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ORIGINAL RESEARCH

# Clinical and Microbiological Characteristics of Carbapenem-Resistant *Klebsiella pneumoniae* Associated Recurrent Urinary Tract Infections

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**Background:** Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a major pathogen responsible for urinary tract infections (UTIs). However, its role and characteristics in recurrent urinary tract infections (rUTIs) remain poorly understood. Investigating its features in rUTIs may provide insights into effective prevention strategies.

**Methods:** We analyzed a cohort of patients with rUTIs caused by *Klebsiella pneumoniae* from April 2020 to April 2024. Antibiotic susceptibility of the isolates was evaluated. Biofilm Formation Assay and *Galleria mellonella* infection models were employed to assess the virulence of the strains. Polymerase Chain Reaction (PCR) and whole-genome sequencing (WGS) were utilized to determine multilocus sequence typing (MLST) and capsular serotyping, as well as to identify resistance genes, virulence genes, and plasmid replicons. Phylogenetic relationships among the isolates were also established.

**Results:** A total of 41 patients with rUTIs were included, with 56.1% caused by CRKP. 97.01% of CRKP carry the $bla_{KPC-2}$ gene. Compared to patients infected with carbapenem-susceptible *Klebsiella pneumoniae* (CSKP), those infected with CRKP had a higher prevalence of underlying diseases and complications. Both groups of strains exhibited a high degree of antibiotic resistance. CRKP strains demonstrated enhanced biofilm formation capacity and greater lethality in *Galleria mellonella* infection models. The predominant phenotype of the CRKP strain was ST11 KL64, whereas the CSKP strain showed multiple phenotypes in different patients. Sequencing analyses revealed that both groups of strains carried a wide range of virulence genes, resistance genes, and plasmid replicons. Among the cases of rUTIs, 31 were identified as relapses caused by the same strain, with no significant differences between the initial and final infection strains.

**Conclusion:** This study demonstrates that patients with rUTIs caused by CRKP present significant complexity in terms of clinical features, strain resistance and virulence properties. When managing UTIs caused by CRKP, special care needs to be taken to manage recurrent infections.

**Keywords:** recurrent urinary tract infections, carbapenem-resistant *Klebsiella pneumoniae*, antibiotic resistance, virulence, whole genome sequencing

## Introduction

UTIs are among the most common bacterial infections, affecting approximately 150 million people globally each year.[1] UTIs can occur across all age groups, from newborns to the elderly, with clinical manifestations ranging from localized infections to complicated conditions such as pyelonephritis and cystitis.[2] Severe cases may lead to renal pelvis and tubule damage, potentially resulting in renal failure, bacteremia, sepsis, or even life-threatening complications.[3]

RUTIs are defined as at least three UTIs in a 12-month period or at least two UTIs in a 6-month period with at least 14 days between infections. Approximately 60% of women will experience at least one UTI during their lifetime, and

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30% to 40% may develop rUTIs, with some experiencing six or more infections annually. Among elderly males, rUTIs are also prevalent due to age-related pathological changes.[4] *Klebsiella pneumoniae* is a significant pathogen responsible for UTIs, second only to Escherichia coli in some regions.[5]

*Klebsiella pneumoniae* is classified as one of the ESKAPE pathogens, which also includes *Enterococcus faecium, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter species*. As a common uropathogen, *Klebsiella pneumoniae* poses a considerable global health threat.[6] Its virulence factors, such as capsules and fimbriae, enhance its ability to colonize and persist in the urogenital tract. Additionally, *Klebsiella pneumoniae* can form biofilms in the urinary tract, shielding itself from the bladder environment and strengthening its colonization ability. These virulence factors and biofilm formation contribute to rUTIs development.[7–9] CRKP further strengthens its survival capacity, increasing its transmissibility.[10] During the acquisition of drug resistance, *Klebsiella pneumoniae* may incorporate virulence-related mobile genetic elements, significantly expanding its adaptability and geographical spread. The resistance mechanisms include the production of carbapenemases, porin gene deletions or mutations, and upregulation of efflux pumps.[11,12] In Asian countries like Vietnam and Laos, CRKP transmission has intensified, with infections being prolonged and difficult to treat.[13] The increasing resistance rate of *Klebsiella pneumoniae* undermines the effectiveness of empirical treatment strategies.

There are many studies on *Escherichia coli* causing rUTIs. For instance, a study in the Netherlands found that *Escherichia coli* could persist in the bladder for extended periods or recolonize the bladder from the intestines, causing rUTIs.[14] Similarly, an Iranian hospital study revealed that nearly all isolated *Escherichia coli* strains exhibited biofilm formation in vitro, which extended their urinary tract presence, thereby exacerbating recurrence rates and treatment challenges.[15] However, research on rUTIs caused by *Klebsiella pneumoniae* remains limited. Additionally, the predominant strains and resistance mechanisms of *Klebsiella pneumoniae* vary regionally.[16] For example, community-acquired rUTIs in Taiwan demonstrated stronger adhesion and invasion abilities in pathogenic *Klebsiella pneumoniae* compared to urinary colonizers.[17] A case study by Michelle Kalu described a rUTI caused by CRKP, in which CRKP adapted to repeated antibiotic exposure through changes in carbapenem resistance and biofilm formation, highlighting its versatility.[18]

Due to the limited reports on rUTIs caused by CRKP and the variations in patient characteristics and CRKP strains across regions, this study statistically analyzed cases of rUTIs in a tertiary hospital in Guizhou. The analysis examined drug resistance, biofilm formation, virulence levels, and gene profiles of CRKP strains responsible for rUTIs. Furthermore, it aimed to determine whether rUTIs in hospitalized patients were caused by the recurrence of the same strain or reinfection by different strains. Ultimately, this research seeks to inform strategies for the prevention and treatment of rUTIs and the optimal use of antibiotics.

