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ORIGINAL ARTICLE

Clinical and Microbiological Characteristics of Bloodstream Infections due to CRKP

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ABSTRACT

Background: **Bloodstream infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) often result in high mortality rates. This study aimed to explore the local epidemiology and risk factors of CRKP bloodstream infections, characterize the resistance and virulence genes of CRKP isolates in this region of China, and identify the circulating hypervirulent *Klebsiella pneumoniae* strains locally.**

Methods: **In a case-controlled retrospective study, we examined the prevalence, patient background, and risk factors for CRKP BSIs.**

Results: **The overall CRKP BSI incidence was 2.2 (54/2,420,826) per 100,000 patient-days. Acute operation (p = 0.012), being a resident in a long-term-care facility (p = 0.019), transfer to ICU within 30 days (p = 0.014), pulmonary diseases (p = 0.022), renal diseases (p = 0.004), liver diseases (p = 0.008), abdominal infections (p = 0.005), gastric tube (p = 0.011), quinolone use (p = 0.029), carbapenem use (p = 0.001), and antifungal agent use prior to culture within 30 days (p = 0.001) were risk factors for CRKP BSIs. In total, 88.9% (48/54) isolates were detected with KPC carbapenemase, followed by NDM carbapenemase at 7.4% (4/54). *YcfM*, *wabG*, and *entB* were present in all strains. The following four virulence genes had a positivity rate of over 90%: *fimH* (98.15%), *mrkD* (96.30%), *uge* (92.60%), and *kpn* (92.60%).**

Conclusions: **Acute operation, being a resident in a long-term-care facility, transfer to ICU within 30 days, pulmonary diseases, renal diseases, liver diseases, abdominal infections, gastric tube, quinolone use, carbapenem use, and antifungal agent use prior to culture within 30 days were risk factors for CRKP BSIs. KPC carbapenemase ranked first in isolates of CRKP BSIs. Virulence genes were widely present.**

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KEYWORDS

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INTRODUCTION

Carbapenems have been regarded as the first line of defense in the treatment of BSIs (bloodstream infections) caused by drug-resistant Gram-negative bacterial infections due to their broad spectrum of activity. Nevertheless, with the extensive use of carbapenems, many bacteria resistant to carbapenems have appeared, and now it presents a particularly critical problem worldwide [1-4]. Among carbapenem-resistant *Enterobacteriaceae*, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a causative organism of nosocomial infections, as well as community-acquired infections, the latter often involving hypervirulent strains [5]. Hypervirulent strains of *Klebsiella pneumoniae* are closely associated with high mortality and poor prognosis [5-7]. Carbapenem-resistant isolates have already emerged as hypervirulent-type *Klebsiella pneumoniae* [8,9].

Here, we conducted a study on CRKP BSIs in Xiamen, a southern area in China, from January 1, 2019, through December 31, 2021, and we considered the following objectives: 1) to study the epidemiology and risk factors of BSIs caused by CRKP in this area, 2) to clarify and identify the resistant and virulent genes that encode for CRKP in this area of China, and 3) to identify the hypervirulent-type *Klebsiella pneumonia* in this area of China.

MATERIALS AND METHODS

Patients and settings

With the intent of examining CRKP BSI prevalence, the patients' background, and the risk factors associated with CRKP BSIs, we conducted a case-control study. A retrospective epidemiologic surveillance study of BSIs caused by CRKP was conducted within a 2,800-bed academic medical center in the southern area of China from January 1, 2019, through December 31, 2021. Cases of CRKP BSIs during the inpatients' hospital stay were classified as the case group. BSI patients who were negative for CRKP but positive for carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) during their hospital stay, during the same study period, were used as the selection pool for the control group. Exclusion criteria were community-acquired BSIs, missing key data, screening samples, and subsequent episodes in the same patient. The same exclusion criteria were applied to cases and controls [10].

CRKP cases, as determined by clinical culture, were selected by review of microbiological reports. All identified inpatients were initially eligible to participate, and their medical charts were reviewed. For inpatients with multiple episodes of BSIs with CRKP, only data relevant to the first episode were collected and analyzed.

Cases of CSKP BSIs were randomly selected as a control group from the same clinical departments during the study period. Controls whose records had insufficient information were replaced by other randomly selected controls. For inpatients with multiple episodes of CSKP BSIs, only data relevant to the first episode were collected and analyzed. The age (± 2 years) and genders of the patients were matched to inpatients with CRKP BSIs, and the ratio for the CRKP:CSKP group was 1:2. We set a ratio of 1:2 in this study for two reasons: 1) concern for sufficient numbers in a stratified analysis; and 2) increase in power given the expected prevalence of exposure among the controls [11].

