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Emergence and clinical challenges of ST11-K64 carbapenem-resistant Klebsiella pneumoniae: molecular insights and implications for antimicrobial resistance and virulence in Southwest China

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Abstract

Background In clinical practice, the emergence of ST11-K64 carbapenem-resistant Klebsiella pneumoniae (ST11-K64 CRKP) has become increasingly alarming. Despite this trend, limited research has been conducted to elucidate the clinical and molecular characteristics of these strains.

Objectives This study aimed to comprehensively investigate the clinical characteristics, antimicrobial resistance patterns, resistance and virulence-associated genes, and molecular epidemiology of ST11-K64 CRKP in Southwest China.

Methods A retrospective analysis was performed on patients infected with carbapenem-resistant Klebsiella pneumoniae (CRKP) in a tertiary care hospital between July 2021 and May 2022. A total of 69 CRKP strains were isolated, with clinical data collected for detailed analysis. Laboratory assessments included antimicrobial susceptibility testing, hypermucoviscosity string testing, genotypic characterization of antimicrobial resistance and virulence genes, and multi-locus sequence typing. Statistical analyses were conducted using SPSS, with significance set at $P < 0.05$.

Results Among the 69 CRKP isolates, 36 strains (52.2%) were identified as ST11-K64 CRKP. Hematological diseases were less associated with ST11-K64 CRKP infection compared to non-ST11-K64 strains ($P = 0.012$). However, central intravenous catheter use ($P = 0.001$), mechanical ventilation ($P = 0.002$), tracheal intubation ($P = 0.006$), and tracheotomy ($P = 0.041$) were significantly more common in ST11-K64 CRKP cases. Resistance rates to amikacin ($P < 0.001$), gentamicin ($P = 0.004$), tobramycin ($P = 0.034$), and sulfamethoxazole ($P < 0.001$) were significantly higher in ST11-K64 CRKP. Additionally, resistance-associated genes such as bla_{KPC-2} ($P < 0.001$) and virulence-associated genes including *rmpA* ($P < 0.001$), *iucA* ($P < 0.001$), *rmpA2* ($P < 0.001$), and *iutA* ($P = 0.001$) were detected at significantly higher

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rates in ST11-K64 strains compared to non-ST11-K64 strains. Furthermore, compared to ST11-K47 CRKP, ST11-K64 CRKP harbored more virulence genes, such as *rmpA* ($P=0.007$), *iucA* ($P=0.001$), and *iutA* ($P=0.003$).

Conclusion Our findings underscore the rising prevalence of ST11-K64 CRKP, characterized by high levels of antimicrobial resistance and the presence of potent resistance and virulence genes. This strain poses a significant clinical and therapeutic challenge, necessitating heightened vigilance, stringent infection control measures, and robust clinical management strategies.

Keywords Clinical characterization, ST11-K64, ST11-K47, Virulence genes, Infection

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a formidable pathogen that causes a spectrum of infections ranging from community-acquired illnesses to severe nosocomial infections, such as pneumonia, bacteremia, endophthalmitis, liver abscesses, urinary tract infections, and potentially fatal septic shock [1]. The management of infections caused by drug-resistant *K. pneumoniae* heavily relies on carbapenems, which are considered the antibiotics of last resort. However, the emergence and rapid proliferation of carbapenem-resistant Klebsiella pneumoniae (CRKP) has escalated alarmingly, threatening global health systems [2, 3]. For instance, data from the China Antimicrobial Surveillance Network (CHINET) indicate a sharp rise in resistance rates to meropenem and imipenem, from 2.9% to 3% in 2005 to 26.3% and 25% in 2018, respectively [4]. This dramatic increase underscores the urgent need for effective containment strategies and clinical interventions.

