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ORIGINAL RESEARCH

Epidemiological and Molecular Characteristics of Hypermucoviscous and Hypervirulent *Klebsiella pneumoniae* Isolates in Community Patients in Shanghai, China

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Background: The occurrence and dissemination of hypermucoviscous and hypervirulent *Klebsiella pneumoniae* (hm-hvKp) isolates in clinical settings are a critical public health problem in the world. However, the data on these isolates in community populations are limited. This study aims to understand the prevalence and molecular characteristics of hm-hvKp isolates in community patients in Shanghai, China.

Methods: In 2018, an active surveillance system focused on hm-hvKp in community diarrhoeal cases was implemented in Pudong New Area, Shanghai, China, involving 12 sentinel hospitals. The antimicrobial susceptibility of hm-hvKp isolates from fecal samples was tested, and whole-genome sequencing (WGS) was performed to predict the serotypes and sequence types and to identify antimicrobial resistance determinants, virulence determinants, and phylogenetic clusters.

Results: The overall prevalence of hm *K. pneumoniae* isolates was 2.48% (31/1252), with the proportions of 1.76% (22/1252) for hm-hvKp and 0.72% (9/1252) for hm not hv *K. pneumoniae*. The prevalence of hm-hvKp isolates among different age groups and different months was statistically significant. All the 22 hm-hvKp isolates were susceptible to 20 antimicrobial agents and only carried bla_{SHV} gene, and KL1 and KL2 accounted for eight (36.36%) cases and seven (31.82%) cases, respectively. The eight ST23/KL1 isolates belonged to the predominant CG23-I clade, which typically possessed the virulence determinants profile of *rmpA*/*rmpA2-iro-iuc-ybt-irp-clb*. The five ST86/KL2 isolates were assigned to the global clusters ST86/KL2-1 (n=2), ST86/KL2-2 (n=2), ST86/KL2-3 (n=1), all lack of the *clb* gene. Shanghai ST23/KL1 and ST86/KL2 isolates were closely related to the global isolates from liver abscesses, blood, and urine.

Conclusion: Hm-hvKp is carried by the community population of Shanghai, with ST23/KL1 and ST86/KL2 isolates predominant. Hm-hvKp isolates of different continents, different sources, and different virulence levels were closely related. Ongoing surveillance of hm-hvKp isolates in the community population is warranted.

Keywords: hypermucoviscous, hypervirulent, *Klebsiella pneumoniae*, drug resistance determinants, virulence determinants, whole-genome sequencing

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a gram-negative opportunistic bacterium that can cause nosocomial and community infections[1,2] According to the level of virulence (pathotypes), it can be divided into hypervirulent *Klebsiella*

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pneumoniae (hvKp) and classical *Klebsiella pneumoniae* (cKp). Since the first report of hvKp in Taiwan Province, China, in 1986,[3] infections caused by hvKp have been well documented in Asian Pacific Rim,[4–6] especially in China,[7–9] but still rarely isolated in Europe;[10,11] the typical clinical syndrome involves liver abscess without biliary tract disease, and other manifestations of infection include endophthalmitis and bacteremia.[8,12–16] Since the terms hypermucoviscosity and hypervirulence have often been used synonymously in many studies. However, new evidence has suggested that hypermucoviscosity and hypervirulence are two different phenotypes that should not be used synonymously. Moreover, it is crucial to emphasize that a negative string test is inadequate for determining whether a strain possesses hypervirulence or not.[17] *Klebsiella pneumoniae* with both hypermucoviscous and hypervirulent phenotypes (for practical purposes, we refer to it as hm-hvKp) shows a higher level of virulence than cKp.[18,19]

Previous studies mainly reported nosocomial and extraintestinal infections related to hm-hvKp, which showed the epidemiological characteristics, antimicrobial resistance, and molecular characteristics of hm-hvKp isolates were diverse.[7,20–22] Over the past three decades, KL1 and KL2 have been the most common serotypes in clinical hm-hvKp isolates worldwide, followed by KL57, KL20, and KL5.[10,23] Molecular analysis based on multilocus sequence typing (MLST) or whole-genome sequence (WGS) revealed that most K1 isolates belonged to ST23, whereas KL2 represented different STs, such as ST86, ST65, and ST375.[9,14,15,24] In general, hm-hvKp is susceptible to most antimicrobials. However, with the acquisition of multidrug-resistant (MDR) plasmids, MDR hvKp, such as carbapenem-resistant hvKp (CR-hvKp) or colistin-resistant hv*Kp* (CoR-hvKp), have emerged,[25–28] complicating the course of clinical practice due to their multidrug-resistant and hypervirulent characteristics. In 2016, a fatal outbreak caused by ST11 CR-Kp with hypermucoviscous and hypervirulent (CR-hm-hvKp) was reported in ICU in China for the first time.[29]

Since then, ST11 CR-hm-hvKp has been widely reported in healthcare-associated and hospital-acquired infections all over the world, mostly in China.[26,30–32] Moreover, the circulation of CR-hm-hvKp in hospitals, urban wastewater treatment plants (WWTPs), and adjacent rivers was discovered in eastern China.[33] The rising prevalence and antimicrobial resistance of hm-hvKp are posing a serious challenge to public health.

Although those studies have provided a good understanding of the pathogenesis and epidemiology of hm-hvKp in clinical settings and environments, limited data are available to describe intestinal infection caused by hm-hvKp in community populations. It was supposed that hm-hvKp isolates causing liver abscesses came from the gastrointestinal tract,[34] and animal experiments proved that microorganisms are available to pass through the intestinal mucosa and vascular barrier to cause infections in the liver.[35] Therefore, it is of great importance to understand the prevalence and characteristics of hm-hvKp from the intestinal tract to explore the traceability of hm-hvKp infection.

This study aimed to analyze the prevalence, antimicrobial susceptibility, and molecular characteristics of hm-hvKp isolates in community patients in Shanghai, China. The findings of this study will provide scientific evidence for the prevention and control of hm-hvKp infection and the assessment of disease burden.