Both case and control groups' data were collected from a database of hospital infection monitoring system. This database identified the individual participants during and after data collection. This database drew information from numerous sources, including patients' electronic health records, laboratory, microbiology, and medication administration records, and it included admission and discharge data, age, gender, health care exposure during the prior year (acute care hospitalization, acute operation, dialysis, resident of a long-term-care facility, transfer to ICU (intensive care unit) within 30 days and receipt of corticosteroids), underlying conditions (diabetes mellitus, hypertension, cancer, neurological diseases, blood diseases, pulmonary diseases, renal diseases, liver diseases, heart diseases, abdominal infections, and skin infections), body temperature prior to culture, smoking history, drinking history, blood transfusion history, chemoradiotherapy history, indwelling devices prior to culture (central venous catheter, gastric tube, tracheal cannula, and urinary catheter), laboratory findings (white blood cell levels, platelet levels, C-reactive protein levels, procalcitonin levels, total protein levels, and albumin levels), antimicrobial use prior to culture within 30 days (quinolone use, cephalosporin use, carbapenem use, tetracycline use, oxazolidinone use, glycopeptides use, clistin use, metronidazole use, aminoglycosides use, macrolides use, and trimethoprim-sulfamethoxazole use), antifungal agent use prior to culture within 30 days, length of hospital stay, and discharge disposition. Each patient's first positive culture was used to remove patients with multiple cases [12]. For identifying possible risk factors of CRKP BSIs, patients' demographic characteristics and medical conditions were collected from the electronic sources mentioned above by comparing the CRKP and CSKP groups.

This study was approved by the local Ethics Committee of Xiamen Hospital of Traditional Chinese Medicine (2024-K046-01) and complied with the Declaration of Helsinki (2008).

Definition of CRKP
A case of CRKP BSIs was defined as the first positive clinical culture from blood, with one or more of the following criteria: 1) minimum inhibitory concentrations (MICs) for meropenem ≥ 4 μg/mL, 2) MICs for imipenem ≥ 4 μg/mL, 3) MICs for ertapenem ≥ 2 μg/mL, and/or 4) MICs for doripenem ≥ 4 μg/mL.

Microbiological investigations
Species identification was performed with the Vitek 2 Compact automatic microbial analyzer (BioMerieux, Marcy - l'Etoile, France) and confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Zybio Inc., Chongqing, China).

Molecular detection
Multiplex polymerase chain reactions (PCRs) were used to detect the presence of carbapenemase genes (*bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{OXA-48-like}$) and virulence genes (*rmpA*, *wcaG*, *uge*, *fimH*, *mrkD*, *ycfM*, *entB*, *iutA*, *k2A*, *PEG-344*, *kpn*, *magA*, *wabG*, *iorN*, and *allS*); the primers are listed in Supplemental Table S1 [13-16]. PCR products were sequenced, and the nucleotide and the deduced protein sequences were analyzed with software programs that were available from the National Center of Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov).

Antimicrobial susceptibility testing
With regard to the antimicrobial susceptibility test, minimal inhibitory concentrations (MICs) of ceftazidime, cefepime, cefotaxime, ceftriaxone, piperacillin/tazobactam, doripenem, aztreonam, amikacin, gentamycin, tobramycin, ciprofloxacin, levofloxacin, trimethopri-sulfamethoxazole, and tigecycline were determined with the Vitek 2 Compact automatic microbial analyzer (BioMrieux, Marcy-l'Etoile, France) according to the CLSI guidelines. In addition, MICs of ertapenem, imipenem, meropenem, ceftazidime/avibactam, and colistin-polymyxin-B were determined using E-test strips (BioMerieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. Ertapenem, imipenem, meropenem, doripenem, and ceftazidime/avibactam MICs were interpreted according to the CLSI guidelines. The interpretive criteria for colistin-polymyxin-B was based on the breakpoints of EUCAST. And the interpretive criteria for tigecycline was based on the breakpoints of the Food and Drug Administration (FDA).

MALDI-TOF MS
Autof (Autof KIT): The *Klebsiella pneumoniae* isolates were plated on Columbia blood agar (bioMérieux, Marcy l' Étoile, France) and incubated for 18 hours to 24 hours at 37°C. Isolated colonies of each strain were selected and used for MALDI-TOF MS identification using the MALDI-TOF MS (Zybio Inc., Chongqing, China), as previously described [17]. The obtained spectra were manually selected into the EX 3000-Smartspec software (Zybio Inc., Chongqing, China). Then, the selected spectra could be clustered and calculated to form a dendrogram. Cluster analysis was performed by spectra compared to each other in the database, according to the manufacturer's instructions (Zybio Inc., Chongqing, China). Each dendrogram with differences and similarities in relative terms represented one fungus isolate, and the abscissa represented the distance of the isolates' relationships. According to the type assignment, the closer it is, the more likely it is of the same type. Hierarchical clustering of the spectra was performed by applying the Ward algorithm and cosine distance between all pairs of peak profiles [18].

