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ORIGINAL RESEARCH

# Molecular Characteristics and Phenotypical Analysis of Carbapenem-Resistant *K. Pneumoniae* in the Lüliang Region, Shanxi Province

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**Introduction:** To explore the resistance characteristics and molecular features of carbapenem-resistant *K. pneumoniae* (*CRKP*) isolates prevalent in the Lüliang region, 81 *CRKP* isolates were collected from three hospitals in the Lüliang region, Shanxi Province.
**Methods:** The resistance of these *CRKP* isolates to 11 antibiotics was determined using the disk diffusion method, and antimicrobial resistance encoding genes and virulence genes were detected by PCR. The mucoid phenotype of the *CRKP* isolates was examined via the string test, and bacterial biofilm formation ability was measured using the crystal violet staining method.
**Results:** The resistance rates of the 81 *CRKP* isolates to the 11 antibiotics ranged from 62.96% to 100%, with a multidrug resistance rate of 83.95%. The resistance genes $bla_{SHV}$, $bla_{TEM}$ and $bla_{KPC}$ were the most widely distributed, with a detection rate of 100%. Among the 81 *CRKP* isolates, 70 had the ability to form biofilms, and 58 presented highly mucoid phenotypes. The virulence genes *rmpA2, peg-344*, and *fimH* presented high carriage rates of 92.59%, 91.36%, and 88.89%, respectively. The carriage rate of *IroB* was low, at 20.99%. Among these genes, *fimH, rmpA2*, and *iucA* were associated with biofilm formation, while *markD* and *fimH* were associated with a highly mucoid phenotype, and the highly mucoid phenotype was strongly correlated with the biofilm formation ability.
**Conclusion:** This study revealed that the *CRKP* strains isolated in the Lüliang region of Shanxi Province were strongly resistant and that this resistance was related to virulence characteristics. Therefore, antibiotic management should be strengthened in clinical practice to control the prevalence of *CRKP* in this region.
**Keywords:** *CRKP*, resistance, virulence, biofilm, ucoid phenotypes

## Introduction

*Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic pathogen bacterium that is widely present in hospital environments and isolates of this bacterium have developed resistance to multiple antibiotics, causing severe community-associated infections and posing a significant threat to human health.[1] *K. pneumoniae* often resides in the human intestine, and can cause infections in the respiratory system, urinary system, and skin and soft tissues, especially in patients with compromised immune systems.[2,3] Currently, cephalosporins and carbapenems are commonly used to treat *K. pneumoniae* infections in clinical settings. However, the extensive use of these antibiotics has led to the emergence of multidrug-resistant and pan-resistant *CRKP* isolates. In particular, the detection rate of carbapenem-resistant KP has increased in recent years,[4] making the prevention and treatment of the associated diseases more challenging.

The resistance mechanisms of *CRKP* are diverse, with the main mechanism being the production of carbapenemases,[5] which can hydrolyze various carbapenem antibiotics, including meropenem, imipenem, and ertapenem. In 1997, the first *CRKP* strain was discovered in North America,[6] and since then, reports of *CRKP* infections have increased globally.[7] The spread of *CRKP* has become a severe problem, especially in some regions where the resistance rate of *CRKP* is very high, exceeding 50%.[8] In China,

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according to the report of the National Antimicrobial Resistance Monitoring System (China Antimicrobial Resistance Surveillance System, CARSS), the prevalence and characteristics of *CRKP* vary across different regions. Therefore, understanding the phenotypic and molecular characteristics of *CRKP* isolates from specific regions is highly useful for guiding clinical drug use. According to an analysis by the CARSS, the resistance rate of *CRKP* has increased in recent years. However, there are few reports on the prevalence characteristics, resistance status, and virulence-related characteristics of *CRKP* in the Lüliang region of Shanxi Province. This study aims to provide effective theoretical and data support for the prevention and treatment of *CRKP* infections in this region by conducting research on the prevalence characteristics, phenotypic resistance, resistance genes, and virulence genes of *CRKP* in the Lüliang region of Shanxi Province.

# Materials and Methods

## Strain Source

In this study, 81 *CRKP* isolates were collected from three hospitals (Fenyang Hospital of Shanxi Province, Fenyang City People's Hospital, and Fenyang City Maternal and Child Health Care Hospital) in the Lüliang region of Shanxi Province (Figure 1) between December 2022 and December 2023. The clinical information of the patients, including symptoms and strain sources, was collected on the basis of the patient's medical record number. These data were obtained with the consent of the hospital. The *CRKP* isolates were obtained according to the "National Clinical Laboratory Procedures". The isolates were sequentially labeled KP1-KP81. The control strains used for the clinical susceptibility test were *Escherichia coli* ATCC25922 and *K. pneumoniae* ATCC-BAA-1705H and ATCC-BAA-1706, which were used as positive and negative control strains for the mCIM test, respectively.

