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ORIGINAL RESEARCH

Risk Factors and Molecular Epidemiology of Infections Among Patients with Intestinal Colonization by Carbapenem-Resistant *Enterobacteriaceae*: A Retrospective Study of Active Screening in a Tertiary Hospital

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Backgroud: To investigate the molecular epidemiology of intestinal colonization by carbapenem-resistant *Enterobacteriaceae* (CRE) and identify risk factors for subsequent infection, providing evidence for early risk stratification and targeted prevention.

Methods: From August 2023 to August 2024, we retrospectively enrolled CRE-positive patients identified through active rectal swab screening at the First Affiliated Hospital of Kunming Medical University and monitored them for subsequent infections. Colonizing and infecting isolates were collected and tested for carbapenem-resistance genes, major virulence genes, capsular serotypes, and were subjected to multilocus sequence typing (MLST). Clinical data were integrated and multivariate logistic regression was performed to identify risk factors associated with secondary infections.

Results: Among 8,088 patients who underwent active intestinal CRE screening, the positivity rate was 0.53% (43/8,088). Among patients with colonization, the incidence of secondary infection was 37.2% (16/43). All patients with secondary infections were colonized and infected with *Klebsiella pneumoniae*, with the lower respiratory tract, bloodstream, and urinary tract being the primary infection sites. Multivariable analysis showed that having more than three comorbidities was an independent risk factor for hospital-acquired infection among colonized patients (odds ratio [OR]=0.118; 95% CI:0.017–0.812; P=0.030). The carriage rate of bla_{KPC} was 77.8% among colonizing strains and 100% among infecting strains. Among virulence genes, *aerobactin, allS*, and *peg344* were significantly more prevalent in infecting strains (P<0.05). Homology analysis revealed that, except for one patient, the colonizing and infecting isolates in patients with secondary infections were highly homologous ST11-KL64, KPC-producing *K. pneumoniae*.

Conclusion: Although the intestinal colonization rate of CRE was relatively low, the risk of secondary infection remained substantial. Bacterial genetic traits and host conditions contribute to secondary infections. Establishing surveillance systems based on clinical and molecular epidemiology coupled with intensified screening in high-risk departments may help identify high-risk patients early and enable proactive interventions to reduce CRE-related secondary infections.

Keywords: intestinal colonization, carbapenem-resistant *Enterobacteriaceae*, active screening, virulence genes, clonal homology

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) have been designated "critical priority" pathogens by the World Health Organization because of their severe antimicrobial resistance, high pathogenicity, rapid dissemination, and

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significant public health impact.[1] CRE Infections are often associated with limited treatment options, prolonged hospitalization, poor clinical outcomes, and mortality rates as high as 40%–50%.[2] The gastrointestinal tract serves as the primary ecological reservoir for CRE. Colonization is promoted by healthcare exposure, compromised host immunity, and extensive antibiotic use, and may lead to subsequent extraintestinal infections—including bloodstream and respiratory tract infections—through translocation of colonizing strains.[3–5] Growing evidence indicates a high degree of clonal relatedness between colonizing and infecting isolates, underscoring the pivotal role of intestinal colonization as a key source of nosocomial CRE infections.[6,7] Active rectal CRE screening, combined with contact precautions and environmental disinfection, constitutes an effective "surveillance–intervention" strategy to curb nosocomial transmission. However, comprehensive data on the epidemiological characteristics, resistance mechanisms, and virulence profiles of CRE among colonized patients—particularly factors influencing progression from colonization to infection—remain limited.

Therefore, we conducted a comprehensive investigation based on active CRE surveillance and infection monitoring at a tertiary hospital between August 2023 and August 2024. This study systematically examined the epidemiological characteristics and distribution of resistance and virulence genes in colonizing and infecting CRE isolates. We also evaluated risk factors associated with subsequent infections in patients with CRE. The findings are expected to enhance the early identification of high-risk patients, strengthen nosocomial transmission alert systems, and provide an evidence-based foundation for targeted infection control and individualized therapeutic strategies.

Materials and Methods

Strain Collection

Rectal swab samples were collected from CRE-positive patients during active surveillance at the First Affiliated Hospital of Kunming Medical University between August 2023 and August 2024. Infection status was continuously monitored during hospitalization, and CRE strains were isolated from infection-related specimens.

Inclusion criteria: (1) patients who underwent active CRE rectal swab screening within 48 hours of admission; (2) for multiple hospitalizations, only the first positive screening result was included; (3) for patients with two or more positive screenings during one hospitalization, only the first positive sample was analyzed; and (4) infectious episodes meeting the diagnostic criteria for nosocomial infections.[8]

Exclusion criteria: (1) patients unable to undergo screening because of severe diarrhea or similar conditions; (2) specimens of poor quality or contamination; (3) discordance in bacterial species between colonizing and infecting isolates; and (4) repeated isolation of strains from the same anatomical site in a single patient.

Patient Grouping

Patients with CRE detected by rectal swab but without subsequent infection were defined as the colonization group, whereas those who developed clinical infection were defined as the infection group. Colonization-only isolates were obtained from patients who remained colonized without infection, while colonizing isolates from infected patients and infecting isolates were both isolated from patients who subsequently developed CRE infections. All isolates were subjected to antimicrobial susceptibility testing and screening for virulence and resistance genes. In addition, isolates from the infection group were further analyzed by molecular typing to assess their genetic relatedness.