In Europe and the United States, the dissemination of carbapenem resistance has been primarily driven by *K. pneumoniae* sequence type 258 (ST258). In contrast, sequence type 11 (ST11) has emerged as the predominant CRKP lineage in China [5]. ST11 CRKP is recognized as a clinically high-risk clone due to its extraordinary ability to acquire plasmids encoding multidrug resistance and hypervirulence traits [6]. This capacity has positioned ST11 as a formidable challenge for healthcare systems. Research has shown that CRKP strains belonging to the ST11 lineage, particularly those with capsular serotypes K64 and K47, exhibit both extensive drug resistance and an alarming propensity for rapid dissemination in clinical settings, compounding the difficulty of managing nosocomial infections [7–9]. Moreover, the evolutionary trajectory of these strains reveals that ST11-K64, which has gained prominence in recent years, likely originated from the ST11-K47 subclone through homologous recombination involving a ~ 154-kb region encompassing capsular and lipopolysaccharide biosynthesis loci [10]. This genetic shift has endowed ST11-K64 with a broader array of virulence and multidrug resistance plasmids, conferring prolonged survival and increased pathogenicity compared to ST11-K47 [9]. Consequently, the prevalence of ST11-K64 CRKP has shown a worrying upward trend, underscoring its status as a significant public health concern [11–13]. Despite its clinical relevance, the current body of literature on ST11-K64 CRKP remains limited, with gaps in understanding its clinical characteristics, resistance mechanisms, and molecular epidemiology. Addressing these gaps is critical for developing targeted strategies to mitigate its impact.

The present study aims to bridge this knowledge gap by systematically investigating the clinical features, resistance profiles, and molecular characteristics of ST11-K64 CRKP. By analyzing clinical data, antimicrobial susceptibility, and the genetic determinants of resistance and virulence, this research seeks to enhance our understanding of this pathogen and inform effective containment and treatment strategies.

Materials and methods

Study setting and patients

From July 2021 to May 2022, we consecutively collected 69 non-duplicated CRKP isolates at a teaching hospital in Southwest China. Patients were diagnosed with a bacterial infection attributed to CRKP. The inclusion criteria were defined as follows: (1) the first incidence of CRKP infection falling within the study period; (2) symptomatic infection from the site where CRKP was cultured; (3) patients meeting the diagnostic criteria for infection; (4) solely considering the initial occurrence of CRKP. Exclusion criteria included: (1) insufficient clinical data; (2) duplicate isolates from the same patient; (3) duplicate isolates obtained from the clonal dissemination or outbreaks of CRKP in the same ward patients (The clonal dissemination or outbreaks of CRKP usually exhibit high genetic variability and may carry unique genomic features, which may include resistance genes and virulence factors. This can lead to the microbiological and genetic characterization of CRKP that may not represent the general traits of other common strains when analyzed.). Conversely, outpatients and cases with incomplete or unavailable medical records were systematically excluded from the analysis. The protocol for this study was approved by the research administration of Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2022-E433-01). This study used an anonymous way to protect the participants and obtained their permission.

Data collection and definitions

The medical data of 69 CRKP strains were collected from the hospital's electronic health records system. The collected data encompassed essential demographic information (gender and age), intensive care unit (ICU) admissions, prior hospitalizations, length of hospital stay, department, sample types, laboratory examination, underlying comorbidities, infection type, invasive procedures, surgical interventions, antibiotic exposures, chemotherapy usage, and clinical outcomes.

According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), a CRKP strain is defined as a clinical isolate that shows resistance to carbapenem. The definition of ST11-K64 CRKP is CRKP that belongs to ST11 and has capsular serotypes K64. The definition of ST11-K47 CRKP is CRKP that belongs to ST11 and has capsular serotypes K47. The definition of non-ST11-K64 CRKP is CRKP that does not belong to ST11 and does not have capsular serotypes K64.

Sample identification and antimicrobial susceptibility testing

The isolates were identified using the VITEK2 Compact system (BioMérieux, Marcy l'Etoile, France) or the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system (MALDI-TOF/MS; BioMérieux, Lyons, France) according to specific selection criteria. The antibiotic susceptibility tests were conducted on the isolates using either the disk-diffusion method or the VITEK 2 Compact system, with each method specified for specific antibiotics. The results were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), version 2021. In our study, commonly used clinical antibiotics included aztreonam, amikacin, amoxicillin-clavulanic acid, cefepime, cefazolin, ceftriaxone, cefuroxime, cefoxitin, ciprofloxacin, cefoperazone-sulbactam, ceftazidime/avibactam, piperacillin-tazobactam, gentamicin, tobramycin, sulfamethoxazole, levofloxacin, ertapenem, imipenem, meropenem, and piperacillin. Furthermore, *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922 were used as the quality control bacterial strains.

