Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Clinical Characteristics and Whole-Genome Sequencing Analysis of Carbapenem- and Colistin-Resistant Hypervirulent *Klebsiella pneumoniae*

Jingwen Zhang[1,2], Yanghua Xiao[1,2], Feng Nie[1,2], Ping Li[1,2], Tianxin Xiang[1]

[1]Jiangxi Provincial Key Laboratory of Prevention and Treatment of Infectious Diseases, Jiangxi Medical Center for Critical Public Health Events, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330052 People's Republic of China; [2]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, People's Republic of China

Correspondence: Tianxin Xiang; Ping Li, Email ndyfy02258@ncu.edu.cn; ndyfy10356@ncu.edu.cn

Background: Colistin-resistant hypervirulent carbapenem-resistant *Klebsiella pneumoniae* (ColR-hv-CRKP) has emerged as a critical clinical challenge, yet its clinical and genomic characteristics remain poorly understood.

Methods: From 2021 to 2024, 326 non-duplicate hv-CRKP isolates were collected from patients with confirmed clinical infections at a tertiary hospital in China. Antimicrobial susceptibility was determined by broth microdilution. Clinical data were retrospectively analyzed, and logistic regression identified risk factors for ColR-hv-CRKP infection. Whole-genome sequencing was performed to characterize molecular characteristics and colistin resistance-associated mutations.

Results: Ninety-two isolates (92/326, 28.22%) were colistin-resistant by broth microdilutionby. Patients with ColR-hv-CRKP infection were more likely to have a history of ICU admission, prolonged hospitalization, and invasive procedures, were more likely to have isolates recovered from sputum specimens, and had more frequent prior exposure to colistin (all $P < 0.05$). Multivariable analysis identified prior colistin administration (OR = 4.58, 95% CI 2.41–8.69) and sputum specimen source (OR = 2.44, 95% CI 1.35–4.42) as independent risk factors for ColR-hv-CRKP infection. Whole-genome sequencing revealed that most ColR-hv-CRKP isolates belonged to ST11 (90/92, 97.83%), mainly KL64 (52/92, 56.52%) or KL25 (36/92, 39.13%). Carbapenem resistance was primarily mediated by bla_{KPC-2} (91/92, 98.91%), while no *mcr* genes were detected. Whole-genome sequencing analysis showed that most ColR-hv-CRKP isolates (81/92, 88.04%) harbored colistin resistance-associated mutations. *pmrB* alterations were the most frequent, detected in 45.65% (42/92) of isolates, predominantly Thr157Pro (18/42) and Ser205Pro (8/42) substitutions. *mgrB* mutations occurred in 35.87% (33/92) of isolates, predominantly due to insertional inactivation (27/33, 81.82%) mediated by ISKpn26 and IS903B elements. Phylogenetic analysis revealed that all ST11-KL25 ColR-hv-CRKP isolates clustered into a highly homogeneous group with minimal intra-lineage diversity and displayed significantly smaller pairwise SNP distances than ST11-KL64 /KL107 isolates ($P < 0.0001$).

Conclusion: Colistin resistance in hv-CRKP was mainly associated with *pmrB* and *mgrB* mutations in the dominant ST11-KL25 and ST11-KL64 lineages. Prior colistin exposure was the key clinical risk factor, highlighting the selective pressure of colistin use and the urgent need for strengthened antimicrobial stewardship.

Keywords: *Klebsiella pneumoniae*, colistin, resistance, whole-genome sequencing, clinical characteristics

Introduction

Klebsiella pneumoniae is an important opportunistic pathogen within the Enterobacteriaceae family and a major cause of nosocomial infections.[1] It is generally categorized into classical *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKP) based on distinct genetic and phenotypic characteristics. cKP primarily affects immunocompromised individuals and readily acquires antimicrobial resistance determinants, contributing to the widespread emergence of carbapenem-resistant

https://doi.org/10.2147/IDR.S582373
Received: 18 November 2025
Accepted: 26 February 2026
Published: 7 May 2026

K. pneumoniae (CRKP).[2] In contrast, hvKP is defined by the presence of specific virulence-associated genes, including *peg-344, rmpA, rmpA2, iroB*, and *iucA*, which confer enhanced invasiveness, enabling severe community- and hospital-acquired infections in otherwise healthy hosts, often involving multiple organs.[3,4] The convergence of hypervirulence and carbapenem resistance has led to the rise of hypervirulent carbapenem-resistant *K. pneumoniae* (hv-CRKP), a formidable "dual threat" that combines high virulence with limited therapeutic options.[5] These strains significantly complicate clinical treatment and are associated with increased morbidity and mortality.[6,7]

Polymyxins, including polymyxin B and polymyxin E (colistin), are nonribosomal peptide antibiotics that have historically been reserved as one of the last-line treatment options for carbapenem-resistant Gram-negative bacterial infections because of concerns regarding neurotoxicity and nephrotoxicity.[8] However, the global emergence of colistin-resistant hv-CRKP (ColR-hv-CRKP) further exacerbates the clinical burden.[9,10] It is well established that resistance to polymyxins in Gram-negative bacteria is primarily mediated by modifications to lipopolysaccharide (LPS), which increase the net positive charge on the bacterial surface.[11] This mechanism is known to reduce electrostatic interaction between LPS and the cationic colistin, thereby diminishing antimicrobial efficacy.[12,13] Previous molecular studies have demonstrated several resistance mechanisms, including mutations or constitutive activation of two-component regulatory systems (TCSs) such as PhoP/Q, PmrA/B, and CrrAB; inactivation or mutation of the negative regulator *mgrB*; and the horizontal transfer of *mcr* genes.[14,15] Despite these insights, comprehensive data on the clinical and genomic features of ColR-hv-CRKP remain limited, and the specific chromosomal mutations contributing to colistin resistance in the hv-CRKP isolates have not been systematically characterized. Therefore, the primary objective of this study was to identify clinical risk factors associated with ColR-hv-CRKP infection. The secondary objectives were to characterize the genomic diversity of ColR-hv-CRKP isolates and to elucidate the chromosomal mechanisms underlying colistin resistance.

In this study, we investigated the prevalence, clinical characteristics, and risk factors associated with ColR-hv-CRKP infections. We also performed genomic analyses of 92 ColR-hv-CRKP isolates to characterize their molecular features, phylogenetic relationships, resistance gene profiles, and chromosomal mutations related to colistin resistance.

Materials and Methods

Isolate Collection and Identification

A total of 326 non-duplicate clinical isolates of hypervirulent carbapenem-resistant *K. pneumoniae* (hv-CRKP) and corresponding clinical data were collected from the Clinical Microbiology Laboratory of the First Affiliated Hospital of Nanchang University between January 2021 and December 2024. The isolates were obtained from various clinical specimens, including sputum, blood, bronchoalveolar lavage fluid (BALF), ascites, pus, secretion, urine and drainage. Only the first isolate per patient during the study period was included to avoid duplication. Bacterial identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Carbapenem and colistin resistance was determined according to the Clinical and Laboratory Standards Institute (CLSI M100-34) guidelines,[16] and isolates resistant to at least one carbapenem agent (imipenem (MIC $\geq$ 4 mg/L), meropenem (MIC $\geq$ 4 mg/L), or ertapenem (MIC $\geq$ 2 mg/L)) were defined as carbapenem-resistant. Among these isolates, 92 strains were further identified as colistin-resistant hv-CRKP and selected for subsequent antimicrobial susceptibility testing and whole genome sequencing. Hypervirulence was assessed using a combination of phenotypic and genotypic criteria. The hypervirulent phenotype was initially screened using the string test, with a positive result defined as the formation of a viscous string $\geq$ 5 mm in length when a colony was stretched by an inoculation loop. Isolates that were positive in the string test and harbored known virulence-associated genes (*rmpA, rmpA2, iucA*, and *iroB*) by PCR were defined as hypervirulent.[3] All isolates were stored in Luria-Bertani (LB) broth supplemented with 20% glycerol at –80 °C for further analysis.