Statistical analyses
The incidence of CRKP BSIs were reported as the number of BSIs cases per 100,000 hospital patient-days. Descriptive statistics were used to summarize the clinical and epidemiologic characteristics of CRKP BSIs. Continuous variables were presented as medians with the range or interquartile range. For categorical variables, the percentage of patients or isolates in each category was calculated. The chi-squared test was used to compare categorical variables. The Mann-Whitney U test was used to compare continuous variables. To identify risk factors for isolating CRKP, the chi-squared test was performed. Factors showing p-value < 0.05 were considered candidate predictors that were significantly related to CRKP BSIs and were extracted; following which, multi-variate analysis was performed for these factors using the logistic regression model. All analyses were performed using the IBM SPSS statistical software package version 25 (IBM Corp, Armonk, NY, USA).

RESULTS

Prevalence of CRKP BSIs
The overall CRKP BSIs incidence between January 1, 2019, and December 31, 2021, was 2.2 (54/2,420,826) per 100,000 patient-days. During 2,420,826 patient-days, we found that 293 BSI inpatients with *Klebsiella pneumoniae* isolates were hospitalized, and 54 inpatients with CRKP BSIs were eligible for screening in this study. The overall detection rate of CRKP among KP (*Klebsiella pneumoniae*) BSI inpatients was 19.5% (54/293). During the three study years, the detection rate of CRKP among BSI inpatients caused by KP increased, 16.5% (16/97) in 2019, 11.7% (12/103) in 2020, and 28.0% (26/93) in 2021, respectively. The characteristics of the inpatients are shown in Supplemental Table S2 and included 36 males and 18 females. The median age was 69 years (range 0 - 94 years).

Clinical characteristics and outcomes
We found that 20.4% (11/54) of inpatients had functional status deterioration, as can be seen in Supplemental Table S2. One inpatient in the ICU died within 30 days of admission, which was not due to bloodstream infec-

Table 1. Risk factors for BSIs isolating CRKP.

Variables	Univariate analysis	Multi-variate analysis	p-value
	OR (95% CI)	aOR (95% CI)	
Acute operation	2.800 (1.123 - 6.982)	46.850 (2.341 - 937.536)	0.012
Being a resident in a long-term-care facility	33.927 (4.300 - 267.674)	156.556 (2.335 - 10,496.846)	0.019
Transfer to ICU within 30 days	11.414 (5.193 - 25.090)	16.140 (1.742 - 149.513)	0.014
Cancer	0.509 (0.262 - 0.991)		
Neurological diseases	3.713 (1.580 - 8.725)		
Blood diseases	2.484 (1.045 - 5.906)		
Pulmonary diseases	3.099 (1.573 - 6.107)	8.955 (1.365 - 58.728)	0.022
Renal diseases	3.406 (1.604 - 7.235)	44.122 (3.304 - 589.144)	0.004
Liver diseases	2.000 (1.027 - 3.896)	14.346 (2.030 - 101.397)	0.008
Abdominal infections	5.054 (2.072 - 12.330)	30.514 (2.837 - 328.260)	0.005
Blood transfusion	4.009 (2.010 - 7.998)		
Central venous catheter	4.420 (2.168 - 9.009)		
Gastric tube	14.286 (6.474 - 31.525)	40.489 (2.335 - 702.148)	0.011
Tracheal cannula	5.788 (2.665 - 12.570)		
Urinary catheter	6.914 (3.144 - 15.206)		
White blood cells > 10,000/mm^3	2.094 (1.077 - 4.069)		
Platelets < 100 × 10^9/L	0.375 (0.144 - 0.973)		
Quinolone use	2.110 (1.077 - 4.135)	9.071 (1.247 - 65.971)	0.029
Cephalosporin use	3.166 (1.587 - 6.316)		
Carbapenem use	4.808 (1.597 - 14.473)	122.854 (6.358 - 2,373.879)	0.001
Tetracycline use	6.651 (2.007 - 22.045)		
Oxazolidinone use	4.430 (2.095 - 9.367)		
Glycopeptides use	6.538 (2.367 - 18.062)		
Clistin use	13.375 (1.567 - 114.159)		
Aminoglycosides use	6.625 (1.290 - 34.024)		
Macrolides use	10.918 (1.242 - 95.960)		
Antifungal agent use prior to culture within 30 days	8.357 (3.881 - 17.995)	451.634 (11.707 - 17,423.692)	0.001
Length of stay	2.300 (1.412 - 3.745)		

OR: odds ratio, aOR: adjusted odds ratio, CI: confidence interval.

Table 2. Carbapenemase gene of 54 CRKP isolates.

Carbapenemase	Isolates (n, %)
KPC	48 (88.9)
NDM	4 (7.4)
IMP	0 (0.0)
KPC+NDM	1 (1.9)
NDM+IMP	1 (1.9)
Total	54 (100.0)

CRKP: carbapenem-resistant *Klebsiella pneumoniae.*

Table 3. Carbapenemase of 54 CRKP isolates by year, 2019 - 2021.