String test

The string test was employed to discern the hypermucoviscous phenotype, following the previous description [14]. In brief, after overnight incubation of KP on 5% sheep blood agar plates at 37 °C, a standard inoculating loop is used to extract a filamentous strand of mucus from the bacterial colony. A traction length exceeding 5 mm on the inoculating loop is considered indicative of a positive hypermucoviscous phenotype.

PCR detection

The genomic DNA of the CRKP strains was extracted following the protocol provided by the Biospin Bacteria Genomic DNA Extraction kit (Bioflux, Hangzhou, China). Subsequently, capsular serotype-specific genes (*K64* and *K47*), virulence genes (*peg-344*, *iroB*, *iucA*, *ituA*, *rmpA* and *rmpA2*), and carbapenemase genes(bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}*and* bla_{OXA-48}) were detected by polymerase chain reaction (PCR) using specific primers as previously described in Table S1 [15–17]. The PCR products were visualized by 1% agarose gel electrophoresis. The amplified positive PCR products were then validated through direct DNA sequencing (Sangon Biotech, Shanghai, China). Nucleotide sequences were compared using the Basic Local Alignment Search Tool (BLAST)(http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Multi-locus sequence typing (MLST)

PCR amplification of seven housekeeping genes (*gapA*, *mdh*, *phoE*, *tonB*, *infB*, *pgi*, *and rpoB*) was performed according to the protocol available at http://bigsdb.pasteur.fr/klebsiella/klebsiella.html (Table S1). The PCR amplified products were sequenced at Sangon Biotech in Shanghai, China. Allelic profiling and sequence types (STs) determination were subsequently confirmed using the aforementioned website.

Statistical analysis

Continuous variables were presented as the mean ± standard deviation or the median with interquartile range for normally and non-normally distributed data, respectively. Statistical comparisons between continuous variables were conducted using either Student's t-test or the Mann-Whitney U test, while categorical variables were analyzed through the $\chi 2$ test or Fisher's exact test. A significance threshold of $p<0.05$ was applied to determine statistical significance.

Results

Clinical characteristics of ST11-K64 CRKP infection in patients

During the period from July 2021 to May 2022, a total of 69 patients infected with CRKP isolates were identified in our hospital. According to the detection results of K64 and MLST, CRKP strains were classified as ST11-K64 CRKP (52.2% detection rate) and non-ST11-K64. Detailed demographic information and the clinical factors of the patients with ST11-K64 CRKP infection are shown in Table 1. None of the following demonstrated a significant difference between ST11-K64 CRKP and non-ST11-K64 CRKP groups: age, gender, previous hospitalizations, admission to ICU, length of stay in the hospital, department, sample types, laboratory examination, infection type, antibiotic exposure, and

