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ORIGINAL RESEARCH

Genomic Analysis of Carbapenem-Resistant Hypervirulent *Klebsiella pneumoniae* in a Chinese Tertiary Hospital

Lan Chen[1,*], Ying Zhou[2,*], Shanshan Wang[1], Chunyang Wu[3], Peiyao Zhou[4], Bingjie Wang[2], Zhu Chen[5], Fangyou Yu[2]

[1]Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Ningbo University, Ningbo, Zhejiang, 315010, People's Republic of China; [2]Department of Clinical Laboratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, 200082, People's Republic of China; [3]Department of Respiratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, 325000, People's Republic of China; [4]Department of Laboratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China; [5]Department of Clinical Laboratory Medicine, Ningbo No. 2 Hospital, Ningbo, Zhejiang, 315010, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhu Chen; Fangyou Yu, Email mars853234@163.com; wzjxyfy@163.com

Background: Carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) has become a clinical crisis and is associated with significant morbidity and mortality. The prevalence of CR-hvKP has trended upward since 2010. This study aims to describe the clinical and genomic characteristics of CR-hvKP collected from a tertiary hospital in eastern China, from August 2020 to October 2021.

Methods: We tested the susceptibility to common antibiotics in these isolates to feature the antibiotic-resistant phenotypes. We also applied whole-genome sequencing and core-genome phylogenetic to analysis the genetic features of these isolates. Plasmid replicons were identified by using the PlasmidFinder database, and core-genome phylogenetic analysis by Parsnp database.

Results: All these strains isolated from the patients with serious underlying diseases and poor prognosis. We found all CR-hvKp isolates exhibited a multidrug-resistant (MDR) phenotype. These results revealed that bla_{KPC-2} was the predominant carbapenemases gene (n = 53, 84.1%), and ST11-KL64 CR-hvKP strains dominated, forming a single cluster, and differed by an average of 26 core SNPs. We only found eight ST15 isolates containing KL24 and KL112 type capsules, with the main carbapenem resistance genes being $bla_{OXA-232}$ and bla_{KPC-2}. All ST11-KL64 strains had a series of resistance and virulence genes, along with IncHIB-FIB virulence plasmids and IncFII resistance plasmids, while the prevalence of resistance plasmids like the IncFII plasmid was absence in ST15 isolates.

Conclusion: This suggests that ST11-KL64 CR-hvKP has emerged as the most prevalent hypervirulence and carbapenem-resistant *K. pneumoniae* and may contribute to hospital outbreaks of infection, which required most clinical attention.

Keywords: carbapenem-resistant, hypervirulence, *Klebsiella pneumoniae*, whole-genome sequencing, antimicrobial resistance, virulence genes

Introduction

Klebsiella pneumoniae (KP) is a common opportunistic pathogen in clinical practice, capable of causing infectious diseases in the urinary tract, respiratory tract, blood, and soft tissues.[1] Hypervirulent *Klebsiella pneumoniae* (hvKp) has a higher virulence than KP[2] and can cause severe infectious diseases, including pyogenic liver abscess, endophthalmitis, and meningitis.[3,4]

Although multidrug resistance and hypervirulence were previously thought to follow distinct evolutionary directions with non-overlapping genomic signatures for each phenotype,[5] hvKp has recently garnered more attention due to its increased likelihood of acquiring antimicrobial resistance (AMR) genes, particularly those that code for carbapenemases.[2,6] Carbapenem-resistant hvKp (CR-hvKp) exhibits both hypervirulence and carbapenem resistance phenotypes, making infections caused by these strains difficult to treat with current antibiotics, and should therefore receive greater attention.[7]

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CR-hvKP emerged in the early 2010s and is primarily prevalent in Asia, especially China, but cases have been reported worldwide.[8] In 2016, a lethal outbreak of ST11 CR-hvKP occurred in a Chinese intensive care unit, with 21 ST11 KPC-2-producing CR-hvKP strains isolated from five patients who died during hospitalization.[7] CR-hvKP has spread globally and poses a significant human public health threat.[9] Therefore, the early recognition of these hypervirulent strains, including their resistance determinants, is a priority concern.

