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ORIGINAL RESEARCH

Molecular Epidemiological Characteristics of *bla*$_{IMP-4}$-Carrying *Klebsiella pneumoniae* ST-11 in Hospitalized Patients

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Purpose: To investigate the molecular epidemiology and risk factors of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection.

Patients and Methods: Patient's clinical data and CRKP strains were collected from November 2017 to December 2018 at a tertiary hospital in Wuhan, China. The antimicrobial susceptibilities, carbapenem-resistant genes, multi-locus sequence typing (MLST), homologous analysis, and risk factors for CRKP were determined.

Results: A total of 203 CRKP strains were isolated, and 98.5% (200/203) of patients were nosocomially infected. The mortality rate was 17.7% (36/203). All 203 strains were confirmed as carbapenemases -producing strains. The most predominant carbapenemase gene was *bla*$_{IMP-4}$ (81.3%, 165/203), followed by *bla*$_{KPC-2}$ (25.1%, 51/203) and *bla*$_{NDM-1}$ (23.2%, 47/205). Of the 203 strains, 28 (13.8%) had both *bla*$_{KPC-2}$ and *bla*$_{IMP-4}$ genes, 23 (11.3%) had both *bla*$_{IMP-4}$ and *bla*$_{NDM-1}$ genes, 20 (9.9%) had *bla*$_{KPC-2}$, *bla*$_{IMP-4}$ and *bla*$_{NDM-1}$ three genes. MLST analysis showed that there were 48 ST typologies (including 7 new STs), of which ST-11 was the most prevalent (59.6%, 121/203). Phylogenetic analysis showed that 203 CRKP isolates came from 7 clusters and exhibited a strong correlation with the isolation source. eBURST analyses indicated that CRKP isolates have undergone different evolutionary processes. Patients with ST-11 CRKP underwent more mechanical ventilation (50% vs 32.9%, P=0.020) and gastric catheterization (15.7% vs 6.1%, P=0.042) within 3 months before sample collection, and also had higher drug-resistance rate than non-ST-11 CRKP. Comparing with CSKP (carbapenem-sensitive *Klebsiella pneumoniae*), gastrointestinal disease (odds ratio [OR]=6.168, P=0.003), nosocomial infection (OR=5.573, P=0.012), antibiotic exposure (OR=4.131, P=0.004), urinary catheterization (OR=3.960, P=0.031) and venous/arterial catheterization (OR=2.738, P=0.026) within the preceding 3 months were independent risk factors for CRKP infection.

Conclusion: The IMP-4 was the most predominant carbapenemase and *bla*$_{IMP-4}$ bearing *Klebsiella pneumoniae* ST-11 was spreading in the hospital. Nosocomial infections, antibiotic exposure, and urinary and venous/arterial catheterization within 3 months were the risk factors for developing CRKP infection.

Keywords: *Klebsiella pneumoniae*, carbapenemase, multi-locus sequence typing, MLST, ST-11, IMP-4

Introduction

Klebsiella pneumoniae is one of the most common causes of nosocomial infection. In recent years, the exposure to antibiotics has dramatically increased the drug resistance of *K. pneumoniae*.[1–3] *K. pneumoniae* was first reported to be resistant to

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penicillin in 1960.[4] Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has gradually emerged with the introduction of carbapenems and is now a global health concern.[3,5] Due to their ease of dissemination and the limited therapeutic options, CRKP infections are usually severe and poorly treated, leading to prolonged hospitalization, increased burden of care, increased risk of multiple infections, and higher mortality rates.[6–8] The World Health Organization has identified CRKP as a severe public health threat.[9] In China, the China Antimicrobial Surveillance Network (CHINET) has reported a rise in the rates of *K. pneumoniae* resistance to meropenem from 2.9% in 2005 to 26.3% in 2018, and the resistance rates to imipenem have increased from 3.0% in 2005 to 25% in 2018 (http://www.chinets.com/).

The production of carbapenemases is the chief cause of carbapenem resistance in *K. pneumoniae*. The three major types of carbapenemases include *K. pneumoniae* carbapenemase (KPC), metallo-β-lactamases (MBLs) such as New Delhi metallo-β-lactamase (NDM), Imipenemase metallo-β-lactamase (IMP) and Verona integron-encoded metallo-β-lactamase (VIM), and OXA48 enzymes. Among these, IMP exhibits a relatively low prevalence in clinical *K. pneumoniae*. In China, only a limited number of studies have documented clonal transmission of *IMP-4-producing K. pneumoniae* among children and newborns,[10–12] with only small-scale sample study reporting clonal transmission among adults.[13] The specific transmission routes and molecular epidemiological characteristics of these strains remain unclear.

CRKP epidemics exhibit significant regional variations in bacterial characteristics, patients' baseline characteristics and clinical outcomes, and the distribution of carbapenemases.[14] The prevalence of CRKP also varies across different geographical areas in China.[15–17] Thus continuous monitoring of CRKP in different areas is worthwhile.

This study investigated the clinical and molecular epidemiological characteristics of CRKP and identified risk factors for the development of CRKP at a large teaching hospital in central China. $Bla_{\text{IMP-4}}$-carrying ST-11 *K. pneumoniae* discovered as the main prevalent type.

Materials and Methods

Bacterial Collection, Identification, and Antimicrobial Susceptibility Testing

Samples from patients infected with CRKP (n = 203) were collected at a tertiary hospital in Wuhan, China, between November 2017 and December 2018. Recurrent infections were excluded, and only the first episode of infections caused by CRKP was included in this analysis. In addition, only patients with complete clinical data were included. All of the specimens were inoculated on a blood agar medium and incubated at a temperature of 37°C for a period of 18 to 24 hours. BD Phoenix™100 Automated Microbiology System (BD Ltd. in Franklin Lakes, NJ, USA) was used to identify the strains and determine their susceptibility to various antimicrobial agents. The modified Hodge test was employed to confirm the presence of carbapenemase-producing strains. All results were interpreted according to the guideline provided by the Clinical and Laboratory Standards Institute (CLSI) M100.[18] The disks used for the confirmation test were obtained from Beijing Tiantan Biological Products Corporation in China. *K. pneumoniae* (ATCC700603) served as the control strain to ensure the accuracy of the experiment. All isolates were stored at −80°C for further study. Patient information, including age, gender, history, diagnosis, and outcome, was obtained from electronic medical records. We also collected the clinical data of a sample of carbapenem-susceptible *Klebsiella pneumoniae*(CSKP) patients (n = 67) using a systematic sampling method within a list of all CSKP patients in the same hospital between November 2017 and December 2018. A random starting point was chosen, and every 10th identity card number was added to the target CSKP population list.