Table 1 Clinical characteristics of patients infected with ST11-K64 CRKP

Factors	ST11-K64 CR-KP(*n* = 36)n(%)	non-ST11-K64 CR-KP(*n* = 33)n(%)	*P*-value
Basic data			
Age[a]	57.8 ± 15.5	60.2 ± 21.7	0.591
Male	29(80.5)	25(75.7)	0.629
Previous Hospitalizations	19(52.7)	21(63.6)	0.361
Admission to ICU	28(77.1)	21(63.6)	0.196
Length of stay in hospital[a,] days	44.6 ± 27.6	36.8 ± 26.3	0.237
Department			
ICU	13(36.1)	7(21.2)	0.173
rehabilitation medicine	7(19.4)	5(15.1)	0.638
Respiratory medicine	3(8.3)	8(24.2)	0.071
others	13(36.1)	13(39.4)	0.779
Sample types			
Sputum	20(55.5)	13(39.3)	0.179
Urine	5(13.8)	6(18.1)	0.627
Bronchoalveolar lavage fluid	4(11.1)	4(12.1)	0.896
Blood	1(2.8)	4(12.1)	0.135
others	6(16.6)	6(18.1)	0.381
Laboratory examination			
C-reaction protein (> 10 mg/L)	30(83.3)	24(72.7)	0.167
Neutrophils(>7.5*10^9 /l)	20(55.5)	12(36.3)	0.110
Procalcitonin(> 0.5ng/ml)	19(52.7)	17(51.5)	0.916
White blood cells(>10.0*10^9 /l)	17(47.2)	11(33.3)	0.241
Underlying diseases			
Pulmonary disease	31(86.1)	28(84.8)	0.882
Cardiovascular disease	23(63.8)	15(45.4)	0.124
Factors	ST11-K64 CR-KP(*n* = 36)n(%)	non-ST11-K64 CR-KP(*n* = 33)n(%)	*P*-value
Kidney diseases	23(63.8)	20(60.6)	0.779
Cerebrovascular disease	22(61.1)	13(39.3)	0.071
Hepatobiliary and Pancreatic Diseases	18(50.0)	20(60.6)	0.376
Hypertension	17(47.2)	19(57.5)	0.457
Hematological diseases	11(30.5)	20(60.6)	**0.012**
Diabetes	7(19.4)	9(27.2)	0.441
Malignant tumors	4(11.1)	6(18.1)	0.405
Infection type			
Pneumonia	24(66.7)	17(51.5)	0.200
Bacteremia	1(2.8)	4(12.1)	0.135
Urinary infection	5(13.8)	6(18.1)	0.627
intracranial infection	3(8.3)	1(3.0)	0.332
Invasive procedures and devices			
Central intravenous catheter	31(86.1)	16(48.4)	**0.001**
Drainage tube	31(86.1)	22(66.6)	0.056
Mechanical ventilation	31(86.1)	17(51.5)	**0.002**
Urinary catheter	30(83.3)	21(63.6)	0.063
tracheal intubation	25(69.4)	12(36.3)	**0.006**
tracheotomy	16(44.4)	7(21.2)	**0.041**
Surgery	15(41.6)	14(42.4)	0.949
Bone marrow biopsy	4(11.1)	4(12.1)	0.896
Antibiotic exposure			
β-lactam-β-lactamase inhibitors	29(80.5)	28(84.8)	0.638
Carbapenem antibiotic	21(58.3)	20(60.6)	0.848
Chemotherapy	13(36.1)	10(30.3)	0.609
Fluoroquinolones	7(19.4)	12(36.3)	0.073
Tigecycline	7(19.4)	6(18.1)	0.893

Table 1 (continued)

Factors	ST11-K64 CR-KP(*n* = 36)n(%)	non-ST11-K64 CR-KP(*n* = 33)n(%)	*P*-value
Cephalosporins	6(16.6)	11(33.3)	0.109
Aminoglycosides	5(13.8)	8(24.2)	0.272
Glycopeptides	5(13.8)	4(12.10)	0.828
Outcomes			
Positive outcome	21(58.4)	20(60.6)	0.848
Negative outcome	15(41.6)	13(39.4)	0.848

If not otherwise stated, data are reported using frequency and percentage

[a] Age, and length of stay in hospital as mean and standard deviation (SD)