Figure 1. The location of the hospital where the samples were collected. The red dots represent the areas where the samples were collected.

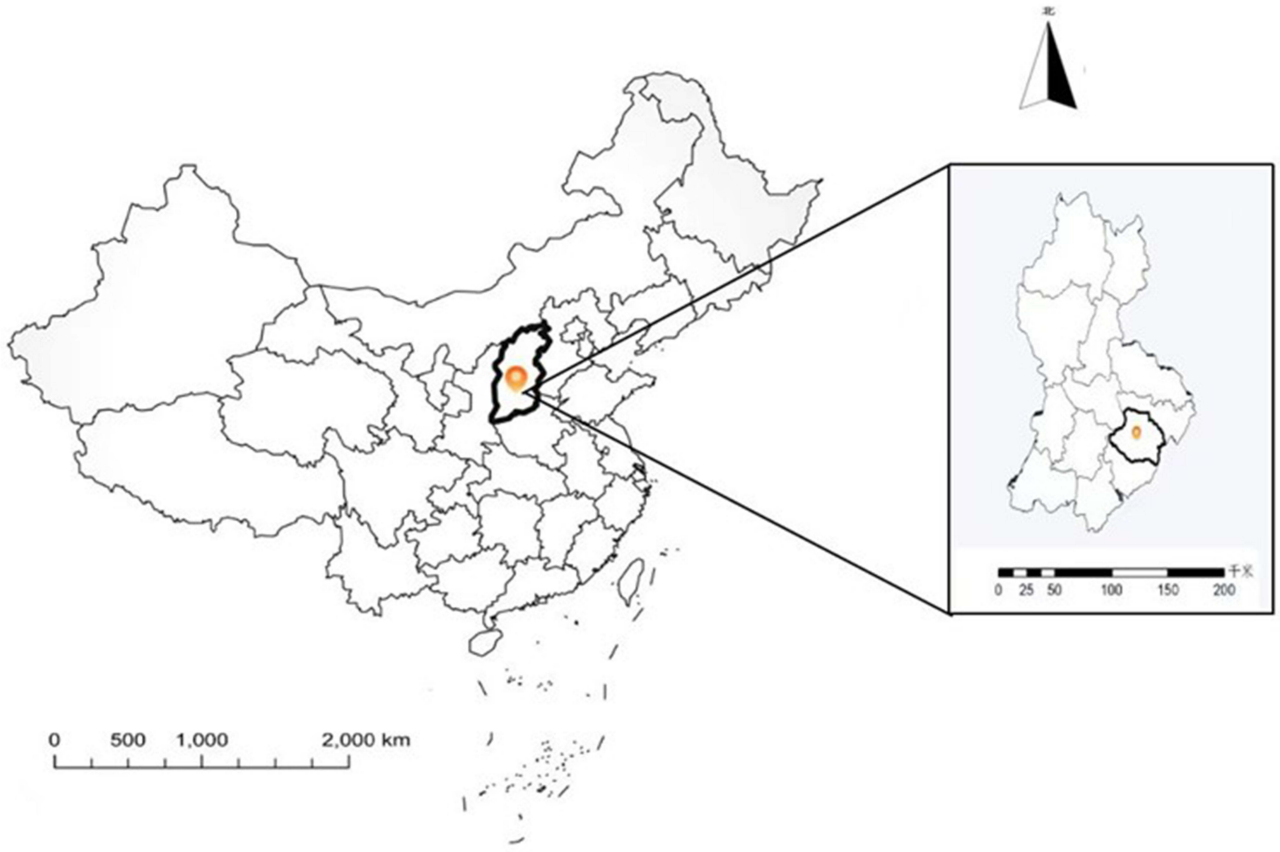


**Figure 1** The source locations of the 81 carbapenem-resistant K. pneumoniae isolates.
**Notes**: The red dots represent the hospital where the samples were collected.

