Antibiotic Resistance, Molecular Characteristics and Risk Factors of Carbapenem-Resistant Klebsiella pneumoniae in Clinical Isolates

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Background: The global epidemic of carbapenem-resistant Klebsiella pneumonia (CRKP) has become a significant public health challenge. This study aimed to investigate the antibiotic resistance and molecular characteristics of CRKP and the clinical characteristics of infected patients.

Methods: Sixty-two clinically isolated CRKP strains were collected for the first time from the First Affiliated Hospital of Zhejiang Chinese Medical University in Zhejiang Province. The carbapenemase gene, virulence-associated gene, capsular serotype gene and fenestra protein gene were detected by PCR. Univariate logistic regression and multivariate logistic regression analyses were performed to predict the risk factors for the prognosis of CRKP infection.

Results: All CRKP isolates were resistant to meropenem, piperacillin-tazobactam, and ceftazidime (100%, 62/62), and all but one CRKP isolate was resistant to imipenem and cefepime (96.8%, 61/62). The rate of colistin resistance was the lowest (11.9%, 8/62). For CRKP in the ICU, the rates of resistance to various antibiotics were significantly higher than those in general ward patients. Fifty strains carried the carbapenemase gene \( \text{bla}_{\text{KPC}} \), and 3 strains carried both the \( \text{bla}_{\text{KPC}} \) and \( \text{bla}_{\text{NDM}} \) genes. The virulence genes \( \text{wabG}, \text{vca}, \text{entB}, \text{ureA} \) and \( \text{fimH} \) were detected in more than 90% of the 62 CRKP strains. Two strains had \( \text{OmpK35}, \text{OmpK36} \) and \( \text{Hcp} \) gene deletions. The \( \text{bla}_{\text{KPC}}, \text{rmpA} \) and \( \text{rmpA2} \) genes had the highest positive rate in blood samples, and \( \text{bla}_{\text{NDM}} \) had the highest positive rate in stool samples. Multivariate analysis showed that pulmonary disease affected the prognosis of CRKP infection.

Conclusion: The prevalence and molecular characteristics of CRKP clinical isolates in Zhengjiang Province in China were described, and the antibiotic resistance rate was higher. Additionally, relevant genes of CRKP strains and clinical characteristics of patients are related to the progression and prognosis of CRKP infection.

Keywords: Klebsiella pneumoniae, carbapenem-resistant, antibiotic resistance, carbapenemase, virulence gene, risk factor

Introduction

Klebsiella pneumoniae (KP) is an Enterobacteriaceae pathogen associated with hospital and community-acquired infections, such as pneumonia, blood infection, and urinary tract infection.1 Carbapenem antibiotics are widely used to treat patients with KP infection due to their stable effects on β-lactamase.2 Antibiotic resistance and widespread dissemination of CRKP have become some of the most difficult challenges in clinical infection therapy. However, carbapenem-resistant Klebsiella pneumonia (CRKP) has become a severe threat in both hospitals and communities due to the widespread use of antibiotics. According to previous reports, the production of carbapenemase is the most common mechanism of carbapenem resistance among KP isolates.3 Molecular methods have detected that the majority of CRKP isolates contain a carbapenemase gene, such as the K. pneumoniae carbapenemase (\( \text{bla}_{\text{KPC}} \)) or New-Delhi-metallo-β-lactamase (\( \text{bla}_{\text{NDM}} \)) genes.4 CRKP has been reported in almost all regions of China and is traditionally believed to not be highly virulent.5 However, the recent emergence of highly virulent Klebsiella pneumoniae (hvKP) in mainland China has attracted much attention because it can cause highly invasive infections,
such as severe pneumonia, liver abscesses, meningitis and dophthalmitis.⁶,⁷ hvKP tends to exhibit high viscosity, probably associated with specific virulence genes and capsular polysaccharides.⁸,⁹ The string test is commonly used to identify hyperviscosity phenotypes. Genetic determinants of high virulence usually reside in mobile genetic elements, such as the high virulence plasmid-related genes \( rmpA, rmpA2, \) and \( iutA \).¹⁰

