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ORIGINAL RESEARCH

Characterization of Carbapenem-Resistant *Klebsiella pneumoniae* in a Tertiary Hospital in the Eastern Coastal Region of Zhejiang, China: Detection of a Novel ST6853 Clone

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Purpose: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is linked to hospital-acquired infections and associated with significant mortality rates, presenting a serious risk to public health. This research aimed to examine the in-hospital epidemiology and antimicrobial resistance of CRKP isolates, offering insights for the management and control of CRKP infections.

Patients and Methods: In total, 76 carbapenem-resistant *Klebsiella pneumoniae* isolates were collected from inpatients at the First Affiliated Hospital of Ningbo University from May 2022 and February 2024. The isolates were mainly acquired from sputum, urine, secretions, and blood. Antimicrobial susceptibility testing was performed using the VITEK 2 Compact system or the Kirby-Bauer disk-diffusion method. In addition to susceptibility testing, phylogenetic analysis, and serotype analysis, whole genome sequencing was conducted to identify genes associated with antibiotic resistance.

Results: Antimicrobial susceptibility testing showed that all 76 CRKP isolates were resistant to most common antibiotics. The CRKP isolates mainly carried *Klebsiella pneumoniae* carbapenemase, and the predominant gene was $bla_{\text{KPC-2}}$. Homology analysis found that the major sequence type (ST) was ST11, as a single isolate was identified as an ST11 single-locus variant (ST6853), followed by ST15 and ST48. Serotype analysis found that the major serotype was K64, followed by K24 and K62.

Conclusion: The CRKP isolates in our study exhibited resistance to the majority of commonly used antibiotics. The $bla_{\text{KPC-2}}$ gene was identified as the predominant carbapenemase. The major ST of CRKP found in our hospital was ST11, with K64 and O2a being the prevalent predicted serotypes. These findings should prove useful for the control and management of CRKP infections, and provided guidance for clinical antibiotic therapy.

Keywords: *Klebsiella pneumoniae*, carbapenem resistant, antimicrobial resistance, bacterial infections, nosocomial infections

Introduction

Klebsiella pneumoniae is an encapsulated, non-motile, Gram-negative bacterium belonging to the family Enterobacteriaceae, which is widely distributed in the environment and usually colonizes the oropharynx and intestinal mucosal surfaces of humans.[1,2] *K. pneumoniae* is an opportunistic pathogen and a common cause of community- and hospital-acquired pneumonia, urinary tract infections, surgical wound infections, pyogenic liver abscesses, and endogenous endophthalmitis.[3] Carbapenems are a class of very effective antibiotic agents characterized by a broad spectrum of antibacterial activity, stability against β-lactamases, and few side effects that are often used as "last-line agents" or "antibiotics of last resort" for infections of severely ill patients and drug-resistant bacteria.[4] However, due to the extensive use of antibiotics, including carbapenems, antibiotic resistance has become a notable global health concern.

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In particular, infections caused by carbapenem-resistant *K. pneumoniae* (CRKP) are particularly concerning because of the elevated mortality rates, especially among immunocompromised and critically ill patients.[5]

In this study, CRKP isolates were collected from patients hospitalized in the First Affiliated Hospital of Ningbo University for analysis of in-hospital epidemiology and antibiotic susceptibility, in addition to whole genome sequencing (WGS) to identify genes associated with antibiotic resistance, phylogenetic analysis, and serotype analysis as a reference for the prevention and control of clinical CRKP infections and the rational use of antimicrobials.

Materials and Methods

Bacterial Isolation

CRKP isolates (N = 76) were collected from the sputum (n = 44), feces (n = 11), blood (n = 6), urine (n = 6), bronchoalveolar lavage fluid (n = 3), pus (n = 3), and other sites (n = 3) of unduplicated patients in the intensive care unit (ICU; n = 41), medicine ward (n = 31), or surgery ward (n = 4) of the First Affiliated Hospital of Ningbo University (Ningbo, China) between May 2022 and February 2024. The specimens were collected by nurses, transported to the clinical laboratory, and inoculated on appropriate plates based on the source (Table 1).

Respiratory specimens were co-inoculated on blood agar as well as vancomycin-containing chocolate agar (Autobio Biotech, China). Faecal specimens were streaked on blood agar with an imipenem disk positioned at the primary-secondary streak junction, and carbapenem-non-susceptible Gram-negative bacterial isolates were selected based on their growth patterns. Confirmed CRKP isolates were cryopreserved from purified subcultures of selective media.

Table 1 Demographic and Clinical Characteristics of Patients and Specimens

Characteristic (unit)	Numerical Data, n (%)
Average age (years)	70.6
Median age (years)	72.0
Interquartile range (years)	61.0–85.5
Category by age (years), n (%)	
<20	1 (1.3%)
20–40	2 (2.6%)
41–60	16 (21.1%)
61–80	34 (44.7%)
81–100	23 (30.3%)
Gender, n (%)	
Male	56 (73.7%)
Female	20 (26.3%)
Source, n (%)	
Sputum	44 (57.9%)
Faeces	11 (14.5%)
Blood	6 (7.9%)
Urine	6 (7.9%)
Bronchoalveolar lavage fluid	3 (3.9%)
Pus	3 (3.9%)
Others	3 (3.9%)
Colonization rate in respiratory specimens, n (%)	29 (38.2%)
Time from hospital admission to isolate collection, n (%)	
< 48 hours	12 (15.8%)
> 48 hours	64 (84.2%)

(*Continued*)

Table 1 (Continued).

