

Epidemiological Profile of Hypervirulent *Klebsiella pneumoniae* Isolates: A Multicenter Cross-sectional Study in Hebei Province, China

MingZhi Guo

the Second Hospital of Hebei Medical University

KaiYue Xu

the Second Hospital of Hebei Medical University

Pu Qin

the Second Hospital of Hebei Medical University

Cuixin Qiang

the Second Hospital of Hebei Medical University

Jing Yang

the Second Hospital of Hebei Medical University

Yanan Niu

the Second Hospital of Hebei Medical University

Weigang Wang

the Second Hospital of Hebei Medical University

Ye Gan

the Second Hospital of Hebei Medical University

Min Zhao

the Second Hospital of Hebei Medical University

Qiuyue Huo

the Second Hospital of Hebei Medical University

Qianqing Li

the Second Hospital of Hebei Medical University

Huimin Yang

the Second Hospital of Hebei Medical University

Jiafeng Zhao

the Second Hospital of Hebei Medical University

Zijun Geng

the Second Hospital of Hebei Medical University

Chang Lv

the Second Hospital of Hebei Medical University

Yanwei Li

the Second Hospital of Hebei Medical University

Xiang Wang

the Second Hospital of Hebei Medical University

Zhirong Li

the Second Hospital of Hebei Medical University

Jianhong Zhao

zhao.jh_2002@hebmh.edu.cn

the Second Hospital of Hebei Medical University

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Abstract

Objective

To investigate the clinical and molecular epidemiological characteristics of hypervirulent *Klebsiella pneumoniae* (hvKP) in Hebei Province, providing a theoretical basis for curbing its transmission and reducing the associated disease burden.

Methods

A total of 344 non-duplicate *Klebsiella pneumoniae* (KP) strains, along with their antimicrobial susceptibility results and clinical information of infected patients, were collected from 27 hospitals across different regions in Hebei Province. Multilocus sequence typing (MLST) and *wzi* serotyping of hvkp strains were further performed by PCR and Sanger sequencing.

Results

Among the 344 KP isolates, hvKP strains accounted for 25.3% (87/344). Zhangjiakou and Tangshan Cities exhibited the highest hvKP detection rate (44.4%, 8/18), while Shijiazhuang City had the lowest (14.7%, 14/95). ICU (19.5%, 17/87) and respiratory medicine department (13.8%, 12/87) were the top two source departments for hvKP infections. Respiratory disorders (32.2%, 28/87) comprised the most prevalent underlying disease in infected patients, followed by neurological disorders (28.7%, 25/87). Compared to non-hvKP cases, 86.2% (75/87) of hvKP patients exhibited favorable outcomes, with no statistically significant difference between the two groups. The resistance rates of hvKP demonstrated lower resistance rates to most antimicrobials compared with classical *K. pneumoniae* (cKP) ($P < 0.05$). Among hvKP isolates, ST23-K1 is the predominant genotype-serotype combination, with K1 (39.1%, 34/87), K2 (23.0%, 20/87), and K57 (21.8%, 19/87) as the major serotypes.

Conclusion

HvKP exhibits a high clinical isolation rate in Hebei Province, China. Enhanced clinical monitoring and the development of rational intervention strategies are essential to prevent the spread and epidemic of this pathogen.

Introduction

Klebsiella pneumoniae (KP) is a opportunistic pathogenic Gram-negative bacillus^[1], which is widely colonized in the human intestinal tract and respiratory tract. KP can cause a variety of severe infections in immunocompromised individuals or hospitalized patients, including hospital-acquired pneumonia,

urinary tract infections, sepsis, and catheter-related bloodstream infections [2,3]. In recent years, there have been an increasing number of research and clinical reports on hypervirulent *Klebsiella pneumoniae* (hvKP). Compared with classic *Klebsiella pneumoniae* (cKP), hvKP often exhibits a unique hypermucoviscosity phenotype and harbors related virulence factors, such as the mucin-regulating genes *ompA* and *ompA2*, as well as the iron acquisition-associated genes *magA* and *iroBCDN*, enabling it to cause severe infections in immunocompetent individuals [4,5]. Infections caused by hvKP can lead to a range of clinical manifestations, such as invasive liver abscess, meningitis and lung abscess, with an associated mortality rate up to 15%–30% [6].

The prevalence of hvKP exhibits obvious geographical variations and temporal trends. In July 2024, the World Health Organization (WHO) issued a warning stating that the global transmission risk of hvKP is moderate [7]. In particular, the ST23-K1 strain of hvKP has been detected in 12 countries and regions, indicating that the threat of hvKP to public health is escalating [7]. Analysis showed that the global prevalence rate of hvKP increased from 27.7% in 2006–2018 to 38.5% in 2019–2024 [8]. With the widespread use of broad-spectrum antibiotics, certain carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strains have acquired mobile genetic elements carrying virulence genes, thereby evolving into carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP), which has now emerged as a global public health concern [9]. Notably, the global infection rate of CR-hvKP increased from 9.5% in 2016–2018 to 16.5% in 2019–2024 [8]. This upward trend highlights that the prevalence of hvKP represents not only a regional issue, but also a common challenge for global public health.

To date, epidemiological studies on hvKP have been conducted across various regions in China; however, data specific to Hebei Province remain limited. Given its status as a populous province neighboring the Beijing city, capital of China, and its role as a pivotal transportation hub, **characterizing the epidemiology of hvKP in Hebei** holds profound significance for regional hvKP prevention and control. Therefore, this study aims to characterize the prevalence and epidemiological features of hvKP in Hebei, providing a scientific basis for regional surveillance and control strategies.

Materials and Methods

1.1 Strain Sources and Clinical Data

KP strains were collected from 27 hospitals across 11 cities in Hebei Province between August 23 and September 20, 2024. For duplicate strains from the same patient, only the first isolate was retained, while contaminated and duplicate strains were excluded from subsequent analyses. All isolates were identified as KP using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Following isolation and purification, strains were stored at -80°C until further analysis.

Patient clinical information was retrospectively collected, including gender, age, admitting department, date of isolation, underlying diagnoses (e.g., diabetes mellitus, hypertension, pulmonary diseases, hepatobiliary diseases), and clinical outcome. Clinical outcome information was retrospectively

collected at two time points, including the initial discharge outcome recorded on October 17, 2024, and an updated outcome status obtained on January 10, 2025. These data were combined to define the final clinical outcome.