Clinical Data Collection

Clinical and microbiological data were retrospectively retrieved from the Laboratory Information System (LIS) and Electronic Medical Record (EMR) of the First Affiliated Hospital of Kunming Medical University, for all patients screened for intestinal CRE between August 2023 and August 2024. Extracted information included microbiological results, antimicrobial administration records, surgical and procedural notes, discharge summaries, and nursing documentation.

The following variables were assessed:

(1) Demographic and hospitalization details: Age, sex, admission route (emergency vs transfer), admission department, ICU admission, length of hospital stay, and primary diagnosis.
(2) Underlying diseases and comorbidities: Conditions were categorized by organ system (cardiovascular, respiratory, neurological, gastrointestinal, cerebrovascular, genitourinary, endocrine, and immune). Chronic illnesses such as diabetes, hypertension, chronic kidney disease, and malignancy were recorded as comorbidities. The number of comorbidities and the proportion of patients with ≥ three comorbidities were documented.
(3) Prior healthcare exposure: hospitalization within 6 months, surgery within 30 days, or previous ICU admission before colonization detection.
(4) Invasive procedures and device use: presence of a central venous catheter, urinary catheter, endotracheal intubation or tracheostomy, mechanical ventilation, enteral feeding tube, or drainage devices.
(5) Antimicrobial therapy: systemic antimicrobial agents administered after CRE detection were categorized by class (carbapenems, cephalosporins, fluoroquinolones, aminoglycosides, polymyxins, tigecycline, and ceftazidime–avibactam), as well as antifungal and antiviral agents.

All variables were recorded relative to the date of the first CRE-positive rectal swab. Data accuracy was verified independently by two reviewers to ensure data integrity.

Bacterial Identification and Antimicrobial Susceptibility Testing

Identification of colonizing and infecting isolates was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, USA).[9] Antimicrobial susceptibility testing was conducted according to the 2023 guidelines of the Clinical and Laboratory Standards Institute (CLSI). The Kirby-Bauer disk diffusion method was used for most antibiotics, while the minimum inhibitory concentrations (MICs) of tigecycline, polymyxin B, and ceftazidime-avibactam were determined by broth microdilution. Antimicrobial susceptibility results were interpreted according to the criteria recommended in the CLSI document M100-S33.[10] For polymyxin B, interpretation followed the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org), and for tigecycline, breakpoints established by the United States Food and Drug Administration (FDA)[11] were used. *Escherichia coli* ATCC 25922, obtained from the National Center for Clinical Laboratories in China, served as the quality control strain.

Detection of Resistance Genes, Virulence Genes, and Capsular Serotypes

Bacterial genomic DNA was extracted using the boiling method as previously described.[12] Two fresh single colonies were selected and suspended in sterile distilled water or TE buffer to a total volume of 1000μL. The suspension was incubated at 100°C in a metal heat block for 10 minutes and immediately placed on ice for 2–5 minutes to facilitate debris settling and protect the DNA integrity. The mixture was then centrifuged at 13,000g for 5 min. The supernatant (avoiding the pellet) was carefully collected as the DNA template, aliquoted if necessary and stored at −20°C. Polymerase chain reaction (PCR) was used to amplify resistance and virulence genes, and multiplex PCR assays were performed to determine capsular serotypes (KL). Screened resistance genes included bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{VIM}, and $bla_{\text{OXA-48}}$. The detected virulence genes included mucoviscosity-associated genes (*rmpA, rmpA2, magA*); genes involved in lipopolysaccharide synthesis (*wabG, uge*); fimbrial-associated genes (*fimH, mrkD*); siderophore system genes (*iroB, iroN, iutA, iucA, entB, aerobactin*); urease regulation genes (*allS*), silver resistance genes (*silS*), and hypervirulence plasmid marker genes (*peg344*). The capsular serotypes included KL1, KL2, KL47, and KL64. Primer sequences and reaction conditions were optimized according to previously published studies.[13–16] Positive PCR products were verified using agarose gel electrophoresis and visualized by gel imaging. The products were sequenced by Beijing Biomed Gene Technology Company, and the resulting sequences were compared with reference sequences using the BLAST program to determine genotypes.

Multilocus Sequence Typing (MLST)

MLST analysis was performed according to the primer sequences and reaction conditions provided in the *Klebsiella pneumoniae* MLST database (https://bigsdb.pasteur.fr/klebsiella/). Seven housekeeping genes (*gapA, mdh, phoE, tonB, infB, pgi*, and *rpoB*) were amplified using PCR for sequence typing.

Based on the distribution patterns of virulence and resistance genes and capsular serotypes, a minimum spanning tree (MST) was constructed using MLST data to illustrate the phylogenetic relationships among isolates. The analysis was performed in R (v4.4.2) with the "ape", "igraph", and "ggtree" packages, and the tree was visualized with annotations of gene and serotype information. In addition, a heatmap clustering tree was generated using "pheatmap" and "ComplexHeatmap" to reveal co-occurrence patterns of virulence and resistance genes across isolates.