Characteristic (unit)	Numerical Data, n (%)
Origin, n (%)	
Emergency ICU*	20 (26.3%)
ICU 2	9 (11.8%)
ICU 1	6 (7.9%)
ICU 3	6 (7.9%)
Geriatric medicine 1	5 (6.6%)
Hematology 2	5 (6.6%)
Laminar air flow ward	4 (5.3%)
Geriatric medicine 3	3 (3.9%)
Pulmonary and critical care medicine	3 (3.9%)
International healthcare center	2 (2.6%)
Nephropathy	2 (2.6%)
Other surgery ward	2 (2.6%)
Cardiology	1 (1.3%)
Gastroenterology	1 (1.3%)
Gastrointestinal surgery	1 (1.3%)
Geriatric medicine 2	1 (1.3%)
Hematology 1	1 (1.3%)
Infectious diseases	1 (1.3%)
Internal medicine	1 (1.3%)
Pediatrics	1 (1.3%)
Thoracic surgery	1 (1.3%)

Abbreviation: *ICU: Intensive care unit.

Inclusion criteria for CRKP:

1. *K. pneumoniae* identification was jointly confirmed by MALDI-TOF MS and WGS;
2. Antimicrobial susceptibility testing results classified as intermediate or resistant to ertapenem or imipenem per CLSI M100 Ed35 guidelines;
3. First isolate from each patient.

Pathogen Culture

Clinical specimens were cultured on Columbia blood agar plates (respiratory specimens were co-cultured on vancomycin-containing chocolate agar plates) at 35°C and growth was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, bioMérieux, Marcy-l'Étoile, France).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed with the VITEK® 2 Compact fully automated bacterial analysis system and GN334 reagent (bioMérieux, Marcy-l'Étoile, France). Ceftazidime/avibactam (CZA) testing was performed exclusively by Kirby-Bauer. The testing procedures followed the CLSI M100 Ed35 guidelines.

WGS and Multilocus Sequence Typing (MLST)

In this study, WGS of the isolates was performed by Hangzhou Digital-Micro (Hangzhou, China). Revived isolates were identified as *K. pneumoniae* by MALDI-TOF MS, with antimicrobial susceptibility testing confirming resistance phenotypes of archived strains. WGS excluded *Klebsiella variicola* and *Klebsiella quasipneumoniae* within the *K. pneumoniae* complex. MLST of seven conserved housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB*) of the CRKP isolates was confirmed with ResFinder software (http://genepi.food.dtu.dk/resfinder). Phylogenetic analysis

was conducted based on the sequences of the seven conserved housekeeping genes using IQ-TREE software (version 2.0.3; https://github.com/Cibiv/IQ-TREE/releases).

Serotype Analysis

In this study, the K locus, O locus, predicted capsule type, and predicted O type of the CRKP isolates were identified with the Pathogenwatch genomic surveillance tool (https://pathogen.watch).

Results

Clinical Manifestation

The demographic and clinical characteristics of all patients and specimens are presented in Table 1. The average patient age was 70.6 ± 16.8 (range, 12–98) years and 75.0% were aged > 60 years. Furthermore, the ratio of male to female patients was about 3:1. The primary sources of the isolates were sputum, feces, blood, urine, bronchoalveolar lavage fluid, and pus. The most significant sources of the isolates were sputum (57.9%), feces (14.5%), blood (7.9%), and urine (7.9%). A total of 47 respiratory specimens were collected (including sputum and bronchoalveolar lavage fluid), with 29 showing colonization, accounting for 38.2% of all specimens. A total of 11 faecal specimens were collected, all of which were considered colonizing bacteria. The corresponding six patients underwent decolonization treatment with antibiotics. The patients were mainly admitted to the ICU, geriatric medicine, hematology, laminar air flow ward, pulmonary and critical care medicine, international healthcare center, and nephrology. The four most common wards were the emergency intensive care unit (26.3%), intensive care unit 2 (11.8%), intensive care unit 1 (7.9%), and intensive care unit 3 (7.9%).