1.2 Methods

1.2.1 Age Grouping

Patients were categorized into four age groups according to the criteria established by the China Antimicrobial Resistance Surveillance System (CARSS) ^[10]: neonatal group (age ≤ 28 days), pediatric group (age > 28 days and ≤ 14 years), adult group (age > 14 years and ≤ 65 years), and elderly group (age > 65 years). Analyses were performed separately for each age group.

1.2.2 Virulence Determination of Strains

PCR was employed to detect the virulence genes *rmpA*, *rmpA2*, *iroB*, *iucA*, and *peg-344*. KP strains positive for all these five virulence genes were defined as hvKP ^[11, 12, 13]. Primers were synthesized by Shanghai Biological Engineering Co., Ltd., with sequences listed in Table 1.

Table 1
Primer Sequences for Virulence Genes, *wzi* Gene, and Other Genes

Primer Name	Primer Sequence(5'~3')
rmpA-F	ACTGGGCTACCTCTGCTTCA
rmpA-R	CTTGCATGAGCCATCTTTCA
rmpA2-F	CTTTATGTGCAATAAGGATGTT
rmpA2-R	CCTCCTGGAGAGTAAGCATT
iucA-F	CTCTTCCCGCTCGCTATACT
iucA-R	GCATTCCACGCTTCACTTCT
iroB-F	CCGCAAAGAGACGAACCGCCTT
iroB-R	CGGGCAATCCCCGCTTTGACTT
peg344-F	CTTGAAACTATCCCTCCAGTC
peg344-R	CCAGCGAAAGAATAACCCC
16S RNA-F	AGAGTTTGATCCTGGCTCAG
16S RNA-R	ACGGCTACCTTGTTACGACTT
wzi-F	GTGCCGCGAGCGCTTTCTATCTTGGTATTCC
wzi-R	GAGAGCCACTGGTTCCAGAATTACCGC
rpoB-F	GGCGAAATGGCWGAGAACCA
rpoB-R	GAGTCTTCGAAGTTGTAACC
gapA-F	TGAAATATGACTCCACTCACGG
gapA-R	CTTCAGAAGCGGCTTTGATGGCTT
Mdh-F	CCCAACTCGCTTCAGGTTTCAG
Mdh-R	CCGTTTTTCCCCAGCAGCAG
Pgi-F	GAGAAAAACCTGCCTGTACTGCTGGC
Pgi-R	CGCGCCACGCTTTATAGCGGTTAAT
PhoE-F	ACCTACCGCAACACCGACTTCTTCGG
PhoE-R	TGATCAGAACTGGTAGGTGAT
InfB-F	CTCGCTGCTGGACTATATTCG
InfB-R	CGCTTTCAGCTCAAGAACTTC

Primer Name	Primer Sequence(5'~3')
TonB-F	CTTTATACCTCGGTACATCAGGTT
TonB-R	ATTCGCCGGCTGRGCRGAGAG

The PCR reaction mixture (25 µL total volume) consisted of 12.5 µL of 2× Taq Master Mix, 1 µL of forward primer, 1 µL of reverse primer, 8.5 µL of ddH₂O, and 2 µL of DNA template. Amplification conditions were as follows: initial denaturation at 95°C for 2 min; followed by 24 cycles of 95°C for 30 s, 55°C for 40 s, and 72°C for 1 min; and a final extension at 72°C for 10 min ^[14].

Electrophoresis: Approximately 8 µL of each PCR product was loaded onto a 1% agarose gel and electrophoresed at 120 V for 30 min. Gels were subsequently visualized using a gel imaging system to confirm the presence of specific amplification bands.

1.2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed in participating hospitals using automated systems, including VITEK 2 (bioMérieux), Meihua MA120, and Zhuhai DL-96. Susceptibility to ceftazidime/avibactam (CZA) was determined by the disk diffusion method. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Interpretations of resistant (R), intermediate (I), and susceptible (S) were based on the 2024 CLSI M100 breakpoints. Strains resistant to any of ertapenem (ETP), meropenem (MEM), or imipenem (IPM) were classified as CRKP.

1.2.4 Capsular Serotyping

Genomic DNA was extracted from strains using the rapid boiling lysis method. Capsular serotyping was predicted by *wzi* gene sequencing ^[15]. Primers for *wzi* amplification are listed in Table 1. The PCR reaction mixture was prepared as described in Section 1.2.2. Amplification conditions were as follows: initial denaturation at 94°C for 2 min; followed by 30 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. The amplified products were sent to Shanghai Bioengineering Co., Ltd. for unidirectional sequencing, and the data were submitted to the website of Pasteur Institut (<https://bigsd.bpasteur.fr>) for alignment to obtain the *wzi* typing and corresponding capsular type of the strains.

1.2.5 Multilocus Sequence Typing (MLST)

The seven housekeeping genes of KP (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) were amplified by PCR ^[16]. The PCR reaction mixture was prepared as described in Section 1.2.2.. Amplification conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 20 s, annealing at 50°C for 30 s (with annealing temperatures of 60°C for *gapA* and 45°C for *tonB*), and 72°C for 30 s; and a final extension at 72°C for 5 min. The amplified products were sent to Shanghai Bioengineering Co., Ltd.

for unidirectional sequencing, and the sequencing results were uploaded to the website of Pasteur Institut (<https://bigsd.b.pasteur.fr>) for alignment and analysis.

1.2.6 Statistical Analysis

Antimicrobial susceptibility data for KP were analyzed using SPSS version 25.0 (IBM Corp.) and WHONET version 5.6 software. Detection rates and proportions of hvKP were described using frequencies and percentages. Differences in hvKP proportions between groups were compared using the χ^2 test. A $P < 0.05$ was considered statistically significant. Data visualization was performed using the online platform ChiPlot (<https://www.chiplot.online>).

Results

2.1 Overview of KP Isolates

In total, 388 KP strains were collected from 27 hospitals across 11 cities in Hebei Province during August 23–September 20, 2024. After excluding 44 strains that were contaminated or duplicated, 344 strains were ultimately included in the subsequent genomic analyses. Analysis of the five virulence genes revealed that 87 strains defined as hvKP, while the remaining 257 strains were categorized as cKP. The overall detection rate of hvKP was 25.3% (87/344).