Materials and Methods

Case Recruitment

A cross-sectional surveillance of *K. pneumoniae* isolates in diarrhoeal outpatient cases was conducted in 12 sentinel hospitals (three tertiary hospitals, seven secondary hospitals, and two primary hospitals) from January 1st 2018 to August 31st 2018 in Pudong New Area of Shanghai, China (Figure 1). Pudong New Area is the largest district (over 1210 square kilometers) of Shanghai Municipality, which locates in a latitude of 31.14N and longitude of 121.81E. There are 12 subdistricts and 24 towns in Pudong New Area, with more than 5.5 million residents. The 12 sentinel hospitals were chosen based on their location, catchment area, and patient volume. Diarrhoea was defined as three or more loose stools within the previous 24 hours according to the guidelines of the Global Enteric Multicentre Study (GEMS).[36] The first 3–6 outpatient cases meeting the definition of diarrhoea were investigated and sampled in each sentinel hospital every week. All recruited patients and/or their parents/guardians were interviewed by trained doctors or nurses using a standard questionnaire in hospitals. Demographic (eg, gender, date of birth, and address) information was collected and recorded. Patients referred from other hospitals or who had an experience of hospitalization or travelled abroad within the past 3 months were excluded.

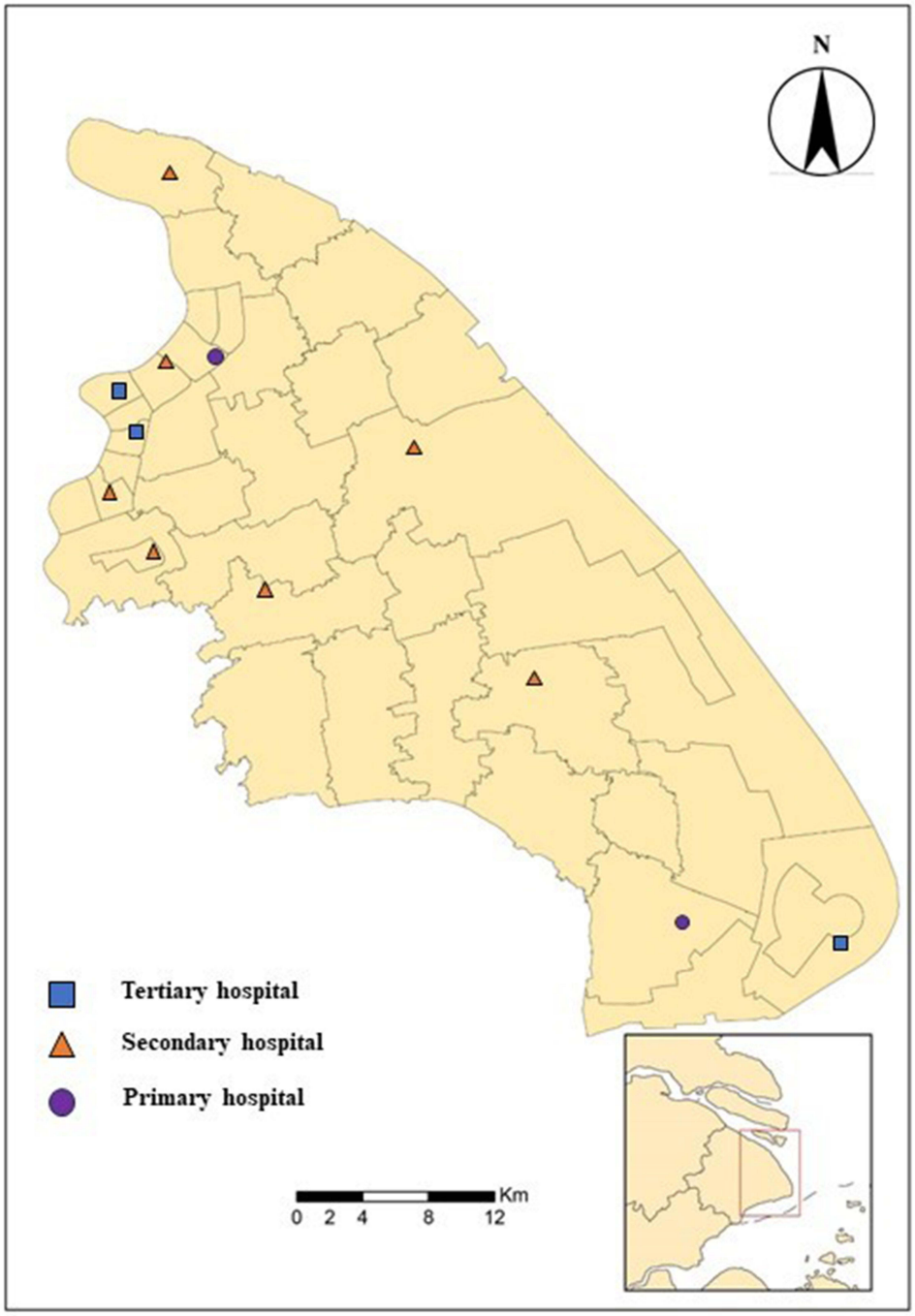

Figure 1 Distribution of 12 sentinel hospitals in Pudong New Area of Shanghai, China.

Sample Collection and Transport

Fresh fecal specimens were collected in sterilized containers when the recruited patients visited a doctor and then transferred to the Pudong New Area Center for Disease Control and Prevention (PDCDC) laboratory for *K. pneumoniae* testing within 24 hours.

Culture and Confirmation of Hm-hvKp

Fecal samples were directly inoculated on MacConkey agar (OXOID, UK) and cultured at 36 ± 1°C for 24 hours.[37] Suspected *Klebsiella spp*. colonies were firstly identified by both VITEK 2 Compact (BioMérieux, France) and real-time

fluorescence quantitative PCR (Mabsky, China); then, hmKp complex isolates were confirmed as previously described.[38] In short, the string test was used for hmKp complex screening, and the positive isolates (formation of viscous strings >5 mm in length when a loop was used to stretch the colony on agar plate) were further confirmed with whole-genome sequencing using the Kleborate software, which included assembly quality, species, antimicrobial resistance determinants, serotype prediction, multilocus sequence typing (MLST), and acquired virulence determinants.[39] The isolates, which were identified as *K. pneumoniae* with aerobactin virulence determinants *iuc* positive,[22,23,40] were confirmed as hm-hvKp, while *iuc*-negative *K. pneumoniae* were hm not hv*K. pneumoniae* (Figure 2).

