicrob Agents Chemother*. 2019;63(2). doi:10.1128/AAC.00779-19.
12. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *enterobacteriaceae* worldwide. *Clin Microbiol Infect*. 2014;20(9):821–830. doi:10.1111/1469-0691.12719
13. Arabaghian H, Salloum T, Alousi S, et al. Molecular characterization of carbapenem resistant *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* isolated from Lebanon. *Sci Rep*. 2019;9(1):531. doi:10.1038/s41598-018-36554-2
14. Cheng P, Li F, Liu R, et al. Prevalence and molecular epidemiology characteristics of carbapenem-resistant *Escherichia Coli* in Heilongjiang Province, China. *Infect Drug Resist*. 2019;12:2505–2518. doi:10.2147/IDR.S208122
15. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. 26Th Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
16. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, Valid From 2017-01-01. Available from: Httpwww.Eucast.OrgClinical_Breakpoints. Accessed February 24, 2017.
17. Dallenne C, Da CA, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother*. 2010;65(3):490–495. doi:10.1093/jac/dkp498
18. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70(1):119–123. doi:10.1016/j.diagmicrobio.2010.12.002
19. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol*. 2002;40(6):2153–2162. doi:10.1128/JCM.40.6.2153-2162.2002
20. Plante LA. Management of sepsis and septic shock for the obstetrician-gynecologist. *Obstet Gynecol Clin North Am*. 2016;43(4):659–678. doi:10.1016/j.ogc.2016.07.010
21. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrob Agents Chemother*. 2005;49(2):760–766. doi:10.1128/AAC.49.2.760-766.2005
22. Joseph S, Forsythe S. Multilocus Sequence Typing (MLST) for Cronobacter Spp. *Methods Mol Biol*. 2017;1616:241–248.
23. Shoaei P, Shojaei H, Khorvash F, et al. Clostridium difficile infection in cancer patients with hospital acquired diarrhea at the teaching hospitals in Iran: multilocus Sequence Typing Analysis (MLST) and antimicrobial resistance pattern. *Ann Ig*. 2019;31(4):365–373. doi:10.7416/ai.2019.2298
24. Fupin H, Demei Z, Fu W, Et A. Report of CHINET antimicrobial resistance surveillance program in 2014. *Chin J Infect Chemother*. 2015;401–410.
25. Uechi K, Tada T, Shimada K, et al. Emergence of a carbapenem-resistant and colistin-heteroresistant *Enterobacter Cloacae* clinical isolate in Japan. *J Infect Chemother*. 2019;25(4):285–288. doi:10.1016/j.jiac.2018.09.004

26. Wang Q, Wang X, Wang J, et al. Phenotypic and genotypic characterization of carbapenem-resistant *Enterobacteriaceae*: data from a longitudinal large-scale CRE study in China (2012–2016). *Clin Infect Dis*. 2018;67(suppl_2):S196–S205. doi:10.1093/cid/ciy660
27. Miltgen G, Bonnin RA, Avril C, et al. Outbreak of IMI-1 carbapenemase-producing colistin-resistant *Enterobacter cloacae* on the French Island of Mayotte (Indian Ocean). *Int J Antimicrob Agents*. 2018;52(3):416–420. doi:10.1016/j.ijantimicag.2018.05.015
28. Cai Y, Chen C, Zhao M, et al. High prevalence of metallo-beta-lactamase-producing *Enterobacter cloacae* from three tertiary hospitals in China. *Front Microbiol*. 2019;10:1610. doi:10.3389/fmicb.2019.01610
29. Jin C, Zhang J, Wang Q, et al. Molecular characterization of carbapenem-resistant *Enterobacter cloacae* in 11 Chinese cities. *Front Microbiol*. 2018;9:1597. doi:10.3389/fmicb.2018.01597
30. Nabarro L, Shankar C, Pragasam AK, et al. Clinical and bacterial risk factors for mortality in children with carbapenem-resistant *enterobacteriaceae* bloodstream infections in India. *Pediatr Infect Dis J*. 2017;36(6):e161–e166. doi:10.1097/INF.0000000000001499
31. Alves PH, Boff RT, Barth AL, Martins AF. Synergy of Polymyxin B, tigecycline and meropenem against carbapenem-resistant *Enterobacter Cloacae* complex isolates. *Diagn Microbiol Infect Dis*. 2019;94(1):81–85. doi:10.1016/j.diagmicrobio.2018.11.011
32. Medeiros GS, Rigatto MH, Falci DR, Zavascki AP. Combination therapy with Polymyxin B for carbapenemase-producing *Klebsiella pneumoniae* bloodstream infection. *Int J Antimicrob Agents*. 2019;53(2):152–157. doi:10.1016/j.ijantimicag.2018.10.010
33. MacNair CR, Stokes JM, Carfrae LA, et al. Overcoming *Mcr-1* mediated colistin resistance with Colistin in combination with other antibiotics. *Nat Commun*. 2018;9(1):458. doi:10.1038/s41467-018-02875-z