Carbapenemase	2019 (n, %)	2020 (n, %)	2021 (n, %)	Total (n, %)
KPC	12 (25.00)	10 (20.83)	26 (54.17)	48 (100.00)
NDM	2 (50.00)	2 (50.00)	0 (0.00)	4 (100.00)
IMP	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
KPC + NDM	1 (100.00)	0 (0.00)	0 (0.00)	1 (100.00)
NDM + IMP	1 (100.00)	0 (0.00)	0 (0.00)	1 (100.00)
Total	16 (29.63)	12 (22.22)	26 (48.15)	54 (100.00)

CRKP: carbapenem-resistant *Klebsiella pneumoniae.*

Table 4. Virulence genes of 54 CRKP isolates.

Virulence gene	Isolates (n, %)
wcaG	0 (0.00)
k2A	0 (0.00)
magA	0 (0.00)
iroN	0 (0.00)
allS	0 (0.00)
PEG-344	8 (14.81)
iutA	12 (22.22)
rmpA	25 (46.30)
kpn	50 (92.59)
uge	50 (92.59)
mrkD	52 (96.30)
fimH	53 (98.15)
ycfM	54 (100.00)
wabG	54 (100.00)
entB	54 (100.00)

wcaG*: guanosine diphosphate-beta-L-fucose synthetase, *k2A*: speciffc to K2 capsule serotype, *magA*: mucoviscosity associated gene A, *iroN*: salmochelin, *allS*: allantoin, *PEG-344*: putative transporter, *iutA*: ferric aerobactin, *rmpA*: regulator of mucoid phenotype A, *kpn*: FimH-like adhesion, *uge*: uridine diphosphate galacturonate 4-epimerase, *mrkD*: type 3 ffmbrial adhesion, *fimH*: type 1 ffmbrial adhesin, *ycfM*: outer membrane lipoprotein, *wabG*: biosynthesis of the core lipopolysaccharide, *entB*: enterobactin, CRKP: carbapenem-resistant *Klebsiella pneumoniae.

tion but due to multiple organ failure caused by cancer, same as for the one inpatient that died in the CSKP group. CRKP group had higher medical expenses than CSKP group, as shown in Supplemental Table S2 ($p < 0.001$).

Analysis of risk factors of BSI inpatients' isolated CRKP

The results of univariate analysis using the chi-squared test in BSI inpatients with CRKP are shown in Table 1. There were twenty-eight parameters associated with BSI-isolated CRKP, namely acute operation during prior year ($p = 0.023$), resident of a long-term-care facility during prior year ($p < 0.001$), transfer to ICU within 30 days ($p < 0.001$), cancer ($p = 0.046$), neurological diseases ($p = 0.002$), blood diseases ($p = 0.031$), pulmonary diseases ($p = 0.001$), renal diseases ($p = 0.001$), liver diseases ($p = 0.040$), abdominal infections ($p < 0.001$), blood transfusion ($p < 0.001$), central venous catheter ($p < 0.001$), gastric tube ($p < 0.001$), tracheal cannula ($p < 0.001$), urinary catheter ($p < 0.001$), white blood cells > 10,000/mm^3 ($p = 0.025$), platelets < 100×10^9/L ($p = 0.039$), quinolone use ($p = 0.028$), cephalosporin use ($p = 0.001$), carbapenem use ($p =$