Whole Genome Sequencing

All the hmK.p complex isolates were selected for genome sequencing. DNA libraries were constructed using the KAPA SYBR® FAST qPCR kits (Kapa Biosystems, USA) and sequenced using the Illumina HiSeq PE150 sequencing platform (Illumina, USA). Paired-end reads (150 bp) were generated, and the sequencing depth was ≥100x. Low-quality reads were filtered, and clean reads were available for further analysis. The raw data were assembled using A5-MiSeqv20150522[41] and

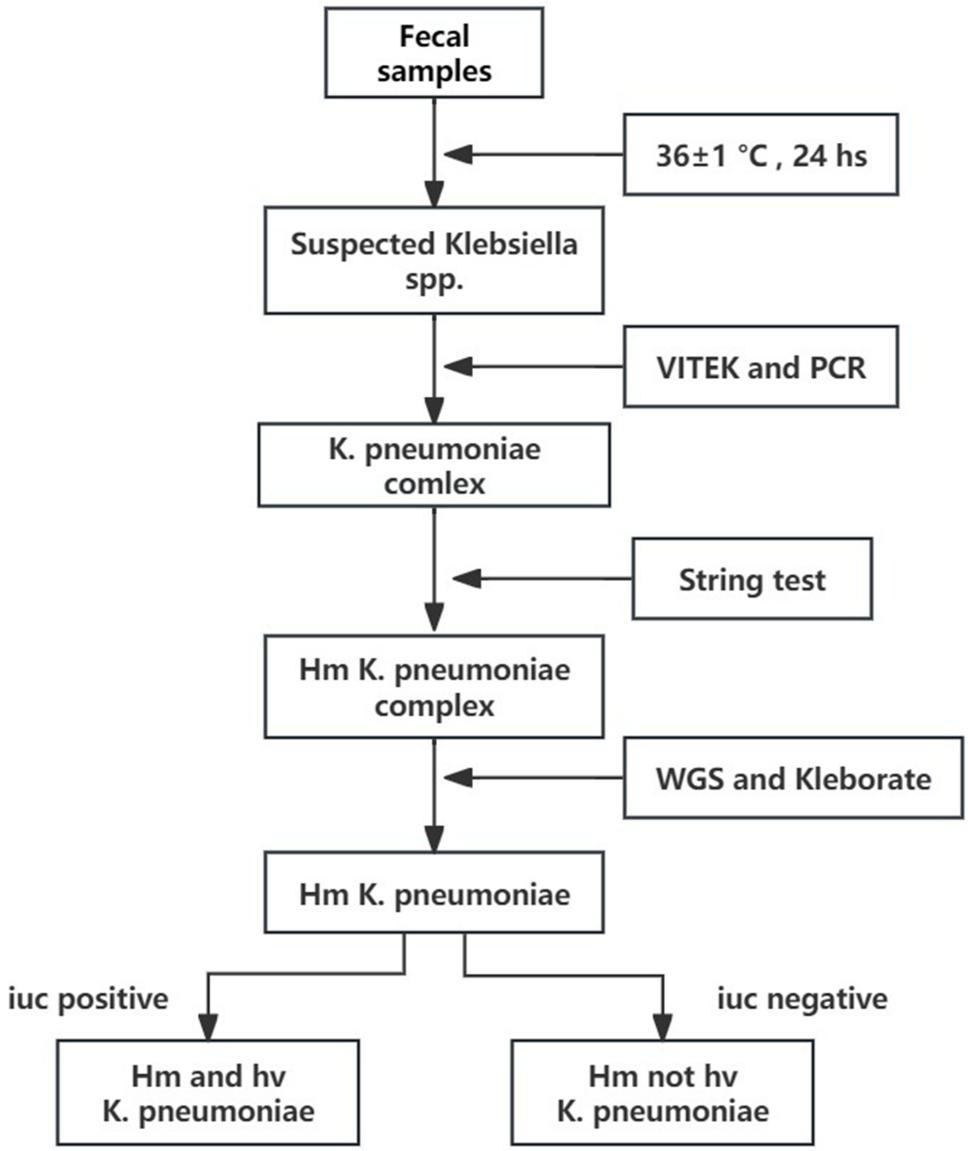

Figure 2 Culture and confirmation of hm and hv*K. pneumoniae* isolates and hm not hv*K. pneumoniae* isolates.

SPAdesv3.9.0[42] to construct contigs and scaffolds. All the assembled genomes were evaluated using CheckM,[43] and the qualified genomes should have completeness over 95% and contamination less than 5%.

Antimicrobial Susceptibility Testing (AST)

To assess the susceptibility of *K. pneumoniae* isolates to 20 antimicrobial agents of nine categories, the minimum inhibitory concentrations (MICs) were determined using the broth microdilution method, and breakpoints were used according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (M100-Ed33, 2023). *Escherichia coli* (ATCC25922) and *Klebsiella pneumoniae* (ATCC700603) were used as reference strains for quality control. The MIC ranges (μg/mL) of 20 antimicrobials used in our study were as follows: Amoxicillin/Clavulanate (2/1~64/32); Ampicillin/Sulbactam (2/1~64/32); Cefoxitin (2~64); Ceftazidime (0.5~16); Cefotaxime (0.25~8); Cefepime (0.25~16); Amikacin (4~128); Gentamicin (1~32); Kanamycin (8~64); Nalidixic acid (4~64); Levofloxacin (0.125~8); Ciprofloxacin (0.03~32); Tetracycline (1~32); Minocycline (1~32); Doxycycline (0.5~16); Meropenem (0.06~4); Imipenem (0.25~8); Trimethoprim/sulfamethoxazole (0.25/4.75~8/152); Chloramphenicol (2~64); Aztreonam (1~32).

Kleborate Analysis

All the *K. pneumoniae* genomes were analyzed with Kleborate software[39] to obtain the distribution of antimicrobial resistance determinants, serotype prediction, MLST, and acquired virulence determinants.