Bold values in the *P*-value column indicated statistical significance

Table 2 Resistance profile of ST11-K64 CRKP strains

Antibiotic agent	ST11-K64 CR-KP(*n* = 36)n(%)	non-ST11-K64 CR-KP(*n* = 33)n(%)	*P*-value
ceftazidime/avibactam	7(19.4)	12(36.3)	0.116
amikacin	35(97.2)	15(45.4)	**0.000**
gentamicin	35(97.2)	24(72.7)	**0.004**
tobramycin	35(97.2)	27(81.8)	**0.034**
sulfamethoxazole	35(97.2)	16(48.4)	**0.000**
Cefperazone-Sulbactam	35(97.2)	32(96.9)	0.293
piperacillin-tazobactam	36(100)	32(96.9)	0.293
cefepime	36(100)	30(90.9)	0.064
cefoxitin	36(100)	35(97.2)	0.293
aztreonam	36(100)	31(93.9)	0.134
levofloxacin	36(100)	33(100)	/
cefazolin	36(100)	33(100)	/
ceftriaxone	36(100)	33(100)	/
amoxicillin-clavulanic acid	36(100)	33(100)	/
ertapenem	36(100)	33(100)	/
imipenem	36(100)	33(100)	/
ciprofloxacin	36(100)	33(100)	/
cefuroxime	36(100)	33(100)	/
meropenem	36(100)	33(100)	/
ceftazidime	36(100)	33(100)	/
piperacillin	36(100)	33(100)	/

Bold values in the *P*-value column indicated statistical significance

outcomes. Furthermore, no significant differences were observed among most of the underlying conditions, such as pulmonary disease, cardiovascular disease, kidney disease, cerebrovascular disease, hepatobiliary and pancreatic diseases, hypertension, diabetes, and malignant tumors ($P>0.05$). However, a significant difference was found for hematological diseases(30.5 vs. 60.6%, $P=0.012$). Additionally, central intravenous catheter (86.1 vs. 48.4%, $P=0.001$), mechanical ventilation (86.1 vs. 51.5%, $P=0.002$), tracheal intubation (69.4 vs. 36.3%, $P=0.006$), and tracheotomy (44.4 vs. 21.2%, $P=0.041$) were more prevalent in the ST11-K64 CRKP group than in the non-ST11-K64 CRKP group, whereas the other invasive procedures and devices showed no significant differences.

Antimicrobial susceptibility of ST11-K64 CRKP strains

The Table 2 shows the antimicrobial susceptibility profiles of the ST11-K64 CRKP, and non-ST11-K64 strains. All ST11-K64 CRKP strains exhibited high resistance to multiple antibiotics, including β -lactam-β-lactamase inhibitors, Cephalosporins, Carbapenem antibiotic, Aminoglycosides, Fluoroquinolones, Penicillins, sulfamethoxazole, and aztreonam. Nevertheless, their resistance towards ceftazidime/avibactam was notably lower. In comparison, the resistance rates of amikacin(97.2 vs. 45.4%, $P=0.000$), gentamicin(97.2 vs. 72.7%, $P=0.004$), tobramycin(97.2 vs.81.8%, $P=0.034$), and sulfamethoxazole(97.2 vs. 48.4%, $P=0.000$) were significantly higher in the ST11-K64 CRKP strains than non-ST11-K64 strains.

The results of the string test, Carbapenemase and Virulence-Associated genes

Table 3 compares hypermucoviscosity, carbapenemase genes, and virulence genes between ST11-K64 CRKP strains and non-ST11-K64 strains. All strains of ST11-K64 CRKP were found to harbor bla_{KPC}, whereas the presence of bla_{NDM}, bla_{IMP}, bla_{VIM}*and* bla_{OXA-48} was not observed. ST11-K64 CRKP carried the most virulence-associated genes *rmpA* and *iucA*, followed by *rmpA2* and *iutA*, with only 4 strains exhibiting hypermucoviscosity. Additionally, the detection rates of bla_{KPC}(100 vs. 45.4%, $P=0.000$), *rmpA*(61.1 vs. 18.1%, $P=0.000$), *iucA*(61.1 vs. 18.1%, $P=0.000$), *rmpA2*(58.3 vs. 21.2%, $P=0.000$), and *iutA* (55.5 vs. 18.1%, $P=0.001$) were significantly higher in the ST11-K64 CRKP than in the non-ST11-K64group.