In recent years, the detection rate of CRKP with high virulence and high drug resistance has continued to increase, and the horizontal movement of its drug resistance genes can cause the proliferation of drug-resistant strains. Therefore, early detection and monitoring of CRKP colonization or infection and research on the occurrence and development of its drug resistance mechanism are essential to control the occurrence and spread of bacterial drug resistance.¹¹ Furthermore, an epidemiological analysis of CRKP is imperative. Effective monitoring of these isolates can limit the spread of antibiotic resistance. In hospital treatment, multiple risk factors can affect the prognosis of CRKP infection, and analysis of risk factors for CRKP infection is necessary to help clinicians take effective preventive measures in the early stage.¹² This study investigated the clinical characteristics, drug resistance and molecular characteristics of CRKP clinical strains and the risk factors for CRKP infection. The goal of this study was to provide a reference for the diagnosis and treatment of clinical infection and rational drug use and to provide information to help reduce the possibility of strain outbreaks.

**Materials and Methods**

**Clinical Data Collection**

All infected patients (the clinical source of 62 CRKP isolates) characteristics were extracted and are presented in Table 1. The survey items included demographic data, such as age, sex and basic medical history; hospitalization characteristics, including sample type and separation department; and treatment with invasive surgery, including tracheal intubation or tracheotomy, arteriovenous catheterization, indwelling catheter, etc. The prognosis of the patient was based on the patient’s condition at discharge: those who died of irreversible organ failure and did not improve at discharge were considered to have a poor prognosis. The consent was waived by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University (2019-KL-090-01). The clinical samples were part of the routine hospital laboratory procedure, and noninfected patients were removed. The study was performed in accordance with the guidance of the Declaration of Helsinki and relevant regulations.

**Sample Collection and Antibiotic Susceptibility Test**

Sixty-two CRKP strains were isolated for the first time from The First Affiliated Hospital of Zhejiang Chinese Medical University from August 2019 to May 2020, and the specimens of noninfected patients were removed. The antibiotics commonly used clinically were tested for antimicrobial susceptibility through the micro broth dilution method and the Vetek 2 Compact system (Biomerieux, Marcy L’Etoile, France). Antibiotic susceptibility results refer to the unified standards of the Clinical and Laboratory Standards Institute (https://clsi.org/standards/products/microbiology/documents/, Published in 2022). Tigecycline MICs were interpreted using EUCAST (http://www.eucast.org) MIC breakpoint standards, and CRKP was defined as resistance to meropenem, imipenem or ertapenem (minimum inhibitory concentration ≥ 4 µg/mL). All CRKP strains were reconfirmed by the paper diffusion method to avoid Vetek system errors¹³ (according to CLSI: the diameter of the meropenem and imipenem inhibition zone is ≤ 19 mm, and the diameter of the ertapenem inhibition zone is ≤ 18 mm (antibiotics from Oxoid UK). Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) were used as quality control strains for the antibiotic susceptibility test.

**String Test**

The isolates of Klebsiella pneumoniae on the agar plate were stretched through the inoculation ring. A viscous silk with a length of >5 mm was produced in all three experiments, the drawing test was considered positive, and the strain belonged to the hyperviscosity phenotype.¹⁴
DNA Extraction and Application of Genes