# Materials and Methods

## Patient Information and Strain Collection for rUTIs

From April 2020 to April 2024, we conducted a study at a tertiary hospital in Guizhou, China, collecting samples from patients with rUTIs caused by *Klebsiella pneumoniae*. According to the diagnostic criteria for rUTIs, patients must have had at least three UTIs within a 12-month period or at least two UTIs within a 6-month period with at least 14 days between infections.[4] Samples with incomplete patient information or those containing two or more bacterial or fungal species in the same urine specimen were excluded.

The isolates were identified using a Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (BioMérieux, France). *Klebsiella pneumoniae* ATCC 700603 (Microbiologics, USA) was used as the quality control strain.[19]

## Antibiotic Susceptibility Testing and Carbapenemase Screening

Antibiotic susceptibility of *Klebsiella pneumoniae* was assessed using the minimum inhibitory concentration (MIC) method on the VITEK 2 automated microbiology system (BioMérieux, France). Tested antibiotics included cefoxitin, cefuroxime, ceftriaxone, ceftazidime, cefepime, aztreonam, amikacin, levofloxacin, ertapenem, meropenem, and imipenem. *Klebsiella pneumoniae* ATCC 700603 served as the quality control strain. Results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[20]

## Biofilm Formation Assay

The biofilm formation assay was performed using 1% crystal violet staining, with absorbance measured at 570 nm. Biofilm generation capacity was calculated using the formula: ODc = average OD of the negative control + (3 × SD of the negative control). Based on this, strains were classified as follows: Strong biofilm producer (OD > 4 × ODc), Moderate biofilm producer (4 × ODc ≥ OD > 2 × ODc), Weak biofilm producer (2 × ODc ≥ OD > ODc), non-biofilm producer (OD ≤ ODc). *Klebsiella pneumoniae* NTUH-K2044 (Microbiologics, USA) and LB broth were used as positive and negative controls, respectively. Each experiment was performed in triplicate.[21]

## Galleria Mellonella Assay

The virulence of the collected *Klebsiella pneumoniae* strains was evaluated using the *Galleria mellonella* infection model. Overnight bacterial cultures were adjusted to a 0.5 McFarland concentration (approximately $1 \times 10^8$ CFU/mL) in saline. Ten microliters of the bacterial suspension were injected into *Galleria mellonella* larvae (250–350 mg; Guilin Jiacheng Co., Ltd)., which were incubated at 37°C in darkness for 72 hours. Larval survival was monitored at 12-hour intervals. Saline was used as the negative control, and *Klebsiella pneumoniae* NTUH-K2044 served as the positive control. Each experiment was repeated three times.[22]

## Virulence Gene Identification

PCR was used to detect nine virulence factors associated with *Klebsiella pneumoniae*, including *iroB* (siderophore synthesis), *peg344, rmpA, rmpA2* (capsule overexpression), *mrkD* (type 3 fimbriae), *entB, ybtS* (iron uptake), *fimH* (type 1 fimbriae), and *wcaG* (capsule fucose and endotoxin synthesis). PCR products were analyzed by electrophoresis to identify target bands.[23]

## String Test

A single bacterial colony was picked from an agar plate using an inoculation loop and lifted vertically. A positive string test was defined as a string >5 mm in length, indicating a hypermucoviscous phenotype of Klebsiella pneumoniae.[24]

## Capsular Serotyping

PCR was conducted to determine the capsular serotypes of *Klebsiella pneumoniae* strains, focus-ing on the following serotypes: K1, K2, K5, K20, K54, K57, and K64.[25] Strains outside these sero-types were further characterized using WGS.

## Genome Sequencing

Genomic DNA was extracted using a bacterial DNA extraction kit (Beijing Solarbio Science & Technology Co., Ltd). Whole-genome sequencing was performed on the Illumina platform PE150 (Beijing Novogene). Assembly was achieved using SOAPdenovo (version 2.04) and SPAdes, with fragments below 500 bp filtered out.[26] Resistance and virulence genes were identified using the ResFinder and VFDB databases, respectively. Plasmid replicon types were classified using the PlasmidFinder v2.1 database.[27,28] Capsular typing was determined via Kaptive, while MLST was performed using MLST software based on seven housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB*).[29] SNP analysis was conducted with Snippy v4.6.0, using the initial isolate from each patient as a reference.[30] Phylogenetic relationships were analyzed using RAxML v8.2.4, and trees were visualized with the Interactive Tree of Life (iTOL) tool.[31]

## Statistical Analysis

Clinical data were analyzed using GraphPad Prism 9.0. Normally distributed data were expressed as mean ± standard deviation, while non-normally distributed data were represented as median (interquartile range). Differences between two independent samples were analyzed using the Wilcoxon signed-rank test. Categorical data were presented as frequency (percentage) and compared using the Chi-square test or Fisher's exact test. A p-value < 0.05 was considered statistically significant.[32]

# Results

## Clinical Characteristics of Patients Statistical Analysis

Between April 2020 and April 2024, a total of 589 *Klebsiella pneumoniae* isolates were obtained from urine samples. After reviewing medical records and excluding cases of UTIs involving multi-ple bacterial species, 41 patients were identified with rUTIs caused by *Klebsiella pneumoniae*, resulting in a total of 114 isolates.

Carbapenem susceptibility screening showed that, among the 41 patients with rUTIs, 23 (56.1%) were infected with CRKP strains, and 18 (43.9%) were infected with CSKP strains. There were 67 CRKP isolates from patients with CRKP-associated rUTIs and 47 CSKP isolates from patients with CSKP-associated rUTIs.

A comparison of clinical characteristics between the two patient groups revealed that those with rUTIs caused by CRKP were older and had significantly more underlying conditions, including cardiac insufficiency, neurodegenerative diseases, severe pneumonia, and COPD. Regarding complications, patients with CSKP-associated rUTIs exhibited higher rates of hydronephrosis and neurogenic bladder. Additionally, CRKP-associated rUTIs were linked to complications such as respiratory failure, electrolyte imbalance, and hypoalbuminemia. Hypertension was prevalent in both groups, and urinary catheter rates were similarly high (Table 1).