Detection of Carbapenemase Genes of CRKP Isolates

Bacterial DNA was prepared using the bacteria DNA extraction kit (TIANamp Bacteria DNA kit, China) according to the manufacturer's instructions. All the samples underwent tests to detect the existence of common carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA}) using polymerase chain reaction (PCR) methods. The oligonucleotide primers specific for carbapenemase genes in the PCR assays were designed in this study according to previous studies[19–21] and are listed in Table 1. DNA sequencing was performed by Beijing Tsingke Biotech Co., Ltd. (Tianjin, China). The amplification system contained 9.5 μL ddH_2O, 12.5 μL multiplex buffer, 1 μL primer1 (10μM), 1 μL primer2 (10μM), and 1 μL template for a final volume of 25 μL. The PCR amplification conditions were as followings: 94°C for 5 minutes (pre-denaturation); 94°C for 30

Table 1 Primers Designed in This Study for Screening the Carbapenemase Genes of CRKP

Ambler Classification	*bla* Genes Group	Oligonucleotide Sequence 5'—3'
A	bla_{KPC}	F: GCTACACCTAGCTCCACCTTC
		R: ACAGTGGTTGGTAATCCATGC
B	bla_{IMP}	F: CTACCGCAGCAGAGTCTTTG
		R: AACCAGTTTTGCCTTACCAT
	bla_{VIM}	F: ATGGTGTTTGGTCGCATATC
		R: TGGGCCATTCAGCCAGATC
	bla_{NDM}	F: GGCGGAATGGCTCATCACGA
		R: CGCAACACAGCCTGACTTTC
D	bla_{OXA}	F: TTGGTGGCATCGATTATCGG
		R: GAGCACTTCTTTTGTGATGGC

seconds (denaturation), 56°C for 30 seconds (annealing), and 72°C for 1 minute (extension) (35 cycles); and followed by a final extension at 72°C for 10 minute. Amplified PCR products stained with GelRed were separated by electrophoresis on 1.5% (w/v) agarose (1st base) at 100 V for 30–40 minutes. The molecular size of fragments generated by electrophoresis was determined by comparison to 2-kb DNA ladders (Dalian TaKaRa Corporation), and band patterns were captured under an ultraviolet illuminator. Then, amplified PCR products were sequenced by Sanger sequencing, and the complete sequences were compared with those reported in GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/).

Multilocus Sequence Typing (MLST) Analysis and Phylogenetic Tree Construction

Clonal relationships analysis of CRKP strains was performed by MLST. Bacterial DNA extraction was performed as previously described to detect carbapenemase genes.

The housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB, and tonB*) of *K. pneumoniae* were amplified by PCR, and the alleles and STs were determined using online database tools (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The BioNumerics software (version 7.6) was used to construct phylogenetic trees based on MLST sequences using the maximum likelihood method with 1000 bootstrap replicates. To explore patterns of evolutionary descent among isolates, we used global optimal e-BURST (goeBURST) analysis of PHYLOViZ 2.0 software to classify the STs of all strains into clonal complexes (CC), groups, and singletons. The software's default settings follow the strictest criteria: if two STs differ by only one allele, they are considered single-locus variants (SLVs); if they differ by two alleles, they are considered double-locus variants (DLVs). Only SLVs can form a group. If a group contains enough STs, and one of the STs has the highest number of corresponding SLVs, that ST can be provisionally considered a likely ancestral ST, and the group can be regarded as a CC. STs that do not belong to any group are classified as singletons.

Statistical Analysis

Percentages and frequencies were used to describe categorical variables. The Chi-square test or Fisher's exact test was used to compare categorical variables. Risk factors for the development of CRKP were analyzed using binary logistic regression. Variables with a P value of <0.05 in the univariate analysis were included in logistic regression models for multivariable analysis. In multivariable logistic regression, risk factors with P-values <0.05 and the odds ratios (ORs) >1 were considered as independent risk factors. A P value of <0.05 was considered to be statistically significant. Analyses were performed using SPSS 26.0 for MacIntosh (SPSS Inc., Chicago, IL, USA).

Ethics Approval and Informed Consent

The study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the Tongji Medical College Ethics Committee, Huazhong University of Science and Technology (2018-S356). Due to the retrospective nature of the study, patient consent for inclusion was waived. We ensure that the use of anonymized data posed minimal risk to patient privacy.

Results

Patient Characteristics

A total of 203 patients with CRKP infection were included in this study, with a median age of 56.0 (interquartile range, [IQR], 38.0–67.0) years, and 136 (67.0%) were men. Of these, 98.0% (199/203) patients had at least one comorbidity, and the common comorbidities were cardiovascular disease (41.4%, 84/205), followed by gastrointestinal disease (36.9%, 75/205) and cerebrovascular disease (31.5%, 64/205). 98.5% (200/203) of the patients were nosocomially infected with CRKP, and 5 (2.5%) were admitted to the relevant department after CRKP infection was detected during outpatient visits. Within 3 months prior to sample collection, 68.5% (139/203) patients had a history of hospitalization, 61.1% (124/203) patients had intensive care unit (ICU) stay, 88.7% (180/203) were exposed to antibiotics, and 89.2% (181/203) patients had undergone invasive procedures. Among those who had undergone invasive procedures, 65.5% (133/203) patients had received venous/arterial catheterization, 63.1% (128/203) had indwelling urinary catheters, 54.2% (110/203) had indwelling gastric catheters, and 43.3% (88/203) underwent invasive mechanical ventilation. The median hospital stay days were 23.0 (14.0–36.0) days. The mortality rate among patients with CRKP was 17.7% (36/205) (Table 2).