Antimicrobial Susceptibility Testing

Initial antimicrobial susceptibility testing for all isolates was performed using the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile, France). Isolates exhibiting resistance to any carbapenem antibiotic (imipenem, meropenem, or ertapenem) and to colistin, based on VITEK 2 interpretation, were designated as putative ColR-hv-CRKP isolates. Subsequently, the antimicrobial susceptibility of the 92 ColR-hv-CRKP isolates was determined using the broth microdilution method for the

following antibiotics: imipenem (1–64 μg/mL), meropenem (1–64 μg/mL), cefotaxime (1–64 μg/mL), ceftriaxone (1–64 μg/mL), ceftazidime (1–64 μg/mL), cefazolin (1–64 μg/mL), levofloxacin (0.5–32 μg/mL), ciprofloxacin (0.5–32 μg/mL), aztreonam (1–64 μg/mL), ticarcillin/clavulanate (16/2-128/2 μg/mL), piperacillin/tazobactam (2/4-128/4 μg/mL), ceftazidime/avibactam (2/4-128/4 μg/mL), tobramycin (1–64 μg/mL), amikacin (2–128 μg/mL), doxycycline (1–64 μg/mL), minocycline (1–64 μg/mL), tigecycline (2–16 μg/mL), trimethoprim/sulfamethoxazole (2/38-8/152 μg/mL), and colistin (1–64 μg/mL). Antibiotic powders were obtained from commercial sources (MedChemExpress, USA), and serial twofold dilutions were prepared according to the manufacturer's instructions. All testing procedures and result interpretations were conducted according to the CLSI M100-Ed34.[17] According to CLSI breakpoints for *K. pneumoniae*, isolates with colistin MICs > 2 mg/L were defined as resistant. *K. similipneumoniae* ATCC 700603, *K. pneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922 were used as the quality-control strain for all assays.

Clinical Data Collection and Definitions

Clinical and demographic data were retrospectively collected from electronic medical records. Patients were eligible for inclusion if they were hospitalized at the First Affiliated Hospital of Nanchang University between January 2021 and December 2024 and had a confirmed hv-CRKP isolate isolated from a clinical specimen. Patients of all age groups were included. Only cases considered to represent infection were enrolled. Infection was defined based on compatible clinical manifestations together with microbiological evidence, as determined by treating physicians and confirmed by medical record review. Patients were excluded if they had multiple hospital admissions during the study period, in which case only the first admission was included, if medical information or key clinical data were incomplete, or if the isolate was considered a contaminant. Surgery was defined as any operative procedure conducted under general or regional anesthesia within 90 days before isolate collection. Invasive procedures encompassed mechanical ventilation, central venous catheterization, urinary catheterization, tracheostomy, and drainage interventions. Prolonged hospitalization was defined as a continuous hospital stay lasting more than 30 days prior to the recovery of the isolate.

DNA Extraction and Whole Genome Sequencing

Genomic DNA was extracted from overnight cultures of colistin-resistant isolates using the TIANamp Bacteria DNA Kit (Tiangen Biotech, China) following the manufacturer's protocol. DNA quality and concentration were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and 1% agarose-gel electrophoresis. Whole-genome sequencing (WGS) was performed on the Illumina NovaSeq 6000 platform (Illumina, USA) generating 150 bp paired-end reads. Raw sequencing reads were trimmed using Trimmomatic v0.39 to remove low-quality bases and adapter sequences.[17] High-quality clean reads were then assembled de novo using SPAdes v4.0.0.[18] Assembly quality was evaluated with QUAST v5.2.0.[19] Genome annotation was performed on the high-quality assemblies using Bakta v1.6.0.[20] In silico multilocus sequence typing (MLST) was carried out using mlst v2.11.0, with allele profiles retrieved from the PubMLST database (https://pubmlst.org/). Capsular polysaccharide (K) and lipopolysaccharide (O) locus types were identified using Kaptive v2.0.7.[21] Antimicrobial resistance genes, including mobilized colistin resistance genes (*mcr-1* to *mcr-10*) using ResFinder v4.1, and virulence genes were identified using the Virulence Factor Database (VFDB),[22,23] respectively. The virulence scores of the strains were assigned using the Kleborate tool (https://github.com/katholt/Kleborate).[24]

Phylogenetic Analysis of Colistin-Resistant Hv-CRKP Isolates

Core-genome single-nucleotide polymorphisms (SNPs) were identified using Snippy v4.6.0 (https://github.com/tseemann/snippy) with default parameters, employing the ST11 *K. pneumoniae* reference strain HS11286 (GCF_000240185.1) as the reference genome. The resulting core genome alignment was filtered to remove recombinant regions using Gubbins v3.2.1 to ensure accurate phylogenetic inference.[25] SNP-sites v2.5.1 was then used to extract biallelic SNP positions from the recombination-free alignment.[26] Pairwise SNP distances between isolates were computed with snp-dists v0.8.2 (https://github.com/tseemann/snp-dists), and isolates differing by ≤ 16 core SNPs were defined as potential clonal clusters.[27] A maximum-likelihood phylogenetic tree was reconstructed from the filtered core-SNP alignment using IQ-TREE2 v2.2.6, with automatic model selection and 1,000 ultrafast bootstrap replicates.[28] The final tree was visualized and annotated with Interactive Tree of Life (iTOL) (https://itol.embl.de).[29]

Identification of Colistin Resistance-Associated Mutations

To investigate the molecular mechanisms of colistin resistance, each genome was aligned against the colistin-susceptible *K. pneumoniae* reference strain MGH 78578 (RefSeq accession GCF_000016305.1) and further compared with the colistin-susceptible reference HS11286 (RefSeq accession GCF_000240185.1) to exclude polymorphisms unrelated to colistin resistance. Snippy v4.6.0 (https://github.com/tseemann/snippy) was used with default parameters to identify single-nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations within the PmrA/B, PhoP/Q, and *mgrB* loci. Variants were subsequently annotated with SnpEff v5.1 to assess their predicted functional impact.[30] Insertion sequences (IS) were identified using the online tool ISfinder (https://www-is.biotoul.fr/).

Statistical Analysis

Statistical analyses were performed using SPSS (version 26.0, IBM Corp). Categorical variables were compared using Chi-square or Fisher's exact test, while continuous variables were analyzed using Student's *t*-test or Mann–Whitney *U*-test as appropriate. Risk factors for ColR-hv-CRKP infection were identified through the above differential analysis, with variables showing P <0.05 subsequently included in multivariate logistic analysis. A P value < 0.05 was considered statistically significant. GraphPad Prism 8 was used for data visualization.

Results

Clinical Characteristics of Patients Infected with ColR-Hv-CRKP

A total of 326 non-duplicate hv-CRKP isolates were collected from the First Affiliated Hospital of Nanchang University between 2021 and 2024. Among these, 92 isolates (92/326, 28.22%) demonstrated resistance to colistin and were classified as ColR-hv-CRKP, whereas 234 isolates (71.78%) remained susceptible to colistin and were classified as colistin-susceptible hv-CRKP (ColS-hv-CRKP). The demographic and clinical characteristics of patients infected with ColR-hv-CRKP and ColS-hv-CRKP are summarized in Table 1. Patients with ColR-hv-CRKP infection were more frequently admitted to the ICU (53.26% vs 33.76%, $P = 0.013$) and had a higher prevalence of prior ICU stays (89.13% vs 76.90%, $P = 0.013$). Sputum specimens accounted for a higher proportion in the ColR-hv-CRKP group than in the ColS-hv-CRKP group (78.26% vs 57.69%, P = 0.001). Prolonged hospitalization (> 30 days; 64.13% vs 49.60%, P = 0.019) and invasive procedures (94.57% vs 86.70%, P = 0.049) were significantly more common among ColR-hv-CRKP cases. However, the proportions of patients with hospitalization within the previous month or long-term steroid/immunosuppressant exposure did not differ between groups. Prior antibiotic exposure differed significantly between the two groups (χ^2-test, P < 0.001). Carbapenem-based regimens were more frequently used in the ColS-hv-CRKP group than in the ColR-hv-CRKP group (54.27% vs. 39.13%). In contrast, prior exposure to colistin-based regimens was more common in the ColR-hv-CRKP group compared with the ColS-hv-CRKP group (36.96% vs. 8.97%). Multivariable analysis identified prior colistin administration (OR = 4.58, 95% CI 2.41–8.69) and sputum specimen source (OR = 2.44, 95% CI 1.35–4.42) as independent risk factors for ColR-hv-CRKP infection (Table 2). Other variables, including prolonged hospitalization, ICU admission, or prior invasive procedures, were not independently associated after multivariate logistics regression analysis.