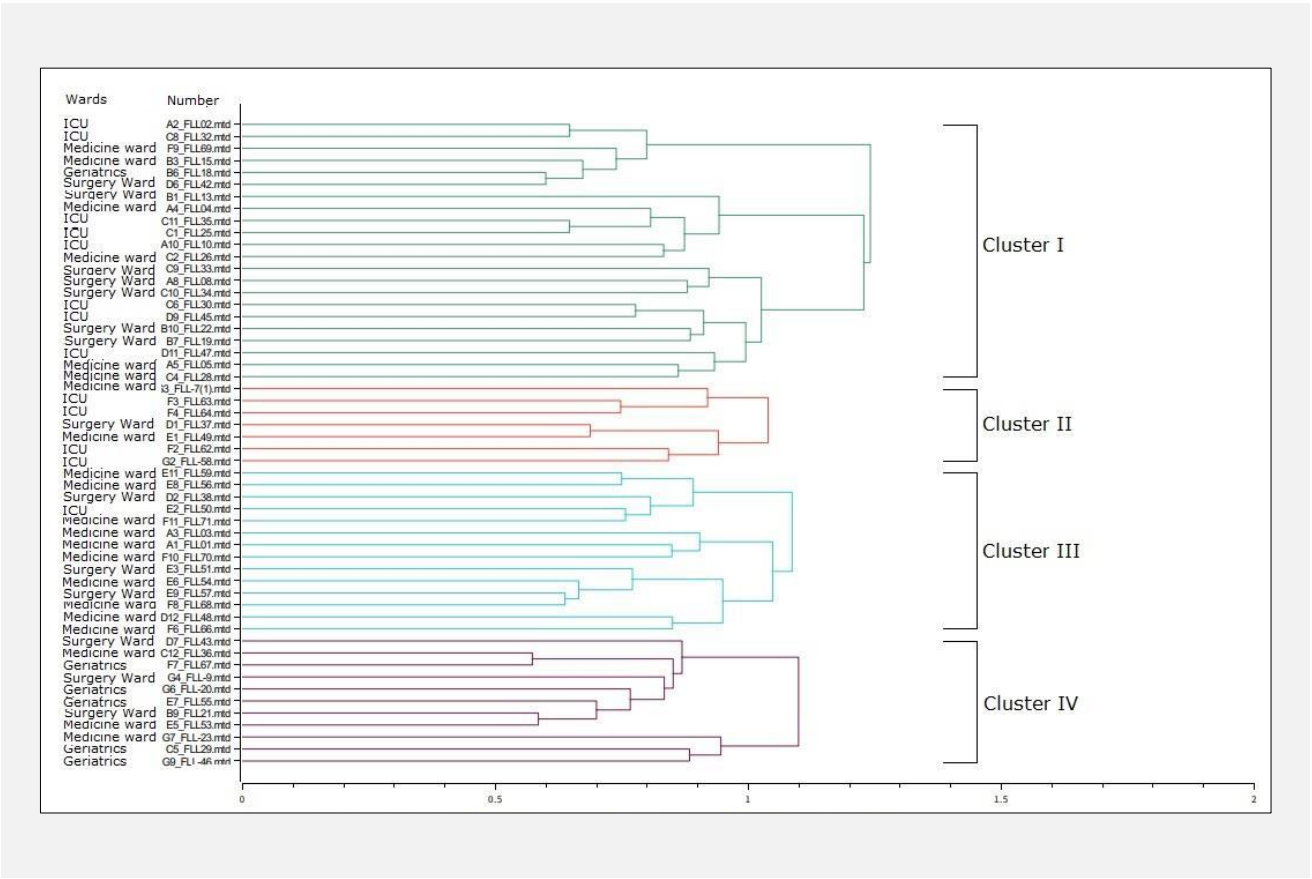

Figure 1. Hierarchical clustering of CRKP isolates by MALDI-TOF MS.

0.005), tetracycline use (p = 0.002), oxazolidinone use (p < 0.001), glycopeptides use (p < 0.001), colistin use (p = 0.009), aminoglycosides use (p = 0.029), macrolides use (p = 0.027), antifungal agent use prior to culture within 30 days (p < 0.001), and length of stay (p < 0.001).

Multivariate logistic regression analysis was applied to analyze the prognostic significance of these 28 factors, revealing that acute operation (p = 0.012, odds ratio: 46.850, 95% confidence interval [CI]: 2.341 - 937.536), resident of a long-term-care facility (p = 0.019, odds ratio: 156.556, 95% confidence interval [CI]: 2.335 - 10,496.846), transfer to ICU within 30 days (p = 0.014, odds ratio [OR]: 16.140, 95% confidence interval [CI]: 1.742 - 149.513), pulmonary diseases (p = 0.022, odds ratio OR: 8.955, 95% confidence interval [CI]: 1.365 - 58.728), renal diseases (p = 0.004, odds ratio [OR]: 44.122, 95% confidence interval [CI]: 3.304 - 589.144), liver diseases (p = 0.008, odds ratio [OR]: 14.346, 95% confidence interval [CI]: 2.030 - 101.397), abdominal infections (p = 0.005, odds ratio [OR]: 30.514, 95% confidence interval [CI]: 2.837 - 328.260), gastric tube (p = 0.011, odds ratio [OR]: 40.489, 95% confidence interval [CI]: 2.335 - 702.148), quinolone use (p = 0.029, odds ratio [OR]: 9.071, 95% confidence interval [CI]: 1.247 - 65.971), carbapenem use (p = 0.001, odds ratio [OR]: 122.854, 95% confidence interval [CI]: 6.358 - 2,373.879), and antifungal agent use prior to culture within 30 days (p = 0.001, odds ratio [OR]: 451.634, 95% confidence interval [CI]: 11.707 - 17,423.692) were indeed independent risk factors for patients isolating CRKP (Table 1).

Antimicrobial susceptibility testing

Antimicrobial susceptibility results for CRKP are listed in Supplemental Table S3. All isolates showed non-susceptibility to carbapenems and cephalosporins. Colistin-polymyxin-B and tigecycline retained excellent activity, with a susceptibility rate of 100%. The susceptibility rate of ceftazidime/avibactam remained fully consistent with the KPC-carbapenems-producing *Klebsiella pneumoniae* strains.