2.2 Regional Distribution of Strains

The distribution characteristics of KP isolate sources in each city of Hebei Province were shown in Fig. 1. In terms of the detection rate of hvKP, among the 11 cities, Zhangjiakou City and Tangshan City had the highest detection rates, both reaching 44.4% (8/18); followed by Baoding City at 42.9% (12/28), Hengshui City at 30.8% (4/13), and Qinhuangdao City at 25.8% (8/31); Shijiazhuang City had the lowest detection rate of hvKP, at 14.7% (14/95). However, these differences were not statistically significant ($P > 0.05$).

2.3 Distribution of Strains by Specimen Type and Department

Among the 87 hvKP strains, sputum were the most frequent specimens source, accounting for 70.1% (61/87), followed by blood and urine specimens at 6.9% (6/87) and 5.7% (5/87) respectively. Other specimen types accounted for 17.2% (15/87) of the total. Regarding department distribution, the 87 hvKP isolates primarily originated from the intensive care unit (ICU; 19.5%, 17/87), respiratory medicine (13.8%, 12/87), and general surgery and neurosurgery (both 8.0%, 7/87 each). There were no statistically significant differences in specimen types or department distribution between hvKP and cKP isolates ($P > 0.05$ for both; Table 2).

Table 2
Sources and Composition Ratios of Bacterial Strains

Variable	hvKP(n = 87)n(%)	cKP(n = 257)n(%)	KP	χ^2	<i>P</i>
Specimen Type				6.223	0.101
Sputum	61(70.1)	155(60.3)	216(62.8)		
Blood	6(6.9)	30(11.7)	36(10.5)		
Urine	5(5.7)	35(13.6)	40(11.6)		
Other	15(17.2)	37(14.4)	52(15.1)		
Department Distribution				3.667	0.453
ICU	17(19.5)	70(27.2)	87(25.3)		
Respiratory Medicine	12(13.8)	23(9.0)	35(10.2)		
General Surgery	7(8.0)	26(10.1)	33(9.6)		
Neurosurgery	7(8.0)	21(8.2)	28(8.1)		
Other	44(50.6)	117(45.5)	161(46.8)		

2.4 Clinical Characteristics of KP-Infected Patients

Among patients from whom hvKP isolates were recovered, males predominated, accounting for 72.4% (63/87), whereas females comprised 27.6% (24/87). With respect to age distribution, the elderly group represented the largest proportion at 63.2% (55/87), followed by the adult group at 36.8% (32/87). Analysis of underlying comorbidities revealed that respiratory system diseases were the most common among patients with hvKP infection, accounting for 32.2% (28/87), followed by neurological diseases (25/87, 28.7%) digestive system diseases (11.5%, 10/87), and cardiovascular system diseases (8.0%, 7/87). No statistically significant differences were observed in the distribution of clinical characteristics between patients infected with hvKP and those infected with classical *Klebsiella pneumoniae* (cKP) ($P > 0.05$). Regarding clinical outcomes, 86.2% (75/87) of patients with hvKP infection showed clinical improvement, whereas 13.8% (12/87) experienced clinical deterioration. There was no statistically significant difference in prognosis between the hvKP and cKP groups ($P > 0.05$; Table 3).

Table 3
Characteristics of Patients: Age, Gender, Medical History, and Clinical Outcomes

Characteristics	hvKP(n = 87)n(%)	cKP(n = 257)n(%)	KP	χ^2	P
Gender				3.855	0.05
Male	63(72.4)	156(60.7)	219(63.7)		
Female	24(27.6)	101(39.3)	125(36.3)		
Age Group				1.764	0.414
Neonatal group	0	0	0		
Pediatric group	0	2(0.8)	2(0.6)		
Adult group	32(36.8)	110(42.8)	142(41.3)		
Elderly group	55(63.2)	145(56.4)	200(58.1)		
Disease Classification				5.484	0.63
Neurological diseases	25(28.7)	66(25.7)	91(26.5)		
Respiratory diseases	28(32.2)	62(24.1)	90(26.2)		
Digestive system diseases	10(11.5)	35(13.6)	45(13.1)		
Cardiovascular diseases	7(8.0)	15(5.8)	22(6.7)		
Other	17(19.5)	79(30.7)	96(27.6)		
Clinical Outcome*					
Improvement	75(86.2)	216(84.0)	291(84.6)		
Deterioration	12(13.8)	41(16.0)	53(15.4)		
*Clinical outcomes were categorized based on discharge disposition. Improvement was defined as discharge with clinical improvement or complete recovery, whereas deterioration included clinical worsening, discharge against medical advice due to severe illness, transfer to a higher-level medical institution, or death.					

2.5 Comparison of Antimicrobial Resistance Between hvKP and cKP

Significant differences in resistance were observed between hvKP and cKP to the following antimicrobials: piperacillin, cefoperazone/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam, ampicillin/sulbactam, gentamicin, tobramycin, ciprofloxacin, chloramphenicol, ceftazidime, cefazolin, cefuroxime, cefotaxime, ceftazidime, cefepime, ceftriaxone, imipenem (IPM), meropenem (MEM), ertapenem (ETP), nitrofurantoin, aztreonam, and trimethoprim/sulfamethoxazole.

hvKP isolates exhibited significantly lower resistance rates to most antimicrobials than cKP ($P < 0.05$; Table 4). In addition, 4 CR-hvKP strains were identified among the hvKP isolates, of which two were resistant to IPM and ETP, and the remaining two were resistant to IPM and MEM.