## Antibiotic Susceptibility Testing

CRKP strains causing rUTIs demonstrated high resistance rates to all 11 antibiotics tested. Re-sistance rates for FOX, CXM, CRO, FEP, ATM, ETP, IPM, and MEM were 100%. Sensitivity or intermediate responses were observed only for

**Table 1** Comparison of Clinical Characteristics Between Patients with rUTIs Caused by CRKP and CSKP

| Characteristics | CRKP (n=23) | | CSKP (n=18) | | *p*-value |
|---|---|---|---|---|---|
| | Frequency | Rate | Frequency | Rate | |
| Age (years) | 81 (65.5–88.5) | | 53 (45.5–69.5) | | 0.0026 |
| Sex Male | 17 | 73.91% | 13 | 72.22% | 0.9035 |
| Female | 6 | 26.09% | 5 | 27.78% | |
| Underlying medical conditions | | | | | |
| DM | 9 | 39.13% | 4 | 22.22% | 0.2482 |
| HTN | 14 | 60.87% | 7 | 38.89% | 0.1623 |
| Cardiac insufficiency | 11 | 47.83% | 2 | 11.11% | 0.0122 |
| Neurodegenerative disease | 9 | 39.13% | 1 | 5.56% | 0.0130 |
| Severe pneumonia | 16 | 69.57% | 1 | 5.56% | <0.0001 |
| COPD | 9 | 39.13% | 1 | 5.56% | 0.0130 |
| Comorbidities | | | | | |
| History of urinary incontinence | 7 | 30.43% | 6 | 33.33% | 0.8431 |
| Urethral catheter | 20 | 86.96% | 14 | 77.78% | 0.4382 |
| Ureteral abnormalities | 2 | 8.70% | 5 | 27.78% | 0.1071 |
| Kidney failure | 3 | 13.04% | 3 | 16.67% | 0.7446 |
| Kidney stone | 3 | 13.04% | 4 | 22.22% | 0.4382 |
| Renal insufficiency | 3 | 13.04% | 4 | 22.22% | 0.4382 |
| Kidney cysts | 7 | 30.43% | 4 | 22.22% | 0.5559 |
| Hydronephrosis | 1 | 4.35% | 5 | 27.78% | 0.0352 |
| Neurogenic bladder | 1 | 4.35% | 8 | 44.44% | 0.0021 |
| Respiratory failure | 16 | 69.57% | 2 | 11.11% | 0.0002 |
| Anaemia | 6 | 26.09% | 7 | 38.89% | 0.3820 |
| Electrolyte imbalances | 11 | 47.83% | 3 | 16.67% | 0.0368 |
| Hypoalbuminemia | 13 | 56.52% | 2 | 11.11% | 0.0027 |
| Dyslipidemia | 5 | 21.74% | 2 | 11.11% | 0.3694 |
| Deficiencies in action | 11 | 47.83% | 9 | 50.00% | 0.8901 |