Phylogenetic Analysis

Phylogenetic analysis based on single-nucleotide polymorphisms (SNPs) was conducted using snippy software, with 31 genomes (22 hm-hvKp and nine hm not hv *K. pneumoniae*) collected in this study to be mapped to hypervirulent *K. pneumoniae* reference genome NTUH-K2044 (GenBank: GCA_000009885.1) and SGH10 (GenBank: CP025080.1).[44,45] To put our hm-hvKp isolates into the context of the global hvKp population, 406 ST23/KL1 genomes and 167 ST86/KL2 genomes from the BIGSdb-Pasteur database and NCBI database were retrieved (Figure S1 and Table S1) and used for constructing the phylogenetic trees of global ST23/KL1 and ST86/KL2 genomes, respectively. SGH10 (GenBank: CP025080.1) and KPN55602 (GenBank: CP042977.1) were used as reference genome for ST23/KL1 and ST86/KL2 isolates, respectively.[44,46] iTOL software was used for visualization of the phylogenetic trees.[47]

Statistical Analysis

Categorical variables were summarized in numbers (percentages) and analyzed using the chi-square test or Fisher's exact test. Continuous variables are presented as the median (range). A two-sided test was applied, and results with $P < 0.05$ were considered statistically significant. All analyses were performed using SPSS Statistics 26 (IBM Corporation, NY, USA).

Results

Characteristics of the Patients

A total of 1252 patients were enrolled in this study, including 682 (54.47%) males and 570 (45.53%) females. The age of the patients ranged from 7 days old to 90 years old, with a median age of 18 (interquartile range [IQR], 2–40) years old (Table 1).

Among the 1252 cases, 122 isolates of *K. pneumoniae* complex were identified, of which 31 (25.41%) isolates were hm *K. pneumoniae*, and three isolates (2.46%) were hm *K. quasipneumoniae*. The overall prevalence of hm *K. pneumoniae* isolates was 2.48% (31/1252), with the proportions of 70.97% (22/31) for hm-hvKp and 29.03% (9/31) for hm not hv *K. pneumoniae*. Among the 22 patients with hm-hvKp, 15 (68.18%) were male and 7 (31.82%) were female. The age of the patients ranged from 1 year old to 87 years old, with a median age of 32.5 (IQR 11–60) years old. The prevalence of hm-hvKp isolates was higher in the male than in the female (2.20% vs 1.23%), but the difference was not statistically significant (P=0.19). The prevalence of hm-hvKp isolates among different age groups was statistically significant (P=0.05), and a linear increase was also found among different age groups (P=0.01). The difference in the prevalence of hm-hvKp isolates

Table 1 Basic Characteristics of the Recruited Cases

Characteristic	No. of Cases	Proportion (%)
Total	1252	100
Sex		
Male	682	54.47
Female	570	45.53
Age		
0–4	466	37.22
5–19	167	13.34
20–59	440	35.14
≥ 60	179	14.30
Month		
January	114	9.11
February	69	5.51
March	150	11.98
April	154	12.30
May	138	11.02
June	140	11.18
July	235	18.77
August	252	20.13

among different months was statistically significant (P=0.005), with the highest prevalence in February (8.70%, 6/69). No statistically significant difference was found in sex, age groups, and months in hm not hv *K. pneumoniae* isolates, respectively (Table 2).

Antimicrobial Susceptibility Testing and Resistance Determinants Detection

All the 22 hm-hvKp isolates were susceptible to 20 antimicrobial agents (Table 3), and all the 22 isolates only carried bla_{SHV} gene, which encode β-lactamase resistance. No other resistance determinants were found. Similar characteristics were found in the nine hm not hv *K. pneumoniae* isolates. All the hm not hv *K. pneumoniae* isolates were susceptible to 20 antimicrobial agents except for one isolate (PDKP18045) that was resistant to Amoxicillin/Clavulanate, Ampicillin/Sulbactam and Cefoxitin, while intermediate to Levofloxacin and Ciprofloxacin, as well. Eight isolates only carried bla_{SHV} gene, and one isolate (PDKP18045) carried bla_{SHV}, bla_{DHA}, *str, qnr*, and *sul* genes.

Serotypes and MLST

Among the 22 hm-hvKp isolates, six serotypes were found (Figure S2A). KL1 and KL2 were predominant, accounting for eight (36.36%) cases and seven (31.82%) cases, respectively, followed by KL57 (13.64%, n=3). Other serotypes included KL20 (9.10%, n=2), KL5 (4.55%, n=1), and KL64 (4.55%, n=1). MLST of the 22 isolates revealed 11 different sequence types (STs); all the KL1 isolates belonged to ST23, whereas the KL2 isolates represented ST86 (n=4), ST375 (n=2), and ST380 (n=1).

Table 2 Prevalence of hm *K. pneumonia*, hm-hvKp, and Hm Not Hv *K. pneumoniae* Infections

Characteristic	No. of cases	No. of hm *K. pneumoniae* Infections (Prevalence, %)	*P*	No. of hm-hvKp Infections (Prevalence, %)	*P*	No. of hm not hv *K. pneumoniae* Infections (Prevalence, %)	*P*
M(IQR)[a]		36(11–60) years old		32.5(11–60) years old		36(3–63) years old	
Sex			0.26		0.19		1.00
Male	682	20(2.93)		15(2.20)		5(0.73)	
Female	570	11(1.93)		7(1.23)		4(0.70)	
Age			0.09		**0.05**		0.92
0–4	466	6(1.28)		3(0.64)		3(0.64)	
5–19	167	4(2.40)		3(1.80)		1(0.60)	
20–59	440	13(2.95)		10(2.27)		3(0.68)	
≥ 60	179	8(4.47)		6(3.35)		2(1.12)	
Month			**0.003**		**0.005**		0.46
January	114	6(5.26)		4(3.51)		2(1.75)	
February	69	6(8.70)		5(7.25)		1(1.45)	
March	150	2(1.34)		1(0.67)		1(0.67)	
April	154	1(0.65)		1(0.65)		0	
May	138	2(1.45)		2(1.45)		0	
June	140	6(4.28)		5(3.57)		1(0.71)	
July	235	6(2.56)		3(1.28)		3(1.28)	
August	252	2(0.80)		1(0.40)		1(0.40)	

Notes: [a]Indicated median (interquartile range); bold numerical text indicated statistically significant.