Table 4
Antimicrobial Resistance Profiles of hvKP and cKP

Antimicrobial Agent	Resistance Rate(%)		χ^2	<i>P</i>
	hvKP	cKP		
Piperacillin	15.63	47.13	9.79	< 0.05
Cefoperazone/Sulbactam	5.80	16.92	5.251	< 0.05
Ceftazidime-Avibactam	0.00	0.00	-	-
Amoxicillin/Clavulanic Acid	3.33	45.21	16.858	< 0.001
Piperacillin/Tazobactam	4.65	18.18	9.194	< 0.05
Ampicillin/Sulbactam	12.12	43.08	20.703	< 0.001
Amikacin	3.66	9.17	2.589	0.108
Gentamicin	6.06	28.78	14.444	< 0.001
Tobramycin	3.70	34.29	19.051	< 0.001
Ciprofloxacin	12.90	45.23	21.062	< 0.001
Chloramphenicol	0.00	24.36	6.33	< 0.05
Ceftazidime	4.60	26.55	18.608	< 0.001
Cefazolin	8.51	44.03	19.346	< 0.001
Cefuroxime	11.59	46.24	25.952	< 0.001
Cefotetan	0.00	8.54	2.373	0.123
Cefotaxime	11.11	54.05	14.921	< 0.001
Cefoxitin	4.76	19.49	5.098	< 0.05
Cefepime	5.75	26.82	16.775	< 0.001
Ceftriaxone	5.26	38.39	14.829	< 0.001
Tigecycline	5.41	3.52	0.277	0.598
Minocycline	6.25	20.61	3.626	0.057
Imipenem	4.60	12.65	4.417	< 0.05
Meropenem	2.30	12.25	7.319	< 0.05
Ertapenem	2.30	14.29	7.726	< 0.05
Colistin	0.00	3.23	0.658	0.417
Nitrofurantoin	12.50	32.95	3.866	< 0.05

Antimicrobial Agent	Resistance Rate(%)		χ^2	<i>P</i>
	hvKP	cKP		
Aztreonam	5.33	36.15	26.059	< 0.001
Trimethoprim/Sulfamethoxazole	11.90	40.88	12.01	< 0.05

2.6 Distribution of MLST and Serotypes of hvKP

Among the 87 hvKP strains, 18 sequence types (STs) were identified in total, with ST23 being the predominant type (39.1%), as shown in Table 5. Through *wzi* gene sequencing and database comparison, 85 out of these strains were matched to the corresponding capsular serotypes, while the remaining 2 strains (ST893 and ST6618) had no corresponding K serotype in the reference database. Among the 87 strains, the K1 serotype was the most prevalent, accounting for 39.1% (34/87), followed by the K2 serotype at 23.0% (20/87) and the K57 serotype at 21.8% (19/87) (Fig. 2). The proportions of other serotypes were all less than 5%, as shown in Table 5. Combined analysis of ST types and capsular serotypes showed that ST23-K1 combination accounted for 37.9% (33/87) of all hvKP strains (Table 5 and Fig. 3). The K2 serotype was associated with multiple ST types, including ST65, ST86 and ST375, while the K57 serotype correlated with ST218, ST412 and ST592. Of the 4 CR-hvKP strains in this study, the corresponding ST-serotype combinations were ST23-K1, ST412-K57, ST485-K5, and ST1265-K1, respectively (Table 5).

Table 5
Correspondence Between MLST Types and Capsular Serotypes in hvKP

Sequence Type	Capsular Serotype	Number of Isolates(n = 85)	Percentage(%)
23	1	33	37.9
29	54	3	3.4
36	62	1	1.1
37	12	1	1.1
65	2	8	9.2
86	2	7	8.0
218	57	3	3.4
374	2	1	1.1
375	2	4	4.6
412	57	11	12.6
485	5	1	1.1
592	57	5	5.7
660	16	2	2.3
896	3	1	1.1
1049	5	3	3.4
1265	1	1	1.1

Discussion

The infection rate of hvKP is increasing globally, and its widespread prevalence has evolved into a worldwide public health problem ^[17, 18]. In China, the prevalence of hvKP is driven by multiple factors such as the extensive use of antibiotics, population mobility and the uneven distribution of medical resources. There is an urgent need to carry out more epidemiological studies to formulate targeted prevention and control strategies ^[19]. This study investigated and analyzed data including the detection rate, antimicrobial resistance, ST type, and serotype distribution of hvKP from multicenter samples in Hebei Province, so as to elucidate the regional epidemiological features of this pathogen.

The prevalence of hvKP varies across different regions of the world. A study showed that the global prevalence of hvKP is approximately 25.7%, with the lowest prevalence in the Americas (4.9%) and the highest in Africa (27.2%). Further analysis revealed that the prevalence is the lowest in Mexico (1.1%) and the highest in Denmark (83.3%) ^[20]. In this study, 87 strains of hvKP were isolated from 344

collected strains of KP, with a detection rate of 25.29%, which is consistent with the reports from other regions of China, such as 23.6% in Shanghai^[21] and 27.5% in Taiwan^[22]. However, some regions of China have reported notably higher or lower detection rates of hvKP, such as 73.91% in Wuhan, 57.14% in Shanghai and 8.33% in Zhejiang^[19]. Similarly, significant regional variations in hvKP detection rates were observed among different prefecture-level cities in Hebei Province. Zhangjiakou City and Tangshan City had the highest detection rate (44.4%), while Shijiazhuang City had the lowest (14.7%). This disparity may be attributed to regional differences in medical infrastructure and infection control awareness. Although variations were observed among different cities, these differences were not statistically significant. This finding suggests the absence of evident geographic clustering within Hebei Province during the study period. In addition, regional differences in detection rates may also be associated with variations in antibiotic usage patterns, sources of clinical samples, and geographic and climatic conditions^[23, 24]. For example, the incidence of KP bloodstream infections has been reported to increase significantly during warmer months^[25]. Further detailed investigations are required to clarify the specific factors underlying these observations.

In the present study, the detection of hvKP was mainly from the ICU, followed by Department of Respiratory Medicine and General Surgery. This might be associated with the fact that the ICU mainly admits critically ill and elderly patients with compromised immune resistance, along with the application of various invasive treatments and long-term use of large doses of broad-spectrum antibiotics. Patients in the ICU are at a higher risk of nosocomial infections^[26]. Therefore, during treatment, in addition to strictly implementing aseptic techniques and minimizing invasive procedures, attention should also be paid to the rational use of antimicrobial agents and monitoring of bacterial resistance^[26].