## Carbapenemase Phenotype Confirmation

The modified carbapenem inactivation method (mCIM) recommended by the CLSI2020[9] was used to detect carbapenemase in the 81 clinical *CRKP* isolates. The specific method was as follows: A full loop of the test colony was inoculated into 2 mL of tryptic soy broth (TSB, Beijing Solarbio Science & Technology China) and vortexed for 15s to mix. A 10 μg meropenem disk was placed into the tube with sterile forceps, fully immersed in the bacterial suspension, sealed, and incubated at 35 ± 2 °C for 4 hours. *Escherichia coli* ATCC 25922 was prepared as a 0.5 McFarland bacterial suspension, inoculated onto Mueller-Hinton agar (MHA, Beijing Solarbio Science & Technology China) solid medium, and dried for 5 minutes. The meropenem disk was removed from the tube with sterile forceps, squeezed dry, and placed on the previously prepared MHA medium. The plate was incubated at 35 ± 2 °C for 18–24 hours, and the inhibition zone diameter was measured with a caliper. The results were interpreted according to the carbapenemase confirmation criteria of the CLSI 2020 version: (1) colonies with an inhibition zone diameter of 6–15 mm or scattered colonies within a 16–18 mm inhibition zone were considered carbapenemase positive. (2) colonies with an inhibition zone diameter ≥ 19 mm were considered carbapenemase negative. (3) The two scenarios can be classified as indeterminate for carbapenemase production: an inhibition zone diameter of 16–18 mm or ≥19 mm combined with scattered colonies within the inhibition zone.

## Determination of the Antibiotic Resistance Phenotype of Carbapenem-Resistant K. Pneumoniae

The susceptibility of the 81 *CRKP* isolates to 11 antibiotics was determined via the disk diffusion method recommended by the CLSI. These antibiotics included ceftazidime (CAZ, 30 μg), cefoperazone (CFP, 30 μg), ceftriaxone (CRO, 30 μg), imipenem (IPN, 10 μg), meropenem (MEM, 10 μg), ciprofloxacin (CIP, 5 μg), levofloxacin (LVF, 5 μg), amikacin (AK, 30 μg), gentamicin (CN, 30 μg), trimethoprim-sulfamethoxazole (SXT, 25 μg), and ampicillin (AMP, 25 μg). The susceptibility results were interpreted according to the CLSI 2020 standards. *CRKP* isolates resistant to three or more classes of antibiotics were considered multidrug resistant.[10]

## Examination of the Mucoid Phenotype of Carbapenem-Resistant K. Pneumoniae

The mucoid phenotype of the *CRKP* isolates was examined via the string test. The specific procedure was as follows: *CRKP* isolates were transferred to Columbia blood agar plates and incubated at 37 °C for 16–20 hours. A loopful of the colony was lifted with a loop, and if a mucous filament longer than 0.5 cm formed, the strain was considered hypermucoviscous (HM); otherwise, it was considered non-hypermucoviscous (NO HM).[11]

## Determination of the Biofilm Formation Ability of Carbapenem-Resistant K. Pneumoniae

The biofilm formation ability of *K. pneumoniae* in vitro was measured via the crystal violet staining method in a 96-well plate, as referenced in the literature.[1] The specific operation steps were as follows: A standard 96-well microtiter plate was prepared, with 200 μL of tryptic soy broth added to each well. A single colony of the fresh isolate was inoculated into the broth medium and grown with shaking 120 r/min at 37 °C for 16–18 hours. Then, 2 μL of the bacterial suspension was added to 200 μL of broth medium, with uninoculated broth medium serving as the blank control. The culture was incubated at 37 °C for 48 hours. After incubation, the optical density (OD1) at 570 nm was measured using a microplate reader, with the blank control representing the OD10. The medium was gently aspirated, and the wells were washed three times with sterile physiological saline to remove extracellular planktonic bacteria. The plate was then dried in an oven at 60 °C for 20 minutes. Two hundred microliters of crystal violet solution was added to each well, and the samples were stained for 20 minutes. After staining, the crystal violet solution was aspirated, and the wells were washed four times with sterile physiological saline to remove the dye from the well walls, followed by drying the 96-well plate in a sterile hood for 30 minutes. To each well, 200 μL of 95% ethanol was added, the mixture was gently shaken for 30 minutes at 60 r/min, and the optical density (OD2) at 570 nm was measured, with the blank control as the OD20. The biofilm formation ability was calculated using the formula B = (OD2 - OD20) / (OD1 - OD10).Strains with B < 0.1 were

considered nonadherent; strains with $B \geq 0.1$ were considered adherent; strains with $0.1 < B \leq 1.0$ were considered moderately adherent; and strains with $B > 1.0$ were considered strongly adherent.

## Detection of Resistance and Virulence Genes

Bacterial genomic DNA was extracted via the boiling method.[12] Using this DNA as a template, PCR (Applied Biosystem America) was used to amplify the resistance and virulence genes of *CRKP*. The resistance genes included carbapenemase genes (*$bla_{KPC}$, $bla_{NDM}$, $bla_{OXA-48}$, $bla_{IMP}$*), ESBL enzyme-encoding genes *($bla_{CTX-M}$, $bla_{TEM}$, $bla_{SHV}$)*, quinolone resistance genes *(qnrB, oqxA)*, and aminoglycoside resistance genes (*aac*). The virulence genes included adhesion genes (*fimH, markD*), mucoid phenotype-related genes (*rmpA, rmpA2, magA, IroB, peg-344*), and iron carrier genes *(iucA)*. The PCR primer information for the resistance and virulence genes is shown in Table 1.