Distribution and Molecular Characteristics of ColR-Hv-CRKP Isolates

A total of 92 ColR-hv-CRKP isolates were identified between 2021 and 2024, including 14 (15.22%) isolates in 2021, 17 (18.48%) in 2022, 42 (45.65%) in 2023, and 19 (20.65%) in 2024 (Figure 1A). Most ColR-hv-CRKP isolates were recovered from the Intensive Care Unit (49/92, 53.26%), followed by Neurosurgery (16/92, 17.39%), Orthopedics (7/92, 7.61%) (Figure 1B). Regarding specimen types, the majority of isolates originated from sputum (72/92, 78.26%), while a minority were isolated from blood (5/92, 5.43%) (Figure 1C). To investigate the molecular characteristics of ColR-hv-CRKP, whole-genome sequencing was performed on all 92 isolates. MLST analysis revealed that the vast majority (90/92, 97.83%) belonged to sequence type ST11, the predominant high-risk clone associated with carbapenem resistance in China, while the remaining two isolates were classified as ST15. Capsular typing identified two major capsule loci: KL64 (52/92, 56.52%) and KL25 (36/92, 39.13%), with two isolates each belonging to KL107 (2/92, 2.17%) and KL2 (2/92, 2.17%). Virulence gene profiling showed that all isolates carried the hypermucoviscosity-associated regulator *rmpA2* and

Table 1 Demographic and Clinical Characteristics of Patients Infected with ColR-Hv-CRKP and ColS-Hv-CRKP

Demographic	ColR-hv-CRKP Infection Group (n = 92)	ColS-hv-CRKP Infection Group (n = 234)	P Value
Age, years (mean ± SD)	60.4±15.8	58.2±16.6	0.266
Gender			
Male	62 (67.39)	167 (71.37)	0.477
Female	30 (32.61)	67 (28.63)	
Wards			
ICU	49 (53.26)	79 (33.76)	**0.013**
Neurosurgery	16 (17.39)	45 (19.23)	
Orthopedics	7 (7.61)	18 (7.69)	
Emergency Medicine	6 (6.52)	15 (6.41)	
Public Health	4 (4.35)	8 (3.42)	
Respiratory Medicine	3 (3.26)	19 (8.12)	
Hematology	2 (2.17)	6 (2.56)	
Rehabilitation Medicine	2 (2.17)	3 (1.28)	
Other wards	3 (3.26)	41 (17.52)	
Comorbidity			
Hypertension	32 (34.78)	97 (41.45)	0.251
Diabetes	13 (14.13)	34 (14.53)	0.935
Stroke	10 (10.87)	22 (9.40)	0.700
Kidney Dysfunction	6 (6.52)	10 (4.27)	0.316
Tumor	5 (5.43)	12 (5.13)	0.774
Specimen source (sputum vs. non-sputum)			
Sputum	72 (78.26)	135 (57.69)	**0.001**
Non-sputum	20 (21.74)	99 (42.31)	
Previous medical history			
ICU admission	82 (89.13)	180 (76.90)	**0.013**
Hospitalization within the past month	16 (17.39)	31 (13.25)	0.300
Prolonged hospitalization	59 (64.13)	116 (49.60)	**0.019**
Surgery within 90 days	58 (63.04)	122 (52.14)	0.07
Invasive procedure	87 (94.57)	203 (86.70)	**0.049**
Long-term exposure of steroids or immunosuppressants	8 (8.70)	18 (7.69)	0.86
Colistin administration	34 (36.96)	21 (8.97)	**< 0.001**
Carbapenem administration	65 (70.65)	155 (66.24)	0.4
Prior antibiotic exposure before isolate isolation			
Non-carbapenem β-lactam-based regimens	17 (18.48)	65 (27.78)	**< 0.001**
Carbapenem-based regimens	36 (39.13)	127 (54.27)	
Colistin-based regimens	34 (36.96)	21 (8.97)	
Non-β-lactam-based regimens	3 (3.26)	8 (3.42)	
Not treated with antibiotics	2 (2.17)	13 (5.56)	
APACHE II	17 (13.00,25.75)	20 (5.00,27.00)	0.772

Notes: Bold values indicate statistically significant results (P < 0.05). For risk-factor analysis, specimen source was additionally dichotomized as sputum versus non-sputum.

the aerobactin biosynthetic cluster (*iucABCD*) along with its receptor gene *iutA*. Kleborate analysis indicated that all isolates belonged to the iuc1 lineage, and the majority exhibited virulence scores of ≥3 (90/92, 97.83%), with both ST15 isolates displaying the highest score of 5. Notably, 90 out of 92 isolates (97.83%) did not carry the salmochelin operon (*iroBCDN*), whereas both ST15 isolates harbored this operon. Plasmid replicon typing revealed that IncFII (pHN7A8) was the dominant plasmid backbone, detected in most isolates (90/92, 97.83%).

Table 2 Multivariate Analysis of Risk Factors for ColR-Hv-CRKP Infection

Risk Factors	B	S.E.	Wals	P	Exp(B)	95% CI for Exp(B)	
						Low	High
Prolonged hospitalization	0.542	0.279	3.767	0.052	1.719	0.995	2.970
ICU admission	0.788	0.419	3.545	0.060	2.199	0.968	4.996
History of invasive procedures	0.456	0.533	0.731	0.393	1.577	0.555	4.482
Colistin administration	1.521	0.302	8.742	**<0.001**	4.576	2.410	8.686
Sptum source specimen	0.894	0.302	8.742	**0.003**	2.444	1.352	4.421

Notes: Bold values indicate statistically significant results (P < 0.05). Variables with a P-value < 0.05 in the univariate analysis (Table 1) were included in the multivariate logistic regression model. An odds ratio (OR) > 1 indicates an increased risk of ColR-hv-CRKP infection.
Abbreviations: B, regression coefficient; S.E, standard error; CI, confidence interval.

Phylogenetic Analysis of ColR-Hv-CRKP Isolates

To explore the genetic relationships among the ColR-hv-CRKP isolates, a core-genome phylogenetic tree was generated based on SNP alignments (Figure 2). Notably, ST11-KL25 clade comprised all 36 isolates of this capsule type and formed a highly homogeneous monophyletic cluster with minimal intra-clade diversity and strong bootstrap support. These KL25 isolates were predominantly recovered from the ICU (22/36, 61.11%) and neurosurgery (6/36, 16.67%) wards and were mainly collected during 2023. In contrast, the KL64 isolates exhibited greater genetic heterogeneity and were distributed across multiple subclades. The two ST15 isolates carrying the KL2 capsule locus were phylogenetically distinct from the ST11-KL64-dominated lineages and formed a small, independent branch on the periphery of the tree.

To investigate genetic relatedness of ColR-hv-CRKP within the hospital environment, we performed core-genome SNP analysis and visualized pairwise SNP distances in heatmaps stratified by capsular type (Figure 3). Using a conservative threshold of ≤16 SNPs to define putative recent transmission events, several clusters of highly related isolates were identified in both groups. In the KL64/KL107 group (Figure 3A), multiple closely related isolates exhibited minimal SNP divergence. Notably, the KL25 group showed tighter genetic clustering (Figure 3B). A particularly homogeneous cluster of 11 isolates differed by no more than 11 SNPs (Figure 3B), suggesting recent hospital-associated spread from a common source. Two additional clusters containing 11 and 7 isolates, respectively, also exhibited SNP distances within the ≤13 SNP range. Overall, KL25 isolates exhibited significantly smaller pairwise SNP distances than KL64/KL107 isolates (median 15 [IQR 11–19] vs 24 [IQR 10–60]; Mann–Whitney *U*-test, $P < 0.0001$) (Supplementary Figure S1).

Resistance Phenotypes and Resistance Gene Profiles Among ColR-hv-CRKP Isolates

Antimicrobial susceptibility testing of the 92 ColR-hv-CRKP isolates demonstrated universal resistance to a wide spectrum of β-lactam antibiotics, including cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), and cefazolin (CZO) (Figure 4). In addition, all isolates exhibited resistance to β-lactam/β-lactamase inhibitor combinations ticarcillin/

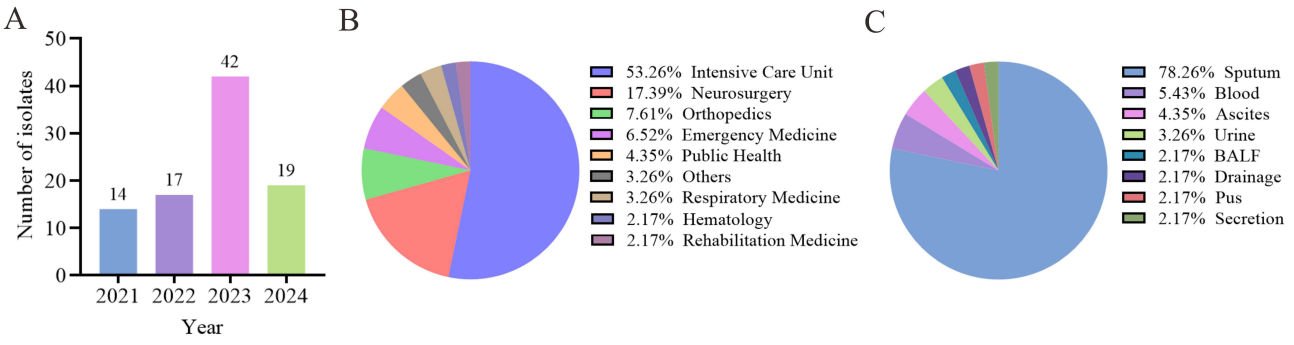

Figure 1 Distribution of 92 colistin-resistant hypervirulent carbapenem-resistant *K. pneumoniae* (ColR-hv-CRKP) isolates. (**A**) Annual number of ColR-hv-CRKP isolates collected from 2021 to 2024. (**B**) Departmental distribution of the ColR-hv-CRKP isolates. (**C**) Specimen-type distribution of the ColR-hv-CRKP isolates.
Abbreviation: BALF, bronchoalveolar lavage fluid.