Susceptibility Testing Results

The susceptibility testing results of all 76 CRKP isolates are shown in Table 2 and Figure 1. All isolates were multi-drug resistant. The resistance rates to amoxicillin/clavulanic acid, cefepime, cefoperazone/sulbactam, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, ertapenem, imipenem, piperacillin/tazobactam and levofloxacin were 98.7%, 92.1%, 100.0%, 100.0%, 98.7%, 98.7%, 100.0%, 100.0%, 98.7%, 100.0%, and 82.9%, respectively. While the resistance rates to

Table 2 Resistance of the CRKP Isolates to Commonly Used Antibacterial Drugs

Drug	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	SDD* n (%)
Beta-lactams				
Amoxicillin/Clavulanic Acid	1 (1.3)	/	75 (98.7)	/
Cefepime	/	/	70 (92.1)	6 (7.9)
Cefoperazone/Sulbactam	/	/	76 (100.0)	/
Cefoxitin	/	/	76 (100.0)	/
Ceftazidime	/	1 (1.3)	75 (98.7)	/
Ceftazidime/Avibactam	55 (72.4)	1 (1.3)	20 (26.3)	/
Ceftriaxone	1 (1.3)	/	75 (98.7)	/
Cefuroxime	/	/	76 (100.0)	/
Ertapenem	/	/	76 (100.0)	/
Imipenem	1 (1.3)	/	75 (98.7)	/
Piperacillin/Tazobactam	/	/	76 (100.0)	/
Aminoglycosides				
Amikacin	58 (76.3)	/	18 (23.7)	/
Quinolones				
Levofloxacin	4 (5.3)	9 (11.8)	63 (82.9)	/
Tetracyclines				
Tigecycline	76 (100.0)	/	/	/
Sulfonamides and Trimethoprim				
Cotrimoxazole	29 (38.2)	/	47 (61.8)	/

Abbreviation: *SDD, susceptible-dose dependent.

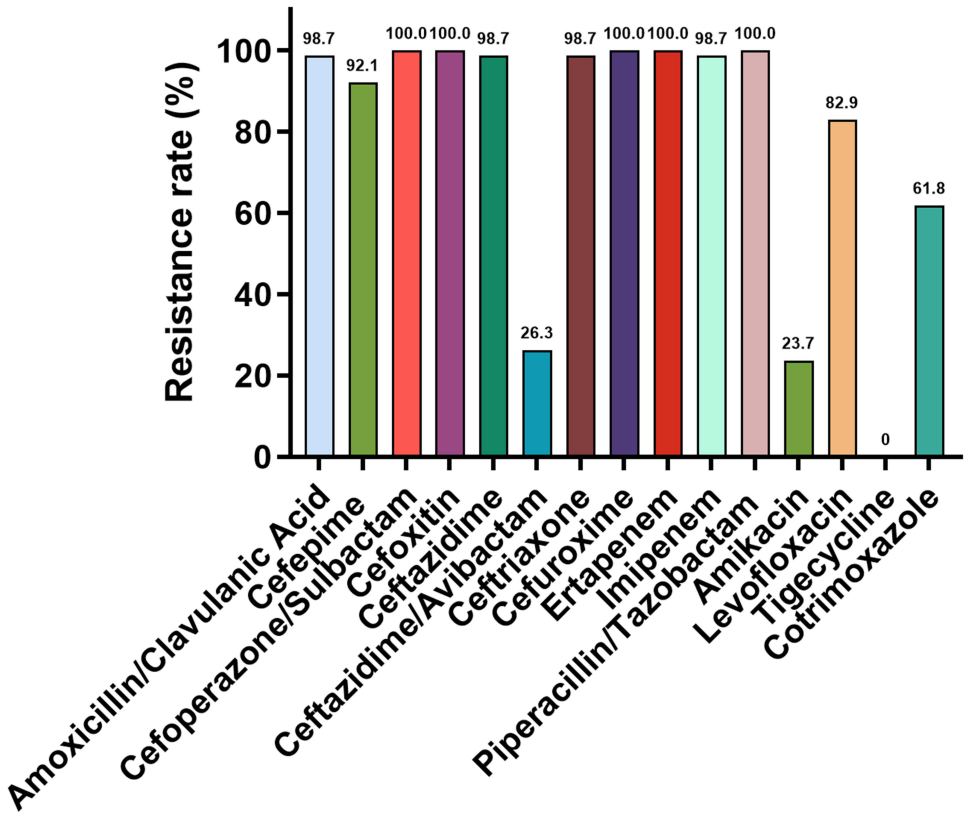

Figure 1 Susceptibility of CRKP to antibiotics.

amikacin and cotrimoxazole were 23.7% and 61.8%. The resistance rate to ceftazidime/avibactam was 26.3%, the susceptibility rate was 72.4%, and the intermediate rate was 1.3%. All isolates were susceptible to tigecycline.

Resistance Gene Analysis Results

In total, 111 genes associated with resistance to beta-lactamase, aminoglycosides, chloramphenicol, trimethoprim, fosfomycin, quinolones, tetracyclines, sulfonamides, macrolides, rifampicin, and colistin were identified by WGS analysis of 76 CRKP isolates (Table 3 and Table 4).

Among the isolates with only one carbapenemase gene, $bla_{\text{KPC-2}}$ was predominant and detected in 43 (56.6%) of the CRKP isolates, followed by $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-1}}$ in 12 (14.5%) and six (6.6%) isolates, respectively. Only three isolates carried the $bla_{\text{OXA-10}}$ gene, while 1 carried the $bla_{\text{IMP-4}}$ gene. Among the CRKP isolates carrying two carbapenemase genes, 7, 1, 1, 1, and 1 carried the $bla_{\text{KPC-2}}$+$bla_{\text{OXA-10}}$, $bla_{\text{KPC-2}}$+$bla_{\text{NDM-5}}$, $bla_{\text{KPC-2}}$+$bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$+$bla_{\text{OXA-1}}$, and $bla_{\text{IMP-4}}$+$bla_{\text{OXA-1}}$ genes, respectively. Notably, 2 CRKP isolates carried the $bla_{\text{KPC-14}}$ gene (Figure 2).