The aim of our study was to determine the current occurrence of CR-hvKp in a tertiary hospital in eastern China. We analyzed the clinical outcomes through reviewing medical history and identified different serotypes, virulence-associated markers, and antimicrobial drug resistance genes among the CR-hvKp isolates by using whole genome sequencing (WGS).

Materials and Methods

Bacterial Strains

To explore the characteristics of the carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) isolates, we collected 63 CR-hvKP isolates from 54 patients with underlying diseases and poor prognosis in a tertiary hospital in Ningbo, Zhejiang Province, China, from August 2020 to October 2021. The strains from different isolation sites of the same patient were included in this study. All the information about the patients were listed in Table 1. The speciation was determined by Matrix-Assisted Laser Flight Desorption/ Ionisation Time of (MALDI-TOF MS). Based on the antibiotic susceptibility results and the patient's prognosis, we initially determined whether the infected bacteria were CR-hvKP, and then further confirmed based on the results of whole-genome sequencing.

Antimicrobial Susceptibility Test

We performed bacterial antimicrobial susceptibility testing using VITEK2 system. MICs were measured for cefoxitin, ceftriaxone, aztreonam, cefepime, imipenem, tobramycin, gentamicin, amikacin, ciprofloxacin, levofloxacin, piperacillin/tazobactam, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole. For MIC determination, *E. coli* ATCC 25922 was used as a quality control strain, which was purchased from National Center for Clinical Laboratories and kept by our laboratory. CLSI2022-M100-ED31was used to determine the interpretative breakpoints.[10]

Whole-Genome Sequencing and Bioinformatics Analysis

The genomic DNA of these CR-hvKP was extracted using a commercial DNA extraction kit (Qiagen, Germany). The genome sequencing was then performed by the Illumina NovaSeq 6000 platform, with 2×150 bp paired-end reads. The multilocus sequence typing (MLST), capsular type, resistance and virulence determinants were determined by the Kleborate (version 0.3.0) (https://github.com/katholt/Kleborate/). Plasmid replicons were identified using the PlasmidFinder database using the minimum coverage and minimum identities of 90% (https://cge.cbs.dtu.dk/services/PlasmidFinder/).

Results

Clinical Characteristics of Patients Infecting with CR-hvKp

We collected 63 isolates of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) from 54 patients at a tertiary hospital in China. This study included strains from different isolation sites of the same patient, with 9 patients having 2 isolates from different specimens (Table 1). The patients were predominantly males (81.5%, n = 44) with a median age of 66 years. They were mainly from different ICU wards in the hospital (23/54 42.6%), followed by the emergency intensive care unit (EICU; 6/54, 11.1%), gerontology (5/54; 9.3%), hematology (4/54; 7.4%), neurosurgery (3/54; 5.5%), emergency ward (3/54; 5.5%) and other wards (10/54; 18.6%). Among the 63 CR-hvKP isolates, a variety of clinical specimens were involved, including sputum (37/63, 58.7%), blood (11/63, 17.5%), urine (4/63, 6.3%), stool (4/63, 6.3%), and other specimens (7/63, 11.2%). Notably, some patients had poor prognoses, with 20 patients (37.0%) experiencing delirium during discharge, 4 patients still requiring treatment, and 2 patients dying during their hospitalization. Due to the multidrug resistance and higher virulence of infections caused by CR-hvKP isolates, distressing clinical outcomes were more likely to occur.