Chromosomal DNA was extracted using a TIANamp Bacteria Genomic DNA Kit (Tiangen Biotech Co. Ltd, Beijing, China). PCR amplifications were performed for carbapenemase genes (\textit{bla}\textsubscript{KPC}, \textit{bla}\textsubscript{NDM}, \textit{bla}\textsubscript{OXA-48}, \textit{bla}\textsubscript{VIM}, \textit{bla}\textsubscript{GIM}, \textit{bla}\textsubscript{SIM}, and \textit{bla}\textsubscript{IMP}), virulence genes (\textit{magA}, \textit{uge}, \textit{aerobactin}, \textit{wcaG}, \textit{mrkD}, \textit{wabG}, \textit{fimH}, \textit{rmpA}, \textit{ycf}, \textit{urea}, \textit{ybtA}, \textit{kuB}, \textit{iorN}, \textit{iutA}, \textit{rmtB}, and \textit{alls}), capsular serotype-specific genes (K1, K2, K5, K20, K54, and K57), fenestra protein genes (\textit{ompk35} and \textit{ompk36}) and a bacterial pathogenicity-associated gene (\textit{hcp}) that encodes a haemolysin-coregulated protein, and the primers are listed in Table S1\textsuperscript{9,11,15–18}. The PCR conditions were as follows: 95 °C predenaturation for 5 minutes; 95 °C denaturation for 10 seconds, 50–62°C annealing for 20 seconds, 72 °C extension for 20 seconds, a total of 35 cycles; 72°C end extension for 40s and storage at 4 °C. The amplified products were electrophoresed in a 1% agarose gel, and the PCR-positive products were sent to Shanghai Shenggong Company for sequencing. The sequencing results were performed in GenBank for BLAST comparison to confirm the genotype.

### Statistical Analysis

The continuous data are described as the mean and standard deviation, and classification data are expressed in quantity and percentage. The resistance rate between ICU and general ward patients was compared using the \(\chi^2\) test. The positive rate of genes

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**Table 1 Clinical Patient Data**

|                      | N=62 (%) |
|----------------------|----------|
| **Age(y)**           |          |
| Mean                 | 69       |
| Range                | 27–90    |
| **Gender(n)**        |          |
| Male                 | 46(74.2%)|
| Female               | 16(25.8%)|
| **Basic medical history(n)** |                        |
| Lung disease         | 33(53.2%)|
| Hypertension         | 32(51.6%)|
| Heart system disease | 24(38.7%)|
| Cerebrovascular system disease | 19(30.6%) |
| Kidney diseases      | 17(27.4%)|
| **Department(n)**    |          |
| ICU                  | 41(66.1%)|
| Haematology department | 14(22.6%) |
| Cadre ward           | 2(3.2%)  |
| Oncology department  | 1(1.6%)  |
| Respiratory medicine | 2(3.2%)  |
| Neurology            | 2(3.2%)  |
| **Specimens(n)**     |          |
| Stool                | 20(32.3%)|
| Sputum               | 17(27.4%)|
| Blood                | 14(22.6%)|
| Sterile body fluids  | 7(11.3%) |
| Urine                | 3(4.8%)  |
| Wound secretions     | 1(1.6%)  |
| **Invasive procedures(n)** |                |
| Yes                  | 39(62.9%)|
| No                   | 23(37.1%)|
| **Poor prognosis**   |          |
| Yes                  | 40(64.5%)|
| No                   | 22(35.5%)|
among different strains was compared by the $\chi^2$ test for categorical variables. A univariate logistic regression model was analysed by the $\chi^2$ test, and multivariate analysis was performed by logistic regression analysis. P<0.05 (two-sided) was considered statistically significant. All statistical analyses were performed using SPSS 25.0 software (IBM, Armonk, NK, USA).

**Results**

**Clinical Characteristics**

The 62 patients infected with CPKP were mainly males (74.2%, 46/62), with a median age of 69 years. In the basic medical history, the incidence of pulmonary disease and hypertension were 53.2% (33/62) and 51.6% (32/62), respectively. The incidences of heart system, cerebrovascular system and kidney diseases were 38.7% (24/62), 30.6% (19/62) and 27.4% (17/62), respectively. For the distribution of departments, the ICU had the highest detection rate (66.1%, 41/62), followed by the haematology department (22.6%, 14/62), cadre ward (3.2%, 2/62), respiratory medicine and neurology departments (3.2%, 2/62), and oncology department (1.6%, 1/62). For sample type, the stool detection rate was the highest (32.3%, 20/62), followed by sputum (27.4%, 17/62), blood (22.6%, 14/62), sterile body fluids (11.3%, 7/62), urine (4.8%, 3/62) and wound secretions (1.6%, 1/62). Among the 62 CRKP-infected patients, 39 underwent invasive procedures, and 40 patients had a poor prognosis (Table 1).