Sequence Alignment and Phylogenetic Analysis

Comparisons of the obtained sequences to the MLST database (https://pubmlst.org/) identified 23 sequence types (STs) (Figures 3 and 4). Phylogenetic analysis (Figure 3) revealed that these STs formed two distinct groups: group 1 consisted of 21 STs (ST11, ST1326, ST14, ST147, ST15, ST211, ST23, ST307, ST35, ST37, ST45, ST464, ST48, ST485, ST611, ST656, ST660, ST6853, ST873, ST889, and ST997), whereas group 2 was composed of two STs (ST355 and ST571). The most abundant STs of the CRKP isolates in our hospital were ST11 (43.4%, 33/76), ST15 (7.9%, 6/76), ST48 (7.9%, 6/76), ST307 (6.6%, 5/76), and ST37 (5.3%, 4/76). Notably, among the isolates, only ST6853 was identified as the ST11 single-locus variant.

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Table 3 Distribution of Beta Lactam-Resistant Genotypes of the CRKP Isolates

Classification of Antimicrobial Drugs	Drug-Resistant Genotype	Strains	Drug-Resistant Genotype	Strains
Beta-lactams	*bla*$_{\text{CTX-M-14}}$	2	*bla*$_{\text{SHV-145}}$	1
	bla$_{\text{CTX-M-147}}$	1	*bla*$_{\text{SHV-155}}$	11
	bla$_{\text{CTX-M-15}}$	6	*bla*$_{\text{SHV-158}}$	23
	bla$_{\text{CTX-M-199}}$	1	*bla*$_{\text{SHV-159}}$	23
	bla$_{\text{CTX-M-27}}$	1	*bla*$_{\text{SHV-172}}$	17
	bla$_{\text{CTX-M-3}}$	3	*bla*$_{\text{SHV-179}}$	1
	bla$_{\text{CTX-M-55}}$	3	*bla*$_{\text{SHV-182}}$	23
	bla$_{\text{CTX-M-65}}$	19	*bla*$_{\text{SHV-185}}$	1
	bla$_{\text{CTX-M-9}}$	1	*bla*$_{\text{SHV-190}}$	1
	bla$_{\text{CTX-M-99}}$	1	*bla*$_{\text{SHV-191}}$	1
	bla$_{\text{DHA-1}}$	2	*bla*$_{\text{SHV-194}}$	1
	bla$_{\text{IMP-4}}$	2	*bla*$_{\text{SHV-199}}$	1
	bla$_{\text{KPC-14}}$	2	*bla*$_{\text{SHV-2}}$	1
	bla$_{\text{KPC-2}}$	53	*bla*$_{\text{SHV-26}}$	1
	bla$_{\text{LAP-2}}$	36	*bla*$_{\text{SHV-27}}$	2
	bla$_{\text{LEN16}}$	1	*bla*$_{\text{SHV-28}}$	12
	bla$_{\text{LEN19}}$	1	*bla*$_{\text{SHV-31}}$	11
	bla$_{\text{LEN2}}$	1	*bla*$_{\text{SHV-33}}$	1
	bla$_{\text{NDM-1}}$	6	*bla*$_{\text{SHV-38}}$	2
	bla$_{\text{NDM-5}}$	12	*bla*$_{\text{SHV-40}}$	1
	bla$_{\text{OKP-A-3}}$	2	*bla*$_{\text{SHV-56}}$	1
	bla$_{\text{OXA-1}}$	2	*bla*$_{\text{SHV-65}}$	1
	bla$_{\text{OXA-10}}$	10	*bla*$_{\text{SHV-67}}$	1
	bla$_{\text{SHV-106}}$	12	*bla*$_{\text{SHV-75}}$	1
	bla$_{\text{SHV-108}}$	1	*bla*$_{\text{SHV-78}}$	1
	bla$_{\text{SHV-11}}$	3	*bla*$_{\text{SHV-79}}$	1
	bla$_{\text{SHV-110}}$	5	*bla*$_{\text{SHV-81}}$	4
	bla$_{\text{SHV-12}}$	14	*bla*$_{\text{SHV-85}}$	1
	bla$_{\text{SHV-129}}$	11	*bla*$_{\text{SHV-89}}$	1
	bla$_{\text{SHV-13}}$	11	*bla*$_{\text{SHV-98}}$	1
	bla$_{\text{SHV-133}}$	1	*bla*$_{\text{TEM-1B}}$	34

Table 4 Distribution of Other Drug-Resistant Genotypes of the CRKP Isolates

Classification of Antimicrobial Drugs	Drug-Resistant Genotype	Strains
Aminoglycosides	*aac(3)-IIa*	1
	aac(3)-IId	5
	aac(6')-Ib3	5
	aac(6')-Ib-cr	5
	aac(6')-Ib-Hangzhou	4
	aadA1	11
	aadA16	6
	aadA2	4
	aadA2b	23
	aph(3')-Ia	5
	aph(3'')-Ib	19
	aph(3')-IIa	1
	aph(6)-Id	21
	armA	2
	rmtB	20
	rmtF	4

(*Continued*)

Table 4 (Continued).