Table 1 Characteristics of Clinical Cases and Isolates

Patients	Years Old	Gender	Isolates	Source	Ward	STs	KL	Virulence Factors	Resistance Genes	Plasmid Replicons	Clinical Outcomes
1	47	Female	FK3004	Sputum	Neurosurgery	ST15	KL24	*iucA, rmpA2*	KPC-2	IncFIB	Improve, discharge
2	22	Male	FK3006	Sputum	ICU-1	ST15	KL112	*iucA, rmpA2*	OXA-232	IncFIB, IncHI1B	Improve, be hospitalized
3	72	Male	FK3009	Blood	Emergency ward	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Improve, be hospitalized
4	51	Male	FK3015	Sputum	ICU-2	ST65	KL2	*iucA, rmpA2, rmpA, iroB*	KPC-3	IncHI1B, IncX5	Delirious, discharge
	51	Male	FK3018	Blood	ICU-2	ST65	KL2	*iucA, rmpA2, rmpA, iroB*	KPC-3	IncHI1B, IncX5	Delirious, discharge
5	33	Male	FK3016	Blood	ICU-2	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Severe, discharge
6	61	Male	FK3021	Sputum	ICU-1	ST15	KL24	*iucA, rmpA2*	KPC-2	IncFIB	Cured, discharge
7	60	Male	FK3023	Sputum	Nephrology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR, IncX3	Improve, discharge
8	65	Female	FK3025	Sputum	Gastrointestinal surgery	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
9	88	Male	FK3033	Sputum	ICU-2	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
10	59	Male	FK3035	Pus	Emergency ward	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
11	82	Male	FK3040	Sputum	EICU	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
12	62	Male	FK3048	Blood	Hematology	ST65	KL2	*iucA, rmpA2, rmpA, iroB*	NDM-1	IncHI1B, IncX3	Improve, discharge
13	92	Male	FK3052	Sputum	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncN, IncR	Continue treatment
	92	Male	FK3121	Sputum	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Continue treatment
14	55	Male	FK3053	Stool	ICU-2	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
15	50	Male	FK3054	Pleural fluid	ICU-2	ST37	KL25	*iucA*	KPC-2	IncFIB	Died
16	76	Female	FK3055	Urine	Urinary Surgery	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
	76	Female	FK3056	Blood	Urinary Surgery	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
17	51	Female	FK3057	Stool	Neurosurgery	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Cured, discharge
18	57	Male	FK3058	Sputum	EICU	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2, NDM-5	IncFII, IncHI1B, IncR	Delirious, discharge
19	68	Male	FK3061	Urine	ICU-1	ST412	KL57	*iucA, rmpA2, rmpA, iroB*	KPC-2	-	Severe, discharge
	68	Male	FK3104	Sputum	ICU-1	ST412	KL57	*iucA, rmpA2, iroB*	KPC-2	-	Severe, discharge
20	70	Male	FK3062	Stool	ICU-2	ST412	KL57	*iucA, rmpA2, rmpA, iroB*	KPC-2	-	Delirious, discharge
	70	Male	FK3064	Blood	ICU-2	ST412	KL57	*iucA, rmpA2, rmpA, iroB*	KPC-2	-	Delirious, discharge
21	87	Male	FK3065	Sputum	Gastroenterology	ST11	KL47	*iucA*	KPC-2	IncFII, IncFIB, IncR	Improve
22	91	Male	FK3101	Sputum	ICU-2	ST37	KL25	*iucA*	KPC-2	IncFIB	Died
23	59	Male	FK3109	Pipe	ICU-2	ST4080	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR, IncX4	Delirious, discharge

(*Continued*)

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Table 1 (Continued).