**Abbreviations**: DM, Diabetes mellitus; HTN, Hypertension; COPD, Chronic obstructive pulmonary disease.

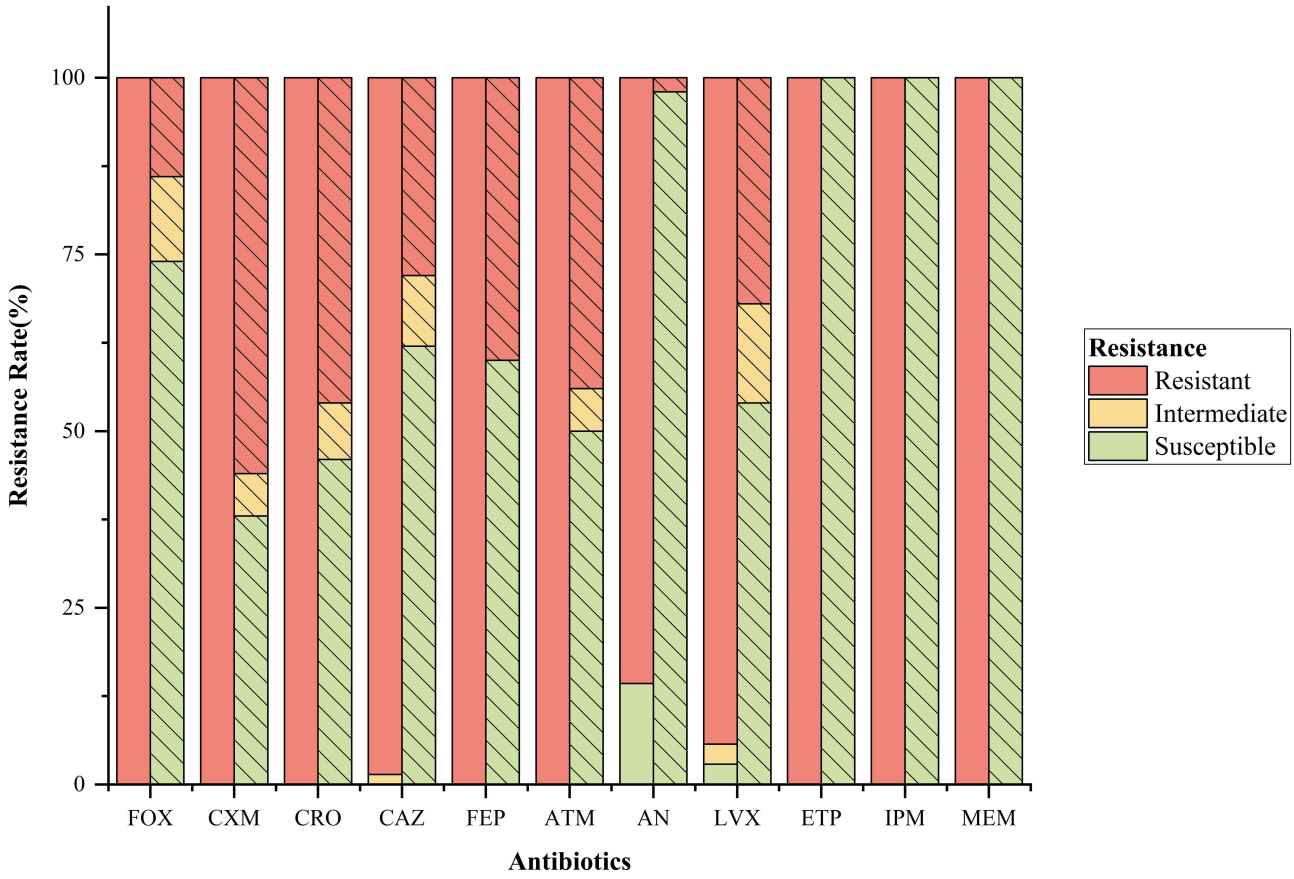


**Figure 1** Antibiotic susceptibility and resistance statistics for CRKP and CSKP strains in rUTIs.
**Notes**: The solid box represents the CRKP; The striped box represents the CSKP. FOX, Cefoxitin; CXM, Cefuroxime; CRO, Ceftriaxone; CAZ, Ceftazidime; FEP, Cefepime; ATM, Aztreonam; AN, Amikacin; LVX, Levofloxacin; ETP, Ertapenem; IPM, Imipenem; MEM, Meropenem.

CAZ, AN, and LVX. In contrast, CSKP strains showed varying resistance levels to eight antibiotics: FOX, CXM, CRO, CAZ, FEP, ATM, AN, and LVX (Figure 1).

## Virulence Comparison

All 114 *Klebsiella pneumoniae* strains isolated from rUTIs exhibited biofilm formation capability. A higher proportion of CRKP strains displayed moderate biofilm formation compared to CSKP strains (p = 0.0336, Figure 2).

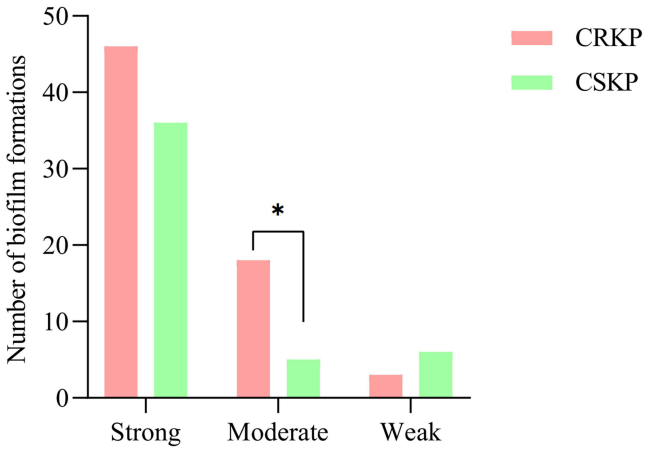


**Figure 2** Biofilm formation ability of CRKP and CSKP in rUTIs.
**Notes**: The statistical method is Chi-square test; The asterisk (*) indicates $p < 0.05$.


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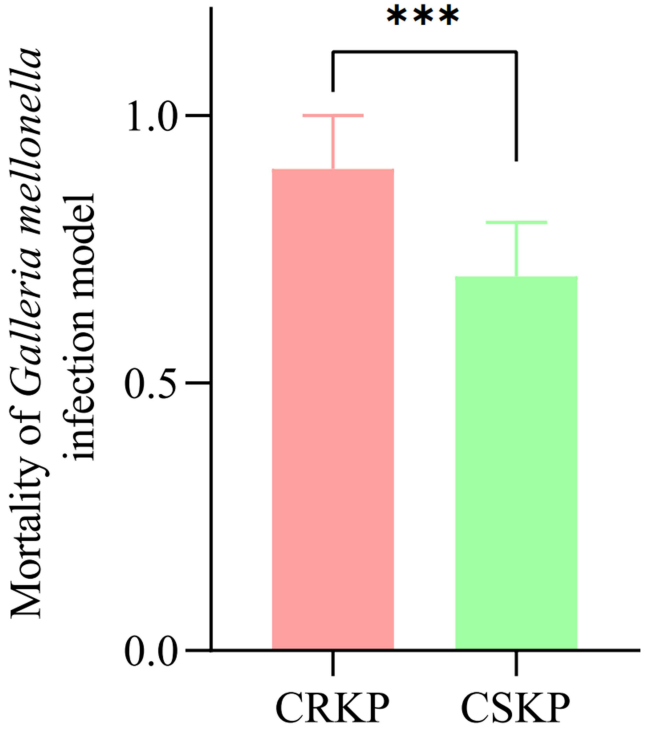


**Figure 3** Mortality comparison in *Galleria mellonella* virulence assay between CRKP and CSKP strains causing rUTIs.
**Notes**: The statistical method is Wilcoxon signed-rank test; The asterisk (***) indicates $p < 0.0005$.

The *Galleria mellonella* infection model was used to evaluate virulence levels of 67 CRKP strains and 47 CSKP strains from rUTIs. Over a 72-hour observation period, CRKP strains caused significantly higher mortality rates in *Galleria mellonella* larvae compared to CSKP strains (p = 0.0003, Figure 3).

## PCR Screening for Virulence Gene Carriage

PCR analysis was conducted to compare the virulence genes of *Klebsiella pneumoniae* strains isolated from rUTIs. Both groups exhibited 100% carriage rates for *entB* and *mrkD*. However, CRKP strains showed higher carriage rates for virulence genes *peg344, rmpA, rmpA2*, and *ybtS*. The *fimH* virulence gene was prevalent in both groups, while the carriage rate of *wcaG* remained low (Table 2).