https://doi.org/10.2147/IDR.S582373

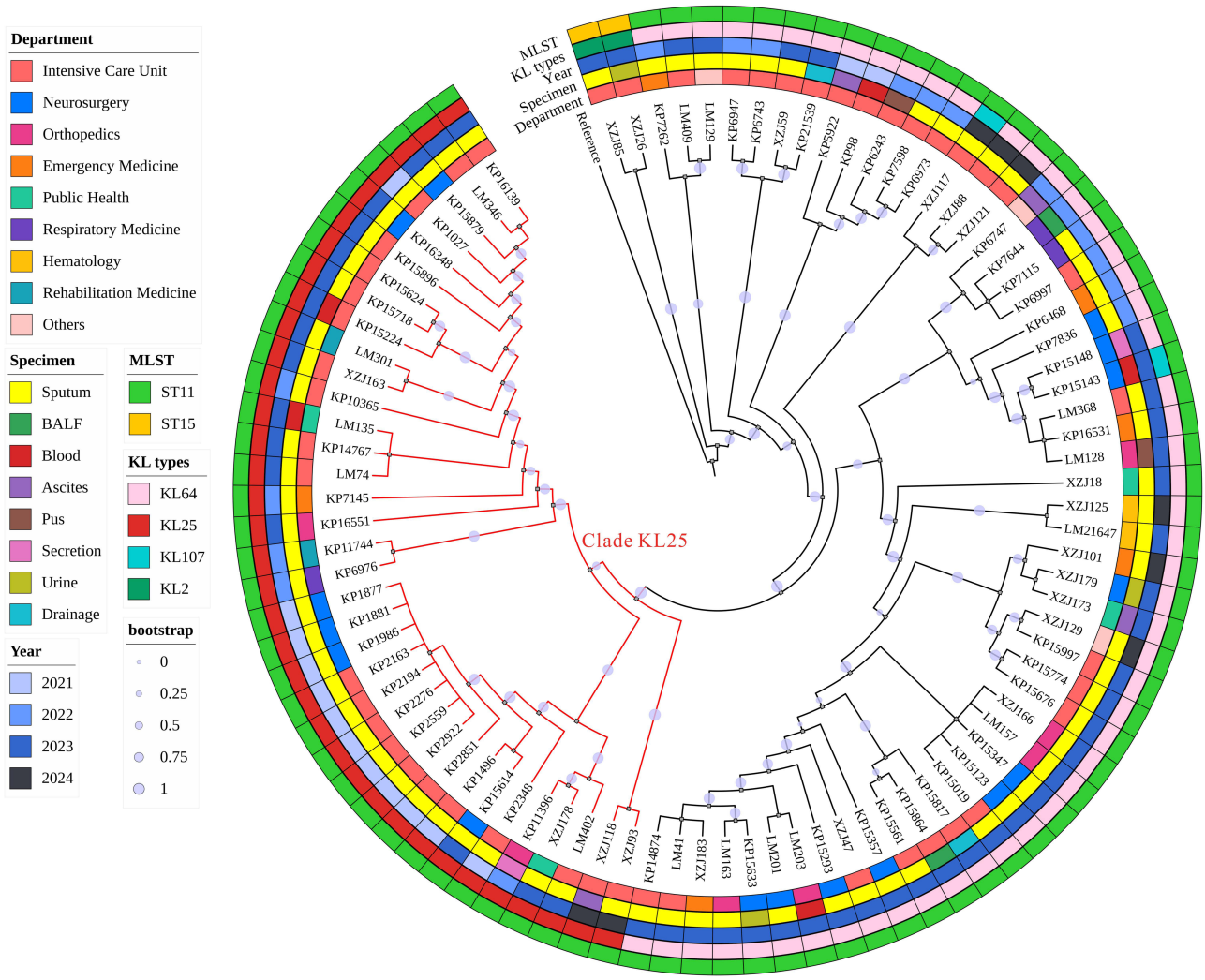

Figure 2 Maximum-likelihood phylogenetic tree based on core-genome SNPs of 92 ColR-hv-CRKP isolates, with metadata annotations indicating capsular type (KL type), specimen source, hospital department and year of isolation. Bootstrap support values are represented by the size of circles at branch nodes. Red branches represent KL25 isolates.

clavulanate (TCC) and piperacillin/tazobactam (TZP), and to the monobactam aztreonam (ATM). Nearly all isolates (91/92, 98.91%) were resistant to the fluoroquinolones levofloxacin (LEV) and ciprofloxacin (CIP). Aminoglycoside resistance was also prevalent, with 84 out of 92 isolates (91.30%) resistant to tobramycin (TOB) and amikacin (AMK). Most isolates were resistant to tetracyclines (doxycycline and minocycline); however, susceptibility to tigecycline (TGC) was retained in 76 isolates (82.61%). Resistance to ceftazidime/avibactam (CZA) was observed in 23 isolates (25.00%). The MIC values, along with MIC_{50} and MIC_{90}, for all tested antimicrobial agents are provided in Supplementary Table S2.

Whole-genome sequencing revealed that carbapenem resistance was primarily mediated by bla_{KPC-2}, detected in 98.91% (91/92) of isolates; one isolate carried bla_{KPC-71}, and another isolate co-harbored bla_{NDM-5} (Figure 4). A variety of other β-lactamase genes were prevalent, including $bla_{CTX-M-65}$ (94.57%), bla_{SHV-12} (78.26%), $bla_{SHV-182}$ (19.57%), bla_{TEM-1B} (92.39%), and $bla_{CTX-M-3}$ (3.26%). Genes conferring resistance to other antimicrobial classes were also widespread. The aminoglycoside resistance gene *rmtB* was present in 92.39% of isolates, while *aadA2* and *aadA16* were detected in 81.52% and 3.26%, respectively. The quinolone resistance gene *qnrS1* was found in 95.65% of isolates, and *aac(6′)-Ib-cr* in 3.26%. The tetracycline resistance gene was was present in *tet(A)* (94.57%). The chloramphenicol resistance gene *catA2* was present in 53.26% of isolates, and the *floR* gene in 3.26%. Trimethoprim resistance genes

https://doi.org/10.2147/IDR.S582373

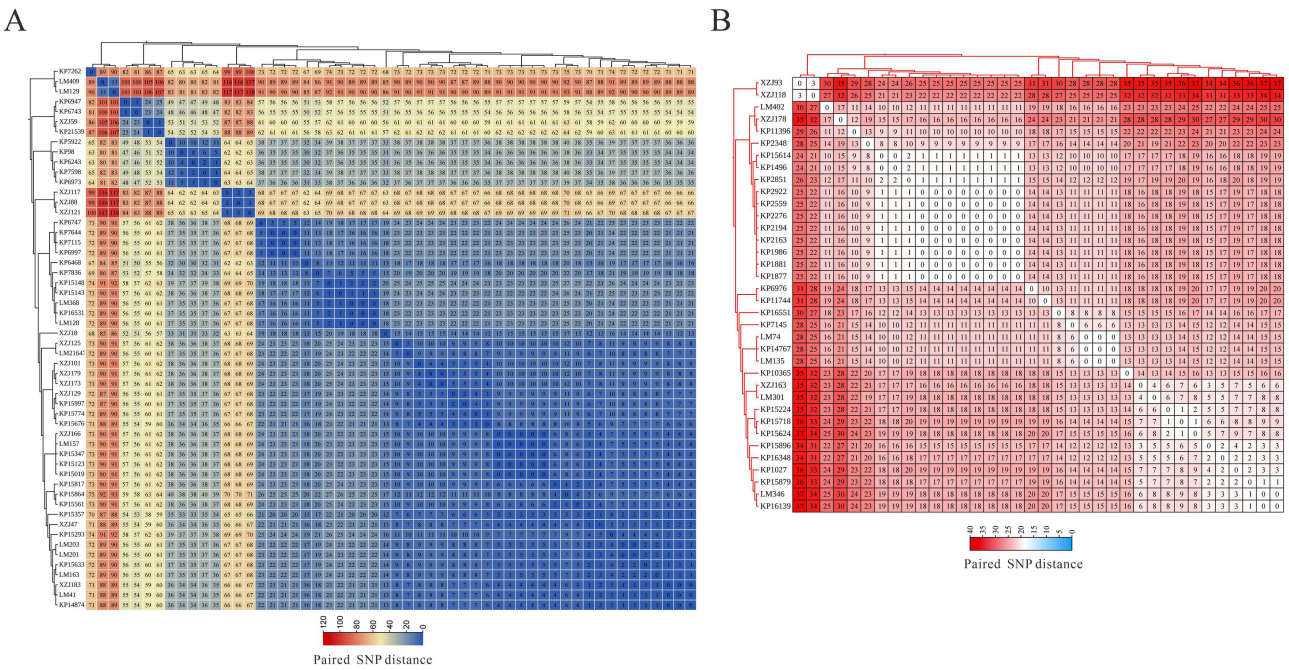

Figure 3 SNP analysis of ColR-hv-CRKP isolates. Heatmaps showing pairwise SNP distances between (**A**) ST11-KL64/KL107 isolates and (**B**) ST11-KL25 isolates. The color scale represents the number of SNP differences.