## Statistical Analysis

GraphPad Prism software version 9.0 was used to conduct all the statistical comparisons. A nonparametric *t*-test and one-way ANOVA were used to compare the different groups. A Tukey post-hoc test was used to determine pairwise differences where appropriate. p-value of 0.05 or less was considered to be indicative of statistical significance. Pearson correlation analysis was used to analyze the correlations between phenotypic and molecular characteristics.

**Table 1** PCR Primer Sequences for Resistance and Virulence Genes

| Gene Type | Gene Name | Primer Sequence | Product Size (bp) | Annealing Temperature (°C) |
|---|---|---|---|---|
| Carbapenemase | $bla_{KPC}$ | F: TGTCACTGTATCGCCGTC<br>R: TTACTGCCCGTTGACGCC | 882 | 57.0 |
| | $bla_{NDM}$ | F: CCAGCTCGCACCGAATG<br>R: AACGCCGCACCAAACG | 564 | 58.8 |
| | $bla_{OXA-48}$ | F: ACATAAATCACAGGGCGTAG<br>R: TATAGTCACCATTGGCTTCG | 500 | 51.4 |
| | $bla_{IMP}$ | F: CTTGATGAAGGCGTTTATGT<br>R: GCCAAGCTTCTATATTTGCGT | 496 | 51 |
| ESBLs Enzyme | $bla_{CTX-M}$ | F: TCGGGAGGCAGACTGGGTGT<br>R: CCTTAGGTTGAGGCTGGGTGA | 688 | 57.7 |
| | $bla_{TEM}$ | F: TCGCCGCATACACTATTCTCAG 14<br>R: ACGCTCACCGGCTCCAGATTTAT | 445 | 55.1 |
| | $bla_{SHV}$ | F: ATGCGTTATATTCGCCTGTG 14<br>R: TGCTTTGTTATTCGGGCCAA | 753 | 58.4 |
| Quinolone | *qnrB* | F: GCACTGAATTTATCGGCTGTC<br>R: CAACGATGCCTGGTAGTTGT | 500 | 53.4 |
| | *oqxA* | F: TCCATACCAACCTCGTCTCCC<br>R: AGCGTGGCTTTGAACTCTGC | 529 | 59.8 |
| Aminoglycoside | *aac* | F: AGTACAGCATCGTGACCAACA 15<br>R: CTCGAATGCCTGGCGTGTTT | 545 | 55.8 |

(*Continued*)

**Table 1** (Continued).

| Gene Type | Gene Name | Primer Sequence | Product Size (bp) | Annealing Temperature (°C) |
|---|---|---|---|---|
| Mucoid Phenotype | *magA* | F:GGTGCTCTTTACATCATTGC 16<br>R:GCAATGGCCATTTGCGTTAG | 1282 | 58 |
| | *rmpA* | F:ACTGGGCTACCTCTGCTTCA 18<br>R:CTTGCATGAGCCATCTTTCA | 516 | 58 |
| | *rmpA2* | F:CTTTATGTGCAATAAG-GATGTT<br>R:CCTCCTGGAGAGTAAGCATT | 451 | 57 |
| | *IroB* | F:AAGTCAAAGCAGGGGTTGCCCG<br>R:GACGCCGACATTAAGACGCAG | 655 | 51 |
| | *peg-344* | F:AGAAGGCGGCAATCCAAG<br>R:CGGTTCACTTCTTTCACTAGG | 694 | 52 |
| Adhesion | *fimH* | F:TGCTGCTGGGCTGGTCGATG 19<br>R:GGGAGGGTGACGGTGACATC | 688 | 49 |
| | *markD* | F:TTCTGCACAGCGGTCCC 20<br>R:GATACCCGGCGTTTTCGTTAC | 480 | 49 |
| Iron Carrier | *iucA* | F: CCCGCTTCCCTACTTT 21<br>R: ATTCGCTTCGCTGTCC | 575 | 54.8 |

# Results

## Clinical Distribution Characteristics of the 81 CRKP Isolates

The *CRKP* isolates collected in this study were distributed in 13 different departments, with 30 *CRKP* isolates from the ICU, accounting for 37.04%, followed by the respiratory department, with 15 *CRKP* isolates, accounting for 18.52% (15/81), and the neurosurgery and oncology departments, each with 7 *CRKP* isolates, accounting for 8.64%. The distribution of the 81 *CRKP* isolates in the different departments is shown in Table 2. These *CRKP* isolates were isolated mainly from sputum (67.9%, 55/81), blood (12.35%, 10/81), and urine (9.88%), with the specific distributions shown in Table 3.