Molecular epidemiological characteristics ST11-K64 CRKP

The MLST, hypermucoviscosity, carbapenemases genes, virulent genes, K64 and K47 results of 69 strains were revealed in Table S2. MLST showed that the 69 CRKP strains belonged to 14 different STs, among which ST11 was the most prevalent, accounting for 45 (65.2%). Interestingly, K64 was detected in 36 strains, and K47 was

Table 3 The results of the string test, carbapenemase and virulence-Associated genes in ST11-K64 CRKP

Factors	ST11-K64 CR-KP(*n* = 36)n(%)	non-ST11-K64 CR-KP(*n* = 33)n(%)	*P*-value
Carbapenemases genes			
blaKPC–2	36(100)	15(45.4)	**0.000**
blaNDM–1	0	6(18.1)	**0.007**
blaNDM–5	0	3(9.0)	0.064
blaOXA–48	0	2(6.0)	0.134
blaVIM	0	0	/
blaIMP	0	0	/
Virulent genes			
rmpA	22(61.1)	6(18.1)	**0.000**
iucA	22(61.1)	6(18.1)	**0.000**
rmpA2	21(58.3)	7(21.2)	**0.000**
iutA	20(55.5)	6(18.1)	**0.001**
iroB	2(5.5)	5(15.1)	0.187
peg-344	1(2.7)	3(9.0)	0.248
Hypermucoviscosity	4(11.1)	2(6.0)	0.457

Bold values in the *P*-value column indicated statistical significance

Table 4 The results of the string test, carbapenemase, and virulence-associated genes in ST11-K64 CRKP and ST11-K47 CRKP strains

Factors	ST11-K64 CR-KP(*n* = 36)n(%)	ST11-K47 CR-KP(*n* = 9)n(%)	*P*-value
rmpA	22(61.1)	1(11.1)	**0.007**
rmpA2	21(58.3)	2(22.2)	0.053
iucA	22(61.1)	0	**0.001**
iutA	20(55.5)	0	**0.003**
iroB	2(5.5)	0	0.469
peg-344	1(2.7)	0	0.613
Hypermucoviscosity	4(11.1)	0	0.295
blaKPC–2	36(100)	9(100)	/

Bold values in the *P*-value column indicated statistical significance

identified in 9 strains out of the 45 ST11-CRKP strains. After this, we conducted a comparative analysis of the disparities between ST11-K64 and ST11-K47 in terms of these factors. We observed a significant association between *rmpA*(61.1 vs. 11.1%, $P=0.007$), *iucA*(61.1 vs. 0%, $P=0.001$), and *iutA*(55.5 vs. 0%, $P=0.003$) in relation to ST11-K64 (Table 4).

Discussion

The increasing prevalence of ST11-K64 CRKP in recent years presents a critical challenge for clinical management, combining high antimicrobial resistance with enhanced virulence, often resulting in severe outcomes such as sepsis and septic shock [9, 18, 19]. This study aimed to elucidate the clinical and molecular characteristics of ST11-K64 CRKP, providing evidence of its extensive spread in Southwest China and underscoring the urgent need for targeted containment strategies. Our findings demonstrate that ST11-K64 CRKP comprised 52.2% of all CRKP isolates, consistent with previous reports identifying this strain as a dominant nosocomial pathogen [20]. Importantly, hematological diseases were associated with reduced susceptibility to ST11-K64 CRKP infection, whereas invasive procedures, such as tracheal intubation, mechanical ventilation, tracheotomy, and central intravenous catheterization, emerged as significant risk factors.

Since 2015, ST11-K64 CRKP has been primarily studied in terms of genetics, virulence, and evolution, and has gradually become the dominant subclone, posing a serious clinical threat in older patients [7, 9, 21]. Nevertheless, limited research has been undertaken on the clinical manifestations of ST11-K64 CRKP infection. In our study, sputum specimens were the most common samples, and pneumonia was the most frequently observed type of infection aligning with earlier studies, highlighting the role of ST11-K64 CRKP primarily invading the respiratory tract [22]. The dominance of this particular strain in causing respiratory infections highlights the importance of continued surveillance to prevent further spread of ST11-K64 CRKP infections. Furthermore, it is plausible that CRKP obtained from sputum may not definitively be identified as the etiological culprits of pneumonia. Since some strains could potentially denote colonization rather than signify a condition of active infection, data on the clinical characteristics of ST11-K64 CRKP may be slightly biased. However, we still believe this result is of great importance.