**Table 2** Comparison of Virulence Gene Carriage Between CRKP and CSKP Strains in rUTIs

| Virulence gene | CRKP (n=67) | | CSKP (n=47) | | *p*-value |
|---|---|---|---|---|---|
| | Frequency | Rate | Frequency | Rate | |
| *iroB* | 59 | 88.06% | 35 | 74.47% | 0.0604 |
| *peg344* | 63 | 94.03% | 7 | 14.89% | <0.0001 |
| *rmpA* | 64 | 95.52% | 10 | 21.28% | <0.0001 |
| *rmpA2* | 63 | 94.03% | 36 | 76.60% | 0.0067 |
| *entB* | 67 | 100% | 47 | 100% | ns |
| *ybtS* | 67 | 100% | 37 | 78.72% | <0.0001 |
| *mrkD* | 67 | 100% | 47 | 100% | ns |
| *fimH* | 64 | 95.52% | 43 | 91.49% | 0.3773 |
| *wcaG* | 1 | 1.49% | 2 | 4.26% | 0.3643 |

**Abbreviation**: ns, No significance.

## Molecular Characteristics

A total of 114 *Klebsiella pneumoniae* strains were isolated from 41 patients with rUTIs. Among these, 45 strains tested positive in the string test. Specifically, 33 of 67 CRKP strains (49.25%) and 12 of 47 CSKP strains (25.53%) were positive, indicating a significantly higher string test positivity rate in CRKP strains compared to CSKP strains ($p = 0.0108$, Figure 4).

The 114 isolates were classified into 20 sequence types (ST) and 19 capsular types. Five strains were untyped for ST, and six were untyped for capsular type. The most common ST type among CRKP strains was ST11 (60/67, 89.55%). However, some patients exhibited different ST types before and after infection, suggesting recurrent infections were caused by distinct strains. The predominant capsular serotype among CRKP strains was KL64 (59/67, 88.06%), all of which were ST11. The second most common was KL2 (5/67, 7.46%), though these strains were not typed for ST. In contrast, CSKP strains displayed diverse ST and KL types across different patients, suggesting a variety of infection sources (Figure 4).

The phylogenetic tree demonstrated that strains isolated from different infection periods in the same patient generally clustered together. SNP analysis of strains from the same patient, using the initial strain as a reference, revealed an average of fewer than 10 SNPs. This indicates that most recurrent infections were caused by the same strain. However,

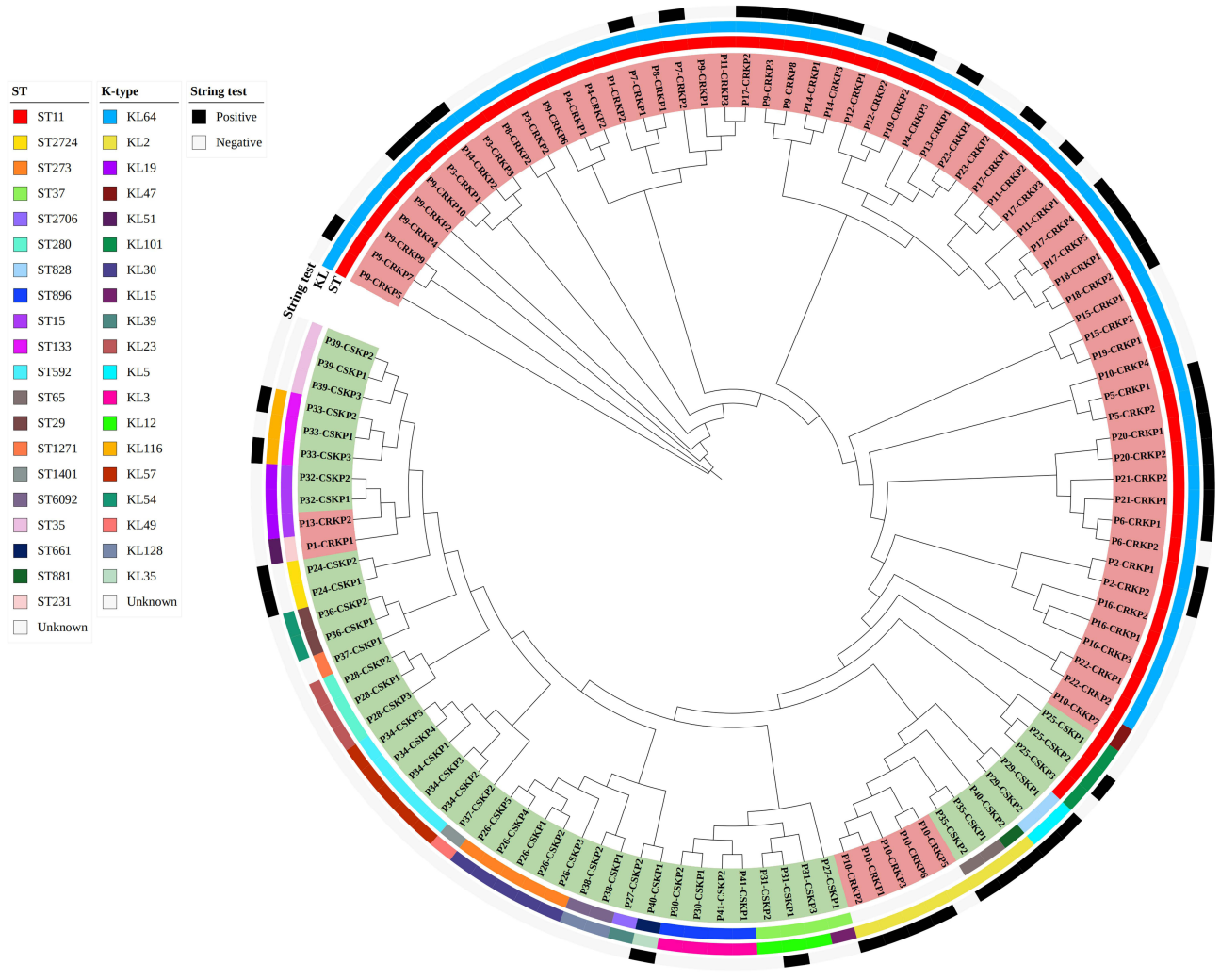


**Figure 4** Phylogenetic tree of 114 *Klebsiella pneumoniae* strains causing rUTIs.
**Notes**: CRKP is indicated by shades of red, CSKP is indicated by shades of green; From the inner to the outer circle, the rings represent MLST typing, capsular serotyping, and string test results.