Table 2 Demographic and Clinical Characteristics of Patients with CRKP Infection

	CRKP Patients (n=203)
Male	136 (67.0%)
Age, years	56.0 (38.0–67.0)
Comorbidities	199 (98.0%)
Lung disease	32 (15.8%)
Cardiovascular disease	84 (41.4%)
Gastrointestinal disease	75 (36.9%)
Urinary system disease	34 (16.7%)
Cerebrovascular disease	64 (31.5%)
Hematological Disease	43 (21.2%)
Endocrine system disease	26 (12.8%)
Rheumatism immunity disease	6 (3.0%)
Malignant solid tumor	27 (13.3%)
Events within 3 months before sample collection	
Previous admission	139 (68.5%)
Prior ICU stay	124 (61.1%)
Prior antibiotic exposure	180 (88.7%)
Prior Invasive procedure	181 (89.2%)
Mechanical ventilation	88 (43.3%)
Laryngoscope/Bronchoscopy/Gastroscope	23 (11.3%)
Thoracic catheterization	32 (15.8%)
Gastric catheterization	110 (54.2%)
Abdominal catheterization	46 (22.7%)
Urinary catheterization	128 (63.1%)
Venous/Arterial catheterization	133 (65.5%)

(*Continued*)

Table 2 (Continued).

	CRKP Patients (n=203)
Outcomes	
Length of hospital stay, days	23.0 (14.0–36.0)
Time from admission to positive culture, days	11.0 (4.0–21.0)
Time from positive culture to discharge, days	11.0 (5.0–19.0)
Recovery	167 (82.3%)
Died	36 (17.7%)

Note: Data are given as median (interquartile range, IQR) or n (%).
Abbreviations: CRKP, Carbapenem-resistant *Klebsiella Pneumoniae*; ICU, intensive care unit.

Drug Resistance Rate and Characteristics of Drug-Resistant Gene in CRKP Strains

All 203 CRKP isolates were confirmed as carbapenemase-producing strains. The highest resistance of all strains was cefazolin (98.5%) excluding intrinsic resistance to ampicillin (100%), followed by piperacillin (97.5%). The highest susceptibility was to tigecycline (100%), followed by minocycline (69.4%) and chloramphenicol (43.9%) (Figure 1).

The results of drug-resistant gene analysis are shown in Figure 2A. The most common drug-resistant gene was $bla_{\text{IMP-4}}$, identified in 81.0% (166/205) of strains, followed by $bla_{\text{KPC-2}}$ was identified in 24.9% (51/205), and $bla_{\text{NDM-1}}$ was identified in 22.9% (47/205) of strains. Among these, 94 (46.3%) strains had only $bla_{\text{IMP-4}}$ gene, 3 (1.5%) had only $bla_{\text{NDM -1}}$gene, 2 (1.0%) had only $bla_{\text{KPC-2}}$ gene, 28 (13.8%) had both $bla_{\text{KPC-2}}$ and $bla_{\text{IMP-4}}$ genes, 23 (11.3%) had both $bla_{\text{IMP-4}}$ and $bla_{\text{NDM-1}}$ genes, 1 (0.5%) had both $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-1}}$ genes, and 20 (9.9%) had three genes: $bla_{\text{KPC-2}}$, $bla_{\text{IMP-4}}$, and $bla_{\text{NDM-1}}$ three genes. None of the isolates carried bla_{OXA} or bla_{VIM} genes.

MLST Analysis

MLST experiments identified a total of 48 distinct STs within the 203 strains. As shown in Figure 2B, the most prevalent STs were ST-11 (59.6%, 121/203), followed by ST-2407 (5.4%, 11/203), ST-15 (3.5%, 7/203), ST-37 (3.0%, 6/203), ST-5254 (2.0%, 4/203), ST-273 (1.5%, 3/203), ST-668 (1.5%, 3/203), and ST-412 (1.5%, 3/203). Additionally, there were also 8 (3.9%) new untitled STs.

Phylogenetic analysis (based on MLST sequences) showed that the 203 CRKP isolates were clustered into different groups which the seven major groups, designated as clades 1 to 7. The phylogenic tree showed that all the ST-11 and ST-

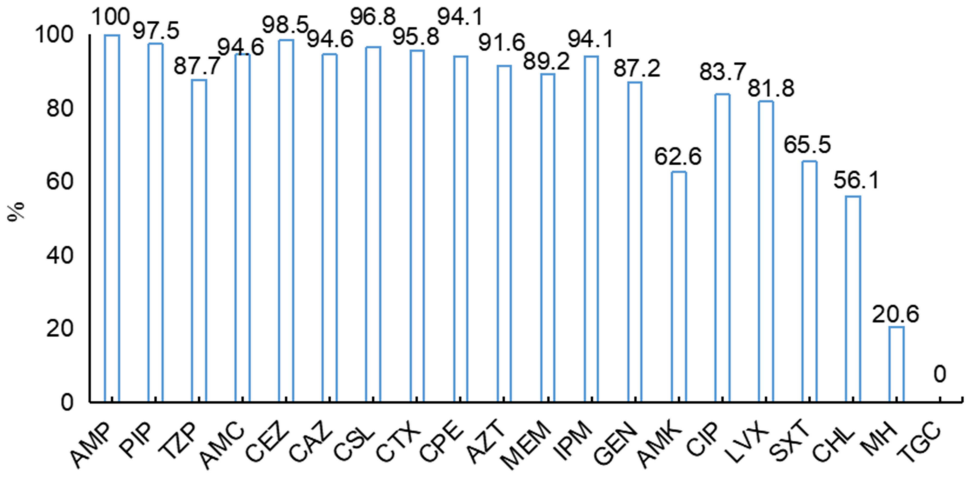

Figure 1 Resistance profiles of CRKP to common antimicrobials.
Abbreviations: AMP, Ampicillin; PIP, Piperacillin; TZP, Piperacillin/tazobactam; AMC, Amoxycillin/clavulanic acid; CEZ, Cefazolin; CAZ, Ceftazidime; CSL, Cefoperazone-sulbactam; CTX, Ceftriaxone; CPE, Cefepime; AZT, Aztreonam; MEM, Meropenem; IPM, Imipenem; GEN, Gentamicin; AMK, Amikacin; CIP, Ciprofloxacin; LVX, Levofloxacin; SXT, Sulfamethoxazole; CHL, Chloramphenicol; MH, Minocycline; TGC, Tigecycline.