Consistent with previous studies, the present study demonstrated that the antimicrobial resistance rate of hvKP to most antimicrobial agents was lower than that of cKP^[27, 28]. It is well established that hvKP is more frequently associated with community-acquired infections, whereas cKP is predominantly linked to hospital-acquired infections^[29]. This epidemiological distinction might result in less exposure of hvKP to the high antibiotic pressure environment in hospitals, thereby reducing the risk of resistance evolution^[29]. Furthermore, the core virulence plasmids of hvKP, such as pLVPK or its homologous plasmids, are all belong to the IncHI1B/IncFIB hybrid incompatibility group. In contrast, the prevalent resistance plasmids of cKP, such as those of the IncFII or IncN type, are incompatible with pLVPK in terms of replication origins and regulatory elements^[30]. This intrinsic genetic incompatibility not only leads to inefficient horizontal transfer of resistance plasmids into hvKP cells but also results in poor post-transfer maintenance stability, thus limiting the stable acquisition of resistance traits by hvKP. The hvKP usually exhibits a hypermucoviscous phenotype, and its capsule may physically impede the uptake of exogenous plasmids, thereby reducing the efficiency of DNA transformation and conjugative transfer to a certain extent^[31]. Meanwhile, the hypermucoviscous phenotype can resist phagocytosis mediated by neutrophils, enhancing the immune evasion ability of the strains in the human body. As a result, the

viability and pathogenicity of hvKP mainly rely on intrinsic virulence factors rather than drug resistance [31].

MLST and *wzi*-based serotyping have been widely used in the molecular epidemiological investigation of KP, and they show high cost-effectiveness in resource-limited settings [32]. Hypervirulent KP strains are predominantly classified into ST23, with their capsular serotypes concentrated in K1, K2, K5, K20, K54 and K57. Strikingly, K1 and K2 are the most common hvKP serotypes in Asia, while their detection rates remain comparatively low in Europe and America [33,-37]. The siderophore systems of K1 and K2 strains can promote the proliferation of bacteria in the host by efficiently acquiring iron elements, thereby further enhancing their pathogenicity [38, 39]. In this study, a total of 18 ST types were detected among the 87 hvKP strains with ST23-K1 being the most prevalent combination(37.9%). The K2 serotype was associated with ST65, ST86 and ST375, indicating that the ST sequence types of the K2 serotype are more diverse. The K57 was predominantly associated with the ST412 clone, which is consistent with previous reports [40]. In China, K57-KP has been found to account for a relatively high proportion among hvKP isolates, second only to the classical dominant serotypes K1 and K2. Notably, it is also one of the capsular serotypes closely associated with pyogenic liver abscess [41]. As reported by Wei et al., compared with K1 and K2 strains, K57-KP carries virulence genes *wcaG*, *allS*, and *magA* at lower rates, while it exhibits higher serum susceptibility, elevated neutrophil phagocytosis efficiency, and stronger biofilm-forming capacity [40].

A total of 4 CR-hvKP strains were identified in the present study, with their genotypes characterized as follows: one strain belonged to ST23-K1, another to ST412-K57, a third to ST485-K5, and the fourth to ST1265-K1. Such substantial diversity in both genetic backgrounds and capsular serotypes implies that the emergence of CR-hvKP in this region is not derived from the clonal expansion of a single dominant strain, but rather is more probably attributed to parallel evolutionary processes occurring via multiple distinct evolutionary trajectories. This finding suggests the independent acquisition of virulence and drug resistance traits across different clonal backgrounds. Moving forward, a dynamic monitoring platform based on whole-genome sequencing should be established to real-time differentiate the conjugation of virulence plasmids and resistance plasmids, as well as key accompanying mutations, thereby enabling precise interruption of CR-hvKP transmission and dissemination.

Although the academic community has conducted extensive investigations hvKP, a unified diagnostic standard for this pathogen has not yet been established. Existing studies have adopted diverse diagnostic criteria: some take positive string test results or corresponding clinical manifestations as the basis for diagnosis, some use the presence of key virulence genes (e.g., *rmpA/rmpA2* and *iucA*) as diagnostic indicators, and others define hvKP based on whole-genome sequencing data. Such inconsistent definitions can lead to considerable variations in the detection rate of hvKP [43]. The diagnostic approach used in this study—defining hvKP by the presence of *iucA*, *iroB*, *peg-344*, *rmpA* and *rmpA2*—also has certain limitations. The virulence regulation of KP is mediated by complex molecular mechanisms: some cKP strains may carry only a subset of virulence genes but still exhibit a

hypervirulent phenotype, whereas some hvKP strains may harbor complete virulence gene clusters without corresponding phenotypic expression of virulence. Therefore, the virulence phenotype of the KP strains included in this study was not experimentally verified. Nevertheless, this limitation does not undermine the overall validity of the statistical results of the present study^[13, 20]. Another limitation is that the study only enrolled KP strains collected from August 2024 to September 2024, and the relatively small sample size may have contributed to the lack of statistically significant differences between hvKP and cKP strains in terms of specimen types, patients' clinical characteristics and clinical prognosis. In future research, it is necessary to include KP strains isolated from more diverse geographical regions and over longer time spans, in order to further explore the differential characteristics between hvKP and cKP strains.

In conclusion, the clinical isolation rate of hvKP is high in Hebei Province, China. Given the high genetic and capsular diversity of hvKP strains observed in this study, it is recommended to immediately establish a province-wide real-time monitoring network for hvKP to facilitate timely tracking of its epidemiological dynamics and effective control of its transmission.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hebei Provincial Hospital of Chinese Medicine (Approval No. HBZY2024-KY-127-01) with a waiver of informed consent and was conducted in accordance with the 1975 Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors report no conflicts of interest in this work.