β-lactamase genes detection

There were 88.9% (48/54) CRKP isolates detected with KPC carbapenemase, followed by NDM carbapenemase, accounting for 7.4% (4/54). Two CRKP strains were detected to produce two types of gene for carbapenemase, one strain producing KPC and NDM, and the other producing NDM and IMP (Table 2). When com-

paring carbapenemase in CRKP isolates from 2019 to 2021, it was found that the detection rates of KPC carbapenemase increased, from 25.00% in 2019 to 54.17% in 2021 (Table 3).

Virulence genes detection

Among 54 strains of CRKP, *YcfM*, *wabG* and *entB* were present in all strains. Four virulence genes with a positivity rate were over 90.00%, which were *fimH* (98.15%), *mrkD* (96.30%), *uge* (92.60%), and *kpn* (92.60%). The positivity rate of *rmpA* was 46.30%, the positive rate of *iutA* was 22.22%, and eight strains of virulence gene *PEG-344* were positive, with a positivity rate of 14.81%. Virulence gene *wcaG*, *k2A*, *magA*, *iroN*, and *allS* were not found (Table 4).

Clonal typing CRKP by MALDI-TOF MS

All 54 CRKP isolates were correctly identified at the species level by MALDI-TOF MS. The hierarchical clustering of MALDI-TOF peak profiles identified four different clusters. CRKP strains from ICU were assigned to cluster I and II. Nearly all CRKP isolates recovered from the geriatrics department were classified into Cluster IV. Most strains from surgery wards were assigned to cluster I. Strains from medicine wards were assigned to cluster I and III (Figure 1).

DISCUSSION

In this study, a total of 54 consecutive, non-repeated BSIs caused by CRKP were reported in three years. During the study period, the detection rate of BSIs caused by CRKP and the detection rates of carbapenemase increased, which is consistent with previous reports [19,20]. KPC carbapenemase ranked first in CRKP isolates, accounting for 88.9%, consistent with the report from Ben-David et al. [21]. As the mortality rate caused by CRKP is high [22], it is urgent to develop scientific measures to prevent and control bloodstream infections caused by CRKP, for example, active screening of CRKP, strengthening of environmental cleaning and disinfection, good hand hygiene, or training and education for healthcare workers.

Previously published reviews have reported several risk factors for CRKP, including severe underlying diseases, use of broad-spectrum antibiotics, use of carbapenem antibiotics, a long hospitalization period, malignancies, hematopoietic stem cell transplantation, tracheotomy, mechanical ventilation, and indwelling catheters [23-25]. In our study, multivariate regression analysis results showed that acute operation, being a resident in a long-term-care facility, transfer to ICU within 30 days, pulmonary diseases, renal diseases, liver diseases, abdominal infections, gastric tube, quinolone use, carbapenem use, and antifungal use prior to culture within 30 days were all risk factors for CRKP BSIs. Notably, *Klebsiella pneumoniae* is part of the normal commensal flora of the digestive tract. If digestive system diseases such as bleeding within the digestive tract, gastric ulcer, and pancreatitis occur, *Klebsiella pneumoniae* may enter the bloodstream and cause BSIs, including CRKP BSIs. As to antimicrobial susceptibility testing, all KPC carbapenems-producing *Klebsiella pneumoniae* strains were sensitive to ceftazidime/avibactam, showing excellent antibacterial activity of ceftazidime/avibactam against CRKP.

These CRKP strains carry virulence-associated genes, which may encode capsules (*magA*, *k2A*, *wcaG*), hypermucoviscosity (*magA*, *rpmA*), adhesins (*fimH*, *mrkD*, *kpn*), lipopolysaccharides (*wabG*, *uge*, *ycfM*), iron acquisition systems (*iutA*, *iroN*, *entB*), and other virulence factors (*allS*, *hly*, *cnf-1*) that enable them to overcome host defenses[26-34]. Moreover, studies [35] have reported that *peg-344*, *iucA*, *rmpA*, and *rmpA2*, and other virulence genes can be used as candidate biomarkers for identifying *Klebsiella pneumoniae* with high virulence with an accuracy > 0.95. Due to the limited number of strains included in this study, more data is needed to support this conclusion. *FimH* and *mrkD* mediate adhesion through encoding *Klebsiella pneumoniae* type 1 and type 3 fimbriae, and *mrkD* may promote development of biofilms. In the present study, *fimH* and *mrkD* were observed with a high positivity rate of over 90%. Therefore, clinicians are advised to consider phenotypes and genotypes of pathogens and to also carefully select an appropriate catheter and more frequent catheter irrigation or replacement to reduce bacterial adhesion and colonization. In the present study, virulence genes were widely present.