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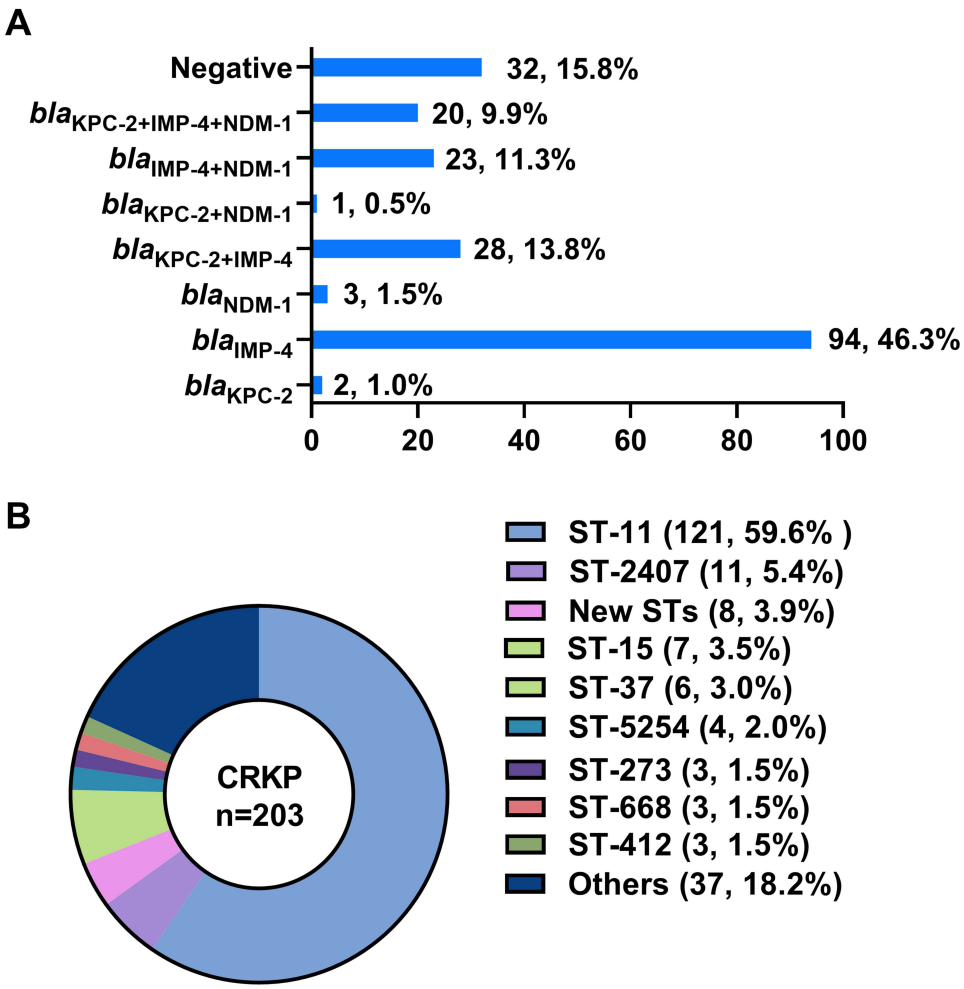

Figure 2 (**A**) Distribution of carbapenemase genes in CRKP strains. (**B**) Distribution of sequence types in CRKP strains. New STs include 7 types of total 8 strains, others include 33 types of total 37 strains. ST, sequence type.

15 isolates clustered in clade 1 but belonged to different subclades. The six ST-37 strains all belonged to clade 4. Clade 5 comprised all ST-2407 which was mainly found in pediatric patients, and most of the ST-2407 cases were found carrying bla_{IMP} (Figure S1). From Figure 3, it can be seen that the 48 ST types of the 203 CRKP strains were divided into 6 CCs and 15 singletons, among which 6 CCs contain 174 strains of CRKP, accounting for 85.71% of the total number. CC1 contains 12 ST types of 136 strains of which ST-11 is the ancestral ST, accounting for 70.00% of all strains, and is a dominant CC of which ST-11 located at the center, so it is believed that ST-11 is the primary founder, and the other ST types in CC1 are evolved from ST-11.

Comparison Analysis of ST-11 and Non-ST-11 CRKP Infections

This analysis included 183 adult patients, of whom 120 patients were with ST-11 CRKP. There was a higher percentage of patients with non-ST-11 CRKP in the cardiology department (4.8% vs 0.0%, P=0.040) compared to those with ST-11 CRKP. There were no significant differences between the two groups in terms of the distribution across other clinic departments, sites of infection, and comorbidities. Patients with ST-11 CRKP had more ICU stay and underwent more mechanical ventilation (51.7% vs 25.4%, P=0.001) and gastric catheterization (58.3% vs 38.1%, P=0.009) in the three months prior to sample collection than patients with non-ST-11 CRKP (Table 3).

Additionally, there were also differences in drug-resistance rates between ST-11 CRKP and non-ST-11 CRKP (Figure 4). Drug-resistance rates of almost all antimicrobial agents, except for minocycline, were higher in the ST-11