Statistical Analysis

Statistical analyses were performed using SPSS version 27.0. Categorical variables are expressed as counts and percentages. The chi-squared (χ^2) test was applied when $n \geq 40$ and $T \geq 1$; otherwise, Fisher's exact test was used when $n < 40$ or $T < 1$. Normally distributed continuous variables are presented as mean ± standard deviation ($\overline{X} \pm S$) and compared using the independent samples *t*-test. Clinical data of patients in the colonization and infection groups were analyzed. Unconditional binary logistic regression analysis was performed using a stepwise regression method to identify the risk factors associated with subsequent infection in CRE-colonized patients. Statistical significance was set at $P < 0.05$.

Results

Clinical Characteristics and Distribution of CRE Isolates

A total of 8,088 patients underwent rectal swab screening between August 2023 and August 2024, and 43 (0.53%) were positive for CRE. Among the positive isolates, 41 (95.4%) were *Klebsiella pneumoniae* and two (4.6%) were *Escherichia coli*. During follow-up, 16 patients with positive rectal swab results subsequently developed infections, and both the colonizing and infecting strains were identified as *K. pneumoniae*, corresponding to an infection rate of 37.21%. *Candida albicans* was detected in respiratory specimens from eight patients; however, based on imaging and laboratory findings, these isolates were determined to represent colonization rather than infection.

Among the 43 patients who tested positive for CRE, 27 (62.8%) were male and 16 (37.2%) were female, with a mean age of 72.49 ± 18.81 years. These patients were primarily located in the Geriatric Intensive Care Unit (25.6%) and the Department of Geriatric Respiratory Medicine (20.9%). The overall distribution of CRE-positive patients, secondary infections, and isolated bacterial species is illustrated in Figure 1.

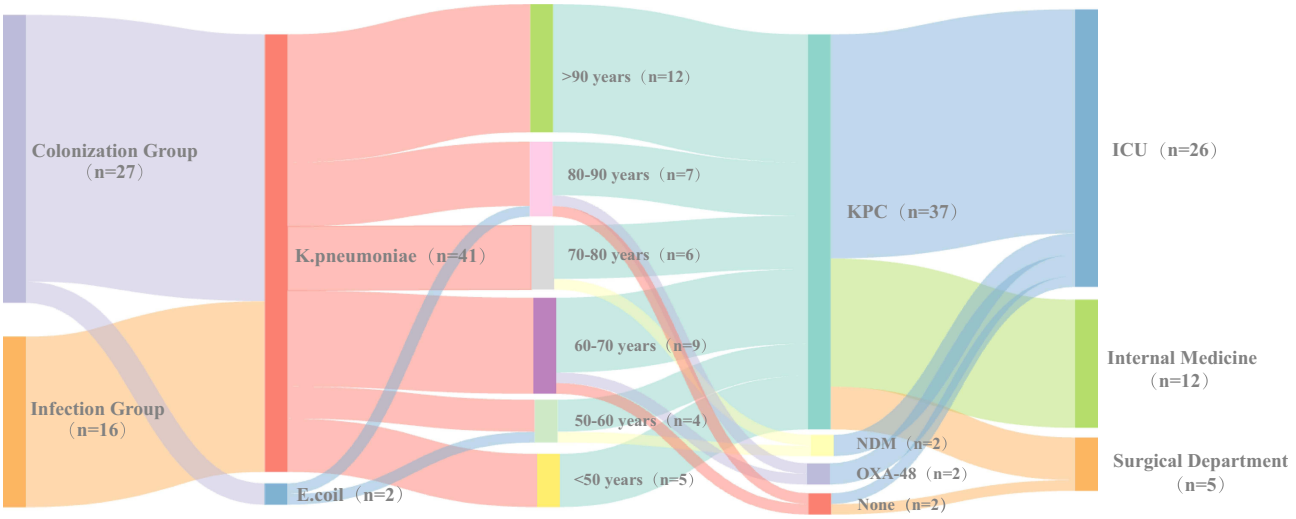

Figure 1 Distribution of CRE Colonization and Infection.

Antimicrobial Susceptibility Profiles

Among the 43 CRE isolates, a high levels of resistance were observed to several commonly used clinical antibiotics, including ampicillin, cefuroxime, cefoperazone/sulbactam, and piperacillin/tazobactam. Resistance rates of the colonizing isolates to tigecycline, polymyxin B, and ceftazidime/avibactam were 0%, 7.4%, and 7.4%, respectively, whereas the corresponding rates for the infecting isolates were 6.3%, 12.5%, and 0%, respectively. Except for minocycline (P= 0.010), no statistically significant differences in antimicrobial susceptibility were observed between the colonizing and infecting isolates (P> 0.05) (Table 1 and Table S1).

Clinical Risk Factor Analysis

Among the 16 patients colonized with CRE, 24 infection episodes occurred at different sites. The most common types of infection were lower respiratory tract (58.3%), bloodstream (20.8%), and urinary tract infections (16.7%). Of the infected patients, seven died in the hospital or were discharged against medical advice. Patients in the colonization group had a more frequent history of prior hospitalization than those in the infection group (92.6% vs 56.3%; P=0.005). Infected patients were more likely to have multiple underlying comorbidities (93.8% vs 37.0%; P<0.001) and high-risk features, such as frequent exposure to multiple antimicrobial agents (68.8% vs 29.6%; P=0.013) (Table 2).