**Table 2** Distribution of the 81 CRKP Strains Among Clinical Departments

| Department | Number | Proportion (%) |
|---|---|---|
| ICU | 30 | 37.04 |
| Respiratory | 15 | 18.52 |
| Neurosurgery | 7 | 8.64 |
| Oncology | 7 | 8.64 |
| Neurology | 5 | 6.17 |
| Cardiology | 4 | 4.94 |
| Urology | 3 | 3.70 |
| Infectious Diseases | 3 | 3.70 |
| Hematology | 2 | 2.47 |
| General Surgery | 2 | 2.47 |
| Nephrology | 1 | 1.23 |
| Orthopedic Surgery | 1 | 1.23 |
| Hepatobiliary Pancreatic Surgery | 1 | 1.23 |


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**Table 3** Distribution of the 81 CRKP Strains Among Different Sample Types

| Specimen Source | Number | Proportion (%) |
|---|---|---|
| Sputum | 55 | 67.90 |
| Blood | 10 | 12.35 |
| Urine | 8 | 9.88 |
| Ascites | 3 | 3.70 |
| Drainage Fluid | 1 | 1.23 |
| Intravenous Catheter | 1 | 1.23 |
| Secretion | 1 | 1.23 |

## Results of Antimicrobial Susceptibility Testing for the 81 CRKP Isolates

We selected 11 drugs from 4 categories of antibiotics for antimicrobial susceptibility testing. All the *CRKP* isolates were resistant to imipenem, meropenem, ceftazidime, and ceftriaxone. The resistance rate for cefoperazone-sulbactam was 90.12%. The resistance rates for ciprofloxacin and levofloxacin were 97.53% and 96.30%, respectively. The resistance rates for amikacin and gentamicin were 77.78% and 82.72%, respectively. The resistance rate for sulfonamide drugs was relatively low, at 62.96%. The multidrug resistance rate of the 81 *CRKP* isolates was 83.95%. The specific results are shown in Table 4.

## Characteristics of the Resistance Genes Carried by the 81 CRKP Isolates

All 81 *CRKP* isolates prevalent in this region carried the $bla_{SHV}$ and $bla_{TEM}$ genes, and the carriage rate of the $bla_{CTX\text{-}M}$ gene was 55.56%. The number of *CRKP* isolates carrying all three resistance genes was 45, accounting for 55.56% (Table 4). The only gene detected for carbapenem resistance was $bla_{KPC}$. Genes for quinolones resistance were also widely distributed in this sample, with detection rates of 53.09% for *qnrB* and 28.40% and 30.86% for *oqxA* and *oqxB*, respectively. The number of *CRKP* isolates carrying *qnrB* + *oqxA* was 13, the number of isolates carrying *qnrB* + *oqxB* was 15, and the isolates carrying all three resistance genes accounted for 16.05%. The number of *CRKP* isolates carrying the aminoglycoside resistance gene *aac* was 31, accounting for 38.27%. The frequency of antimicrobial resistance encoding genes carried by the 81 CRKP strains is shown in Table 5.

## Distribution of Virulence Phenotypes and Genotypes in the 81 CRKP Isolates

In terms of biofilm formation ability, 86.42% (70/81) of the *CRKP* isolates presented strong biofilm formation ability, whereas 71.60% (58/81) of the *CRKP* isolates presented a highly mucoid phenotype. The amplification of virulence

**Table 4** Resistance of the 81 K. Pneumoniae Strains to 11 Commonly Used Antibiotics

| Antibiotic | Number of Resistant Strains | Resistance Rate (%) |
|---|---|---|
| Amikacin | 63 | 77.78 |
| Ampicillin | 81 | 100.00 |
| Ceftazidime | 81 | 100.00 |
| Ceftriaxone | 81 | 100.00 |
| Cefoperazone | 73 | 90.12 |
| Ciprofloxacin | 79 | 97.53 |
| Gentamicin | 67 | 82.72 |
| Levofloxacin | 78 | 96.30 |
| Meropenem | 81 | 100.00 |
| MDR | 68 | 83.95 |
| Imipenem | 81 | 100.00 |
| Sulfamethoxazole | 51 | 62.96 |