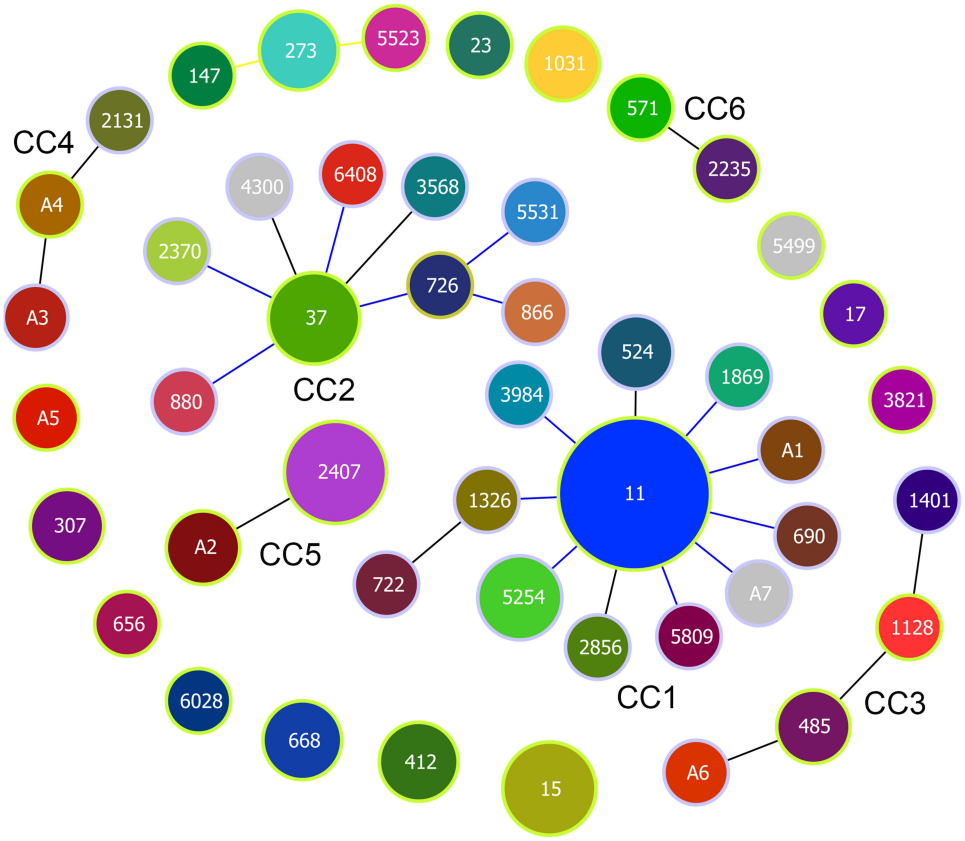

Figure 3 Details of the goeBURST clonal complexes detected in this study. The size of each circle, reflects the number of isolates with that MLST genotype. The lengths of lines are not significant.

group compared to the non-ST-11 group. ST-11 CRKP had a higher rate of resistance and a broader spectrum of resistance compared to non-ST-11 CRKP.

Risk Factors Associated with the Development of CRKP Infection

We also used systematic sampling to collect the clinical data of 67 CSKP patients. A comparison of clinical characteristics between CRKP and CSKP patients is shown in Table 4. In bivariate analysis, CRKP patients showed associations

Table 3 Comparison of Patients (≥18 Years) with ST-11 and Non-ST-11 CRKP Infection

	Total (n=183)	ST-11 (n=120)	Non ST-11 (n=63)	P value
Male	122 (66.7%)	81 (67.5%)	41 (65.1%)	0.741
Age, years	58.0 (46.0–68.0)	58.5 (44.3–71.0)	63.0 (58.0–67.0)	0.789
Comorbidities	179 (97.8%)	117 (97.5%)	62 (98.4%)	1.000
Lung disease	23 (12.6%)	17 (14.2%)	6 (9.5%)	0.368
Cardiovascular disease	73 (39.9%)	51 (42.5%)	22 (34.9%)	0.320
Gastrointestinal disease	60 (32.8%)	38 (31.7%)	22 (34.9%)	0.656
Urinary system disease	34 (18.6%)	26 (21.7%)	8 (12.7%)	0.138
Cerebrovascular disease	55 (30.1%)	38 (31.7%)	17 (27%)	0.512
Hematological Disease	36 (19.7%)	20 (16.7%)	16 (25.4%)	0.158
Endocrine system disease	24 (13.1%)	17 (14.2%)	7 (11.1%)	0.561
Rheumatism immunity disease	6 (3.3%)	3 (2.5%)	3 (4.8%)	0.416
Malignant solid tumor	27 (14.8%)	18 (15%)	9 (14.3%)	0.897

(*Continued*)

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Table 3 (Continued).

	Total (n=183)	ST-11 (n=120)	Non ST-11 (n=63)	P value
Department distribution				
ICU	34 (18.6%)	26 (21.7%)	8 (12.7%)	0.138
Hematology	27 (14.8%)	14 (11.7%)	13 (20.6%)	0.104
Respiratory Medicine	20 (10.9%)	17 (14.2%)	3 (4.8%)	0.053
Neurology	10 (5.5%)	5 (4.2%)	5 (7.9%)	0.316
Infection department	9 (4.9%)	5 (4.2%)	4 (6.3%)	0.497
Urology	6 (3.3%)	3 (2.5%)	3 (4.8%)	0.416
General department	5 (2.7%)	3 (2.5%)	2 (3.2%)	1.000
Cardiology	3 (1.6%)	0 (0.0%)	3 (4.8%)	0.040
Pancreatic surgery	23 (12.6%)	14 (11.7%)	9 (14.3%)	0.612
Cardiovascular Surgery	16 (8.7%)	8 (6.7%)	8 (12.7%)	0.179
Neurosurgery	7 (3.8%)	7 (5.8%)	0 (0.0%)	0.097
Osteology	5 (2.7%)	4 (3.3%)	1 (1.6%)	0.661
Other departments	18 (9.8%)	14 (11.7%)	4 (6.3%)	0.251
Source of infections				
Nosocomial infection	178 (97.3%)	118 (98.3%)	60 (95.2%)	0.341
Lung infection	118 (64.5%)	82 (68.3%)	36 (57.1%)	0.133
Urogenital tract infection	13 (7.1%)	8 (6.7%)	5 (7.9%)	0.768
Digestive tract infection	22 (12%)	14 (11.7%)	8 (12.7%)	0.838
Cardiovascular system infection	1 (0.5%)	0 (0.0%)	1 (1.6%)	0.344
Bloodstream infection	21 (11.5%)	11 (9.2%)	10 (15.9%)	0.176
Reproductive system infection	1 (0.5%)	0 (0.0%)	1 (1.6%)	0.344
Skin and soft tissue infection	5 (2.7%)	4 (3.3%)	1 (1.6%)	0.661
Surgical site infection	2 (1.1%)	1 (0.8%)	1 (1.6%)	1.000
Specimen type				
Sputum	117 (63.9%)	82 (68.3%)	35 (55.6%)	0.087
Chest drainage fluid	2 (1.1%)	0 (0.0%)	2 (3.2%)	0.117
Peritoneal drainage fluid	22 (12%)	13 (10.8%)	9 (14.3%)	0.495
Urine	10 (5.5%)	6 (5.0%)	4 (6.3%)	0.739
Blood	20 (10.9%)	11 (9.2%)	9 (14.3%)	0.292
Wound secretion	11 (6.0%)	8 (6.7%)	3 (4.8%)	0.751
Vomit	1 (0.5%)	0 (0.0%)	1 (1.6%)	0.344
Events within 3 months before sample collection				
Previous admission	130 (71%)	84 (70.0%)	46 (73.0%)	0.669
Prior ICU stay	109 (59.6%)	78 (65.0%)	31 (49.2%)	0.039
Prior antibiotic exposure	162 (88.5%)	109 (90.8%)	53 (84.1%)	0.176
Prior Invasive procedure	161 (88.0%)	107 (89.2%)	54 (85.7%)	0.495
Mechanical ventilation	78 (42.6%)	62 (51.7%)	16 (25.4%)	0.001
Laryngoscope/Bronchoscopy/Gastroscope	21 (11.5%)	16 (13.3%)	5 (7.9%)	0.276
Thoracic catheterization	31 (16.9%)	19 (15.8%)	12 (19.0%)	0.582
Gastric catheterization	94 (51.4%)	70 (58.3%)	24 (38.1%)	0.009
Abdominal catheterization	44 (24%)	30 (25%)	14 (22.2%)	0.676
Urinary catheterization	123 (67.2%)	85 (70.8%)	38 (60.3%)	0.150
Venous/Arterial catheterization	115 (62.8%)	76 (63.3%)	39 (61.9%)	0.849
Outcomes				
Length of hospital stay, days	21 (14–37)	21 (14–37)	24 (15–36)	0.787
Time from admission to positive culture, days	12 (4–22)	12 (5–22)	8 (2–22)	0.392
Time from positive culture to discharge, days	11 (5–18)	10 (4–19)	11 (6–18)	0.416
Recovery	150 (82.0%)	98 (81.7%)	52 (82.5%)	0.884
Died	33 (18%)	22 (18.3%)	11 (17.5%)	0.884