Classification of Antimicrobial Drugs	Drug-Resistant Genotype	Strains
Chloramphenicol	*catA1*	1
	catA2	25
	catB3	6
	cmlA1	11
	floR	14
Trimethoprim	*dfrA1*	3
	dfrA12	1
	dfrA14	37
	dfrA27	6
Fosfomycin	*fosA*	17
	fosA3	2
	fosA6	61
Quinolones	*OqxA*	46
	OqxB	46
	qnrB1	2
	qnrB4	2
	qnrB52	1
	qnrB6	1
	qnrB91	1
	qnrS1	49
Tetracyclines	*tet(A)*	44
	tet(D)	2
Sulfonamides	*sul1*	36
	sul2	42
	sul3	1
Macrolides	*erm(B)*	1
	mef(B)	1
	mph(A)	12
	mph(E)	2
	msr(E)	2
Rifampicin	*ARR-2*	13
	ARR-3	8
Polymyxins	*mcr-1.1*	1

Serotype Analysis

The most common predicted capsule type (K locus type) among the 76 CRKP isolates was K64 (KL64, 36 strains), which accounted for 47.4% of the total, followed by six isolates each of K24 (KL24) and K62 (KL62), 3 of K43 (KL43), and two of K25 (KL25) and K53 (KL53), while all other types accounted for only one isolate each. The most common predicted O type was O2a (37 strains), accounting for 48.7%, followed by O1 (19 strains) at 25.0%. The most common O locus type was O1/O2v1 (54 isolates), accounting for 71.1%, followed by O1/O2v2 (10 isolates) at 13.2% (Figure 4).

Discussion

K. pneumoniae has long been acknowledged as a pathogenic organism, first identified as a causative agent of pneumonia by Carl Friedländer in 1882, and it continues to be among the most prevalent nosocomial pathogens worldwide, with an increasing annual incidence.[6,7] *K. pneumoniae*, a common cause of antimicrobial-resistant opportunistic infections of hospitalized patients, is naturally resistant to penicillins and often acquires resistance to multiple antimicrobials. However, knowledge of the ecology, population structure, and pathogenicity of *K. pneumoniae* remains relatively limited.[8] Over the past decade, *K. pneumoniae* has emerged as a major clinical and public health threat due to the

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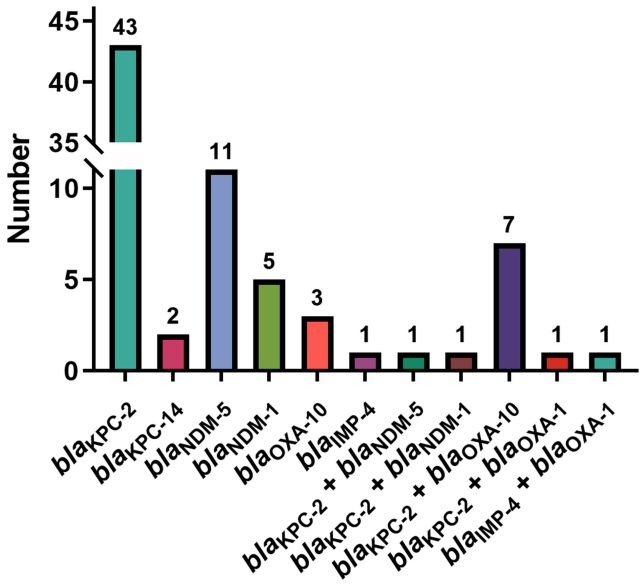

Figure 2 Carbapenemase genes of CRKP.