**Table 5** The Frequency of Antimicrobial Resistance Encoding Genes Carried by the 81 CRKP Strains

| Resistance Gene | Number of Positive Strains (Strains) | Detection Rate(%) |
|---|---|---|
| **ESBLs** | | |
| $bla_{SHV}$ | 81 | 100 |
| $bla_{TEM}$ | 81 | 100 |
| $bla_{CTX\text{-}M}$ | 45 | 55.56 |
| $bla_{SHV}$ +$bla_{TEM}$ | 81 | 100 |
| $bla_{SHV}$ +$bla_{CTX}$ | 45 | 55.56 |
| $bla_{TEM}$ +$bla_{CTX}$ | 45 | 55.56 |
| $bla_{SHV}$ +$bla_{TEM}$ +$bla_{CTX}$ | 45 | 55.56 |
| **Carbapenem** | | |
| $bla_{NDM}$ | 0 | 0 |
| $bla_{IMP}$ | 0 | 0 |
| $bla_{OXA\text{-}48}$ | 0 | 0 |
| $bla_{KPC}$ | 81 | 100 |
| **Quinolone** | | |
| *qnrB* | 43 | 53.09 |
| *oqxA* | 23 | 28.40 |
| *oqxB* | 25 | 30.86 |
| *qnrB*+ *oqxA* | 13 | 16.05 |
| *qnrB*+ *oqxB* | 15 | 18.52 |
| *oqxA*+ *oqxB* | 23 | 28.40 |
| *qnrB*+ *oqxA*+ *oqxB* | 13 | 16.05 |
| **Aminoglycoside** | | |
| *aac* | 31 | 38.27 |

genes revealed that the detection rates of the mucoid phenotype genes *rmpA2* and *peg-344* were the highest, accounting for 92.59% and 91.36%, respectively, followed by *magA* and *rmpA*, accounting for 82.72% and 83.95%, respectively. The carriage rate of *IroB* was low, with this gene detected in only 17 of the *CRKP* isolates detected. The genes encoding type I fimbriae (*fimH*) and type III fimbriae (*markD*) had high carriage rates of 88.89% and 65.43%, respectively; the gene encoding the iron siderophore aerobactin (*iucA*) had a detection rate of 83.95%. Table 6 shows the distribution of virulence phenotypes and genotypes among the 81 *CRKP* isolates.

**Table 6** Distribution of Virulence Phenotypes and Genotypes in the 81 CRKP Strains

| Virulence Phenotype and Gene | Positive Strains (Strains) | Positive Rate (%) |
|---|---|---|
| Virulence Phenotype | | |
| *BF* | 70 | 86.42 |
| *HMV* | 58 | 71.60 |
| Virulence Gene | | |
| *magA* | 67 | 82.72 |
| *rmpA* | 68 | 83.95 |
| *rmpA2* | 75 | 92.59 |
| *IroB* | 17 | 20.99 |
| *peg-344* | 74 | 91.36 |

(*Continued*)


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**Table 6** (Continued).

| Virulence Phenotype and Gene | Positive Strains (Strains) | Positive Rate (%) |
|---|---|---|
| *fimH* | 72 | 88.89 |
| *markD* | 53 | 65.43 |
| *iucA* | 68 | 83.95 |

**Abbreviations**: BF, biofilm; HMV, hypermucoviscous phenotype.

## Correlation Analysis

On the basis of the Pearson correlation coefficient, we analyzed the correlations among the resistance phenotype, virulence phenotype, resistance genes, and virulence genes of the 81 *CRKP* isolates to determine whether there were potential connections among these factors. The correlation analysis results are shown. The formation of *CRKP* biofilms was significantly positively correlated with the levels of *fimH* (r = 0.689), *rmpA2* (r = 0.490), *iucA* (r = 0.453), and HMV (r = 0.643). The highly mucoid phenotype of *CRKP* was significantly positively correlated with the levels of markD (r = 0.643), BF (r = 0.643), and fimH (r = 0.414). In the resistance analysis, the distribution of multidrug-resistant bacteria was strongly positively correlated with the resistance to cefoperazone/sulbactam (r = 0.757) and sulfamethoxazole (r = 0.570) and weakly negatively correlated with the resistance to ciprofloxacin and levofloxacin. Moreover, we found that multidrug resistance was correlated with the levels of resistance genes carried by bacteria, but the correlation coefficient R value was small. Multidrug resistance was negatively correlated with the formation of bacterial biofilms (r = −0.182). The *CRKP* phenotypes and genotypes were correlated to some extent, as shown in Figure 2.