Note: Data are given as median (interquartile range, IQR) or n (%).
Abbreviation: ICU, intensive care unit.

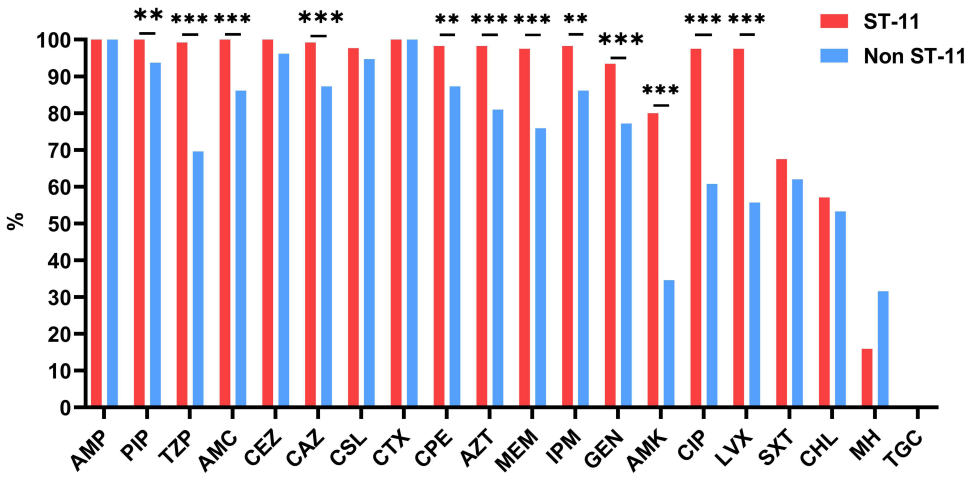

Figure 4 Comparison of resistance rates to common antimicrobials between ST-11 and non ST-11 CRKP strains.
Note: P value of <0.05 is considered to be statistically significant, **P < 0.01, ***P < 0.001.
Abbreviations: AMP, Ampicillin; PIP, Piperacillin; TZP, Piperacillin/tazobactam; AMC, Amoxycillin/clavulanic acid; CEZ, Cefazolin; CAZ, Ceftazidime; CSL, Cefoperazone-sulbactam; CTX, Ceftriaxone; CPE, Cefepime; AZT, Aztreonam; MEM, Meropenem; IPM, Imipenem; GEN, Gentamicin; AMK, Amikacin; CIP, Ciprofloxacin; LVX, Levofloxacin; SXT, Sulfamethoxazole; CHL, Chloramphenicol; MH, Minocycline; TGC, Tigecycline.

with comorbidities, especially gastrointestinal disease, urinary system disease, and cerebrovascular disease, stay in the ICU and cardiology departments, nosocomial infection, lung infection, sputum specimen, previous admission, prior ICU stay, prior antibiotic exposure, prior invasive procedure especially mechanical ventilation, non-invasive ventilation, laryngoscope/bronchoscopy/gastroscope, gastric catheterization, urinary catheterization, venous/arterial catheterization, length of hospital stay, and died outcome. In the multivariate analysis, gastrointestinal disease (OR=6.168, P=0.003), nosocomial infection (OR=5.573, P=0.012), and antibiotic exposure (OR=4.131, P=0.004), as well as urinary catheterization (OR=3.960, P=0.031), and venous/arterial catheterization (OR=2.738, P=0.026) within the prior 3 months before sample collection were independent risk factors significantly associated with CRKP infection.

Table 4 Analysis of Risk Factors of Patients (≥18 Years) with CRKP Infection

Variables	Univariate Analysis			Multivariable Analysis	
	CRKP (n=183)	CSKP (n=67)	*P* value	*P* value	OR (95% CI)
Male	122 (66.7%)	50 (74.6%)	0.229		
Age, years	58.0 (46.0–68.0)	61.0 (54.0–70.0)	0.307		
Comorbidities	179 (76.2%)	56 (83.6%)	**< 0.001**	0.403	3.224 (0.208–50.101)
Lung disease	23 (12.6%)	6 (9.0%)	0.429		
Cardiovascular disease	73 (39.9%)	29 (43.3%)	0.629		
Gastrointestinal disease	60 (32.8%)	6 (9.0%)	**< 0.001**	0.003	6.168 (1.880–20.238)
Urinary system disease	34 (18.6%)	3 (4.5%)	0.005	0.273	3.273 (0.393–27.259)
Cerebrovascular disease	55 (30.1%)	8 (11.9%)	0.003	0.848	1.167 (0.241–5.656)
Hematological Disease	36 (19.7%)	8 (11.9%)	0.155		
Endocrine system disease	24 (13.1%)	14 (20.9%)	0.129		
Rheumatism immunity disease	6 (3.3%)	1 (1.5%)	0.678		
Malignant solid tumor	27 (14.8%)	11 (16.4%)	0.746		
Department distribution					
ICU	34 (18.6%)	1 (1.5%)	0.001	0.732	1.572 (0.119–20.854)
Hematology	27 (14.8%)	9 (13.4%)	0.792		
Respiratory Medicine	20 (10.9%)	4 (6.0%)	0.238		

(*Continued*)

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Table 4 (Continued).