Table 3 Antimicrobial Susceptibility of 22 hm-hvKp Isolates and the Nine hm Not hv*K. pneumoniae* Isolates

Antimicrobial Category	Antimicrobial Agent	Range (μg/mL)	Intermediate n (%)		Resistant n (%)	
			hm-hvKp	hm not hv*K. pneumoniae*	hm-hvKp	hm not hv*K. pneumoniae*
β-Lactam/β-lactamase inhibitor combinations	Amoxicillin/Clavulanate	2/1~64/32	0	0	0	1(11.11)
	Ampicillin/Sulbactam	2/1~64/32	0	0	0	1(11.11)
Cephems	Cefoxitin	2~64	0	0	0	1(11.11)
	Ceftazidime	0.5~16	0	0	0	0
	Cefotaxime	0.25~8	0	0	0	0
	Cefepime	0.25~16	0	0	0	0

(*Continued*)

Table 3 (Continued).

Antimicrobial Category	Antimicrobial Agent	Range (μg/mL)	Intermediate n (%)		Resistant n (%)	
			hm-hvKp	hm not hv*K. pneumoniae*	hm-hvKp	hm not hv*K. pneumoniae*
Aminoglycosides	Amikacin	4~128	0	0	0	0
	Gentamicin	1~32	0	0	0	0
	Kanamycin	8~64	0	0	0	0
Quinolones and fluoroquinolones	Nalidixic acid	4~64	0	0	0	0
	Levofloxacin	0.125~8	0	1(11.11)	0	0
	Ciprofloxacin	0.03~32	0	1(11.11)	0	0
Tetracyclines	Tetracycline	1~32	0	0	0	0
	Minocycline	1~32	0	0	0	0
	Doxycycline	0.5~16	0	0	0	0
Carbapenems	Meropenem	0.06~4	0	0	0	0
	Imipenem	0.25~8	0	0	0	0
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	0.25/4.75~8/152	0	0	0	0
Phenicols	Chloramphenicol	2~64	0	0	0	0
Monobactams	Aztreonam	1~32	0	0	0	0

In the nine hm not hv *K. pneumoniae* isolates, eight serotypes and nine STs were found (Figure S2B). KL57 was found in two isolates, while KL2, KL5, KL103, KL107, KL114, KL117, and KL163 each were represented by one isolate. Nine STs comprised ST412, ST1035, ST86, ST4746, ST1213, ST2693, ST42-2LV, ST1103, and ST2405, with each represented by one isolate.

Virulence Determinants

Seven groups of common virulence determinants were found in the 22 hm-hvKp *K. pneumoniae* isolates. Twenty-two (100%) isolates harbored the genes *rmpA* (encoding mucus phenotype regulatory protein), *iuc* (aerobactin), and *iro* (encoding salmochelin). Eighteen (81.82%) isolates harboured the *rmpA2* gene (encoding mucus phenotype regulation). Fourteen (63.64%) isolates harboured *ybt* and *irp* genes (encoding yersiniabactin). Ten (45.45%) isolates harboured the *clb* gene (encoding colibactin). None of the virulence determinants *rmpA2*, *iuc*, and *clb* were found in the nine hm not hv *K. pneumoniae* isolates.

Phylogenetic Analysis

According to the SNP-based phylogenetic analysis, the 31 *K. pneumoniae* isolates (22 hm-hvKp and nine hm not hv *K. pneumoniae*) were divided into four clades (Figure 3). Clade 1 was composed of 16 hm-hvKp and seven hm not hv *K. pneumoniae* isolates, while Clade 2, Clade 3, and Clade 4 each comprised two hm-hvKp isolates. Among the Clade 1, there were two dominant hm-hvKp clusters. One cluster was composed of eight ST23/KL1 isolates, which shared the same profile of antimicrobial resistance determinants (bla_{SHV}) and virulence determinants (*rmpA-rmpA2-iro-iuc-ybt-clb-irp*). Another cluster was composed of five ST86/KL2 isolates with different profiles of virulence determinants, all without the *clb* gene.