MALDI-TOF MS is currently considered an essential tool in clinical microbiology because it has the potential to improve patient prognosis and decrease the length of hospitalization [36]. Bacterial typing is an important method to identify the route of pathogen transmission. In this study, MALDI-TOF MS correctly identified 100% of the *Klebsiella pneumoniae* isolates. Hierarchical clustering of MALDI-TOF peak profiles identified four distinct clusters.

There were three limitations of this study. First, this was a retrospective study with a relatively small study population, and the mortality rate was found to be very low. Second, information on the clinical characteristics and outcomes could not be completely acquired due to the limitations that are inherent in a retrospective clinical study. Third, this was a case-controlled design in which the level of risk factors was not equal to the expected level commonly seen in the population.

CONCLUSION

Acute operation, being a resident in a long-term-care facility, transfer to ICU within 30 days, pulmonary diseases, renal diseases, liver diseases, abdominal infections, gastric tube placement, quinolone use, carbapenem use, antifungal agent use within 30 days prior to culture were indeed independent risk factors for CRKP

BSIs. KPC carbapenemase ranked first in CRKP BSI isolates. Virulence genes were widely present.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

References:

1. Cantón R, Akóva M, Carmeli Y, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 2012;18(5):413-31. (PMID: 22507109)

2. Asai N, Sakanashi D, Suematsu H, et al. The epidemiology and risk factor of carbapenem-resistant enterobacteriaceae colonization and infections: Case control study in a single institute in Japan. J Infect Chemother 2018;24(7):505-9. (PMID: 29548627)

3. McConville TH, Sullivan SB, Gomez-Simmonds A, et al. Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent risk of infection and 90-day mortality in critically ill patients, an observational study. Plos One 2017;12(10):e0186195. (PMID: 29023567)

4. Yigit H, Queenan AM, Anderson GJ, et al. Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 2001;45(4):1151-61. (PMID: 11257029)

5. Choby JE, Howard-Anderson J, Weiss DS. Hypervirulent Klebsiella pneumoniae - clinical and molecular perspectives. J Intern Med 2020;287(3):283-300. (PMID: 31677303)

6. Fang C-T, Chuang Y-P, Shun C-T, Chang S-C, Wang J-T. A Novel Virulence Gene in Klebsiella pneumoniae Strains Causing Primary Liver Abscess and Septic Metastatic Complications. J Exp Med 2004;199(5):697-705. (PMID: 14993253)

7. Sellick JA, Russo TA. Getting hypervirulent Klebsiella pneumoniae on the radar screen. Curr Opin Infect Dis 2018;31(4):341-6. (PMID: 29847328)

8. Lin Y-T, Cheng Y-H, Chuang C, et al. Molecular and Clinical Characterization of Multidrug-Resistant and Hypervirulent Klebsiella pneumoniae Strains from Liver Abscess in Taiwan. Antimicrob Agents Chemother 2020;64(5):e00174-20. (PMID: 32152079)

9. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis 2013;13(9):785-96. (PMID: 23969216)

10. Yuan Y, Wang J, Yao Z, et al. Risk Factors for Carbapenem-Resistant Klebsiella pneumoniae Bloodstream Infections and Outcomes. Infect Drug Resist 2020;13:207-15. (PMID: 32158236)

11. Hennessy S, Bilker WB, Berlin JA, Strom BL. Factors influencing the optimal control-to-case ratio in matched case-control studies. Am J Epidemiol 1999;149(2):195-7. (PMID: 9921965)

12. Fang L, Xu H, Ren X, et al. Epidemiology and Risk Factors for Carbapenem-Resistant Klebsiella Pneumoniae and Subsequent MALDI-TOF MS as a Tool to Cluster KPC-2-Producing Klebsiella Pneumoniae, a Retrospective Study. Front Cell Infect Microbiol 2020;10:462. (PMID: 33042858)

13. Candan ED, Aksoz N. Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol 2015;62(4):867-74. (PMID: 26637376)

14. Liao W, Huang N, Zhang Y, et al. Comparison of Carbapenem-Resistant Klebsiella pneumoniae Strains Causing Intestinal Colonization and Extraintestinal Infections: Clinical, Virulence, and Molecular Epidemiological Characteristics. Front Public Health 2021;9:783124. (PMID: 34926395)

15. Wajima T, Sugawara T, Umeda Y, Hagimoto A, Tanaka E, Nakaminami H. Molecular characterisation of carbapenem- and tigecycline-resistant Klebsiella pneumoniae strains isolated from blood and bile samples. J Infect Chemother 2022;28(2):187-91. (PMID: 34688546)

16. Zhang S, Zhang X, Wu Q, et al. Clinical, microbiological, and molecular epidemiological characteristics of Klebsiella pneumoniae-induced pyogenic liver abscess in southeastern China. Antimicrob Resist Infect Control 2019;8:166. (PMID: 31673355)