The clinical characteristics associated with infections caused by ST11-K64 CRKP are vital for the prevention of these infections and the administration of effective antimicrobial therapies. Hematological diseases were associated with reduced susceptibility to ST11-K64 CRKP infection. This finding revealed that different subtypes of CRKP may exhibit variations in their pathogenicity and influence on host resistance. ST11-K64 CRKP, as a specific clone of *K. pneumoniae*, may possess unique biological characteristics or pathogenic mechanisms that contribute to its relatively lower propensity to cause bloodstream infections. Moreover, this phenomenon could be correlated with the patient's preexisting conditions, immunological profile, or therapeutic protocols [23]. Interestingly, tracheal intubation, mechanical ventilation, tracheotomy, and central intravenous catheter were risk factors for ST11-K64 CRKP infection. These findings align with earlier studies and highlight the role of invasive interventions in breaching immune barriers, facilitating colonization by virulent strains [24–27]. These data suggest that strict aseptic techniques and regular infection risk assessments are critical for mitigating

nosocomial transmission of ST11-K64 CRKP, particularly in critically ill patients undergoing invasive procedures.

Mechanisms of carbapenem resistance in CRKP include the synthesis of carbapenemase enzymes, plasmid-mediated AmpC enzymes, extended-spectrum β-lactamases (ESBLs), over-expressed efflux pumps, and loss of porins [28, 29]. Our study focused on carbapenemase genes, identifying bla_{KPC-2}, bla_{NDM-1}, bla_{NDM-5}, and bla_{OXA-48} with proportions of 79%, 8.7%, 4.3%, and 2.9%, respectively. This is consistent with a previous conclusion that bla_{KPC-2} was the most prevalent carbapenemase gene in the CRKP isolates [30]. Furthermore, the antimicrobial resistance profiles of ST11-K64 CRKP further underscore its clinical threat. The bla_{KPC-2} gene was detected in 100% of ST11-K64 CRKP isolates, a significantly higher prevalence than in non-ST11-K64 strains. Resistance rates to aminoglycosides and sulfamethoxazole were notably high, alongside complete resistance to most common antibiotics, consistent with prior research [31–33]. Another study also showed that ST11-K64 CRKP exhibited an exceptionally high resistance rate (≥87.5%) to 18 common antibiotics [26]. These findings highlight the dire need for alternative therapeutic strategies, as conventional antibiotics are largely ineffective against this strain. The observed low resistance rate to ceftazidime/avibactam (19.4%) offers a potential avenue for treatment; however, the risk of resistance emergence must be carefully monitored.

Virulence-associated plasmids, including pNTUH-K2044, pLVPK, and pLVPK-like, contain notable genetic markers such as *peg-344*, *iroB*, *iucA*, *iutA*, *rmpA*, *and rmpA2* [34–36]. The role of virulence-associated plasmids in the pathogenicity of ST11-K64 CRKP cannot be overstated. This study identified a significantly higher prevalence of genes such as *iucA*, *iutA*, *rmpA*, and *rmpA2* in ST11-K64 CRKP compared to non-ST11-K64 strains. These genes contribute to hypermucoviscosity, iron acquisition, and enhanced survival, increasing the pathogenic potential of the strain. Although ST11-K64 CRKP strains were shown to possess several genes associated with hypervirulence, the proportion exhibiting hypermucoviscosity in the string test is very low. This observation aligns with the findings of researchers such as Yang Xuemei et al., indicating that the absence of hypervirulence in CRKP carrying virulence plasmids is primarily due to mutations in the rmpA and rmpA2 genes, leading to their functional loss [37]. Moreover, some studies suggested that the presence of virulence plasmids does not necessarily correlate with the high adhesive phenotype of Klebsiella pneumoniae [38]. This indicates that genetic traits within the virulence plasmids or bacterial chromosomes also influence the regulation of both high adhesive and highly virulent phenotypes. Compared to the closely related ST11-K47 subclone, ST11-K64 CRKP harbors a more diverse repertoire of virulence determinants, likely reflecting evolutionary adaptation to enhance environmental fitness and clinical impact. This underscores its status as a high-risk clone necessitating rigorous monitoring and containment measures.