dfrA14 and *dfrA27* were present in 91.30% and 3.26% of isolates, respectively. The sulfonamide resistance gene *sul2* was found in 92.39%, and *sul1* in 3.26% of isolates. The macrolide resistance gene *mph(A)* was detected in 3.26% of isolates. Notably, none of the 92 isolates carried colistin resistance genes (*mcr-1* through *mcr-9*), suggesting that colistin resistance in these strains likely arose from chromosomal mutations rather than *mcr*-type gene acquisition. Overall, the resistance genotypes identified showed high concordant with the phenotypic antimicrobial susceptibility profiles. The high prevalence of bla_{KPC-2} and extended-spectrum β-lactamase genes aligned with the widespread resistance to β-lactam agents, while the frequent detection of *rmtB, qnrS1*, and *tet(A)* was corresponded to the observed resistance to aminoglycosides, fluoroquinolones, and tetracyclines, respectively.

MgrB and Two-component Regulatory System Mutations in ColR-hv-CRKP Isolates

To elucidate the genetic mechanisms underlying colistin resistance in the 92 ColR-hv-CRKP isolates, mutations were analyzed in the two-component regulatory systems PmrA/PmrB and PhoP/PhoQ, as well as in their negative regulator MgrB (Supplementary Table S1). As shown in Table 3, most ColR-hv-CRKP isolates (81/92, 88.04%) harbored colistin resistance-associated mutations. No mutations in *mgrB* or the two-component regulatory systems were identified in the remaining 11 isolates (11.96%). *mgrB* alterations were identified in 33 isolates (35.87%), predominantly attributable to insertional inactivation by various insertion sequences (ISs), including ISKpn26 (14/92, 15.22%), IS903B (5/92, 5.43%), ISKpn14 (5/92, 5.43%), and ISkpn18 (3/92, 3.26%). In addition, nonsense mutations introducing premature stop codons were detected, comprising Gln30* (3/92, 3.26%) and Trp6* (2/92, 2.17%). A single missense substitution, Cys16Arg, was also identified in one isolate (1.09%). Mutations within the PmrA/PmrB system were comparatively frequent. PmrA substitutions were observed in 10 isolates (10.87%), including Gly53Ser (7/92, 7.61%), Gly53Val (2/92, 2.17%), and Gly53Ala (1/92, 1.09%). Multiple amino acid changes were detected in *pmrB*, with Thr157Pro being the most prevalent (19.57%), followed by Ser205Pro (8/92, 8.70%), Pro95Leu (4/92, 4.35%), Ser203Pro (4/92, 4.35%), Ala246Thr (2/92, 2.17%), Asp150His (2/92, 2.17%), Asp150Val (1/92, 1.09%), Asp150Tyr (1/92, 1.09%), and Ser270Arg (1/92, 1.09%). One frameshift mutation, Leu339fs, was also detected (1/92, 1.09%). In the *phoQ* gene, three missense variants were identified: Arg16Cys, Asn378Tyr, and Val450Asp, each detected in one isolate (1.09%). However, no mutations were detected in the *phoP* gene in any of the isolates.

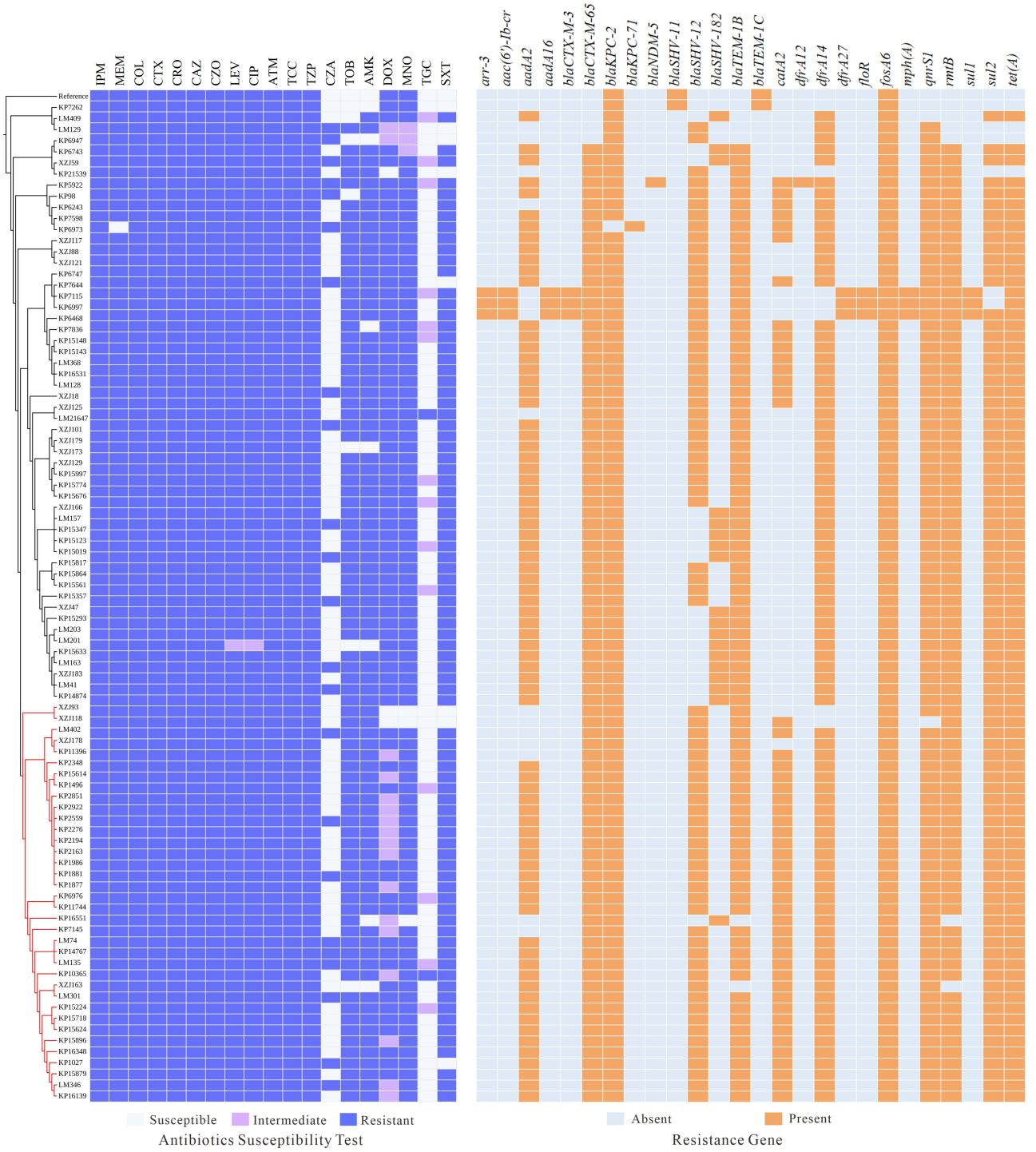

Figure 4 Antibiotic susceptibility profiles and resistance gene distribution among 92 ColR-hv-CRKP isolates. The left panel shows antimicrobial susceptibility testing results for various antibiotics. The right panel displays the presence or absence of resistance genes detected by whole-genome sequencing.