**Antibiotic Sensitivity Results**

The detailed antibiotic resistance results of the 62 CRKP clinical isolates are shown in Table 2. All strains were resistant to meropenem (100%, 62/62), piperacillin-tazobactam (100%, 62/62), and ceftazidime (100%, 62/62), and all but one strain was resistant to imipenem (96.8%, 61/62) and cefepime (96.8%, 61/62). Meanwhile, as shown in Figure 1, the antibiotic resistance rates of CRKP in ICU patients for ciprofloxacin, levofloxacin, amikacin, aztreonam and tigecycline were significantly higher than those in general ward patients ($P=0.004$, $P=0.002$, $P<0.001$, $P=0.001$, and $P=0.002$, respectively).

**String Test of Carbapenemase Genes, Virulence Genes, and Capsular Serotype Specific Genes**

The gene results are shown in Table 3. Among the 62 CRKP isolates, 57 isolates carried carbapenemase genes (91.9%, 57/62). The positive rate of $bla_{KPC}$ was the highest (80.6%, 50/62), followed by those of $bla_{NDM}$ (12.9%, 8/62) and

### Table 2 Antibiotic Sensitivity Results of CRKP Isolates

| Antibiotics | Fold Point (µg/mL) | N=62 (Resistance Rate, %) |
|-------------|--------------------|--------------------------|
|             | Sensitive          | Resistance                |
| IPM         | ≤1                 | ≥4                        | 61 (98.4%) |
| MEM         | ≤1                 | ≥4                        | 62 (100%)  |
| CAZ         | ≤4                 | ≥16                       | 62 (100%)  |
| FEP         | ≤2                 | ≥16                       | 61 (98.4%) |
| ATM         | ≤4                 | ≥16                       | 57 (91.9%) |
| AMK         | ≤16                | ≥64                       | 43 (69.4%) |
| TGC*        | ≤1                 | ≥2                        | 54 (87.3%) |
| CIP         | ≤1                 | ≥4                        | 58 (93.5%) |
| LEV         | ≤2                 | ≥8                        | 60 (96.8%) |
| Col         | ≤2                 | ≥4                        | 8 (11.9%)  |
| TOB         | ≤4                 | ≥16                       | 52 (83.9%) |
| TZP         | ≤16                | ≥128                      | 62 (100%)  |
| SXT         | ≤38                | ≥76                       | 54 (87.1%) |

*Note:* *Tigecycline MICs were interpreted using EUCAST MIC breakpoints.

**Abbreviations:** IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; AMK, amikacin; TGC, tigecycline; CIP, ciprofloxacin; LEV, levofloxacin; COL, colistin; SCR, cefoperazone-sulbactam; TOB, tobramycin; TZP, piperacillin-tazobactam; SXT, cotrimoxazole.
Among the virulence genes, the positive rates of *ycf, entB*, and *ureA* were the highest (96.8%, 60/62), followed by those of *WabG* (93.5%, 58/62), *Uge* (91.9%, 57/62), and *fimH* (90.3%, 56/62). Among the 62 CRKP isolates, 1 isolate was the K5 serotype. The other 61 strains did not have the common capsular serotypes (K2, K5, K20, K54, and K57). Among the 62 CRKP isolates, 2 isolates had a hypermucoid phenotype (3.2%, 2/62).

**HCP and Fenestra Protein Gene Test**

In this study, 2 of 62 CRKP isolates lost the bacterial pathogenicity-associated gene *Hcp* (3.2%, 2/62). Seven of the 62 CRKP isolates lost the fenestra gene, *Ompk35* or *Ompk36* (11.29%, 7/62), and 2 isolates lost both the *Ompk35* and *Ompk36* genes.

**Comparison of Molecular Types in Different Specimen Types**

We compared the positive rate of carbapenemase genes (*bla*KPC and *bla*NDM) and virulence genes (*rmpA* and *rmpA2*) in the specimen types. The positive rate of *bla*KPC in blood samples was higher than that in stool and sputum samples, although statistical analysis showed no significant difference. In stool samples, the positive rate of *bla*NDM was highest (25%). Moreover, the positive rates of the virulence genes *rmpA* and *rmpA2* in blood were both higher than those in stool and sputum samples (Table 4).