Patients	Years Old	Gender	Isolates	Source	Ward	STs	KL	Virulence Factors	Resistance Genes	Plasmid Replicons	Clinical Outcomes
24	65	Male	FK3111	Sputum	Hematology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR	Delirious, discharge
	65	Male	FK3163	Sputum	Hematology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR	Delirious, discharge
25	93	Male	FK3113	Bile	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Continue treatment
26	68	Female	FK3115	Blood	ICU-1	ST412	KL57	*iucA, rmpA2, rmpA, iroB*	KPC-2	-	Delirious, discharge
27	93	Male	FK3118	Sputum	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
28	83	Male	FK3119	Blood	ICU-1	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
29	64	Male	FK3122	Sputum	EICU	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2, NDM-5	IncFII, IncHI1B, IncR	Delirious, discharge
30	77	Female	FK3124	Sputum	EICU	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
31	93	Male	FK3125	Sputum	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Continue treatment
32	51	Male	FK3140	Sputum	ICU-1	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
33	92	Male	FK3154	Sputum	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Continue treatment
	92	Male	FK3159	Secretion	Gerontology	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2, OXA-232	IncFII, IncFIB, IncHI1B, IncR	Continue treatment
34	83	Male	FK3155	Sputum	ICU-1	ST11	KL64	*iucA*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
35	73	Male	FK3157	Sputum	ICU-2	ST412	KL57	*iucA, rmpA2, rmpA, iroB*	KPC-2	-	Improve, discharge
36	40	Female	FK3160	Stool	Hematology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
	40	Female	FK3165	Blood	Hematology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
37	89	Female	FK3161	Sputum	ICU-1	ST15	KL112	*iucA, rmpA2*	OXA-232	IncFIB, IncHI1B	Delirious, discharge
38	56	Male	FK3164	Sputum	Neurosurgery	ST420	KL20	*iucA, rmpA2, rmpA, iroB*	KPC-2	IncFIA, IncFIB, IncFII, IncHI1B	Improve, discharge
39	58	Female	FK3166	Sputum	Emergency ward	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
40	73	Male	FK3168	Urine	Cancer Radiochemotherapy Department	ST37	KL25	*iucA*	KPC-2	IncFIB	Improve, discharge
41	43	Female	FK3169	Sputum	ICU-2	ST15	KL112	*iucA, rmpA2*	OXA-232	IncFIB, IncHI1B	Uncured
	43	Female	FK3195	Cerebrospinal fluid	ICU-2	ST15	KL112	*iucA, rmpA2*	OXA-232	IncFIB, IncHI1B	Uncured
42	58	Male	FK3170	Sputum	ICU-1	ST15	KL112	*iucA, rmpA2*	OXA-232	IncFIB, IncHI1B	Delirious, discharge
43	61	Male	FK3171	Blood	Hematology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
44	54	Male	FK3174	Blood	ICU-2	ST37	KL25	*iucA*	KPC-2	IncFIB, IncR	Delirious, discharge
45	64	Male	FK3175	Sputum	ICU-2	ST11	KL64	*iucA, rmpA2, rmpA*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
46	55	Male	FK3181	Sputum	Nephrology	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge

47	68	Male	FK3184	Urine	Endocrinology	ST11	KL47	*iucA, rmpA2*	NDM-5	IncFII, IncFIB, IncHI1B, IncR, IncX3	Improve, discharge
48	64	Male	FK3186	Sputum	ICU-2	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncHI1B, IncR	Improve, discharge
49	67	Male	FK3187	Sputum	ICU-1	ST11	KL64	*iucA*	KPC-2	IncFII, IncHI1B, IncR	Delirious, discharge
50	43	Male	FK3188	Pus	EICU	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR	Uncured
51	85	Male	FK3191	Sputum	EICU	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR	Delirious, discharge
52	63	Male	FK3194	Sputum	Cardiology	ST15	KL24	*iucA, rmpA2*	KPC-2	IncFIB	Improve
53	63	Male	FK3196	Sputum	Thoracic surgery	ST505	KL64	*iucA, rmpA, iroB*	OXA-181	IncFIA, IncFIB, IncHI2A, IncHI2, IncX3	Uncured
54	63	Male	FK3198	Sputum	Orthopaedic Sports Medicine	ST11	KL64	*iucA, rmpA2*	KPC-2	IncFII, IncFIB, IncHI1B, IncR	Cured

Abbreviations: EICU, emergency intensive care unit; ICU, intensive care unit.