Abbreviations: Antibiotic abbreviations: IPM, imipenem; MEM, meropenem; COL, colistin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; CZO, cefazolin; LEV, levofloxacin; CIP, ciprofloxacin; ATM, aztreonam; TCC, ticarcillin/clavulanate; TZP, piperacillin/tazobactam; CZA, ceftazidime/avibactam; TOB, tobramycin; AMK, amikacin; DOX, doxycycline; MNO, minocycline; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.

Discussion

The prevalence of colistin resistance among hv-CRKP isolates in this study reached 28.22%, which is relatively higher than the rates reported in other regions, where it generally remains below 20%.[9,31] This disparity likely reflects regional

https://doi.org/10.2147/IDR.S582373

Table 3 Mutations Identified in the Two-Component Regulatory Systems and Their Regulator (MgrB) Among 92 ColR-hv-CRKP Isolates

Gene	Mutation Type	Nucleotide and Amino-Acid Change	Isolates, n (%)
mgrB	Insertion sequence	ISKpn26 insertion	14 (15.22%)
		IS903B insertion	5 (5.43%)
		ISKpn14 insertion	5 (5.43%)
		ISKpn18 insertion	3 (3.26%)
	Stop_gained	c.88C > T (p.Gln30*)	3 (3.26%)
		c.17G>A (p.Trp6*)	2 (2.72%)
	Missense_variant	c.46T > C (p.Cys16Arg)	1 (1.09%)
phoQ	Missense_variant	c.46C > T (p.Arg16Cys)	1 (1.09%)
		c.1132A>T (p.Asn378Tyr)	1 (1.09%)
		c.1349T > A (p.Val450Asp)	1 (1.09%)
pmrA	Missense_variant	c.157G > A (p.Gly53Ser)	7 (7.61%)
		c.158G > T (p.Gly53Val)	2 (2.17%)
		c.158G > C (p.Gly53Ala)	1 (1.09%)
pmrB	Missense_variant	c.469A > C (p.Thr157Pro)	18 (19.57%)
		c.613T > C (p.Ser205Pro)	8 (8.70%)
		c.284C > T (p.Pro95Leu)	4 (4.35%)
		c.607T > C (p.Ser203Pro)	4 (4.35%)
		c.736G>A (p.Ala246Thr)	2 (2.17%)
		c.448G > C (p.Asp150His)	2 (2.17%)
		c.449A > T (p.Asp150Val)	1 (1.09%)
		c.448G>T (p.Asp150Tyr)	1 (1.09%)
		c.810C>A (p.Ser270Arg)	1 (1.09%)
	Frameshift_variant	c.1016delT (p.Leu339fs)	1 (1.09%)

Notes: Bold text indicates mutations that have been previously reported as resistance-associated variants; Non-bolded text represents the remaining mutations which are putative novel variants. *Indicates a nonsense mutation introducing a premature stop codon.

variations in antimicrobial use and the selective pressure associated with increased colistin administration in our healthcare setting. Whole-genome sequencing revealed that the vast majority of ColR-hv-CRKP isolates belonged to ST11, the predominant high-risk clone in China, representing more than 70% of clinical CRKP isolates nationwide.[32,33]

Capsular typing identified KL64 and KL25 as the dominant capsule loci among the ColR-hv-CRKP isolates. Although KL64 replaced KL47 as the prevailing capsular type in China after 2016, the relatively high proportion of KL25 isolates (39.13%) observed in our study may indicate an emerging subclonal transition.[34] Distinct capsular types are known to confer varying abilities in immune evasion, environmental persistence, and transmissibility.[35] SNP analysis revealed several conserved clusters among KL25 isolates, suggesting possible recent patient-to-patient transmission. Moreover, the narrower SNP distances within the KL25 lineage compared with KL64 may reflect a recent clonal expansion and potentially higher transmissibility in the hospital setting. Consistent with our genomic observations, Wang et al demonstrated that ST11-KL25 strains display enhanced resistance to phagocytosis, superior biofilm formation, and improved survival under antibiotic pressure, features that may explain their epidemiological success.[35] However, the absence of environmental and healthcare-worker sampling in this study limits our ability to definitively determine the transmission routes, and alternative pathways cannot be excluded. The clonal expansion of ST11-KL64 and ST11-KL25 ColR-hv-CRKP strains highlights the urgent need for comprehensive infection-control strategies, including molecular surveillance for early detection, strict adherence to contact-precaution protocols, prudent use and reduced duration of invasive procedures, and implementation of reinforced protective measures for high-risk patient populations.[36]

Genomic analysis revealed that colistin resistance in our ColR-hv-CRKP isolates was primarily mediated by chromosomal mutations rather than *mcr* genes. Mutations in the two-component regulatory systems PmrA/B and PhoP/Q, along with disruptions in the regulator *mgrB*, were identified in 88.04% of isolates. The predominance of the PmrB Thr157Pro substitution (19.57%) is particularly notable and consistent with previous studies identifying this mutation as a recurrent mutation associated with colistin resistance.[37–39] The PmrB alteration modifies lipid A structure by upregulating the *pmrHFIJKLM* operon, thereby reducing the electrostatic interaction between polymyxins and the bacterial outer membrane.[37,40] Another common mechanism identified was ISKpn26-mediated *mgrB* gene inactivation (15.22% of isolates). ISKpn26 was the most prevalent insertion sequence in our collection and has been experimentally demonstrated to disrupt *mgrB* and thereby confer colistin resistance.[41,42] Moreover, ISKpn26-carrying plasmids often harbor carbapenemase genes, contributing to the development of extensively drug-resistant *K. pneumoniae* phenotypes.[43] In addition, ISKpn26-mediated *mgrB* disruption, *mgrB* Gln30**pmrA* Gly53Ser, *pmrA* Gly53Val, *pmrA* Gly53Ala, *pmrB* Thr157Pro, *pmrB* Ser203Pro, *pmrB* Asp150His, and *pmrB* Asp150Tyr have been experimentally validated to contribute to colistin resistance.[37,42,44–49] Several mutations identified in this study, including *mgrB* Trp6**mgrB* Cys16Arg, *phoQ* Arg16Cys, *phoQ* Val450Asp, *phoQ* Asn378Tyr, *pmrB* Pro95Leu, *pmrB* Asp150Val, *pmrB* Ser270Arg, *pmrB* Ala246Thr, and *pmrB* Leu339fs require further experimental validation to clarify their specific contributions to colistin resistance. No *mcr* genes were detected in any of the isolates, a finding that diverges from several international reports.[50,51] This pattern likely reflects the epidemiological context in China, where *mcr*-mediated resistance may be less prevalent due to the ban on colistin as a growth promoter in animal husbandry since 2017.[52] The low prevalence of *mcr* gene in hv-CRKP may be attributed to the associated fitness cost and reduced stability of the plasmid after carrying *mcr*.[53] The diversity of chromosomal mutations observed indicates that multiple evolutionary routes to colistin resistance may exist within the ST11 lineage under similar selective pressures.

Multivariate analysis identified prior colistin exposure as the strongest independent predictor of ColR-hv-CRKP infection (OR = 4.576, $P < 0.001$). This finding is consistent with previous case–control studies demonstrating that colistin exposure independently increases the likelihood of the in vivo emergence of colistin resistance in CRKP and other Enterobacteriaceae.[54,55] Although colistin use imposes substantial selective pressure and should not be underestimated as a driver of resistance, horizontal transmission of ColR-CRKP may also contribute to the increasing detection of these isolates.[56] These observations suggest that colistin resistance may develops through two complementary pathways: selection during polymyxin therapy and transmission between patients, underscoring the importance of both antimicrobial stewardship and rigorous infection-control measures. Isolation from sputum specimens was also independently associated with ColR-hv-CRKP infection (OR = 2.444, P = 0.003), which may reflect the antimicrobial selective pressure associated with pulmonary infections rather than a direct causal relationship between respiratory infection itself and colistin resistance. This association may be partly explained by the suboptimal alveolar concentrations achieved by intravenously administered polymyxins, which can facilitate the emergence of colistin-resistant subpopulations during prolonged therapy. Taken together, these observations support the important role of antimicrobial selective pressure in the development of colistin resistance. Accordingly, these results underscore the importance of targeted antimicrobial stewardship aimed at prudent colistin use, as well as strengthened surveillance of high-risk patients to enable early detection and timely infection-control interventions.[57]

The universal presence of key virulence factors, including the aerobactin biosynthetic cluster (*iucABCD/iutA*) and the hypermucoidy regulator *rmpA2*, confirms the hypervirulent nature of ColR-hv-CRKP isolates. The aerobactin-mediated iron acquisition system, almost universally detected in this collection, is a critical virulence determinant that promotes bacterial growth under iron-limited conditions and facilitates invasive infection. The absence of the salmochelin operon (*iroBCDN*) in ST11-KL64/25 suggests that these isolates may represent a distinct evolutionary lineage from the classical hypervirulent K1 and K2 serotypes, which typically harbor both siderophore systems.[58] The convergence of extensive drug resistance and hypervirulence therefore represents a particularly alarming evolutionary trend in *K. pneumoniae*, potentially enabling these strains to evade antimicrobial therapy while causing more severe and tissue-invasive infections.[59]

This study has several limitations. First, the relatively small number of colistin-resistant isolates and the lack of hospital environmental samples limited our ability to fully elucidate the transmission dynamics. Second, although whole-genome sequencing provided valuable insights into the genetic basis of colistin resistance, future studies should incorporate experimental validation to confirm the functional effects of the identified mutations. Third, colistin resistance in 11 isolates could not be explained by currently recognized mechanisms, highlighting an important knowledge gap and

https://doi.org/10.2147/IDR.S582373

suggesting the existence of additional or unconventional resistance pathways. Fourth, clinical outcomes were not systematically assessed in this study; therefore, the potential association between colistin resistance and patient prognosis could not be determined.