The genomic evolution of ST11-K64 CRKP, as suggested by the shift in dominance from ST11-K47 to ST11-K64, illustrates the dynamic interplay between genetic plasticity and selective pressures in clinical environments [9, 39]. In our study, out of the 69 CRKP strains, 45 (65%) were identified as belonging to the ST11 subtype. Among these, 36 strains were detected as K64 while the remaining 9 strains belonged to K47. Although no significant differences were observed in the String test and carbapenemase genes between ST11-K64 CRKP and ST11-K47 CRKP, a notable difference was found in the presence of *rmpA*, *iucA*, and *iutA*. These findings were consistent with another report suggesting that 82.9% (68/82) of ST11-K47 strains lacked virulence genes, while all the ST11-K64 strains carried *rmpA2*, *iucABCD*, and *iutA* genes [39]. The acquisition of additional resistance and virulence traits by ST11-K64 CRKP enhances its survival and transmissibility, posing significant challenges for healthcare systems. Future studies should prioritize whole-genome sequencing and functional assessments to unravel the molecular mechanisms underpinning these adaptations and inform targeted interventions.

Despite its strengths, this study has limitations. Firstly, the retrospective design, single-center focus, and relatively small sample size restrict the generalizability of the findings. Secondly, this study focused on the risk factors associated with ST11-K64 CRKP infection, while neglecting the treatment and outcome data, which are crucial for a comprehensive analysis of the impact of ST11-K64 CRKP on patient prognosis. Thirdly, we did not conduct antimicrobial susceptibility testing for tigecycline and colistin, so we cannot determine whether their resistance rates were consistent with previous research. Furthermore, this study only compared the differences in the expression of carbapenemase genes and capsular serotypes. In the near future, we will evaluate the mechanisms of carbapenem resistance and define the KL type using whole-genome sequencing and PFGE. And finally, functional assays, such as biofilm formation, antiserum resistance, and virulence studies in animal models, were not performed and should be prioritized in future research to fully elucidate the pathogenic potential of ST11-K64 CRKP.

Conclusions

This study provides valuable insights into the clinical and molecular characteristics of ST11-K64 CRKP in Southwest China, emphasizing its increasing prevalence, high antimicrobial resistance, and presence of

multiple virulence determinants. Invasive interventions, such as tracheal intubation and central venous catheterization, were identified as key risk factors for infection, highlighting the importance of stringent infection control measures. The detection of blaKPC-2 as the predominant carbapenemase gene and the identification of critical virulence genes such as rmpA, rmpA2, iucA, and iutA underscore the multidimensional threat posed by this high-risk clone. These findings contribute to a deeper understanding of the factors driving ST11-K64 CRKP infections and provide a foundation for developing targeted therapeutic and preventive strategies. Future research, including large-scale genomic analyses and functional studies, is imperative to uncover the detailed mechanisms of resistance and virulence in ST11-K64 CRKP. These efforts will guide the development of innovative interventions to combat this emerging pathogen, ultimately enhancing patient outcomes and protecting public health.

Supplementary Information

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Supplementary Material 1

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Author contributions
Conceptualization, L L and M L; Methodology, L L and S L; Software, L L; Validation, L L, H Z and J L, Formal Analysis, L L; Investigation, J L and J G; Resources, M L and S L; Data Curation, H Z; Writing – Original Draft Preparation, L L; Writing – Review & Editing, S L; Visualization, L L; Supervision, L L; Project Administration, S L; Funding Acquisition, M L, L L. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the Declaration of Helsinki and relevant guidelines and regulations. The study was approved by the Ethics Review Committee of the First Affiliated Hospital of Guangxi Medical University (No.: 2022-E433-01). Since no personally identifiable information was used in this analysis, the Ethics Review Committee of the First Affiliated Hospital of Guangxi Medical University waived the requirement for informed personal consent.

Consent for publication
Not applicable。.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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