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Antimicrobial Resistance Phenotype of the CR-hvKp Isolates

To clarify the antibiotic-resistant phenotype of these CR-hvKp isolates, we tested their susceptibility to 13 antibiotics (Table 2). We found that CR-hvKp was resistant to multiple antibiotic classes, with most isolates showing high-level resistance to carbapenems and all β-lactam antibiotics, including ceftriaxone, cefepime, cefoxitin, and aztreonam (Table 2). Resistance to fluoroquinolones was also frequent, with 92.1% and 88.9% of isolates resistant to ciprofloxacin and levofloxacin, respectively. However, the isolates were relatively susceptible to three antimicrobials: gentamicin, tobramycin, and amikacin, with resistance rates of 23.8%, 22.2%, and 15.9%, respectively. All CR-hvKp exhibited a multidrug-resistant (MDR) phenotype showing resistant to three or more antibiotic classes and 27.0% were resistant to all six antimicrobial classes.

Genomic Phylogeny of CR-hvKp Isolates

To gain a deeper understanding of the molecular mechanisms behind these strains, we conducted whole-genome sequencing and core-genome phylogenetic analysis (Figure 1). The results showed that the ST11 CR-hvKP strains dominated, forming a single cluster, and differed by an average of 26 core SNPs, indicating clonal expansion. These ST11 strains contained the KL64 capsule type and had a series of resistance and virulence genes, along with IncHI1B-FIB virulence plasmids and IncFII resistance plasmids (Figure 1). This suggests that ST11-KL64 CR-hvKP has emerged as the most prevalent hypervirulence and carbapenem-resistant *K. pneumoniae* and may contribute to hospital outbreaks of infection.

While ST15 *K. pneumoniae* was identified as the second most frequent CRKP clone in hospital infections after ST11 *K. pneumoniae*, hypervirulent ST15 CRKP was uncommon. We found eight ST15 isolates containing KL24 and KL112 type capsules, with the main carbapenem resistance genes being $bla_{\text{OXA-232}}$ and $bla_{\text{KPC-2}}$. The prevalence of resistance plasmids like the IncFII plasmid did not observed in ST15 isolates, but only the IncHI1B-FIB virulence plasmid in these ST15 clusters.

In addition to the ST11 and ST15 CR-hvKP prevalence clones, we found several hypervirulent clones like ST412 and ST65 that obtained resistance elements to generate hypervirulent CRKP (Figure 1). Although the classical hypervirulent clone ST412 isolates contained the $bla_{\text{KPC-2}}$ gene, we observed no plasmids, indicating that the gene may have been obtained through other mobile elements. For the ST65 strains, the resistance phenotype mainly attributed to another resistance plasmid, the IncX plasmid, harboring $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-3}}$ genes. The co-existence of different plasmids and the diversity of plasmids simultaneously contributed to the transmission of both resistant and hypervirulent phenotypes in CR-hvKP isolates.

Table 2 Antibiotic Susceptibilities of 63 Carbapenem-Resistant *K. pneumoniae*

Antibiotics	Sensitivity (%)	Intermediate (%)	Resistance (%)
Aztreonam	1.6	0.00	98.4
Ciprofloxacin	6.3	1.6	92.1
Levofloxacin	6.3	4.8	88.9
Tobramycin	69.8	8.0	22.2
Gentamicin	73.0	3.2	23.8
Amikacin	80.9	3.2	15.9
Cefoxitin	4.8	9.5	85.7
Ceftriaxone	0.00	0.00	100.0
Cefepime	3.2	1.6	95.2
Imipenem	1.6	1.6	96.8
Piperacillin/Tazobactam	0.0	0.00	100.0
Trimethoprim/Sulfamethoxazole	42.9	-	57.1
Amoxicillin/Clavulanic acid	0.00	1.6	98.4