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exceptions were observed, such as in patients P1 and P13, whose recurrent infections involved different strains (Figure 4).

Sequencing results showed that resistance mechanisms in most patients remained unchanged during recurrent infections. The majority of CRKP strains carried the $bla_{KPC-2}$ gene (65/67, 97.01%). However, some patients harbored strains with different resistance genes, such as P10-CRKP4 carrying the $bla_{KPC-33}$ gene and P1-CRKP1 carrying the $bla_{OXA-232}$ gene (Figure 5).

A total of 114 *Klebsiella pneumoniae* strains were screened for resistance genes related to more than 10 classes of antibiotics, including carbapenems, quinolones, β-lactams, sulfonamides, and aminoglycosides. Among the 67 CRKP strains, the most frequently detected resistance genes were the β-lactamase-encoding gene $bla_{LAP-2}$ (n = 61), the broad-spectrum β-lactamase-encoding gene $bla_{TEM-1}$ (n = 57), and the quinolone resistance gene *qnrS1* (n = 64). Conversely, the 47 CSKP strains harbored a wider variety of resistance genes, such as the


**Figure 5** Distribution of resistance genes, virulence genes, and plasmid replicons in *Klebsiella pneumoniae* strains causing rUTIs.
**Notes**: CRKP is indicated by shades of red, CSKP is indicated by shades of green; Red boxes indicate resistance genes, blue boxes indicate virulence genes, and yellow boxes indicate plasmid replicon types.

rifamycin resistance gene *arr-3* (n = 6), the gentamicin resistance gene *aac (3)-IId* (n = 13), and the chloramphenicol and quinolone resistance gene *oqxB* (n = 6).

Virulence gene analysis revealed that all 114 strains carried enterobactin-related genes (*fepC, entB*), outer membrane protein (*ompA*) genes, and fimbrial structure-related genes (*ecpA/B/D/E/R*). The enterobactin-related gene *fepG* was only identified in CSKP strains. Except for P15-CRKP2, which lacked the fimbrial gene *ecpC*, and P38-CSKP1 and P38-CSKP2, which lacked the enterobactin-related gene *entA*, all other strains contained these genes. CRKP strains displayed higher carriage rates of yersiniabactin-related genes (*fyuA, irp1/2, ybtA/E/P/Q/S/T/U/X*) and aerobactin-related genes (*iucA/B/C, iutA*) compared to CSKP strains.

Plasmid replicon sequencing identified 14 plasmid replicon types in CRKP strains, primarily IncFIB(K) (n = 66), IncR (n = 60), ColRNAI (n = 66), and IncFII (pHN7A8) (n = 60). CSKP strains carried 13 plasmid replicon types, with IncFIB(K) (n = 33) being the most prevalent (Figure 5).

## Before-and-After Comparison

Phylogenetic analysis revealed that recurrent infections in 31 patients were caused by the same strain. Comparisons of the initial and final infection strains for string test results, biofilm formation, *Galleria mellonella* mortality rates, and gene presence showed no differences between the initial and final isolates (Table 3).

**Table 3** Comparison of Characteristics Between Initial and Final Strains in rUTIs

| Characteristics | ALL | Initial Infection | Final Infection | *p*-value |
|---|---|---|---|---|
| | N=62 (%) | N=31 (%) | N=31 (%) | |
| String test | 27 (43.55%) | 13 (41.94%) | 14 (45.16%) | 0.7978 |
| Biofilm-forming capacity | | | | |
| Strong | 45 (72.58%) | 21 (67.74%) | 24 (67.74%) | 0.3931 |
| Moderate | 14 (22.58%) | 9 (29.03%) | 5 (29.03%) | 0.2244 |
| Weak | 3 (4.84%) | 1 (3.23%) | 2 (6.45%) | 0.5540 |
| Mortality of *Galleria mellonella* | 0.9 (0.6–1) | 0.9 (0.7–1) | 0.8 (0.6–1) | 0.3420 |
| Major resistance gene | | | | |
| $bla_{LAP-2}$ | 34 (54.84%) | 17 (54.84%) | 17 (54.84%) | >0.9999 |
| $bla_{TEM-1}$ | 41 (66.13%) | 21 (67.74%) | 20 (64.52%) | 0.7884 |
| *qnrS1* | 43 (69.35%) | 22 (70.97%) | 21 (67.74%) | 0.7830 |
| *sul2* | 36 (58.06%) | 18 (58.06%) | 18 (58.06%) | >0.9999 |
| *rmtB1* | 33 (53.23%) | 17 (54.84%) | 16 (51.61%) | 0.7991 |
| *tet(A)* | 47 (75.81%) | 23 (74.19%) | 24 (67.74%) | 0.7668 |
| *dfrA14* | 31 (50%) | 16 (51.61%) | 15 (48.39%) | 0.7995 |
| Major virulence gene | | | | |
| *fyuA* | 44 (70.97%) | 22 (70.97%) | 22 (70.97%) | >0.9999 |
| *irp1/2* | 44 (70.97%) | 22 (70.97%) | 22 (70.97%) | >0.9999 |
| *iucA/B/C* | 42 (67.74%) | 21 (67.74%) | 21 (67.74%) | >0.9999 |
| *iutA* | 43 (69.35%) | 22 (70.97%) | 21 (67.74%) | 0.7830 |
| *fepG* | 6 (9.68%) | 3 (9.68%) | 3 (9.68%) | >0.9999 |
| *entA* | 60 (96.77%) | 30 (96.77%) | 30 (96.77%) | >0.9999 |
| *ecpC* | 60 (96.77%) | 30 (96.77%) | 30 (96.77%) | >0.9999 |
| Major plasmid replicon | | | | |
| IncFIB(K) | 54 (87.10%) | 27 (87.10%) | 27 (87.10%) | >0.9999 |
| IncR | 40 (64.52%) | 20 (64.52%) | 20 (64.52%) | >0.9999 |
| ColRNAI | 38 (61.29%) | 19 (61.29%) | 19 (61.29%) | >0.9999 |
| IncFII(pHN7A8) | 36 (58.06%) | 18 (58.06%) | 18 (58.06%) | >0.9999 |
| IncN | 2 (3.23%) | 2 (6.45%) | 0 | 0.1506 |
| IncHI1B | 8 (12.90%) | 4 (12.90%) | 4 (12.90%) | >0.9999 |
| IncFII | 4 (6.45%) | 2 (6.45%) | 2 (6.45%) | >0.9999 |