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Author Contribution

M. Guo and K. Xu were responsible for manuscript writing and data collation. P. Qin, C. Qiang, J. Yang, Y. Niu, W. Wang, Y. Gan, M. Zhao, Q. Huo, Q. Li, H. Yang and J. Zhao were responsible for experimental

operation and data collection. Z. Geng, C. Lü, Y. Li and X. Wang participated in experimental design. Z. Li and J. Zhao were responsible for supervision and editing as well as experimental methodology.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1. Primer Sequences for Virulence Genes, *wzi* Gene, and Other Genes

Primer Name	Primer Sequence(5'~3')
rmpA-F	ACTGGGCTACCTCTGCTTCA
rmpA-R	CTTGCATGAGCCATCTTTCA
rmpA2-F	CTTTATGTGCAATAAGGATGTT
rmpA2-R	CCTCCTGGAGAGTAAGCATT
iucA-F	CTCTTCCCGCTCGCTATACT
iucA-R	GCATTCCACGCTTCACTTCT
iroB-F	CCGCAAAGAGACGAACCGCCTT
iroB-R	CGGGCAATCCCCGCTTTGACTT
peg344-F	CTTGAAACTATCCCTCCAGTC
peg344-R	CCAGCGAAAGAATAACCCC
16S RNA-F	AGAGTTTGATCCTGGCTCAG
16S RNA-R	ACGGCTACCTTGTTACGACTT
wzi-F	GTGCCGCGAGCGCTTTCTATCTTGGTATTCC
wzi-R	GAGAGCCACTGGTTCCAGAATTACCGC
rpoB-F	GGCGAAATGGCWGAGAACCA
rpoB-R	GAGTCTTCGAAGTTGTAACC
gapA-F	TGAAATATGACTCCACTCACGG
gapA-R	CTTCAGAAGCGGCTTTGATGGCTT
Mdh-F	CCCAACTCGCTTCAGGTTCAAG
Mdh-R	CCGTTTTTCCCCAGCAGCAG
Pgi-F	GAGAAAAACCTGCCTGTACTGCTGGC
Pgi-R	CGCGCCACGCTTTATAGCGGTTAAT
PhoE-F	ACCTACCGCAACACCGACTTCTTCGG
PhoE-R	TGATCAGAAGTGGTAGGTGAT
InfB-F	CTCGCTGCTGGACTATATTCG
InfB-R	CGCTTTCAGCTCAAGAACTTC
TonB-F	CTTTATACCTCGGTACATCAGGTT
TonB-R	ATTCGCCGGCTGRGCRGAGAG

Table 2. Sources and Composition Ratios of Bacterial Strains

Variable	hvKP(n=87)n(%)	cKP(n=257)n(%)	KP	χ^2	<i>P</i>
Specimen Type				6.223	0.101
Sputum	61(70.1)	155(60.3)	216(62.8)		
Blood	6(6.9)	30(11.7)	36(10.5)		
Urine	5(5.7)	35(13.6)	40(11.6)		
Other	15(17.2)	37(14.4)	52(15.1)		
Department Distribution				3.667	0.453
ICU	17(19.5)	70(27.2)	87(25.3)		
Respiratory Medicine	12(13.8)	23(9.0)	35(10.2)		
General Surgery	7(8.0)	26(10.1)	33(9.6)		
Neurosurgery	7(8.0)	21(8.2)	28(8.1)		
Other	44(50.6)	117(45.5)	161(46.8)		

Table 3. Characteristics of Patients: Age, Gender, Medical History, and Clinical Outcomes

Characteristics	hvKP(n=87)n(%)	cKP(n=257)n(%)	KP	χ^2	<i>P</i>
Gender				3.855	0.05
Male	63(72.4)	156(60.7)	219(63.7)		
Female	24(27.6)	101(39.3)	125(36.3)		
Age Group				1.764	0.414
Neonatal group	0	0	0		
Pediatric group	0	2(0.8)	2(0.6)		
Adult group	32(36.8)	110(42.8)	142(41.3)		
Elderly group	55(63.2)	145(56.4)	200(58.1)		
Disease Classification				5.484	0.63
Neurological diseases	25(28.7)	66(25.7)	91(26.5)		
Respiratory diseases	28(32.2)	62(24.1)	90(26.2)		
Digestive system diseases	10(11.5)	35(13.6)	45(13.1)		
Cardiovascular diseases	7(8.0)	15(5.8)	22(6.7)		
Other	17(19.5)	79(30.7)	96(27.6)		
Clinical Outcome*					
Improvement	75(86.2)	216(84.0)	291(84.6)		
Deterioration	12(13.8)	41(16.0)	53(15.4)		

*Clinical outcomes were categorized based on discharge disposition. Improvement was defined as discharge with clinical improvement or complete recovery, whereas deterioration included clinical worsening, discharge against medical advice due to severe illness, transfer to a higher-level medical institution, or death.

Table 4. Antimicrobial Resistance Profiles of hvKP and cKP

Antimicrobial Agent	Resistance Rate(%)		χ^2	P
	hvKP	cKP		
Piperacillin	15.63	47.13	9.79	<0.05
Cefoperazone/Sulbactam	5.80	16.92	5.251	<0.05
Ceftazidime-Avibactam	0.00	0.00	-	-
Amoxicillin/Clavulanic Acid	3.33	45.21	16.858	<0.001
Piperacillin/Tazobactam	4.65	18.18	9.194	<0.05
Ampicillin/Sulbactam	12.12	43.08	20.703	<0.001
Amikacin	3.66	9.17	2.589	0.108
Gentamicin	6.06	28.78	14.444	<0.001
Tobramycin	3.70	34.29	19.051	<0.001
Ciprofloxacin	12.90	45.23	21.062	<0.001
Chloramphenicol	0.00	24.36	6.33	<0.05
Ceftazidime	4.60	26.55	18.608	<0.001
Cefazolin	8.51	44.03	19.346	<0.001
Cefuroxime	11.59	46.24	25.952	<0.001
Cefotetan	0.00	8.54	2.373	0.123
Cefotaxime	11.11	54.05	14.921	<0.001
Cefoxitin	4.76	19.49	5.098	<0.05
Cefepime	5.75	26.82	16.775	<0.001
Ceftriaxone	5.26	38.39	14.829	<0.001
Tigecycline	5.41	3.52	0.277	0.598
Minocycline	6.25	20.61	3.626	0.057
Imipenem	4.60	12.65	4.417	<0.05
Meropenem	2.30	12.25	7.319	<0.05
Ertapenem	2.30	14.29	7.726	<0.05
Colistin	0.00	3.23	0.658	0.417
Nitrofurantoin	12.50	32.95	3.866	<0.05
Aztreonam	5.33	36.15	26.059	<0.001

Trimethoprim/Sulfamethoxazole	11.90	40.88	12.01	<0.05
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Table 5. Correspondence Between MLST Types and Capsular Serotypes in hvKP