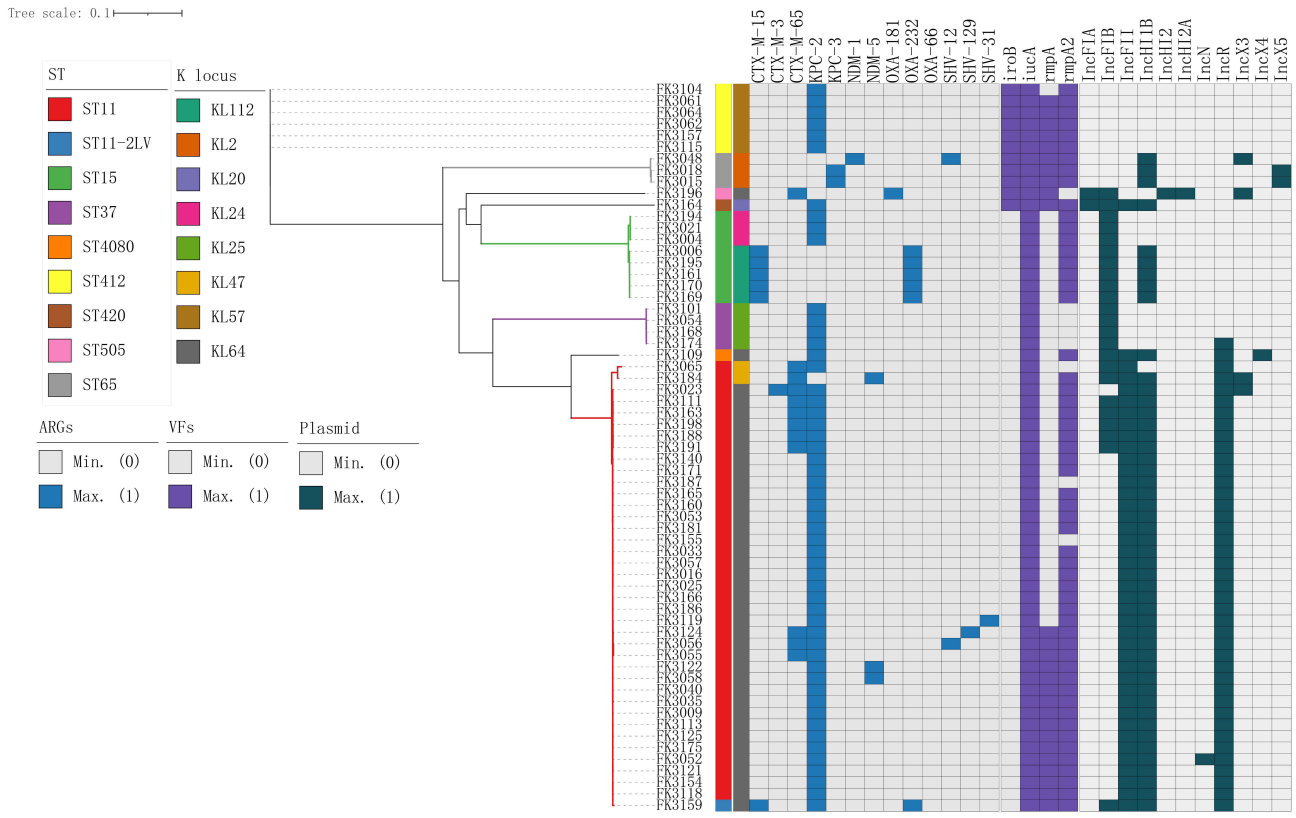

Figure 1 Phylogenetic tree based on core genome alignment of 63 Carbapenem-Resistant Hypervirulent *Klebsiella pneumoniae* in a tertiary hospital in East China. The different STs and KLs is color-coded and illustrated at the tips. The clusters also differed with several colors. The occurrence of resistance and virulence genes, and plasmid replicons were also color-coded. The tree was rooted in the midpoint. Scale bar represents 0.01 mutations per nucleotide position.