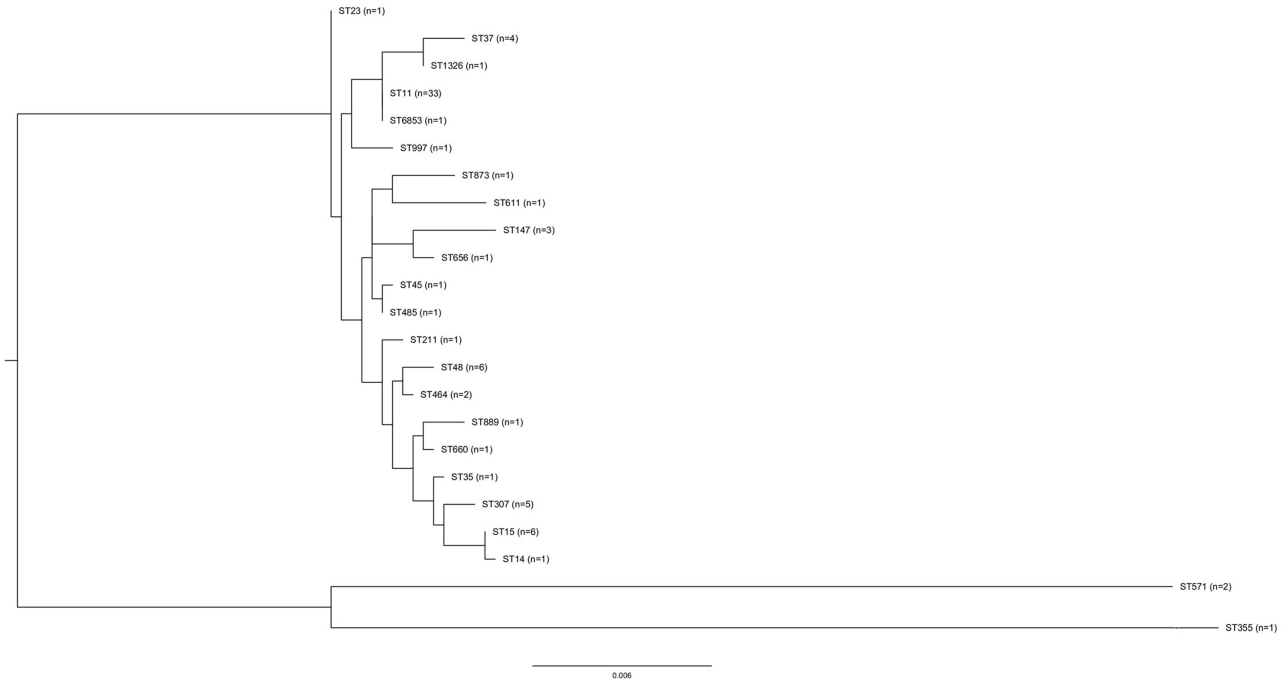

Figure 3 MLST and phylogenetic trees of CRKP.

increasing prevalence of healthcare-associated infections caused by multidrug-resistant strains producing extended-spectrum β-lactamases and/or carbapenemases.[8] As a multidrug-resistant pathogen, *K. pneumoniae* is one of the most common pathogens responsible for pneumonia, urinary tract infections, and bacteremia, thereby presenting a growing challenge for clinicians worldwide. Furthermore, *K. pneumoniae* is a significant contributor to neonatal sepsis, consistently ranking among the top three causative agents in various clinical settings.[9,10] The World Health Organization recognized the increasing prevalence of multidrug-resistant *K. pneumoniae* as a critical public health threat in 2017.[11] In Europe alone, these strains are responsible for over 90,000 infections and more than 7000 deaths annually, accounting for 25% of the total disability-adjusted life years lost to multidrug-resistant bacterial infections.[12] Xu et al[13] reported that the