17. Sun Y, Guo J, Chen R, et al. Multicenter evaluation of three different MALDI-TOF MS systems for identification of clinically relevant filamentous fungi. Med Mycol 2021;59(1):81-6. (PMID: 32437532)

18. Fang L, Liu M, Huang C, et al. MALDI-TOF MS-Based Clustering and Antifungal Susceptibility Tests of Talaromyces marneffei Isolates from Fujian and Guangxi (China). Infect Drug Resist 2022;15:3449-57. (PMID: 35800121)

19. Sun X, Zou X, Zhou B, Yin T, Wang P. Comparison of bloodstream and non-bloodstream infections caused by carbapenem-resistant Klebsiella pneumoniae in the intensive care unit: a 9-year respective study. Front Med (Lausanne) 2023;10:1230721. (PMID: 37795412)

20. Onorato L, Sarnelli B, D'Agostino F, et al. Epidemiological, Clinical and Microbiological Characteristics of Patients with Bloodstream Infections Due to Carbapenem-Resistant K. Pneumoniae in Southern Italy: A Multicentre Study. Antibiotics (Basel) 2022; 11(5):633. (PMID: 35625277)

21. Ben-David D, Kordevani R, Keller N, et al. Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. Clin Microbiol Infect 2012;18(1):54-60. (PMID: 21722257)

22. Giacobbe DR, Marelli C, Cattardico G, et al. Mortality in KPC-producing Klebsiella pneumoniae bloodstream infections: a changing landscape. J Antimicrob Chemother 2023;78(10):2505-14. (PMID: 37606528)

23. Righi E, Peri AM, Harris PN, et al. Global prevalence of carbapenem resistance in neutropenic patients and association with mortality and carbapenem use: systematic review and meta-analysis. J Antimicrob Chemother 2017;72(3):668-77. (PMID: 27999023)

24. Katchanov J, Asar L, Klupp EM, et al. Carbapenem-resistant Gram-negative pathogens in a German university medical center: Prevalence, clinical implications and the role of novel beta-lactam/beta-lactamase inhibitor combinations. Plos One 2018;13(4): e0195757. (PMID: 29649276)

25. Zheng B, Dai Y, Liu Y, et al. Molecular Epidemiology and Risk Factors of Carbapenem-Resistant Klebsiella pneumoniae Infections in Eastern China. Front Microbiol 2017;8:1061. (PMID: 28659886)

26. Hartman LJ, Selby EB, Whitehouse CA, et al. Rapid Real-Time PCR Assays for Detection of Klebsiella pneumoniae with the rmpA or magA Genes Associated with the Hypermucoviscosity Phenotype. J Mol Diagn 2009;11(5):464-71. (PMID: 19644019)

27. Yu W-L, Ko W-C, Cheng K-C, Lee C-C, Lai C-C, Chuang Y-C. Comparison of prevalence of virulence factors for Klebsiella pneumoniae liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. Diagn Microbiol Infect Dis 2008; 62(1):1-6. (PMID: 18486404)

28. Yu WL, Fung CP, Ko WC, Cheng KC, Lee CC, Chuang YC. Polymerase Chain Reaction Analysis for Detecting Capsule Serotypes K1 and K2 ofKlebsiella pneumoniaeCausing Abscesses of the Liver and Other Sites. J Infect Dis 2007;195(8):1235-6. (PMID: 17357063)

29. Behzadi P. Classical chaperone-usher (CU) adhesive fimbriome: uropathogenic Escherichia coli (UPEC) and urinary tract infections (UTIs). Folia Microbiol (Praha) 2020;65(1):45-65. (PMID: 31165977)

30. Turton JF, Perry C, Elgohari S, Hampton CV. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol 2010;59(5):541-7. (PMID: 20110386)

31. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathol Biol (Paris) 2013;61(5):209-16. (PMID: 23218835)

32. Mamlouk K, Boutiba-Ben Boubaker I, Gautier Vr, et al. Emergence and Outbreaks of CTX-M β-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae Strains in a Tunisian Hospital. J Clin Microbiol 2006;44(11):4049-56. (PMID: 16957046)

33. Guiral E, Bosch J, Vila J, Soto S. Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbilol Lett 2011;314(2):170-3. (PMID: 21133987)

34. Sebghati TA, Korhonen TK, Hornick DB, Clegg S. Characterization of the type 3 fimbrial adhesins of Klebsiella strains. Infect Immun 1998;66(6):2887-94. (PMID: 9596764)

35. Harada S, Doi Y. Hypervirulent Klebsiella pneumoniae: a Call for Consensus Definition and International Collaboration. J Clin Microbiol 2018;56(9):e00959-18. (PMID: 29950337)

36. Tsuchida S, Umemura H, Nakayama T. Current Status of Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in Clinical Diagnostic Microbiology. Molecules 2020;25(20):4775. (PMID: 33080897)

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