The Distribution of Antibiotic Resistance Genes and Virulence Factors

The presence of carbapenem-resistant genes in all 63 isolates is concerning, as it limits the options for effective antimicrobial treatment. Most of the isolates carried the bla_{KPC} gene, particularly $bla_{\text{KPC-2}}$, but other carbapenemase genes such as bla_{OXA} and bla_{NDM} were also found. A small proportion of isolates carried multiple carbapenemase genes, which further complicates treatment options. Additionally, a significant proportion of isolates carried beta-lactamase genes.

The hypervirulence of hvKp is due to its unique characteristics, including capsule, lipopolysaccharide, siderophores, and allantoin metabolism. Genetic biomarkers such as *iucA, iroB, rpmA*, and *rmpA2* can be used to identify hvKp strains. All isolates in the study carried the *iucA* gene, which is a critical factor contributing to the high pathogenicity of hvKP. The *iroB* gene was detected in 11 isolates, and the *rpmA* (26/63, 41.3%) and *rmpA2* (55/63, 87.3%) genes were present in a significant proportion of isolates. The combination of these virulence determinants varied among different ST types, leading to different hypervirulence phenotypes.

Overall, the emergence of CR-hvKP strains with limited treatment options and high virulence is a significant public health concern, and further studies are needed to understand the molecular mechanisms behind these strains and develop effective prevention and treatment strategies.

Discussion

HvKp is an emerging pathogen that has been reported in the community setting and has recently caused infections in healthcare settings, with higher virulence and mortality rates.[11,12] In this study, we used whole-genome sequencing (WGS) in conjunction with phenotypic surveillance to characterize CR-hvKP isolates collected from a large tertiary public health hospital in eastern China. This study aims to complement and update the growing body of epidemiological findings on this pathogen.

The majority of CR-hvKP isolates in our study were recovered from patients hospitalized in the ICU, EICU, and hematology departments. Patients hospitalized in these departments are at a greater risk of CR-hvKP infections, mainly due to immune status alterations, the use of broad-spectrum antibiotics, frequent comorbidities, and the use of invasive devices.[13–16] These CR-hvKP isolates showed 27.0% resistance to all six antimicrobial classes. Due to their multidrug resistance and higher virulence, the prognosis of some patients was poor, with 37.0% of patients experiencing delirium at discharge. Therefore, to prevent transmission, we should screen for CRE carriage to enable early detection and implementation of eradication measures.

Our study revealed a high diversity of STs (n=8), with ST11-KL64 *K. pneumoniae* being the predominant bla_{KPC} clone in China. ST11-KL64 CR-hvKP has been identified as a cause of bacterial liver abscess,[17] bacteremia, and other infections, and it is a high-risk clinical pathogen that has gained worldwide attention.[18] Notably, a Chinese report linked pneumonia with high mortality to ST11-KL64 CR-hvKP.[19] Our study results are consistent with these findings, with ST11-KL64 (n=37, 58.7%) accounting for most of the strains. In a multicenter study, ST11 also accounted for most infections (66.6%), followed by ST45 (8.2%), ST15, and ST290 (5.4% each).[20] KPC-producing ST15 *K. pneumoniae* have caused outbreaks.[21] However, a recent Chinese surveillance study reported a lower frequency of ST15 clones,[20] In our study, ST15 was the second most frequent CR-hvKP clone in hospital infections after ST11. Interestingly, we found that ST15-KL112 only carried OXA-232 genes, instead of bla_{KPC-2}. All in all, ST11-KL64 CR-hvKP poses a significant challenge for clinicians in various clinical settings and warrants further attention.