Variables	Univariate Analysis			Multivariable Analysis	
	CRKP (n=183)	CSKP (n=67)	*P* value	*P* value	OR (95% CI)
Neurology	10 (5.5%)	2 (3.0%)	0.523		
Infection department	9 (4.9%)	2 (3.0%)	0.732		
Urology	6 (3.3%)	3 (4.5%)	0.705		
General department	5 (2.7%)	3 (4.5%)	0.445		
Cardiology	3 (1.6%)	8 (11.9%)	0.002	0.535	0.189 (0.001–36.198)
Pancreatic surgery	23 (12.6%)	11 (16.4%)	0.432		
Cardiovascular Surgery	16 (8.7%)	6 (9.0%)	0.958		
Neurosurgery	7 (3.8%)	6 (9.0%)	0.116		
Osteology	5 (2.7%)	3 (4.5%)	0.445		
Other departments	18 (9.8%)	10 (14.9%)	0.258		
Source of infections					
Nosocomial infection	178 (97.3%)	48 (71.6%)	**< 0.001**	0.012	5.573 (1.452–21.384)
Lung infection	118 (64.5%)	32 (47.8%)	0.017	0.573	0.452 (0.029–7.156)
Urogenital tract infection	13 (7.1%)	9 (13.4%)	0.118		
Digestive tract infection	22 (12%)	9 (13.4%)	0.764		
Cardiovascular system infection	1 (0.5%)	0 (0.0%)	1.000		
Bloodstream infection	21 (11.5%)	11 (16.4%)	0.300		
Reproductive system infection	1 (0.5%)	0 (0.0%)	1.000		
Skin and soft tissue infection	5 (2.7%)	5 (7.5%)	0.138		
Surgical site infection	2 (1.1%)	1 (1.5%)	1.000		
Specimen type					
Sputum	117 (63.9%)	30 (44.8%)	0.006	0.345	1.786 (0.543–4.598)
Chest drainage fluid	2 (1.1%)	2 (3.0%)	0.292		
Peritoneal drainage fluid	22 (12%)	9 (13.4%)	0.764		
Urine	10 (5.5%)	8 (11.9%)	0.098	0.786	0.810 (0.178–3.686)
Blood	20 (10.9%)	11 (16.4%)	0.243		
Wound secretion	11 (6.0%)	7 (10.4%)	0.269		
Vomit	1 (0.5%)	0 (3.0%)	1.000		
Events within 3 months before sample collection					
Previous admission	130 (71%)	38 (56.7%)	0.033	0.341	1.77 (0.547–5.73)
Prior ICU stay	109 (59.6%)	15 (22.4%)	**< 0.001**	0.748	0.762 (0.145–4.006)
Prior antibiotic exposure	162 (88.5%)	33 (49.3%)	**< 0.001**	0.009	7.787 (1.654–36.672)
Prior Invasive procedure	161 (88.0%)	5 (7.5%)	**< 0.001**		
Mechanical ventilation	78 (42.6%)	10 (14.9%)	**< 0.001**	0.358	0.269 (0.016–4.416)
Non-invasive ventilation	32 (17.5%)	1 (1.5%)	0.001	0.556	2.959 (0.08–109.496)
Laryngoscope/Bronchoscopy/Gastroscope	21 (11.5%)	0 (0.0%)	0.004	0.094	5.354 (0.890–15.446)
Thoracic catheterization	31 (16.9%)	9 (13.4%)	0.503		
Gastric catheterization	94 (51.4%)	16 (23.9%)	**< 0.001**	0.080	0.306 (0.081–1.153)
Abdominal catheterization	44 (24.0%)	10 (14.9%)	0.121		
Urinary catheterization	123 (67.2%)	17 (25.4%)	**< 0.001**	0.031	3.96 (1.131–13.871)
Venous/Arterial catheterization	115 (62.8%)	18 (26.9%)	**< 0.001**	0.026	2.738 (1.131–6.63)
Length of hospital stay, days	21 (14–37)	16 (11–32)	0.043	0.465	4.345 (0.215–13.445)
Died	33 (18.0%)	1 (1.5%)	0.001		

Note: Data are given as median (interquartile range, IQR) or n (%).

Abbreviations: CRKP, Carbapenem-resistant *Klebsiella Pneumoniae*; CSKP, Carbapenem-susceptible *Klebsiella Pneumoniae*; OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Discussion

CRKP has emerged worldwide as a significant challenge for clinical practice and public health. With the increasingly high resistance rate of *K. pneumoniae* to carbapenems, a comprehensive understanding of CRKP strains in patients is crucial. Our research team has been focusing on the molecular epidemiological characteristics of *K. pneumoniae*,[22,23] and this study we mainly explored the molecular characteristics of CRKP, and identified risk factors for CRKP infection.

This study found that all 203 strains carried carbapenemases, which was consistent with the previous reports in China.[24,25] There are mainly three types of carbapenemases: KPC-type enzymes, metallo-β-lactamases (MBLs) which include VIM, IMP, and NDM, and OXA enzymes. In China, it has been reported that KPC is the most prevalent carbapenemase among clinical isolates of CRKP,[26–28] but the conclusions reported in different regions of China are not consistent. A study reported that the most common carbapenemase gene was NDM in a hospital of Zunyi, China.[25] The present study showed that IMP-4 was the main carbapenemase in the hospital. Studies have shown that IMP is commonly found on the plasmids of IncN, and the horizontal transmission of IncN plasmids leads to sustained outbreaks of IMP in multiple species.[29] Moreover, most bla_{IMP} contain type 1 integrons which are usually embedded in transposons and/or plasmids, facilitating horizontal transfer of genetic material.[30] These genetic backgrounds play a key role in the acquisition and maintenance of resistance in gram-negative bacteria. Gene site mutations can also occur under the selective pressure of antibiotics selection, which is crucial for the spread and development of resistant bacteria. In addition, studies showed that bla_{IMP} often coexists with other resistance genes, such as aacA, catB, and bla_{OXA}.[31] Among the 165 strains carrying the IMP-4 gene in this study, 71 strains carried two or more resistance genes at the same time, indicating the stability and confounding of bla_{IMP}. As a MBL, IMP can be inhibited by ethylenediaminetetraacetic acid (EDTA) but not by clavulanic acid or avibactam. β-Lactam drugs interact with MBLs zinc ions, enabling these enzymes to hydrolyze most cephalosporin and penicillin antibiotics, but not aztreonam. In our research hospital, doctors commonly use ceftazidime avibactam empirically to treat carbapenem-resistant Enterobacteriaceae (CRE) and aztreonam is unavailable in the hospital. This may have contributed to the higher prevalence of IMP-4 and explain the differences between our findings and those of other studies. Our findings may provide valuable insights for guiding clinical antibiotic selection.