**Risk Factors for Poor Prognosis in CRKP-Infected Patients**

The relevant characteristics of 62 CRKP-infected patients were analysed to predict the risk factors for poor prognosis. The univariate analysis showed that male sex, ICU admission, lung disease (primary or secondary diagnosis of lung disease recorded in the medical record), and infection with isolates carrying the *bla*KPC gene were significantly different.
Table 3 The Positive Rate of Genes in 62 CRKP Isolates

| Genes                          | Positive rate (%) |
|-------------------------------|-------------------|
| **Carbapenemase gene**        |                   |
| blaKPC                        | 50 (80.6%)        |
| blaNDM                        | 8 (12.9%)         |
| blaIMP                        | 3 (4.8%)          |
| blaKPC, blaNDM and blaIMP     | 1 (1.6%)          |
| blaKPC and blaNDM             | 3 (4.84%)         |
| blaOKA-48                     | 0                 |
| blaGIM                        | 0                 |
| blaVIM                        | 0                 |
| blaGIM                        | 0                 |
| **Virulence gene**            |                   |
| Uge                           | 57 (91.9%)        |
| WabG                          | 58 (93.5%)        |
| Ycf                           | 60 (96.8%)        |
| entB                          | 60 (96.8%)        |
| urea                          | 60 (96.8%)        |
| fimH                          | 56 (90.3%)        |
| ybtA                          | 52 (83.9%)        |
| iutA                          | 48 (77.4%)        |
| kfuB                          | 9 (14.5%)         |
| mpa                           | 23 (37.1%)        |
| mpa2                          | 31 (50%)          |
| aerobactin                    | 0                 |
| magA                          | 0                 |
| mrkD                          | 0                 |
| wcoG                          | 1 (1.6%)          |
| iroN                          | 1 (1.6%)          |
| alls                          | 1 (1.6%)          |
| **Capsular serotype**         |                   |
| K5                            | 1 (1.6%)          |

Table 4 Comparison of Molecular Types in Different Specimen Types

| Specimen | Specimen | Sputum | Stool | Blood | P*  | χ²  |
|----------|----------|--------|-------|-------|-----|-----|
| **blaKPC** |          |        |       |       |     |     |
| Negative (%) | 3 (17.7) | 15 (88.2) | 2 (11.8) | 1 (7.1) | 0.135 | 4.235 |
| Positive (%)  | 14 (82.3) | 15 (88.2) | 2 (11.8) | 13 (92.9) |       |     |
| **blaNDM** |          |        |       |       |     |     |
| Negative (%)  | 11 (64.7) | 11 (64.7) | 6 (35.3) | 5 (35.7) | 0.229 | 2.966 |
| Positive (%)  | 6 (35.3)  | 5 (25.0)  | 5 (25.0) | 9 (64.3)  |       |     |
| **mpa**      |          |        |       |       |     |     |
| Negative (%)  | 6 (35.3)  | 6 (35.3)  | 10 (50.0) | 4 (28.6) | 0.417 | 1.758 |
| Positive (%)  | 10 (50.0) | 10 (50.0) | 10 (50.0) | 10 (71.4) |       |     |

Note: *Calculated using the χ² test for categorical variables.
between the prognosis groups (Table S2). Furthermore, logistic regression models were performed to predict the correlations of these factors with poor prognosis, and the results showed that pulmonary disease was a potential factor (Table 5).

**Discussion**

In recent years, carbapenem-resistant *Klebsiella pneumonia* has become a severe threat in both hospitals and communities due to the widespread use of antibiotics. Moreover, the emergence of hypervirulent *Klebsiella pneumoniae* is causing an increasing challenge in China. Previous reports have indicated that a majority of CRKP isolates carry resistance genes, and hvKP tends to exhibit high viscosity, probably associated with specific virulence genes and capsular polysaccharides. However, studies on the prevalence of CRKP strains in Zhejiang Province in China are limited. In this study, 62 clinically isolated CRKP strains were collected to investigate the drug resistance, molecular characteristics and clinical characteristics of infected patients.