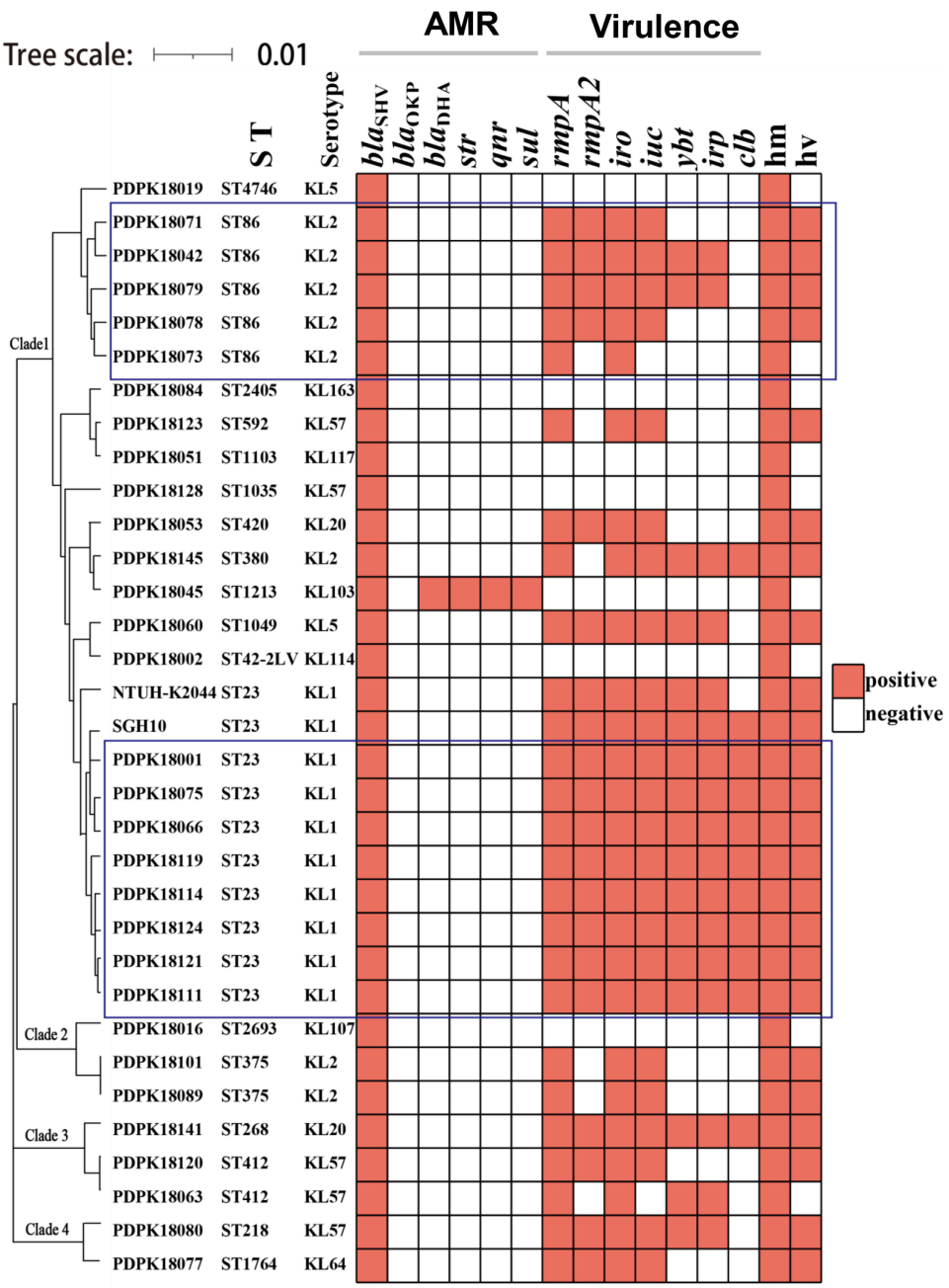

Figure 3 Phylogenetic analysis of 31 *K. pneumoniae* isolates collected in this study. The hvKp genomes NTUH-K2044 and SGH10 were used as reference. AMR, antimicrobial resistance.

To describe the relationship of ST23/KL1 isolates from Shanghai and from other countries, we constructed the phylogenetic tree of 406 global ST23/KL1 genomes (Table S1), including hvKp (n=363) and cKp (n=43). The global ST23/KL1 genomes were divided into two sublineages, and we designated them as CG23-I and CG23-II to be consistent with the findings of Lam et al.[44] The possession of virulence determinants *ybt* and *clb* were obviously higher in the clade CG23-I (98.07% and 98.90%) than in CG23-II (59.09% and 0%). The CG23-I clade was predominant (89.16%, 362/406) and contained seven main clusters (CG23-I-1 to CG23-I-7). Except the cluster CG23-I-5 (France, horse-associated), each cluster was composed of isolates of different continents, different sources, and different virulence levels (hvKp and cKp) (Figure 4). The sublineage CG23-II contained the complete genomes of NTUH-K2044, ED23, and NCTC9494, which

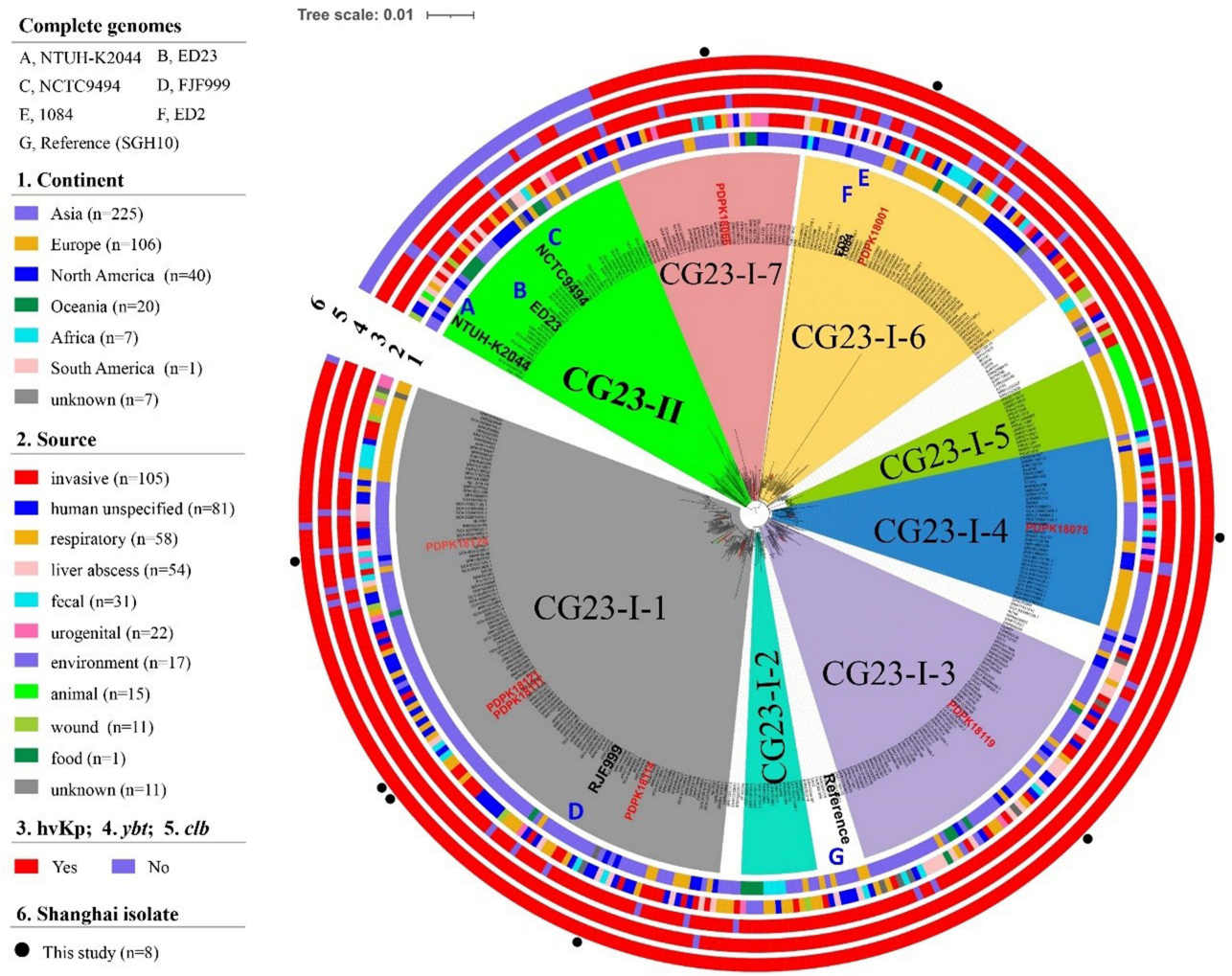

Figure 4 SNP-based phylogenetic analysis of 406 global ST23/KL1 genomes. The complete genome SGH10 (GenBank: CP025080.1) was used as reference genome. The eight Shanghai ST23/KL1 genomes are indicated in red text.