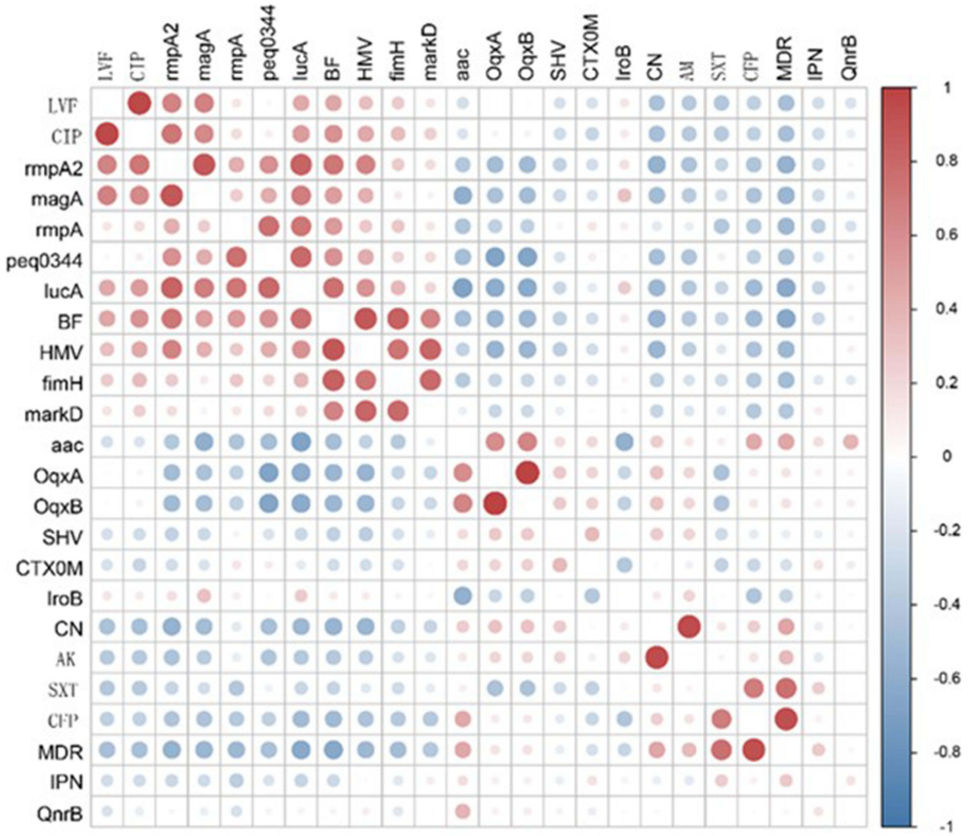


**Figure 2** The correlation analysis among the resistance phenotype, virulence phenotype, resistance genes, and virulence genes of the 81 carbapenem-resistant *K. pneumoniae* isolates based on the Pearson correlation coefficient.

## Discussion

Clinical *CRKP* isolates have spread rapidly worldwide in recent years, posing a severe public health threat. However, there are few reports on the prevalence characteristics, resistance status, and virulence-related characteristics of *CRKP* in the Lüliang region of Shanxi Province. This is the first study providing effective theoretical and data support for the prevention and treatment of *CRKP* infections in this region. The number of clinically isolated *K. pneumoniae* strains has been increasing annually, but reports on the correlation between antimicrobial resistance phenotypes and molecular characteristics are relatively rare. In this study, 81 *CRKP* isolates were isolated from hospitals in Lüliang, Shanxi Province, from January 2021 to December 2023, and their resistance and virulence characteristics were studied. The susceptibility results revealed that the resistance rate of the 81 *CRKP* isolates was very high, with the resistance rate for sulfamethoxazole being 62.96% and the resistance rate for the other 10 antibiotics being greater than 80%. In addition, the MDR rate was 83.95%, indicating that the resistance rate of *K. pneumoniae* isolates prevalent in this region was high and significantly higher than that reported in the literature. Hu and colleagues recently reported that the prevalence of hypervirulent *CRKP* increased in prevalence from 28.2% in 2016 to 45.7% in 2020.[13] We determined that the difference might be related to the molecular characteristics of *K. pneumoniae* prevalent in this region and the antibiotic use practices of the hospital, but further investigations of patient antibiotic use are still needed to determine the reasons for this difference.

Carbapenem antibiotics constitute the last line of defense in the treatment of infections caused by gram-negative bacteria that produce extended-spectrum β-lactamases, and the main mechanism of resistance is the production of carbapenemase, a type of β-lactamase that hydrolyzes various antibiotics, such as penicillins, cephalosporins, and monobactams.[14] The 81 *CRKP* isolates collected in this study were mainly isolated from sputum (67.9%, 55/81), and the samples were obtained from 13 departments, indicating that *CRKP* is widely prevalent in hospitals in this region.A study of the resistance genotypes of the 81 *CRKP* isolates revealed that the main carbapenemase gene prevalent in this region was $bla_{KPC}$, with no detection of $bla_{NDM}$, $bla_{IMP}$, or $bla_{OXA-48}$, which is consistent with relevant reports in the literature.[15] According to the data from the China CRE Network, KPC-type enzymes are the most common carbapenemase carried by *K. pneumoniae*.[16]