34. Martino F, Tijet N, Melano R, et al. Isolation of five enterobacteriaceae species harbouring *blaNDM-1* and *Mcr-1* plasmids from a single paediatric patient. *PLoS ONE*. 2019;14(9):e0221960. doi:10.1371/journal.pone.0221960
35. Wise MG, Estabrook MA, Sahm DF, Stone GG, Kazmierczak KM, Chang Y-F. Prevalence of Mcr-Type genes among colistin-resistant Enterobacteriaceae collected in 2014–2016 as part of the INFORM global surveillance program. *PLoS ONE*. 2018;13(4):e0195281. doi:10.1371/journal.pone.0195281
36. Ku YH, Lee MF, Chuang YC, Yu WL. Detection of plasmid-mediated beta-lactamase genes and emergence of a novel AmpC (CMH-1) in *Enterobacter cloacae* at a Medical Center in Southern Taiwan. *J Clin Med*. 2018;8:1. doi:10.3390/jcm8010008
37. Uzunovic S, Ibrahimagic A, Bedenic B. Antibiotic resistance in *Enterobacter cloacae* strains with derepressed/partly derepressed/inducible AmpC and extendedspectrum beta-lactamases in Zenica-Doboj Canton, Bosnia and Herzegovina. *Med Glas (Zenica)*. 2018;15(1):37–45. doi:10.17392/925-18
38. Babouee FB, Ellington MJ, Hopkins KL, et al. Association of novel nonsynonymous single nucleotide polymorphisms in AmpD with cephalosporin resistance and phylogenetic variations in ampC, ampR, ompF, and ompC in *Enterobacter cloacae* isolates that are highly resistant to carbapenems. *Antimicrob Agents Chemother*. 2016;60(4):2383–2390. doi:10.1128/AAC.02835-15
39. Liu J, Xu Z, Guo Z, et al. Structural investigation of a polysaccharide from the mycelium of *Enterobacter cloacae* and its antibacterial activity against extensively drug-resistant *E. cloacae* producing SHV-12 extended-spectrum beta-lactamase. *Carbohydr Polym*. 2018;195:444–452. doi:10.1016/j.carbpol.2018.04.114
40. Yang L, Wu AW, Su DH, et al. Resistome analysis of *Enterobacter Cloacae* CY01, an extensively drug-resistant strain producing *VIM-1* metallo-beta-lactamase from China. *Antimicrob Agents Chemother*. 2014;58(10):6328–6330. doi:10.1128/AAC.03060-14
41. Zujic AV, Bedenic B, Kocsis E, et al. Diversity of carbapenemases in clinical isolates of *Enterobacteriaceae* in croatia-the results of a multicentre study. *Clin Microbiol Infect*. 2014;20(11):O894–903. doi:10.1111/1469-0691.12635
42. Wailan AM, Paterson DL, Kennedy K, et al. Genomic characteristics of *NDM*-producing *Enterobacteriaceae* isolates in Australia and their *blaNDM* genetic contexts. *Antimicrob Agents Chemother*. 2016;60(1):136–141. doi:10.1128/AAC.01243-15
43. Yu F, Lv J, Niu S, et al. Multiplex PCR analysis for rapid detection of *Klebsiella pneumoniae* carbapenem-resistant (Sequence Type 258 [ST258] and ST11) and hypervirulent (ST23, ST65, ST86, and ST375) strains. *J Clin Microbiol*. 2018;56:9. doi:10.1128/JCM.00731-18
44. Liu C, Qin S, Xu H, et al. Correction: New Delhi Metallo-beta-Lactamase 1(*NDM-1)*, the dominant carbapenemase detected in carbapenem-resistant *Enterobacter cloacae* from Henan Province, China. *PLoS One*. 2015;10(10):e0140726. doi:10.1371/journal.pone.0140726
45. Jiang Y, Jia X, Xia Y. Risk factors with the development of infection with tigecycline- and carbapenem-resistant *Enterobacter cloacae*. *Infect Drug Resist*. 2019;12:667–674. doi:10.2147/IDR.S189941
46. Tian X, Sun S, Jia X, et al. Epidemiology of and risk factors for infection with extended-spectrum Beta-Lactamase-Producing carbapenem-resistant *Enterobacteriaceae*: results of a double case-control study. *Infect Drug Resist*. 2018;11:1339–1346. doi:10.2147/IDR
47. Miao M, Wen H, Xu P, et al. Genetic diversity of Carbapenem-Resistant *Enterobacteriaceae* (CRE) clinical isolates from a tertiary hospital in Eastern China. *Front Microbiol*. 2018;9:3341. doi:10.3389/fmicb.2018.03341
48. Da SK, Varella TR, Bet G, et al. High mortality rate associated with *KPC*-producing *Enterobacter cloacae* in a Brazilian Hospital. *Am J Infect Control*. 2018;46(1):108–110. doi:10.1016/j.ajic.2017.07.011
49. Cheewinmethasiri J, Chittawatanarat K, Chandacham K, Jirapongchareonlap T, Chotirosniramit N. Microbiology, risk factors and mortality of patients with intravenous catheter related blood stream infections in the surgical intensive care unit: a Five-Year, concurrent, case-controlled study. *J Med Assoc Thai*. 2014;97(Suppl 1):S93–101.
50. Tresoldi AT, Padoveze MC, Trabasso P, et al. *Enterobacter cloacae* sepsis outbreak in a Newborn unit caused by contaminated total parenteral nutrition solution. *Am J Infect Control*. 2000;28(3):258–261. doi:10.1067/mic.2000.105286