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## Discussion

*Klebsiella pneumoniae* is one of the ESKAPE pathogens and a priority pathogen identified by the World Health Organization. In many low-income countries, limited treatment options necessitate reliance on empirical approaches for managing Klebsiella pneumoniae infections, exacerbate the severity of these cases. For instance, in Tanzania and Egypt, multidrug-resistant and hypervirulent *Klebsiella pneumoniae* is frequently detected.[6,33] Conversely, in developed countries such as those in Europe, research indicates that the problem of *Klebsiella pneumoniae* infections remains inadequately controlled. A French study identified *Klebsiella pneumoniae* as the leading pathogen in bloodstream infections, accounting for over half of the cases.[34] Similarly, studies in Germany have highlighted its ability to spread not only among humans but also through animals and food.[35] Previous research has suggested that CRKP may evolve in both virulence and resistance during infection, thereby increasing its pathogenicity and complicating treatment.[18] However, most investigations have concentrated on CRKP in bloodstream infections and pyogenic liver abscesses, with relatively limited focus on its role in rUTIs.

In this study, we examined the underlying diseases and complications of patients with rUTIs caused by CRKP and CSKP in a hospital setting. Both groups exhibited high rates of urinary catheter use, a factor that not only heightens the risk of UTIs but also predisposes patients to bloodstream infections. Invasive procedures increase the likelihood of pathogen entry or reduce immune functionality, thereby making patients more vulnerable to infections.[36] While previous studies have reported a predominance of rUTIs among female patients, our findings showed a higher prevalence in males. This discrepancy may be attributed to the prolonged hospitalization of patients and the limited sample size, which skewed the inclusion toward male patients. A study in India suggested that urinary stones in patients with UTIs elevate the risk of recurrent infections.[37] In contrast, our research identified only seven cases with concurrent urinary stones, indicating that factors contributing to rUTIs may vary geographically. Phylogenetic analysis revealed that strains isolated from different time points in patients with rUTIs typically clustered within the same branch, suggesting that rUTIs were predominantly caused by the initial strain of *Klebsiella pneumoniae*. However, a few cases involved reinfection by distinct strains. Specifically, recurrent infections in 31 patients were attributed to the initial strain, with no significant differences in virulence or genetic characteristics observed between initial and final isolates.

Antibiotic susceptibility testing of isolated strains from patients with rUTIs showed that 56.1% of the strains were resistant to carbapenems. Compared to previous studies in China and Iran,[38,39] our findings indicate a higher resistance rate to carbapenems in urine-derived *Klebsiella pneumoniae*. Carbapenems are essential for treating *Klebsiella pneumoniae* infections, and resistance to these antibiotics has become increasingly common. For example, an epidemiological study from the Great Lakes region in the USA reported that approximately half of CRKP isolates were derived from urine.[40] Similarly, a 20-year surveillance project by Castanheira et al, spanning 199 hospitals in 42 countries, found that CRKP accounted for 71.1% of carbapenem-resistant Enterobacteriaceae causing UTIs.[41] In developing countries, carbapenems are often inaccessible due to their high cost and are not used in animals, leading to generally lower resistance rates.[42] However, factors such as diversified resistance mechanisms, cross-resistance among antibiotics, excessive use of β-lactams, and patient-to-patient transmission in hospital settings have contributed to the widespread dissemination of CRKP.[43]

WGS in our study identified $bla_{KPC-2}$ as the primary carbapenem resistance gene. P10-CRKP4 carried $bla_{KPC-33}$, a variant of $bla_{KPC-2}$.[44] SNP analysis revealed significant differences between P10-CRKP4 and other isolates, suggesting acquisition of $bla_{KPC-33}$ through distinct infection pathways. Additionally, P1-CRKP1 carried the $bla_{OXA-232}$ gene, a subtype of $bla_{OXA-48}$, another critical carbapenem resistance determinant. Recent studies in India, Wenzhou, and Yunnan, China, have also identified CRKP strains harboring $bla_{OXA-232}$.[45,46] Our sequencing data revealed that CRKP isolates carried a diverse array of resistance genes, including those for quinolones, β-lactams, sulfonamides, and aminoglycosides. Antibiotic susceptibility testing indicated high resistance rates to cephalosporins, β-lactams, and quinolones, suggesting potential overuse of these antibiotics in treatment. Carbapenemases, such as those encoded by $bla_{KPC-2}$, can hydrolyze almost all β-lactam substrates, including penicillins and cephalosporins. Combined with the co-expression of β-lactam resistance genes, this results in elevated cephalosporin resistance.[47] The resistance rates to cephalosporins vary geographically. For instance, in a Tanzanian hospital, *Klebsiella pneumoniae* exhibited a 91% resistance rate to third-generation cephalosporins,[32] while a Nigerian study reported a resistance rate of 46.5%.[48] Cephalosporins and monobactams inhibit

bacterial cell wall synthesis, while quinolones inhibit DNA synthesis.[49] These antibiotics are widely recommended for UTIs treatment and are extensively used worldwide.[50,51] Plasmid replicon sequencing revealed that CRKP strains predominantly carried IncFIB(K), IncR, ColRNAI, and IncFII(pHN7A8) plasmid replicons. The IncFII(pHN7A8) and IncR replicons are associated with resistance and are commonly found in *Klebsiella pneumoniae* strains harboring $bla_{KPC-2}$.[52] Consistent with this, all $bla_{KPC-2}$-carrying *Klebsiella pneumoniae* isolates in our study contained these plasmid replicons.