Binary logistic regression analysis identified having more than three underlying comorbidities (odds ratio [OR] =0.118, 95% confidence interval [CI]: 0.017–0.812, P=0.030) as independent risk factors for hospital-acquired CRE infection among patients with intestinal colonization (Table S2).

Molecular Epidemiological and Genomic Correlation Analysis

Overall Genetic Profiles of CRE Isolates

PCR-based screening identified major carbapenemase genes among the CRE isolates, with detection rates of bla_{KPC} (77.8%), bla_{NDM} (7.4%), and bla_{OXA-48} (7.4%); carbapenemase-negative isolates accounted for 7.4%. In the infection group, bla_{KPC} was the only carbapenemase detected, with a 100% positivity rate. The carriage rate of bla_{KPC} in the infection group was significantly higher than that in the colonization group (P=0.042), whereas no statistically significant differences were observed for other resistance genes (P>0.05).

Table 1 Antimicrobial Susceptibility Results of Rectal Swabs from 43 CRE Active Screening-Positive Patients

Categories	Antimicrobial Agents	Colonization Group (n=27)		Infection Group (n=16)		x^2	*P*-value
		Resistance Rate [Number (%)]	Susceptibility Rate [Number (%)]	Resistance Rate [Number (%)]	Susceptibility Rate [Number (%)]		
Monobactams	ATM	25 (92.6%)	2 (7.4%)	16 (100%)	0 (0%)	1.243	0.265
Carbapenems	IPM	22 (88.9%)	1 (3.7%)	16 (100%)	0 (0%)	0.656	0.418
	MEM	25 (92.6%)	0 (0%)	16 (100%)	0 (0%)	–	–
	ETP	27 (100%)	0 (0%)	16 (100%)	0 (0%)	–	–
Tetracyclines	DOX	18 (66.7%)	5 (18.5%)	12 (75%)	2 (12.5%)	0.315	0.575
	MN	13 (48.1%)	9 (33.3%)	12 (75%)	0 (0%)	6.676	**0.01**
	TGC	0 (0%)	27 (100%)	1 (6.3%)	15 (93.7%)	1.728	0.189
Polypeptides	POL	2 (7.4%)	25 (92.6%)	2 (12.5%)	14 (87.5%)	0.309	0.578
β-lactam/β-lactamase inhibitors	CZA	2 (7.4%)	25 (92.6%)	0 (0%)	16 (100%)	1.243	0.262

Note: "-" indicates no data; values in bold indicate P< 0.05.

Abbreviations: ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; ETP, Ertapenem; DOX, Doxycycline; MN, Minocycline; TGC, Tigecycline; POL, Polymyxin B; CZA, Ceftazidime-avibactam; CSL, Cefazolin; TZP, Piperacillin-tazobactam; SAM, Ampicillin-sulbactam.

Table 2 Clinical Characteristics Analysis of Colonization Group and Infection Group

Variable		Colonization Group (n=27)	Infection Group (n=16)	x^2/t	*P*-value
Age		71.3±19.903	74.5±17.918	−0.529	0.6
Gender	Male	15	10	0.199	0.655
	Female	12	6		
Department distribution	Intensive Care Unit (ICU)	18	8	1.167	0.28
	Neurosurgery Department	3	2	0.019	0.891
	Internal Medicine Department	6	6	1.166	0.28
6-month medical history	Hospitalization history	25	9	8.018	**0.005**
	ICU hospitalization history	14	6	0.832	0.362
	Surgical history	12	4	1.626	0.202
Underlying diseases	Cardiovascular disease	17	11	0.148	0.7
	Diabetes mellitus	9	7	0.467	0.495
	Malignant tumor	3	2	0.019	0.891
	Coma	4	0	2.613	0.106
	Comorbidity with more than three diseases	10	15	13.277	**<0.001**
Medication history	Proton pump inhibitors	18	13	1.062	0.303
	Glucocorticoid	9	11	5.065	**0.024**
	Carbapenems	8	9	2.978	0.084
	Polymyxin B	3	2	0.019	0.891
	Tigecycline	10	8	0.694	0.405
	Ceftazidime-avibactam	6	6	1.166	0.28
	Antifungal agents	8	8	1.784	0.182
	Antiviral agents	4	4	0.688	0.407
	Use of ≥3 kinds of antimicrobial agents	8	11	6.234	**0.013**
Invasive procedures	Surgery	11	2	3.799	0.051
	Endotracheal intubation	11	6	0.044	0.834
	Urinary catheterization	12	7	0.002	0.965
	Gastric tube	13	3	3.716	0.054
Prognosis	Positive	18	9	0.467	0.495
	Negative	9	7		
Length of hospital stay >30 days		9	8	1.167	0.28

Note: Values in bold indicate P< 0.05.

Virulence gene analysis revealed uniformly high detection rates of *iroN* (100%/100%), *entB* (96.3%/100%), *wabG* (88.9%/100%), *uge* (88.9%/100%), *fimH* (88.9%/100%), and *mrkD* (88.9%/87.5%) in both colonizing and infecting isolates. The carriage rates of *aerobactin, allS*, and *peg344* were significantly higher in infecting isolates than in

colonizating ones (P<0.05). The overall virulence gene profile revealed that most infecting isolates possessed multiple iron-uptake and capsule-associated determinants, possibly facilitating the transition from colonization to infection.