Sequence Type	Capsular Serotype	Number of Isolates (n=85)	Percentage (%)
23	1	33	37.9
29	54	3	3.4
36	62	1	1.1
37	12	1	1.1
65	2	8	9.2
86	2	7	8.0
218	57	3	3.4
374	2	1	1.1
375	2	4	4.6
412	57	11	12.6
485	5	1	1.1
592	57	5	5.7
660	16	2	2.3
896	3	1	1.1
1049	5	3	3.4
1265	1	1	1.1

Figures

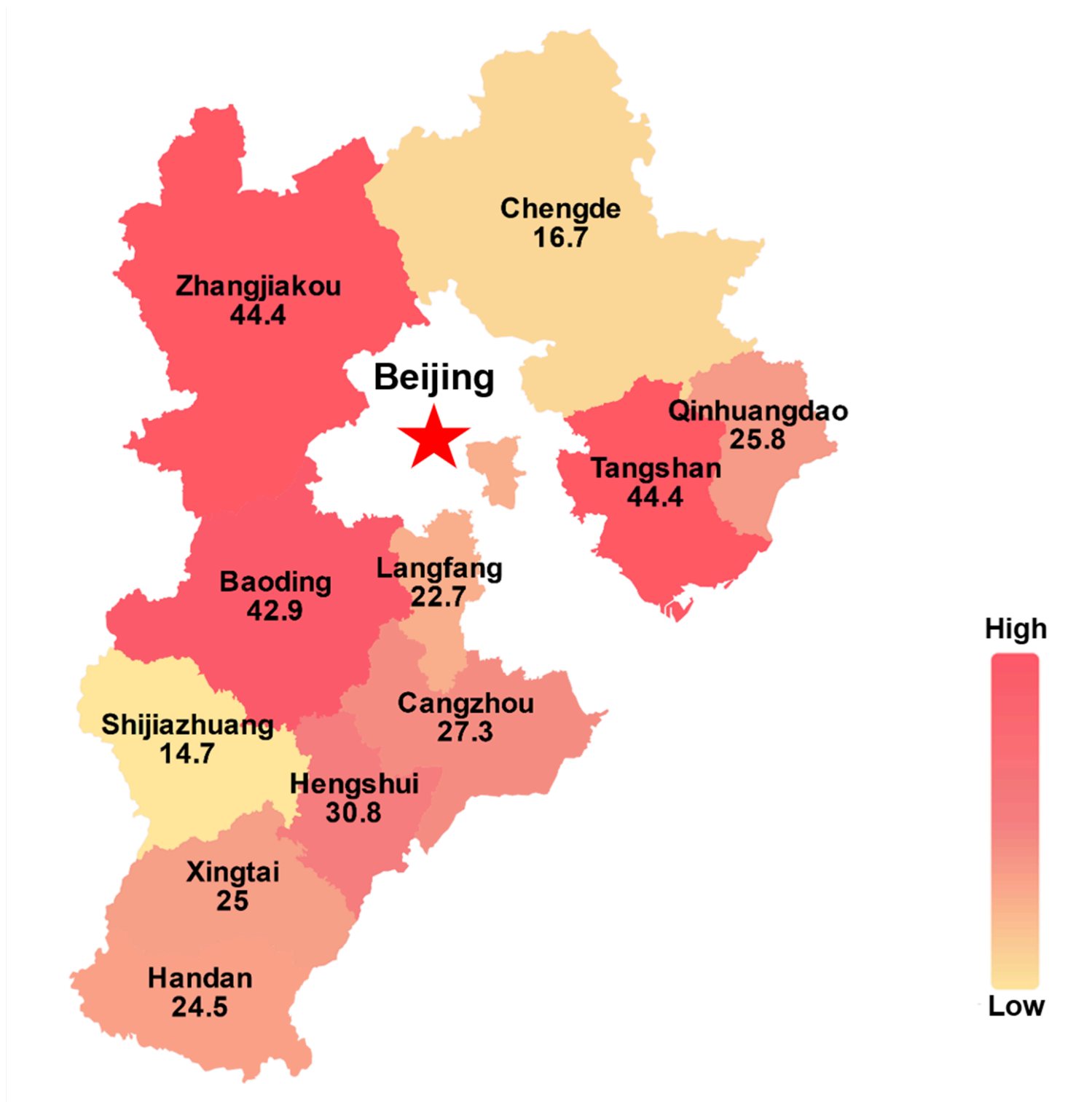


Figure 1

Detection Rate of hvKP in Prefecture-Level Cities of Hebei Province, China.