The emergence of multidrug-resistant bacteria poses a significant challenge in the clinical treatment of *K. pneumoniae* infections. Studying resistance mechanisms is highly important for reducing the mortality rate of multidrug-resistant bacterial infections, improving the spread of resistant bacteria, and delaying the occurrence of pandrug-resistant bacteria. This study revealed that the proportion of multidrug-resistant bacteria was as high as 83.95%, and the distribution of the resistance genes $bla_{CTX-M}$, $bla_{TEM}$, and *aac* were closely related to those of multidrug-resistant bacteria. These three types of genes are plasmid-mediated resistance genes,[17] and 55.56% of the resistant bacteria carried all three genes, which may be crucial for the prevalence and transmission of multidrug-resistant bacteria in this region. Plasmid-mediated quinolone resistance (PMQR) genes are usually carried on plasmids with other antibiotic resistance genes, especially ESBL-encoding genes,[18] and we speculate that the above mentioned prevalent resistance genes in this region are located on and transmitted via the same plasmid, but the specific mechanism needs further in-depth study.

The main virulence factors of *K. pneumoniae* include capsular polysaccharide (CPS), lipopolysaccharide (LPS), adhesins, and siderophores, and these four major virulence factors are also the main factors underlying the characteristic features of hypervirulent *K. pneumoniae* (hvKP).[19] Many types of virulence factors associated with the virulence characteristics have been reported. In this study, 8 virulence genes, associated with mucoid phenotypes, adhesins, and siderophores were selected for amplification. The results revealed that the high mucosity of *CRKP* was strongly correlated with the levels of mainly *rmpA2* (R = 0.474), *markD* (R = 0.643), and *fimH* (R = 0.414), which is consistent with previous research. Among these genes, *rmpA* regulates the expression of capsular polysaccharides and is closely related to the highly mucoid phenotype of *K. pneumoniae*,[20,21] The gene *iucA* encodes the iron siderophore aerobactin, which is closely related to high virulence.[22] This study revealed that the level of *iucA* was closely related to the expression of *peq0344* (r = 0.505), *rmpA2* (r = 0.557), *rmpA* (r = 0.482), *magA* (r = 0.286), and other virulence genes and was strongly correlated with the formation of biofilms (r = 0.453). In this study, the detection rates of the genes encoding type I and type III fimbriae, *fimH* and *mrkD*, were 88.89% and 65.43%, respectively. These genes are related to the formation of bacterial biofilms and have a high detection rate in Enterobacteriaceae, which is consistent with previous reports.[23,24] A total of 86.42% of the *CRKP* isolates in this study were able to form biofilms, and type I and type III fimbriae are important factors for the colonization of *K. pneumoniae*. *fimH*

encodes the FimH adhesin at the tip of type I fimbriae,[25] which is closely related to urinary tract infections,[26] and this study revealed that the biofilm formation ability was strongly correlated with the *fimH* level (r = 0.689); *mrkD* encodes the adhesive protein at the tip of type III fimbriae,[27] which mediates the binding of *K. pneumoniae* with organ cells and lung tissue[27] and is closely related to lung infections. Studies have shown that type III fimbriae play a major role in the formation of biofilms in vitro,[28] and the biofilm formation ability in this study was also strongly positively correlated with the *mrkD* level (r = 0.413). Correlation analysis revealed that the *rmpA2* level was strongly correlated with the *magA* and *iutA* levels (r values of 0.645 and 0.557, respectively), which may be related to these genes being located on the same pLVPK plasmid.[29] Correlation analysis of the resistance and virulence characteristics of *K. pneumoniae* revealed that the resistance phenotype (including multidrug resistance) of the 81 *CRKP* isolates was negatively correlated with the distribution of virulence phenotypes and virulence genes (r < 0), which is consistent with previous reports.[5]

In summary, this study investigated the resistance and virulence characteristics of 81 *CRKP* strains isolated in Lüliang, Shanxi Province. Our data revealed that the resistance rates of *CRKP* in this region are and that multidrug-resistant highly mucoid *CRKP* strains are present in this region. Therefore, in clinical practice, the management of antibiotics should be strengthened to control the prevalence and transmission of *CRKP* in this region.

## Data Sharing Statement

The datasets supporting the conclusions of this article are included within the article.

## Ethics Approval and Consent to Participate

This research was carried out in accordance with the Declaration of Helsinki. The participants were informed of the study, including any benefits or risks involved, and voluntarily provided informed consent. The research was approved by the Ethics Committee of Fenyang College of Shanxi Medical University.

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## Disclosure

The authors declare that they have no competing interests in this work.

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