Among the 62 CRKP-infected patients, 41% were distributed in the ICU, and 14% were distributed in the haematology department. This is probably related to the severe basic diseases that present in those departments and the more invasive treatments administered there. Similarly, Li et al found that ICU admission was a risk factor for CRKP infection. The median age of the CRKP-infected patients was 69 years, and the infections were mainly distributed in the digestive tract, respiratory tract and blood, which indicated that elderly patients and other immunocompromised people are more prone to invasive infections. In this study, the resistance rates of CRKP strains to ceftazidime, cefepime, aztreonam, meropenem, imipenem, cefoperazone and sulbactam were high, and the resistance rate to colistin was the lowest. Moreover, the resistance rate was higher than that in a previous report, probably due to the severe condition of patients and the clinical treatment of multiple types of antibiotics. Therefore, antibiotics should be used appropriately to avoid unnecessary new drug resistance in the clinical treatment of CRKP infection. In this study, the antibiotic resistance rates of CRKP in ICU patients to ciprofloxacin, levofloxacin, amikacin, aztreonam and tigecycline were significantly higher than those in general ward patients, which was probably related to the severe basic diseases of ICU patients and the clinical treatment of multiple antibiotics.

The production of carbapenemase is the most common mechanism of carbapenem resistance among KP isolates. Common carbapenemase genes include *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>IMP</sub>, etc. In our study, 57 strains carried carbapenemase genes, and the most common carbapenemase genes were *bla*<sub>KPC</sub> (80.6%) and *bla*<sub>NDM</sub> (14.0%). A previous study showed that KP carries both *bla*<sub>KPC</sub> and *bla*<sub>NDM</sub> and is resistant to almost all antibiotics. Our research detected three strains carrying both *bla*<sub>KPC</sub> and *bla*<sub>NDM</sub> and one strain carrying both *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub> and *bla*<sub>IMP</sub>. In addition, carbapenemase genes can be spread horizontally between strains through plasmids. This suggests that our test results have value for clinical application in regards to taking effective measures to avoid the outbreak of drug-resistant strains in hospitals through accurate diagnosis of microorganisms, rational use of antibiotics and reduction of invasive procedures. In this study, common carbapenemase genes were not detected in five CRKP strains, which was probably because those strains had other uncommon carbapenemase genes or other resistance mechanisms. In this study, the positive rates of the virulence genes *uge*, *wabG*, *ycf*, *entB*, *ureA*, *fimH* and *yht* were all above 90%, which suggests that these genes may be the main pathogenic factors of CRKP. At present, there is no uniform standard for the hvKP test.

### Table 5 Multivariate Analysis for Prognosis in CRKP-Infected Patients

| Variable              | P    |
|-----------------------|------|
| Gender                | 0.410|
| ICU admission         | 0.071|
| *bla*<sub>KPC</sub>   | 0.663|
| Pulmonary disease     | 0.030*|

**Notes:** *Calculated using a logistic regression model; *P<0.05.
but studies have shown that the presence of the iro, rmpA and rmpA2 genes located on the mobile virulence plasmid can highly predict hvkp.24 rmpA can activate capsule production, resulting in the hypermucoviscosity phenotype, and increase in virulence.25 In this study, the positive rates of rmpA and rmpA2 were 37.1% and 50%, indicating that these strains are super bacteria (with high virulence and high drug resistance) and need more clinical attention.