Conclusions

This study demonstrates that ColR-hv-CRKP is primarily driven by chromosomal mutations, particularly in *mgrB* and the two-component regulatory systems, rather than through acquisition of *mcr* genes. The predominance of the ST11-KL64 and ST11-KL25 lineages, along with evidence of recent clonal expansion and hospital transmission, highlights their epidemic potential. Prior colistin exposure was the strongest independent predictor of ColR-hv-CRKP infection, emphasizing the selective pressure exerted by colistin use. These findings emphasize the critical importance of strengthened antimicrobial stewardship and infection-control measures, as well as continued genomic surveillance and functional validation of resistance mechanisms, to mitigate the further dissemination of these high-risk clones.

Data Sharing Statement

The Illumina sequences of all isolates are available in the NCBI database (Accession number: PRJNA1261662).

Ethical Statement

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University. Written informed consent was obtained from all participants prior to their inclusion in the study. To ensure confidentiality, all patient data were anonymized, and no identifying information is contained within the manuscript.

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.

Funding

This research was supported by the Ganpo Talents Program-Innovative High-End Talent Project (Grant Number gpyc20250230), the Sailing Project for Elite Talents of The First Affiliated Hospital of Nanchang University (Grant Number RSC-0075), the Industry-University-Researcb Innovation Fund for Chinese Universities (Grant Number 2025ZK017), the Youth Talent Scientific Research Cultivation Fund of the First Affiliated Hospital of Nanchang University, China (Grant Number YFYPY202413), and the Science and Technology Research Project of the Jiangxi Provincial Department of Education, China (Grant Number GJJ2400132).

Disclosure

The authors declare no competing interests.

References

1. Effah CY, Sun T, Liu S. et al. *Klebsiella pneumoniae*: an increasing threat to public health. *Ann Clin Microbiol Antimicrob*. 2020;19(1):1. doi:10.1186/s12941-019-0343-8
2. Paczosa MK, Mecsas J. *Klebsiella pneumoniae*: going on the Offense with a Strong Defense. *Microbiol Mol Biol Rev*. 2016;80(3):629–661. doi:10.1128/MMBR.00078-15
3. Russo TA, Olson R, Fang C-T, et al. Identification of Biomarkers for Differentiation of Hypervirulent *Klebsiella pneumoniae* from Classical *K. pneumoniae*. *J Clin Microbiol*. 2018;56(9):e00776. doi:10.1128/JCM.00776-18
4. Choby JE, Howard-Anderson J, Weiss DS. Hypervirulent *Klebsiella pneumoniae* – clinical and molecular perspectives. *J Intern Med*. 2020;287 (3):283–300. doi:10.1111/joim.13007
5. Pu D, Zhao J, Chang K, et al. "Superbugs" with hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*: the rise of such emerging nosocomial pathogens in China. *Sci Bull (Beijing)*. 2023;68(21):2658–2670. doi:10.1016/j.scib.2023.09.040

6. Al Ismail D, Campos-Madueno EI, Donà V, et al. Hypervirulent *Klebsiella pneumoniae* (hvKp): overview, Epidemiology, and Laboratory Detection. *Pathog Immun*. 2025;10(1):80–119. doi:10.20411/pai.v10i1.777
7. Li L, Li S, Wei X, et al. Infection with Carbapenem-resistant Hypervirulent *Klebsiella pneumoniae*: clinical, virulence and molecular epidemiological characteristics. *Antimicrob Resist Infect Control*. 2023;12(1):124. doi:10.1186/s13756-023-01331-y
8. Mohapatra SS, Dwibedy SK, Padhy I. Polymyxins, the last-resort antibiotics: mode of action, resistance emergence, and potential solutions. *J Biosci*. 2021;46(3):85. doi:10.1007/s12038-021-00209-8
9. Liu X, Wu Y, Zhu Y, et al. Emergence of colistin-resistant hypervirulent *Klebsiella pneumoniae* (CoR-HvKp) in China. *Emerg Microbes Infect*. 2022;11(1):648–661. doi:10.1080/22221751.2022.2036078
10. Sabala RF, Fukuda A, Nakajima C, et al. Carbapenem and colistin-resistant hypervirulent *Klebsiella pneumoniae*: an emerging threat transcending the egyptian food chain. *J Infect Public Health*. 2024;17(6):1037–1046. doi:10.1016/j.jiph.2024.04.010
11. Falagas ME, Rafailidis PI, Matthaiou DK. Resistance to polymyxins: mechanisms, frequency and treatment options. *Drug Resist Updat*. 2010;13(4–5):132–138. doi:10.1016/j.drup.2010.05.002
12. Yang S, Wang H, Zhao D, et al. Polymyxins: recent advances and challenges. *Front Pharmacol*. 2024;15:1424765. doi:10.3389/fphar.2024.1424765
13. Ayoub Moubareck C. Polymyxins and Bacterial Membranes: a Review of Antibacterial Activity and Mechanisms of Resistance. *Membranes (Basel)*. 2020;10(8):181. doi:10.3390/membranes10080181
14. Hamel M, Rolain J-M, Baron SA. The History of Colistin Resistance Mechanisms in Bacteria: progress and Challenges. *Microorganisms*. 2021;9(2):442. doi:10.3390/microorganisms9020442
15. Ding Y, Hao J, Xiao W, et al. Role of efflux pumps, their inhibitors, and regulators in colistin resistance. *Front Microbiol*. 2023;14:1207441. doi:10.3389/fmicb.2023.1207441
16. Performance Standards for Antimicrobial Susceptibility Testing. In: *CLSI Document M100*. 34th edn. Clinical and Laboratory Standards Institute 59-68. 2024
17. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–2120. doi:10.1093/bioinformatics/btu170
18. Vasilinetc I, Prjibelski AD, Gurevich A, et al. Assembling short reads from jumping libraries with large insert sizes. *Bioinformatics*. 2015;31(20):3262–3268. doi:10.1093/bioinformatics/btv337
19. Gurevich A, Saveliev V, Vyahhi N, et al. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29(8):1072–1075. doi:10.1093/bioinformatics/btt086
20. Schwengers O, Jelonek L, Dieckmann MA, et al. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microb Genom*. 2021;7(11):000685. doi:10.1099/mgen.0.000685
21. Lam MMC, Wick RR, Judd LM, et al. Kaptive 2.0: updated capsule and lipopolysaccharide locus typing for the *Klebsiella pneumoniae* species complex. *Microb Genom*. 2022;8(3):000800. doi:10.1099/mgen.0.000800
22. Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*. 2020;75(12):3491–3500. doi:10.1093/jac/dkaa345
23. Liu B, Zheng D, Zhou S, et al. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res*. 2022;50(D1):D912–D917. doi:10.1093/nar/gkab1107
24. Lam MMC, Wick RR, Watts SC, et al. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. *Nat Commun*. 2021;12(1):4188. doi:10.1038/s41467-021-24448-3
25. Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res*. 2015;43(3):e15. doi:10.1093/nar/gku1196
26. Page AJ, Taylor B, Delaney AJ, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom*. 2016;2(4):e000056. doi:10.1099/mgen.0.000056
27. Zhang Y, Chen C, Wu J, et al. Sequence-Based Genomic Analysis Reveals Transmission of Antibiotic Resistance and Virulence among Carbapenemase-Producing *Klebsiella pneumoniae* Strains. *mSphere*. 2022;7(3):e0014322. doi:10.1128/msphere.00143-22
28. Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: new Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol Biol Evol*. 2020;37(5):1530–1534. doi:10.1093/molbev/msaa015
29. Letunic I, Bork P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res*. 2024;52(W1):W78–W82. doi:10.1093/nar/gkae268
30. Cingolani P. Variant Annotation and Functional Prediction: snpEff. *Methods Mol Biol*. 2022;2493:289–314.
31. Jian Z, Liu Y, Wang Z, et al. Prevalence and molecular characteristics of colistin-resistant isolates among carbapenem-resistant *Klebsiella pneumoniae* in Central South China: a multicenter study. *Annals of Clinical Microbiology and Antimicrobials*. 2025;24(1):1. doi:10.1186/s12941-024-00769-1
32. Xie M, Yang X, Xu Q, et al. Clinical evolution of ST11 carbapenem resistant and hypervirulent *Klebsiella pneumoniae*. *Commun Biol*. 2021;4(1):650. doi:10.1038/s42003-021-02148-4
33. Liao W, Liu Y, Zhang W. Virulence evolution, molecular mechanisms of resistance and prevalence of ST11 carbapenem-resistant *Klebsiella pneumoniae* in China: a review over the last 10 years. *J Glob Antimicrob Resist*. 2020;23:174–180. doi:10.1016/j.jgar.2020.09.004
34. Zhou K, Xiao T, David S, et al. Novel Subclone of Carbapenem-Resistant *Klebsiella pneumoniae* Sequence Type 11 with Enhanced Virulence and Transmissibility, China. *Emerg Infect Dis*. 2020;26(2):289–297. doi:10.3201/eid2602.190594
35. Gao S, Jin W, Quan Y, et al. Bacterial capsules: occurrence, mechanism, and function. *NPJ Biofilms Microbiomes*. 2024;10(1):21. doi:10.1038/s41522-024-00497-6
36. Chang D, Sharma L, Dela Cruz CS, et al. Clinical Epidemiology, Risk Factors, and Control Strategies of *Klebsiella pneumoniae* Infection. *Front Microbiol*. 2021;12:750662. doi:10.3389/fmicb.2021.750662
37. Jayol A, Poirel L, Brink A, et al. Resistance to colistin associated with a single amino acid change in protein PmrB among *Klebsiella pneumoniae* isolates of worldwide origin. *Antimicrob Agents Chemother*. 2014;58(8):4762–4766. doi:10.1128/AAC.00084-14
38. Cheng Y-H, Lin T-L, Pan Y-J, et al. Colistin Resistance Mechanisms in *Klebsiella pneumoniae* Strains from Taiwan. *Antimicrob Agents Chemother*. 2015;59(5):2909–2913. doi:10.1128/AAC.04763-14