were often used as reference strains of hvKp. The eight Shanghai ST23/KL1 isolates collected in this study were distributed in the clusters CG23-I-1 (n=4), CG23-I-3 (n=1), CG23-I-4 (n=1), CG23-I-6 (n=1), and CG23-I-7 (n=1), closely related to isolates of other sources, such as blood and liver abscess.

Phylogenetic analysis of 167 global ST86/KL2 genomes was also performed (Table S2), involving hvKp (n=140) and cKp (n=27). The global ST86/KL2 genomes were mainly divided into four clusters, ST86/KL2-1 (n=76), ST86/KL2-2 (n=34), ST86/KL2-3 (n=51), and ST86/KL2-4 (n=6), each of which was composed of isolates of different continents, different sources, and different virulence levels (hvKp and cKp) (Figure 5). None of the 167 global ST86/KL2 genomes harbored *clb* gene, while the frequency of the gene *ybt* was 61.68% (103/167), varying in the four clusters, as ST86/KL2-1 (52.63%, 40/76), ST86/KL2-2 (41.17%, 14/34), ST86/KL2-3 (90.20%, 46/51), and ST86/KL2-4 (50%, 3/6). The five ST86/KL2 isolates collected in this study were assigned to the cluster ST86/KL2-1 (n=2), ST86/KL2-2 (n=2), ST86/KL2-3 (n=1), respectively. Except for one isolate (PDPK18071), other four Shanghai ST86/KL2 isolates were each closely related to one isolate from another source, including KPB2523_18 (blood, Russia), KPN55602 (blood, China, Anhui Province), KP19026AIH (Hunman unspecified, Japan), and KPNY42 (urine, China, Hunan Province).

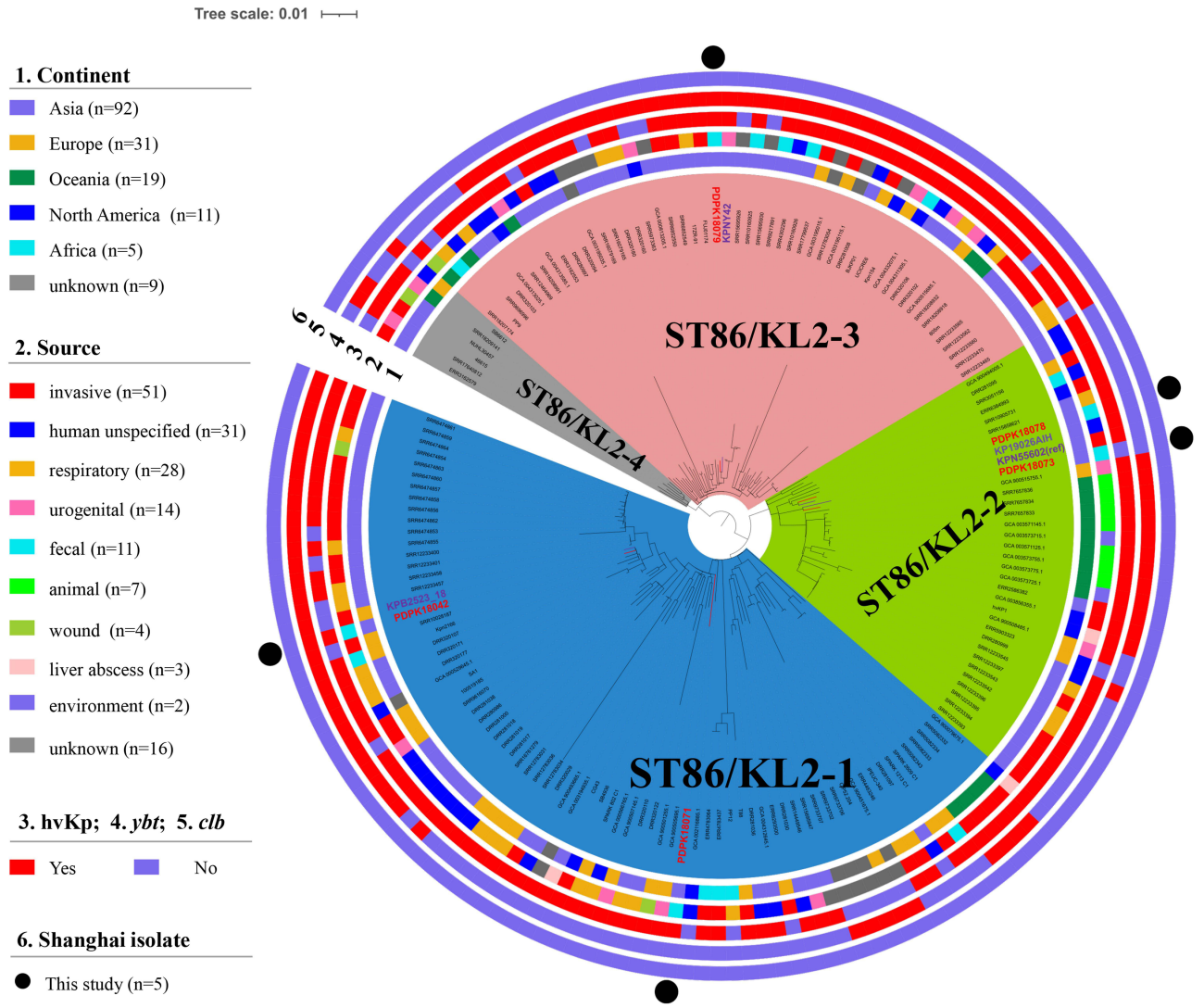

Figure 5 SNP-based phylogenetic analysis of 167 global ST86/KL2 genomes. The complete genome KPN55602 (GenBank: CP042977.1) was used as reference genome. The five Shanghai ST86/KL2 genomes are indicated in red text, while the genomes closest with them are indicated in purple text.