The hypermucoviscous phenotype of KP strains is more likely to cause some special invasive infections, which is usually related to the increased production of capsular polysaccharides and the presence of specific virulence genes. The capsular serotypes of KP can be divided into 82 serotypes.26 In this study, only one K5 strain was detected among the 62 CRKP strains, and a large number of rmpA gene-positive strains did not show a hypermucoid phenotype. This may be due to the reduced expression of the capsule by resistant strains or the presence of other undetected capsule serotypes and regulatory mechanisms. Some studies have shown that the lack of hypermucoviscosity in some rmpA-positive K. pneumoniae strains was associated with a concurrent mutation of rmpA genes.27 Therefore, more studies in other strains are needed to clarify the mechanisms. The type IV secretion system (T6SS) of gram-negative bacteria participates in the formation of bacterial biofilms, resists phagocytosis by macrophages, and induces biological functions, such as apoptosis.28 As the core component of the T6SS, Hcp plays an important role in bacterial pathogenicity.29 There is increasing evidence that the T6SS is related to the pathogenesis of KP.30 In this study, 2 of 62 CRKP isolates did not have Hcp, which may be related to the mechanism of resistance. Seven isolates among the 62 CRKP isolates did not have the fenestra gene, Ompk35 or Ompk36. In addition, we found that 2 isolates without carbapenemase genes also had deletion of Ompk35/Ompk36, suggesting that the loss of outer membrane protein may be related to the mechanism of resistance. This is in line with Sugawa E and Zhang Yong, who found that the existence of Ompk35 and Ompk36 promoted the diffusion of β-lactam drugs in cells.31,32 However, whether that is accompanied by efflux pump expression, ESBL/ AmpC, and high-affinity binding sites requires further study.33

A previous study revealed a strong association between the rmpA gene and hypervirulence,25 and the carbapenemase genes blaKPC and blaNDM were the most common resistance genes in KP isolates. However, the association of carbapenemase and virulence genes with CRKP sources has not been reported. We investigated the potential association between the gene distribution of CRKP among the specimens, and the results showed that the positive rates of blaKPC, rmpA, and rmpA2 in blood samples were the highest, suggesting that highly resistant and virulent strains are more likely to cause blood invasive infections. Bacteraemia is an extreme complication of CRKP infection that commonly occurs with severe invasive injury. Therefore, more samples and further studies on the molecular mechanism of CRKP infection in blood invasion should be performed.

However, a limitation of this study was the lack of in-depth research on the mechanism of drug resistance and virulence. Statistical investigation of risk factors can help clinicians take effective preventive measures in the early stage of infection.34 In this study, the univariate analysis showed that sex, ICU admission, pulmonary disease, and infection with strains carrying the blaKPC gene were statistically significant for the prognosis of CRKP infection. However, multiple regression analysis showed that only pulmonary disease was a potential risk factor for the prognosis of CRKP infection. Notably, there have been few reports about pulmonary diseases as a risk factor for CRKP infection in published studies; thus, this is a clinically significant finding. Klebsiella pneumoniae is part of the normal commensal flora of the respiratory tract and can cause pulmonary infection, including lobar pneumonia, empyema, and lung abscess, when immunocompromised or external infection occurs.7 Patients with a poor prognosis usually have severe infections, long medical treatment times, and even irreversible organ failure. Therefore, patients with such risk factors should receive more attention and effective clinical treatment. However, an in-depth study to explore the possible resistance mechanisms is necessary.

**Conclusion**

The prevalence and molecular characteristics of CRKP clinical isolates in Zhengjiang Province in China were described, and the antibiotic resistance rate was higher than that in a previous report, suggesting that antibiotics should be used appropriately to avoid unnecessary new drug resistance in the clinical treatment of CRKP infection. In addition, blaKPC and blaNDM are the most prevalent carbapenemase genes of K. pneumoniae. The CRKP isolates carrying the virulence
genes *rmpA* and *rmpA2* are more likely to cause invasive blood infection, and the deletion of the *ompk35* and *ompk36* genes may increase drug resistance. Also, lung disease is a potential factor for poor prognosis.

**Data Sharing Statement**

All data generated or analysed during this study are included in this published article.

**Ethics Approval and Consent to Participate**

Compliance with ethical standards: This study was approved by the Medical Ethics Committee of the Hospital (No. 2019-KL-090-01) and was conducted in compliance with ethical, legal, and regulatory norms. The consent was waived by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University due to the retrospective nature of the review. Privacy of the participants was protected and the data was anonymized or maintained with confidentiality.

**Author Contributions**

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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**Disclosure**

The authors report no conflicts of interest in this work.

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