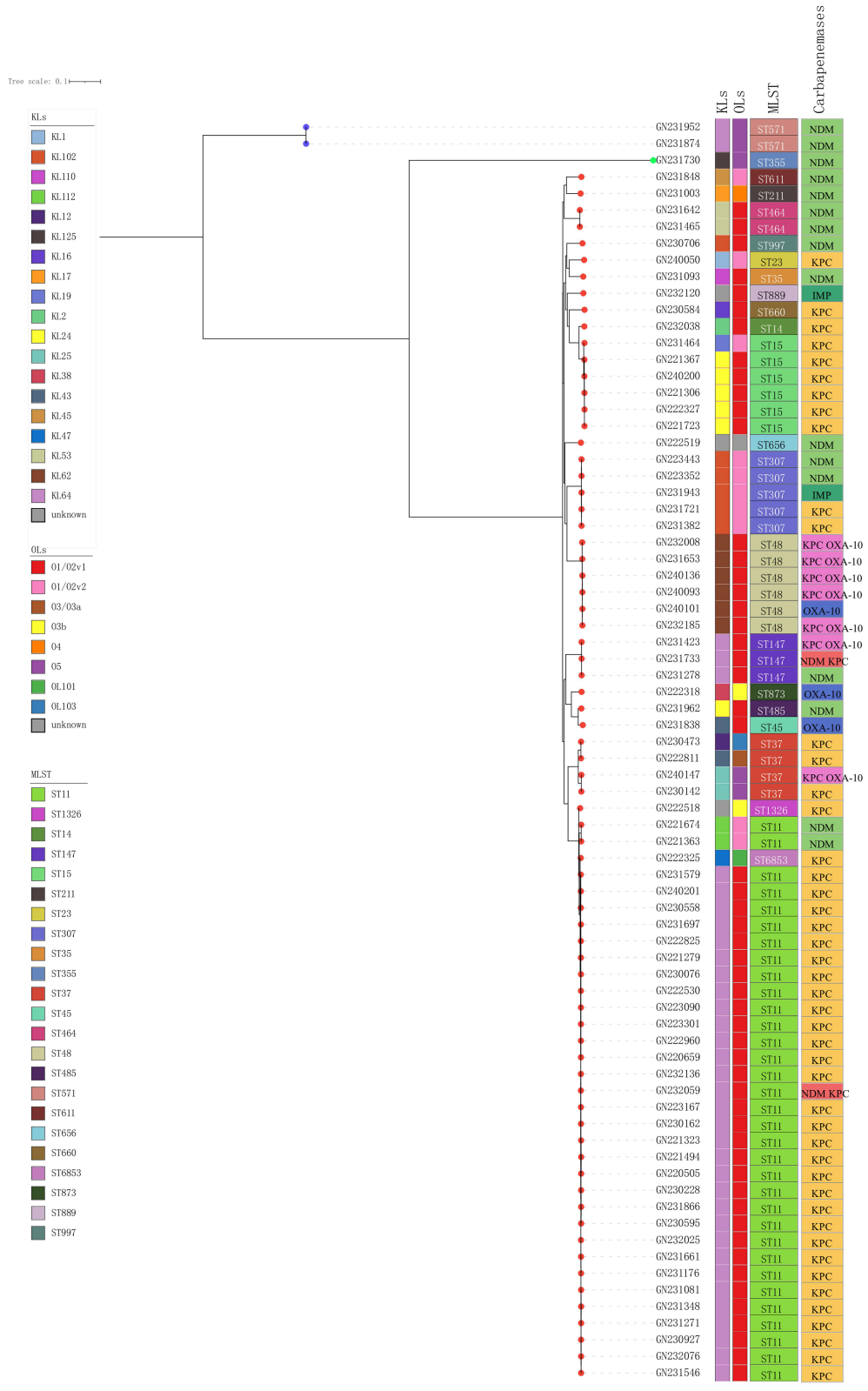

Figure 4 A phylogenetic tree was constructed based on core single nucleotide polymorphisms (SNPs) detected among the sample genomes using the kSNP tool (version 4.0; https://sourceforge.net/projects/ksnp/). Each node in the phylogenetic tree represents a taxonomic unit and the length of the evolutionary branches indicates the genetic distance between samples, where shorter branch lengths signify closer evolutionary relationships. The scale bar represents the unit length of genetic distance between strains, serving as the metric for the phylogenetic tree. Subsequently, the maximum likelihood tree generated with the kSNP tool (version 4.0) based on core SNPs was further visualized and annotated using the Interactive Tree Of Life tool (version 7; https://itol.embl.de/), incorporating the predicted K and O loci, and MLST results for enhanced graphical representation.

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pooled mortality rate was 42.14% among 2462 patients infected with CRKP, as compared to 21.16% of those infected with carbapenem-susceptible *K. pneumoniae*.

In this study, 76 CRKP isolates were collected from clinical patients, which included 57 (75.0%) who were aged >60 years, consistent with an earlier report.[14] Moreover, the 76 CRKP isolates were mainly collected from patients in the ICU (including emergency ICU), geriatric medicine, hematology, laminar air flow ward, pulmonary and critical care medicine, international healthcare center, and nephropathy. Notably, the novel ST6853 clone was detected in emergency ICU patients. ICU patients are at a heightened risk for CRKP infections due to their critical health status, which often necessitates the use of broad-spectrum antibiotics, like carbapenems, which select for CRKP. The frequent use of invasive procedures, such as mechanical ventilation, central venous catheters, and urinary catheters, provides additional routes for bacterial entry and colonization. The ICU environment itself, with the close proximity of patients and frequent movement of healthcare personnel, facilitates the transmission of CRKP.[15,16] Similarly, elderly patients and those with hematological diseases are prone to CRKP infections due to compromised immunity, prolonged use of broad-spectrum antibiotics, and presence of multiple comorbidities. These factors significantly contribute to the elevated incidence of CRKP infections observed in these patient populations within hospital wards.

The CRKP isolates in this study were highly resistant to most antibiotics, including carbapenems, cephalosporins, sulfonamides, quinolones, and β-lactam combined with a β-lactamase inhibitor. CRKP primarily exhibits resistance through the production of *K. pneumoniae* carbapenemase, New Delhi metallo-beta lactamase, Verona integron-mediated metallo-β-lactamase, and OXA-48, which hydrolyze carbapenems and other beta-lactam antibiotics. Additional mechanisms include overexpression of efflux pumps, loss or modification of outer membrane proteins (eg, OmpK35 and OmpK36), alteration of penicillin-binding proteins, and the presence of extended-spectrum beta-lactamases and AmpC beta-lactamases, which collectively contribute to a multidrug-resistant phenotype.[17,18] In this study, carbapenemase genes were detected in all 76 strains, with $bla_{\text{KPC-2}}$ (53 strains, 69.7%) as the most common, follow by $bla_{\text{NMD-5}}$ (12 strains, 15.8%) and $bla_{\text{OXA-10}}$ (10 strains, 13.2%). Among CZA-resistant CRKP strains, 100% carried NDM, as CZA cannot hydrolyze NDM due to the lack of an active-site serine residue.[19] This indicates that the production of class B metallo-β-lactamases was the primary resistance mechanism in this study, which is consistent with previous findings.[20] Two CRKP strains co-produced KPC and NDM, further complicating the resistance mechanisms and clinical treatment strategies. The results indicated that $bla_{\text{KPC-2}}$ was the main carbapenem resistance gene in our hospital, consistent with earlier reports.[21–24] Meanwhile, $bla_{\text{KPC-14}}$, a variant of the KPC family, which shares significant homology with $bla_{\text{KPC-2}}$, was detected in 2 CRKP isolates. KPC-14 is reportedly sensitive to carbapenems but resistant to ceftazidime-avibactam.[25] However, Wang et al reported that the $bla_{\text{KPC-14}}$ gene tends to integrate into other conjugative plasmids via the NTEKPC-Ib element, facilitating the spread of ceftazidime/avibactam resistance,[26] thereby highlighting the potential spread of KPC-14 and the threat of drug resistance, emphasizing the importance of strengthening research and surveillance. While NDM carbapenemases are particularly concerning due to their extensive resistance and rapid dissemination, the NDM-5 variant demonstrates even higher resistance to carbapenems compared to NDM-1.[27] Zhu et al reported that the NDM-5-producing *K. pneumoniae* ST307 isolates exhibits high-level resistance to carbapenems and ceftazidime-avibactam, with the $bla_{\text{NDM-5}}$ located on an IncX3 plasmid of 45,403 bp with a high frequency of conjugative ability.[28] Furthermore, Wei et al reported the emergence of ST789 NDM-5-producing CRKP, which was likely attributed to clonal expansion after acquiring a self-transmissible IncX3 plasmid carrying $bla_{\text{NDM-5}}$, representing a new threat to neonates.[29] While OXA-48-like carbapenemases present a more significant clinical challenge than OXA-10 in *K. pneumoniae*, OXA-10 retains relevance as a commonly encountered β-lactamase. In CRKP, OXA-10 rarely acts as a standalone resistance determinant but is frequently co-produced with other β-lactamase types. Notably, in this study, $bla_{\text{KPC-2}}$ was co-detected in 7 out of 10 CRKP isolates that were positive for $bla_{\text{OXA-10}}$.