The 63 CR-hvKP isolates posed multi-drug-resistant features that exhibited high-level resistance to all β-lactam antibiotics and carbapenems, but remained susceptible to aminoglycosides. All the isolates harboring carbapenemases gene, which make the major contribution to the MDR phenotype. In this study, bla_{KPC-2} genes were predominant among CR-hvKP isolates (84.1%, n = 53), similar finding was noted in Asia and America where bla_{KPC-2} is class A enzymes highly prevalent in CR-hvKP isolates.[22] Moreover, the bla_{KPC-2} genes were distributed across almost all STs, while the diversity of strains carrying OXA-48-like genes was lower. In other countries, such as India, Iran, Russia, and Italy, the majority of CR-hvKP isolates were OXA-48-positive strains.[22] It is important to note that global immigration may lead to changes in the linkages between bacterial resistance mechanisms and regions or cities.[23] Therefore, surveillance of AMR trends should be maintained, and areas with low prevalence cannot be ignored. Furthermore, CR-hvKP strains simultaneously producing two or more carbapenemases can cause serious infectious diseases with high mortality.[22] In our study, two CR-hvKP strains producing bla_{KPC-2} and bla_{NDM-5} carbapenem resistance genes were reported.

All these 63 strains were found to carry virulence genes, including aerobactin (*iucA*), salmochelin (*iroB*), and regulators of mucoid phenotype (*rmpA* and *rmpA2)*. Siderophore systems are crucial for bacterial pathogenicity, enabling them to scavenge iron from host transport proteins, allowing them to survive and proliferate in the host.[24] Aerobactin plays a crucial role in both in vivo and vivo survival of hvKp, compared to other siderophores.[2] Aerobactin has been identified as the most prevalent siderophore in hvKp.[2] These investigations indicate that aerobactin (*iucA*) is the primary determinant of the virulence of hvKP. Our study showed the presence of *iucA* universally among all isolates, and all 63 isolates harbored carbapenemase-encoding genes, indicating the convergence of hypervirulence and multidrug resistance.

While salmochelin (*iroB*) was detected in only 5.4% of bacteremia isolates,[20] it was present in 17.5% of isolates in our study. We also observed a higher frequency of *rmpA* in isolates (41.3%) compared to bacteremia isolates in a previous study (25.2%).[20] The gene encoding aerobactin (*iucA*) was detected in 10.3% of isolates in Singapore, while the remaining virulence genes (*iro, rmpA*, and *rmpA2*) were far less prevalent, occurring in only 2.4% to 4.5% of the isolates.[25] The regulators of mucoid phenotype genes, *rmpA/A2*, were associated with hypermucoviscosity.[26] Strains with *rmpA/A2* were mainly enriched in KL1/KL2 and ST23/ST86/ST65 hypervirulent clones.[26] However, *rmpA/A2* was distributed across most strains in our study, except for the ST37-KL25 strains. The most pandemic linage observed in our study was ST11-KL64, which harbored *iucA, rpmA*, and *rmpA2*.

The copresence of *iucA, iroB, rmpA*, and *rmpA2* was observed in most isolates of ST412-KL57 and ST65-KL2 in our study, except for FK3104. Twenty-seven of the 63 CR-hvKP isolates (42.9%) harbored three or more hypervirulence genes. *K. pneumoniae* isolates carrying hypervirulence genes pose a risk of transmission and constitute a significant public health threat once they exhibit hypervirulence in vitro and vivo.

Conclusion

ST11-KL64 CR-hvKP has emerged as the most prevalent hypervirulence and carbapenem-resistant *K. pneumoniae* and contribute to the transmission of both resistance and hypervirulence phenotype, which required most clinical attention.

Ethics Statement

The research protocol was approved by the Ethics Committee of The First Affiliated Hospital of Ningbo University (2023019A). We confirm that all adult participants gave their informed consent. Guidelines outlined in the Declaration of Helsinki were followed.

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Disclosure

The authors report that there are no competing interests to declare for this work.

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