ST-11 was identified as the most prevalent ST in this study, consistent with previous studies in China.[32–34] ST-11 belongs to the clonal complex group 258 (CC258), which also includes ST-258, ST-512, ST-340 and other subtypes. Globally, ST-258 is the most common type and is widely spread in America and Europe.[35] ST-11 is a single-locus variant of ST-258, with 80% of the genes of ST-258 originating from ST-11. However, the difference between the two lies in the tonB housekeeping gene. ST-11 has been reported in many areas of China, such as Zhejiang, Guangdong, Fujian, and Jiangsu, etc.[36] Beyond China, ST-11 is also a predominant strain in other Asian countries such as Korea,[37] Singapore,[38] and Thailand.[39] However, the reasons for the predominance of ST-11 strains in Asian countries remain unclear. Li's study suggested that under similar environmental conditions (24–28°C and 56% relative humidity), ST-11 CRKP isolates survived for a longer period (32–43 days) compared to non-ST-11 CRKP isolates, which survived for less than 30 days.[40] Longer survival times favor the transmission of the strain.[41] Additionally, hypervirulent ST-11 *K. pneumoniae* is increasingly being reported,[42–44] The ST-11 genome is highly heterogeneous[45] and has been found to harbor various resistance genes (eg, KPC, NDM, and VIM) and virulence genes (eg, irp, ybt, and fyu).[46] Studies have shown that the highly variable region of bacterial capsule polysaccharide genes may be the main evolutionary region of the ST-11 strain, playing a crucial role in its dissemination and determining its virulence and resistance.[47,48] These factors may explain why ST-11 strains can become a dominant type and significantly contribute to spreading resistance genes.

Studies have shown a predominance of ST-11 $bla_{\text{KPC-2}}$ *K. pneumoniae* in China,[49] where this study identified a predominance of ST-11 $bla_{\text{IMP-4}}$ in our research. Zheng's study showed that $bla_{\text{IMP-4}}$-positive isolates were resistant to all β-lactam antimicrobial drugs (namely ampicillin, cefotaxime, meropenem, imipenem, and amoxicillin/clavulanate) and trimethoprim/sulfamethoxazole, classifying them as multidrug-resistant strains.[50] The ST-11 clone is highly transmissible.[48] When an ST-11 clone carries $bla_{\text{IMP-4}}$, its resistance capabilities and transmissibility may be enhanced, potentially contributing to more *K. pneumoniae* infections. While no study has reported on ST-11 *K. pneumoniae* carrying $bla_{\text{IMP-4}}$, however, sporadic studies on other STs of *K. pneumoniae* carrying $bla_{\text{IMP-4}}$ have shown multiple

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resistance genes and high transmission characteristics.[13,51] Therefore, bla_{IMP-4}-carrying ST-11 *K. pneumoniae* could be a continually evolving threat and warrants prospective monitoring.

The phylogenic tree showed that ST-11 and ST-15 isolates belonged to two subclades in clade 1. One study revealed that most carbapenemase-positive *K. pneumoniae* isolates belong to four clonal lineages: ST-11, ST-15, ST-101, and ST-258/512, and their derivatives. These lineages are considered "high-risk" clones, which commonly share a recent ancestor, epidemic success, and a defined geographic distribution.[5] In the present study, all 8 ST-15 cases included 3 were ICU isolates and 2 were hematology isolates, implying that ST-15 may always associate with more severe conditions. Besides, ST-2407 was assigned to another distantly related clades in which 10 out of 11 cases were paediatric isolates and most of the ST-2407 cases harbored bla_{IMP}. A Chinese study on paediatric CRKP also found that ST-2407 was the most frequent MLST, but it also suggested that all CRKP isolates mainly carried the carbapenemases resistance genes bla_{NDM-1} and bla_{KPC-2}, which is inconsistent with our findings.[52] In addition to ST-11, we also identified 7 new STs of CRKP isolates disseminated with the hospital. Although their proportion was extremely small, STs that undergo within-hospital transmission are more difficult to control and are more likely to develop into high-risk clones that warrant monitoring.

In this study, gastrointestinal disease, nosocomial infection, prior antibiotic exposure, urinary catheterization, and venous/arterial catheterization were identified as independent risk factors for CRKP infection. With the advancement of medical technology, invasive operations are increasingly being applied to clinical practice. While these procedures have significant benefits for the diagnosis and treatment of diseases, they also increase the risk of bacterial colonization. Invasive operations lead to congestion, edema, and decreased local immunity of the surrounding tissues, weakening the body's intrinsic barrier against bacteria, and creating conditions for bacterial invasion.[53] A meta-analysis study also suggested that invasive ventilation, central venous catheterization, and prior use of antimicrobials were all independent risk factors for CRKP infection.[54] Therefore, invasive operations should be minimized, whenever clinically feasible, to reduce the risk of CRKP infection when condition permitting.[55]

This study had several limitations, including being a single-center retrospective analysis, which may limit the generalizability of the results to other institutions or geographical areas. Moreover, further studies are needed to investigate the specific mechanisms underlying the transmission of drug-resistant genes and strains.

Conclusions

This study documented the distribution of drug-resistant genes and MLST typing of CRKP and investigated the risk factors of CRKP development. In our findings, identified bla_{IMP-4}-carrying ST-11 *K. pneumoniae* as the most prevalent type, which could represent a continually evolving threat and warrants prospective monitoring. Gastrointestinal disease, nosocomial infection, antibiotic exposure, urinary catheterization, and venous/arterial catheterization within 3 months before sample collection were independent risk factors for CRKP infection. Strict policies for antibiotic use, cautious decisions regarding the implementation of invasive procedures, and careful management of patients with catheters are necessary to prevent CRKP infections.

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Disclosure

The authors report no conflicts of interest in this work.

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