The predominant capsular serotype was KL64, accounting for 69.8% (30/43) of isolates, and its proportion was notably greater in the infection group (93.8%, 15/16) than in the colonization group (P=0.008).

Details regarding resistance genes, virulence genes, capsular serotypes, and MLST types for both groups are summarized in Table 3 and Table S3.

Homology Analysis of Infection Isolates (n=16)

To elucidate the molecular relationship between colonizing and subsequent infecting isolates, homology analyses were conducted on paired isolates obtained from 16 patients who developed secondary CRE infections. The analysis integrated antimicrobial resistance determinants, virulence repertoires, capsular loci, and clonal lineages based on MLST and phylogenetic inference.

Table 3 Detection of Resistance Genes, Virulence Genes and Capsular Serotypes, in Colonization and Infection Groups

		Total (n=43)	Colonization Group (n=27)	Infection Group (n=16)	x^2	*P*-value
Carbapenemase gene	*bla*$_{KPC}$	37	21 (77.8%)	16 (100%)	4.132	**0.042**
	bla$_{NDM}$	2	2 (7.4%)	0	1.243	0.265
	bla$_{OXA-48}$	2	2 (7.4%)	0	1.243	0.265
	Carbapenemase gene not detected	2	2 (7.4%)	0	1.243	0.265
Virulence gene	*rmpA*	20	11 (40.7%)	9 (56.3%)	0.971	0.324
	rmpA2	25	13 (48.1%)	12 (75.0%)	2.976	0.084
	magA	0	0	0	–	–
	wabG	40	24 (88.9%)	16 (100%)	1.911	0.167
	uge	40	24 (88.9%)	16 (100%)	1.911	0.167
	fimH	40	24 (88.9%)	16 (100%)	1.911	0.167
	mrkD	38	24 (88.9%)	14 (87.5%)	1.911	0.167
	iroB	24	13 (48.1%)	11 (68.8%)	1.729	0.189
	iroN	43	27 (100%)	16 (100%)	–	–
	iucA	26	15 (55.6%)	11 (68.8%)	0.732	0.392
	iutA	27	16 (59.3%)	11 (68.8%)	0.387	0.534
	entB	42	26 (96.3%)	16 (100%)	0.607	0.436
	aerobactin	35	19 (70.4%)	16 (100%)	5.824	**0.016**
	alls	20	9 (33.3%)	11 (68.8%)	5.065	**0.024**
	silS	25	13 (48.1%)	12 (75.0%)	2.976	0.084
	peg344	30	15 (55.6%)	15 (93.8%)	6.948	**0.008**
Capsular serotype	*KL64*	30	15 (55.6%)	15 (93.8%)	6.948	**0.008**
	KL47	11	10 (37.0%)	1 (6.3%)	5.002	**0.025**
	KL2	2	2 (7.4%)	0 (0%)	1.243	0.265

Note: "–" indicates no data; values in bold indicate P< 0.05.

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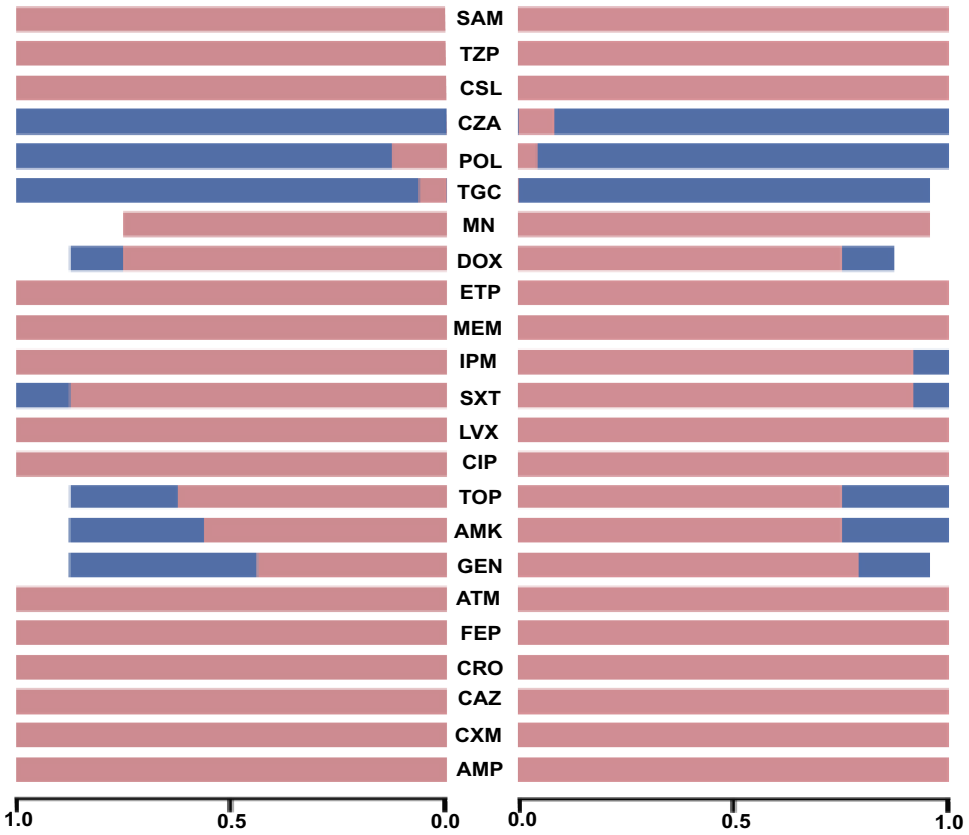