In this study, MLST revealed that the predominant ST among the CRKP isolates was ST11, followed by ST15 and ST48. Globally, ST11 and ST258 are recognized as the most prevalent CRKP clones. Notably, ST11 is the dominant clone in Asia, particularly in China, while ST258 is more frequently reported in the United States and several European countries.[30–32] Chen et al further elucidated the genetic basis of ST258, reporting that the genome of ST258 is a hybrid, with approximately 80% of the genome homologous to ST11 and the remaining 20% to ST442.[33] Li et al identified a novel rare sequence type ST449 among KPC-2-producing CRKP isolates, which uniquely carries only a KPC-

2-positive plasmid without virulence plasmids, contrasting with dominant ST11 clones that co-harbored both resistance and virulence factors.[34] Our study found that ST11 was predominant in the ICU, accounting for 18 isolates, while one ST6853 isolate was detected. Although no prior literature has documented this association, MLST analysis demonstrated that ST6853 is a single-locus variant of ST11 differing at the *phoE* allele. Phylogenetic analysis further revealed a close genetic proximity between these two ST types, leading to the inference that ST6853 likely represents a divergent sublineage derived from the ST11 clonal complex. The emergence of ST6853 underscores the microevolution of *K. pneumoniae* under antibiotic-driven selective pressure, with minor genetic changes in dominant clones, like ST11, giving rise to new subtypes with potentially altered fitness or resistance. While ST11 remains the predominant clone linked to multidrug resistance and hospital-acquired infections, ST6853 represents a notable derivative requiring further investigation. This relationship highlights the genetic adaptability of *K. pneumoniae*, reinforcing the necessity for continuous genomic surveillance and research.

The K serotypes (capsular polysaccharides) and O serotypes (lipopolysaccharides) of *K. pneumoniae* are essential to understand the mechanisms underlying pathogenicity and immune evasion. Both serotypes not only contribute to the virulence of *K. pneumoniae*, but also serve as the foundation for isolate identification through serotyping.[35–37] Capsule genotyping conducted by Hu et al identified 58 distinct K locus types in China, with the majority belonging to KL64 (50.4%) and KL47 (25.9%), and reported that the prevalence of KL64 continues to significantly increase.[38] K1 and K2 are recognized as the predominant serotypes associated with hypervirulent *K. pneumoniae*.[39] In our study, only one isolate of each serotype was identified, originating separately from the ICU and the Department of Geriatric Medicine. The results of this study identified a relationship between MLST and predicted serotypes (Figure 4). We plan on further investigations into the virulence-associated characteristics of *K. pneumoniae* strains.

Conclusion

The CRKP isolates in this study exhibited resistance to the majority of commonly used antibiotics. The bla_{KPC} gene was identified as the predominant carbapenemase. The major sequence type of CRKP found in our hospital was ST11, with K64 and O2a being the prevalent predicted serotypes. These findings highlighted the importance of understanding the prevalence of CRKP to guide antibiotic therapy and strengthen infection control, while helping clinicians to optimize treatment and prevent nosocomial spread.

Ethics Approval

The study involves a retrospective analysis of previously collected strains and data that have been fully anonymized (ie, cannot be directly or indirectly traced back to specific individuals through identifiers). Given the nature of the research (analysis of anonymized strains and data only) and the absence of any personal or sensitive information, the study protocol was submitted to the Ethics Committee of The First Affiliated Hospital of Ningbo University for review and was granted ethical approval (approval no. 2024230RS) along with a waiver of informed consent. All research activities were conducted in strict compliance with the regulations of the Ethics Committee and the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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