Virulence factors play a critical role in bacterial invasion and disease progression. PCR screening detected the *WcaG* gene, associated with bacteremia and regarded as a marker of high virulence, in only a small number of isolates.[53] Genes involved in siderophore and ferric iron uptake enhance bacterial iron acquisition, thereby promoting proliferation within the host. Consistent with the findings of Areli Bautista-Cerón et al, our study revealed a high prevalence of these genes in urine-derived *Klebsiella pneumoniae* strains.[23] Capsule expression-related genes, such as *rmpA* and *rmpA2*, which enhance bacterial growth and counteract host bactericidal substances, were also frequently detected. Similar observations were reported in patients with *Klebsiella pneumoniae* UTIs studies by Jun Li.[54] Analysis of all 114 isolates demonstrated the presence of type 3 fimbriae gene *mrkD*, which enhance bacterial adhesion and invasion of host cells. Adhesion-related factors are vital for bacterial colonization and infection in UTIs.[55] Notably, regional differences in virulence genes were observed. For example, the prevalence of the *rmpA* gene in *Klebsiella pneumoniae* in UTIs from India was only 10.5%,[56] while an Egyptian study reported a *fimH* prevalence of 66.7%.[57] These findings underscore regional variations in the pathogenic mechanisms of *Klebsiella pneumoniae*. Sequencing of virulence genes showed that all 114 isolates carried enterobactin-related gene *fepC*, outer membrane protein gene *ompA*, and fimbrial structure-related genes *ecpA/B/D/E/R*. Additionally, *iucA/B/C* genes, previously linked to high virulence, were more prevalent in CRKP strains than in CSKP strains. Previous reports identified IncFIB(K) as a virulence-associated plasmid.[52] In our study, this plasmid replicon was detected in 86.84% of isolates, suggesting that the strains causing rUTIs may exhibit high virulence.

Biofilm formation plays a significant role in *Klebsiella pneumoniae* invasion of bladder epi-thelial cells and evasion of phagocytes during UTIs.[11] Our study found that all 114 *Klebsiella pneumoniae* strains causing rUTIs had biofilm-forming capabilities, likely due to the expression of *fimH* and *mrkD* virulence genes.[58,59] Similar to our findings, other studies have reported high biofilm formation rates in urine-derived *Klebsiella pneumoniae*.[39,54] Both CRKP and CSKP strains in our study had biofilm-forming capabilities, but CRKP strains had a significantly higher proportion of moderate biofilm formation compared to CSKP strains. This may be related to the expression of *fimH* and *mrkD* virulence genes, influencing biofilm formation. Biofilm formation can reduce antibiotic efficacy, leading to prolonged infection cycles and playing a crucial role in recurrent infections.[60] We used a *Galleria mellonella* model to analyze the virulence levels of CRKP and CSKP strains causing rUTIs. The results showed high mortality rates in *Galleria mellonella* for both CRKP and CSKP strains, with CRKP strains causing significantly higher mortality than CSKP strains. This finding suggests that virulence gene expression may influence strain virulence, consistent with the studies by Shankar[61] and Jun Li.[54] Differences in virulence may also contribute to the higher prevalence of underlying diseases and complications in CRKP-induced rUTIs compared to CSKP, although further confirmation is needed.

Previous reports indicated that KL2 was the main capsule serotype of CRKP in China, but KL64 has recently become the predominant serotype for CRKP.[62] Despite all samples coming from the same hospital, the results showed distinct regional characteristics for ST types. Over 2000 different ST types have been identified globally, with different regions exhibiting different predominant ST types.[63] In European countries, ST258 is the predominant CRKP type,[43] while in China, ST11 is the main type,[64] consistent with our findings. Different ST types of *Klebsiella pneumoniae* may vary in virulence levels,[65] and our results confirmed this. Due to the transmissibility of plasmids, the integration of virulence plasmids among strains is one of the reasons for the high prevalence of ST11 CRKP in China.[66] In our study, the capsular serotypes of ST11 CRKP were mainly KL64, with only one strain identified as KL47. Recent studies have indicated that ST11 KL64 and ST11 KL47 are the predominant CRKP types in China,[67] which is consistent with our findings. Additionally, our study detected one strain each of ST231 and ST15 *Klebsiella pneumoniae*. ST231 *Klebsiella pneumoniae* is primarily prevalent in South and Southeast Asia and has only recently been introduced to China.[46] Meanwhile, ST15 *Klebsiella pneumoniae* has gradually become an emerging international epidemic type, second only to ST11 and ST258.[68] *Klebsiella pneumoniae* has also been detected in animals. Notably, some animal isolates are nearly identical to

human isolates, belonging to the same ST. However, compared to animal isolates, human-derived strains exhibit more pronounced drug resistance patterns.[69]

This study has limitations, as the samples and cases were limited to a tertiary hospital in the capital city of Guizhou Province. Future research could consider collaborating with other institutions to expand the study scope and enhance the generalizability of the findings.

## Conclusion

In conclusion, from the patient's perspective, we found that compared with patients with rUTIs caused by CSKP, those with rUTIs caused by CRKP were older and had a higher prevalence of conditions such as Cardiac insufficiency and Electrolyte imbalances. From the perspective of strains, we observed that CRKP strains exhibited multidrug resistance. CRKP strains showed higher biofilm-forming ability and mortality rate in *Galleria mellonella* compared to CSKP strains. The results of gene sequencing indicated that the main prevalent type of CRKP in our hospital was ST11 KL64, and its main resistance mechanism was the carriage of $bla_{KPC-2}$, while CSKP strains had different types among different patients. In addition, the two groups of strains carried a wide variety of genes, and in this study, most rUTIs were relapses of the same strain. The results of this study will contribute to a better understanding of the clinical characteristics of rUTIs, as well as the microbiological characteristics of *Klebsiella pneumoniae* and the situation of antibiotic resistance in this region.

## Ethics Approval and Patient Consent

The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (No.2022131). We confirm that informed consent obtained from all study participants prior to study commencement, and Guidelines outlined in the Declaration of Helsinki were followed.

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## 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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