Figure 2 Antibiotic Susceptibility Profiles of 16 Pairs of Colonizing and Infecting Strains.
Notes: resistance (red) and susceptibility (blue) rates are shown; intermediate categories are not displayed. Panels depict the colonization group (left) and the infection group (right).
Abbreviations: AMP, Ampicillin; CXM, Cefuroxime; CAZ, Ceftazidime; CRO, Ceftriaxone; FEP, Cefepime; ATM, Aztreonam; GEN, Gentamicin; AMK, Amikacin; TOB, Tobramycin; CIP, Ciprofloxacin; LVX, Levofloxacin; SXT, Sulfamethoxazole/Trimethoprim; IPM, Imipenem; MEM, Meropenem; ETP, Ertapenem; DOX, Doxycycline; MN, Minocycline; TGC, Tigecycline; POL, Polymyxin B; CZA, Ceftazidime-avibactam; CSL, Cefazolin; TZP, Piperacillin-tazobactam; SAM, Ampicillin-sulbactam.

Homology analysis revealed a high degree of concordance between colonizing and infecting isolates across resistance, virulence, and genotypic features. Their antimicrobial susceptibility profiles were almost identical, particularly to β-lactams (AMP, CRO, CAZ) and aminoglycosides (GEN, AMK) (Figure 2). Resistance gene patterns were conserved in 93.8% (15/16) of paired isolates, except for one patient in whom the colonizing strain carried $bla_{\text{KPC-2}}$ while both the infecting and sputum isolates harbored $bla_{\text{OXA-48}}$. Virulence determinants were comparable, with rmpA, rmpA2, mrkD, iroB, iucA, allS, and silS detected in over 80% of isolates, and universal presence of *wabG, uge, fimH, iroN, entB, aerobactin*, and *peg344*. Capsular serotypes between paired isolates were largely identical, with two mismatches (KL64 → KL51; KL47 → KL64). MLST analysis revealed that ST11 was the predominant sequence type, accounting for 95.0% (38/40) of all isolates, followed by ST231. All colonizing isolates from the 16 patients belonged to ST11, whereas one corresponding infecting isolate showed a sequence type shift from ST11 to ST231 (Figure 3). MLST therefore identified ST11 as the dominant epidemic lineage in our center. Minimum-spanning-tree analysis demonstrated tight clustering of isolates within each patient, contrasting with substantial diversity among different patients (Figure 4). Collectively, these results suggest that most infections likely originated from patients' endogenous colonizing strains with minimal genetic divergence during transition to infection.

Discussion

This study provides molecular and epidemiological evidence supporting the progression of intestinal CRE colonization to secondary infection in hospitalized patients. Despite the low overall colonization rate (0.53%), more than one-third of