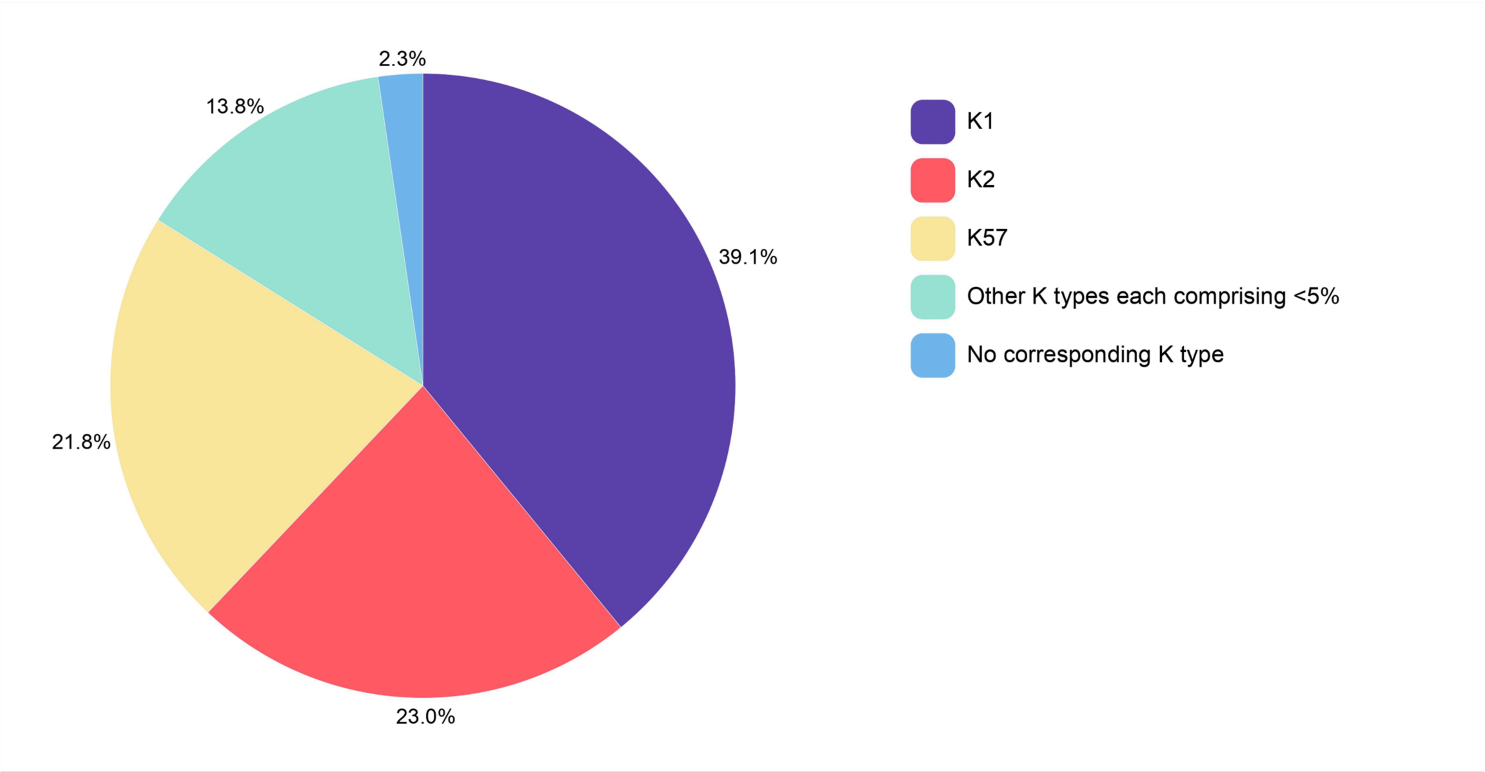


Figure 2

Proportions of capsular serotypes among hvKP strains.

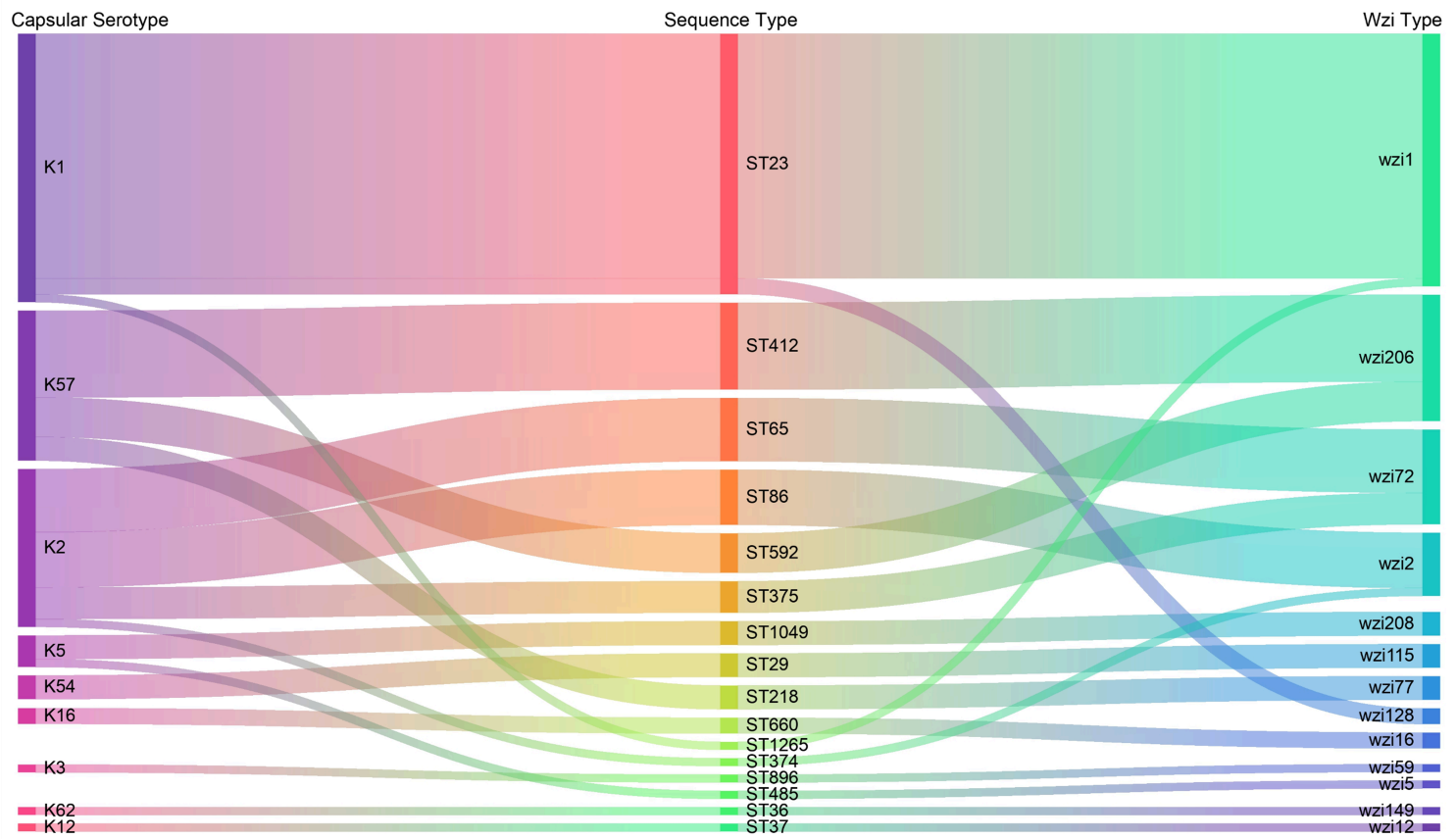


Figure 3

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Relationships among MLST sequence types, *wzi* alleles, and capsular serotypes in hvKP isolates. The Sankey diagram shows the relative abundances of different associations among STs, capsular serotypes, and *wzi* alleles. Flow thickness represents the frequency of each specific combination.