39. Elias R, Spadar A, Phelan J, et al. A phylogenomic approach for the analysis of colistin resistance-associated genes in *Klebsiella pneumoniae*, its mutational diversity and implications for phenotypic resistance. *Int J Antimicrob Agents*. 2022;59(6):106581. doi:10.1016/j.ijantimicag.2022.106581
40. Gunn JS, Ryan SS, Van Velkinburgh JC, et al. Genetic and Functional Analysis of a PmrA-PmrB-Regulated Locus Necessary for Lipopolysaccharide Modification, Antimicrobial Peptide Resistance, and Oral Virulence of Salmonella enterica Serovar Typhimurium. *Infect Immun*. 2000;68(11):6139–6146. doi:10.1128/IAI.68.11.6139-6146.2000
41. Chen X, Li P, Sun Z, et al. Insertion sequence mediating mrgB disruption is the major mechanism of polymyxin resistance in carbapenem-resistant *Klebsiella pneumoniae* isolates from China. *J Glob Antimicrob Resist*. 2022;30:357–362. doi:10.1016/j.jgar.2022.07.002
42. Yang T-Y, Wang S-F, Lin J-E, et al. Contributions of insertion sequences conferring colistin resistance in *Klebsiella pneumoniae*. *Int J Antimicrob Agents*. 2020;55(3):105894. doi:10.1016/j.ijantimicag.2020.105894
43. Fordham SME, Mantzouratou A, Sheridan E. Prevalence of insertion sequence elements in plasmids relating to mgrB gene disruption causing colistin resistance in *Klebsiella pneumoniae*. *Microbiologyopen*. 2022;11(1):e1262. doi:10.1002/mbo3.1262
44. Kong Y, Li C, Chen H, et al. In vivo Emergence of Colistin Resistance in Carbapenem-Resistant *Klebsiella pneumoniae* Mediated by Premature Termination of the mgrB Gene Regulator. *Front Microbiol*. 2021;12:656610. doi:10.3389/fmicb.2021.656610
45. Diene SM, Merhej V, Henry M, et al. The rhizome of the multidrug-resistant Enterobacter aerogenes genome reveals how new "killer bugs" are created because of a sympatric lifestyle. *Mol Biol Evol*. 2013;30(2):369–383. doi:10.1093/molbev/mss236
46. Froelich JM, Tran K, Wall D. A pmrA constitutive mutant sensitizes Escherichia coli to deoxycholic acid. *J Bacteriol*. 2006;188(3):1180–1183. doi:10.1128/JB.188.3.1180-1183.2006
47. Janssen AB, Bartholomew TL, Marciszewska NP, et al. Nonclonal Emergence of Colistin Resistance Associated with Mutations in the BasRS Two-Component System in Escherichia coli Bloodstream Isolates. *mSphere*. 2020;5(2):e00143. doi:10.1128/mSphere.00143-20
48. Xiao Y, Zhang J, Nie F, et al. Epidemiological characterization of chromosome-mediated colistin resistance in hypervirulent carbapenem-resistant *Klebsiella pneumoniae*. *BMC Microbiol*. 2025;26(1):65. doi:10.1186/s12866-025-04615-y
49. Jiménez-Castellanos J-C, Waclaw B, Meynert A, et al. Rapid evolution of colistin resistance in a bioreactor model of infection of *Klebsiella pneumoniae*. *Commun Biol*. 2024;7(1):794. doi:10.1038/s42003-024-06378-0
50. Hatrongjit R, Wongsurawat T, Jenjaroenpun P, et al. Genomic analysis of carbapenem- and colistin-resistant *Klebsiella pneumoniae* complex harbouring mcr-8 and mcr-9 from individuals in Thailand. *Sci Rep*. 2024;14(1):16836. doi:10.1038/s41598-024-67838-5
51. Gharaibeh MH, Alyafawi DA, Elnasser ZA, et al. Emergence of mcr-1 gene and carbapenemase-encoding genes among colistin-resistant *Klebsiella pneumoniae* clinical isolates in Jordan. *J Infect Public Health*. 2022;15(8):922–929. doi:10.1016/j.jiph.2022.07.005
52. Wang Y, Xu C, Zhang R, et al. Changes in colistin resistance and mcr-1 abundance in Escherichia coli of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study. *Lancet Infect Dis*. 2020;20(10):1161–1171. doi:10.1016/S1473-3099(20)30149-3
53. Nang SC, Morris FC, McDonald MJ, et al. Fitness cost of mcr-1-mediated polymyxin resistance in *Klebsiella pneumoniae*. *J Antimicrob Chemother*. 2018;73(6):1604–1610. doi:10.1093/jac/dky061
54. Huang P-H, Cheng Y-H, Chen W-Y, et al. Risk factors and mechanisms of in vivo emergence of colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*. *International Journal of Antimicrobial Agents*. 2021;57(6):106342. doi:10.1016/j.ijantimicag.2021.106342
55. Drozdinsky G, Ben-Zvi H, Kushnir S, et al. Colistin exposure as a risk factor for infections caused by inherently colistin resistant Enterobacteriaceae-a case-control study. *Clin Microbiol Infect*. 2018;24(8):896–899. doi:10.1016/j.cmi.2017.11.022
56. Zarkotou O, Pournaras S, Voulgari E, et al. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing *Klebsiella pneumoniae*: a matched case-control study. *J Clin Microbiol*. 2010;48(6):2271–2274. doi:10.1128/JCM.02301-09
57. European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP), Tsuji BT, Pogue JM, Zavascki AP, et al. International Consensus Guidelines for the Optimal Use of the Polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP). *Pharmacotherapy*. 2019;39(1):10–39. doi:10.1002/phar.2209.
58. Mendes G, Santos ML, Ramalho JF, et al. Virulence factors in carbapenem-resistant hypervirulent *Klebsiella pneumoniae*. *Front Microbiol*. 2023;14:1325077. doi:10.3389/fmicb.2023.1325077
59. Li C, Pan D, Li M, et al. Aerobactin-Mediated Iron Acquisition Enhances Biofilm Formation, Oxidative Stress Resistance, and Virulence of Yersinia pseudotuberculosis. *Front Microbiol*. 2021;12:699913. doi:10.3389/fmicb.2021.699913