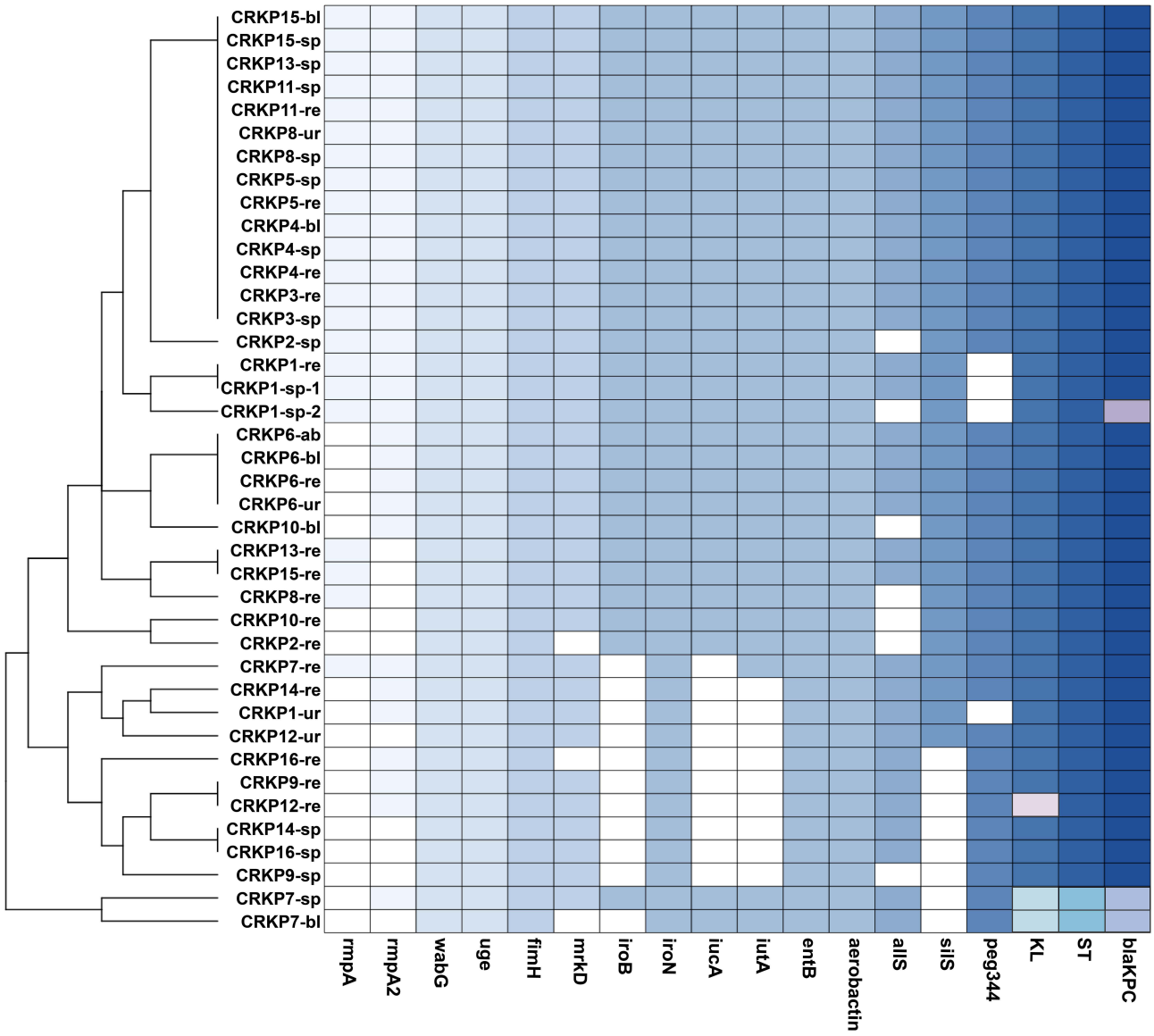

Figure 3 Distribution of Carbapenemase Genes, Virulence Genes, Capsular Serotypes, and ST Types.

Notes: From left to right, categories are encoded by columns: columns 1–15 represent virulence genes; column 16 represents serotype; column 17 represents carbapenemase resistance genes; and column 18 represents Multilocus Sequence Typing (MLST). Color scheme: virulence factors are shown using a light-to-dark blue gradient for different functional modules (capsular polysaccharide, lipopolysaccharide, fimbriae, siderophores, urease, metal resistance, and molecular markers of high--virulence plasmids); serotypes are indicated by dark blue (KL64), light purple (KL47), and light bluish-gray (KL51); MLST is distinguished by dark blue (ST11) and sky blue (ST231); carbapenemases are shown in dark blue (KPC-2), light blue-purple (KPC-33), and light purplish-blue (OXA-48). Samples sharing the same terminal number in the strain ID originate from the same patient, representing colonizing and infecting isolates.

Abbreviations: re, rectal swab; bl, whole blood; ur, urine; sp, sputum; ab,ascites; ST, Sequence Typing; KL, K-locus.

colonized individuals developed infection, most commonly in high-risk geriatric and critical care settings. These findings underscore the urgent need for targeted surveillance and tailored infection control strategies in vulnerable hospital units.

Compared with previous studies focusing[17–20] on high-risk populations such as those in medical high-care wards, immunocompromised patients, and individuals with severe burns, the intestinal CRE colonization rate observed in this study was relatively lower. This difference may be attributed to the hospital-wide surveillance design, which included all hospitalized patients, and provided a more comprehensive and realistic representation of CRE colonization levels across the hospital population. By adopting this approach, our study complements and extends previous high-risk cohort studies, offering new epidemiological evidence on CRE transmission dynamics in real-world hospital settings. These results further emphasize the importance of implementing stratified prevention and control measures, particularly in departments

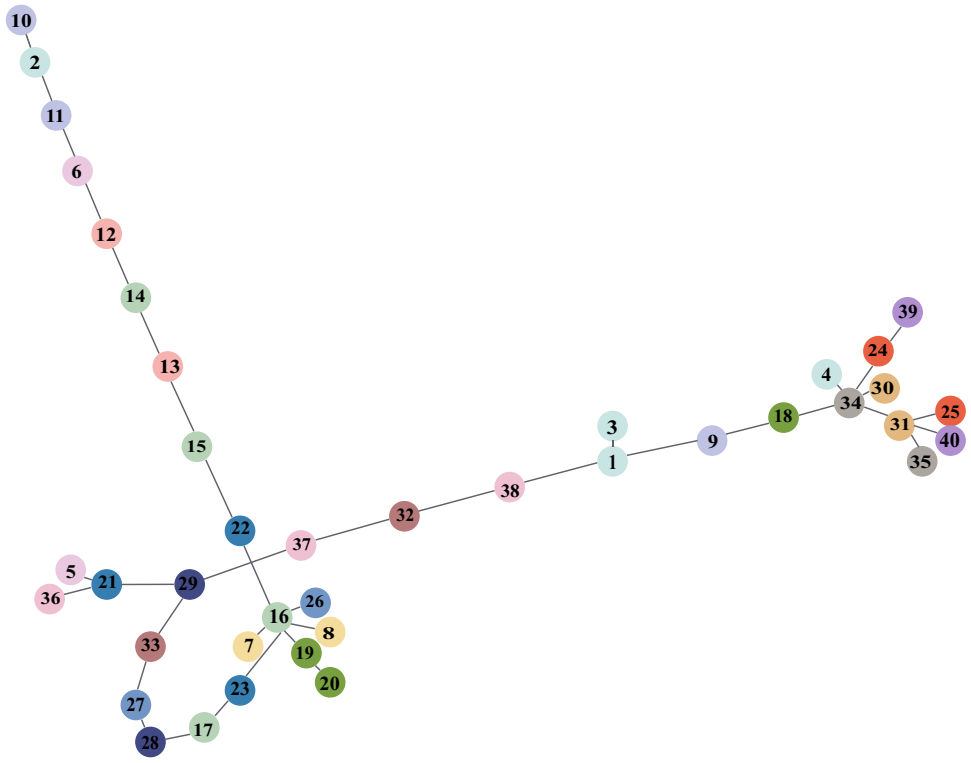

Figure 4 Minimum Spanning Tree of 40 CRKP Strains.
Notes: Nodes of the same color represent colonizing and infecting isolates from the same patients. The numbers correspond to MLST typing results.

with high disease burdens, through active CRE screening and strengthened infection control interventions to interrupt transmission chains and reduce nosocomial infection rates.

Previous studies[21–24] have shown that intestinal CRE colonization can lead to infection via dysbiosis, invasive procedures, and immunosuppression. In the present study, MLST and minimum spanning tree analyses revealed that colonizing and infecting strains from the same patient clustered closely in the phylogenetic tree, indicating high homology and supporting the "colonization-to-infection" hypothesis. Early and appropriate use of antimicrobial therapy can significantly improve clinical outcomes in patients with CRE infections. Our results demonstrated high consistency in resistance profiles between colonizing and infecting isolates for β-lactams (AMP, CRO, CAZ) and aminoglycosides (GEN, AMK), and with 93.8% (15/16) of pairs carrying identical resistance genes. These findings provide valuable guidance for clinical management. Consistent with our results, PEREZ[25] reported an 88% overall concordance between the drug susceptibility profiles of CRE isolates identified by rectal swab screening and those of clinical isolates. Therefore, when infection occurs, susceptibility data from colonizing strains may serve as a crucial reference for empirical antimicrobial therapy, helping to optimize treatment regimens and improve patient outcomes.

Regarding infection characteristics, secondary infections following intestinal CRE colonization are closely associated with invasive procedures and host factors. Interventions such as mechanical ventilation, central venous catheterization, and urinary catheterization can disrupt mucosal barriers, promote gut microbiota translocation, and lead to infections at key sites, including the lower respiratory tract, bloodstream, and urinary tract.[26] In addition, multivariate analysis identified the presence of more than three underlying diseases as independent risk factor for secondary infections among CRE-colonized patients, consistent with findings reported by Migliorini et al[27] and the Wei et al.[28]

Furthermore, this study found significantly higher carriage rates of *aerobactin, allS*, and *peg344* in the infection group than in the colonization group, suggesting that virulence factors may facilitate infection development and progression by enhancing bacterial adhesion, resistance to phagocytosis, and immune evasion. Thus, CRE intestinal colonization is influenced not only by host immunity and clinical interventions but also by the expression of bacterial virulence genes. Future research should further investigate the differences in virulence gene expression and phenotypic characteristics

between colonizing and infecting strains to elucidate specific pathogenic mechanisms. Incorporating virulence gene detection into CRE colonization screening may help achieve more precise infection prevention and control.

Although limited by its single-center and retrospective design, this study was conducted in the largest comprehensive hospital in Yunnan Province, which in 2024 managed 3.75 million outpatient and inpatient visits and 212,000 discharges, providing meaningful regional representativeness and case diversity. Through continuous one-year follow-up of 8,088 patients under a standardized screening protocol, we provide region-specific, systematically validated evidence from southwestern China that contributes to understanding the clinical trajectory of CRE from intestinal colonization to infection. Notably, we identified a patient in whom the colonizing and infecting isolates differed in carbapenem-resistance genes, MLST, and capsular serotypes, suggesting possible inter-site strain replacement or cross-site transmission. This finding underscores the importance of multi-site surveillance and indicates that CRE colonization and infection dynamics may be more complex than previously recognized. Future multicenter prospective studies integrating molecular typing, microbiome analyses, and temporal sampling across multiple body sites are warranted to clarify CRE transmission and evolution pathways. Such integrative approaches may refine risk-prediction models, inform hospital-specific infection control strategies, and support early evidence-based interventions for CRE management.

Conclusion

This study provides a preliminary investigation into the molecular characteristics and risk factors of secondary infections among patients with intestinal CRE colonization, confirming that such infections are closely related to both pathogen genetics and host factors. We recommended establishing comprehensive surveillance systems that integrate clinical and molecular epidemiological data, alongside enhanced screening in high-risk departments, to facilitate timely identification and management of at-risk patients and reduce the incidence of CRE-related secondary infections. Nevertheless, further prospective and randomized controlled studies are required to refine CRE infections prevention strategies, optimize intestinal colonization screening procedures, and explore effective CRE decolonization interventions.

Ethics Approval

This retrospective study used existing data from the Hospital Information System (HIS) of Kunming Medical University. All data were de-identified prior to analysis, and no personally identifiable information was included. Given that only de-identified existing data were used and the study posed no more than minimal risk, the requirement for obtaining individual informed consent was waived by the ethics committee. The study was approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University (Ethics number: (2022) Ethics Review L No. 68) and conducted in accordance with the Declaration of Helsinki.

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Disclosure

The author(s) report no conflicts of interest in this work.

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