Discussion

Our study revealed an overall prevalence of hypermucoviscous (hm) *K. pneumoniae* isolates at 2.48% (31/1252), with 1.76% (22/1252) classified as both hypermucoviscous and hypervirulent (hm-hvKp) and 0.72% (9/1252) as hypermucoviscous but not hypervirulent *K. pneumoniae* (hm not hv *K. pneumoniae*). Notably, individuals aged ≥60 years exhibited a higher proportion (3.35%) of carriage for hm-hvKp. These strains displayed low frequencies of antimicrobial resistance and harbored various virulence determinants. The prevalent types in community patients were identified as ST23/KL1 and ST86/KL2, closely associated with global isolates from liver abscesses, blood, and urine.

In our study, 1.76% of hm-hvKp carriage was found in community patients, which showed the level of hm-hvKp prevalence in Shanghai, China, was different from other countries and districts. Previous studies on the hvKp colonization in the intestinal tract of healthy Chinese and overseas Chinese adults in Asia found a high prevalence of hvKp infection in Japan (16.7%), Singapore (14.9%), Malaysia (14.1%), Hong Kong (12%), Mainland China (11.7%), Taiwan (11.3%), South Korea (4.6%), and Thailand (2.7%).[48,49] A recent survey also revealed a high prevalence of hvKp carriage in the human gut in seven provinces (5.19%) and families in two villages (4%) in China.[50] However, several other studies indicated a much lower prevalence of hvKp colonization in the human gut. A cross-sectional study in Norway showed that hvKp isolates were identified in 0.2% (5/2975) fecal specimens from the adult population.[51] Similarly, another study based on sentinel hospitals in Beijing, China, during 2010–

2019 demonstrated a prevalence of 0.17% (44/25,411) of hvKp infection in outpatient cases.[52] The different prevalence of infection may be mainly related to the different composition of intestinal flora and sanitary conditions of people in various areas. In addition, the differences in the methods used to identify hvKp might play a role in the different hvKp prevalences. It is necessary to enhance the surveillance of hvKp infection using the same identification method in community populations.

Interestingly, we found nine hypermucoviscous *K. pneumoniae* with negative for *rmpA* or *rmpA2* in our study. Similarly, increasing isolates with this phenotype were reported in the past few years,[17,53] and that was also observed in *K. variicola* isolates.[54] This phenomenon questions the existence of another mechanism to express hypermucoviscosity.[54] However, the hypermucoviscous *K. pneumoniae* phenotype without a hypervirulence genotype has been poorly studied, and further studies are needed. We highlight the fact that hypermucoviscous is not equal to hypervirulence. Virulence determinants should also be considered in the detection of hvKp.

In our study, ST23/KL1 and ST86/KL2 accounted for 36.36% and 18.18% of the hm-hvKp isolates, respectively. Several studies demonstrated that ST23/KL1 and ST86/KL2 were the dominant types of hvKp,[9,55] which were associated with life-threatening liver abscesses. Previous studies confirmed that KL1 and KL2 isolates harboured more virulence determinants and more remarkable virulence phenotypes than other serotypes. In this study, we found that the virulence profile of ST23/KL1 isolates was much more uniform than ST86/KL2 isolates. Further phylogenetic analysis, involving over 400 global ST23/KL1 genomes from different kinds of samples, showed that the genomes of the predominant clade CG23-I (~90%) typically possessed the virulence gene profile of *rmpA*/*rmpA2*-*iro*-*iuc*-*ybt*-*irp*-*clb*, which is associated with the hypermucoviscous phenotype, salmochelin, aerobactin, yersiniabactin, and colibactin. Those virulence determinants might be the driving factors for this clade to disseminate all the six inhibited continents and to dominate in the hypervirulent infections globally, especially the colibactin encoded by the *clb* gene.[44] In comparison with ST23/KL1, all the global ST86/KL2 genomes were lack of *clb* gene (colibactin), which might be a reason why the ST86/KL2 was not so common as ST23/KL1 in the clinical infections. All the isolates of ST23/KL1 and ST86/KL2 in our study were phylogenetically closely related to those isolates from liver abscesses, blood, and urine, suggesting the human gut could be the reservoir of hm-hvKp responsible for extraintestinal infections.

Our study revealed a very low antimicrobial resistance in hm-hvKp isolates from community patients, consistent with previous studies.[9,51,52] A previous study in Vietnam demonstrated an inverse relationship between resistome and virulome in hm-hvkp isolates,[5] which was explained by rapid intervention in these cases with appropriate therapy due to severe clinical signs or the death of patients before treatment completion. In our study, this phenomenon can also be accounted for plasmid incompatibilities or a physical barrier due to overexpression of capsule, which decreased the transmission of antimicrobial resistance determinants.[19] However, as multidrug-resistant *K. pneumoniae* has been widely circulating worldwide, multidrug-resistant hm-hvKp isolates from various sources were discovered in recent years.[7,56,57] The hm-hvKp isolates obtain antimicrobial resistance by obtaining antimicrobial resistance plasmids or inserting antimicrobial resistance determinants into virulence plasmids.[58,59] A recent study demonstrated that outer membrane vesicles also delivered carbapenem resistance determinants to hvKp isolates.[60] In China, a fatal outbreak caused by carbapenem-resistant (CR) hm-hvKp in an intensive care unit of a major teaching hospital was reported, with a mortality rate of 100%.[61] Thus, there is an urgent need to perform continuous surveillance on the antimicrobial resistance of hvKp isolates.

CR-Kp is one of the most serious public health problems in the world. Previous studies have found that ST11 was the predominant sequence type of CR-hm-hvKp in China.[8,26,30–32] In this study, none of the ST11 CR-hm-hvKp isolates were found in the fecal samples from community patients, suggesting that the human gut in individuals in the community might not be the major reservoir of CR-hm-hvKp. In contrast, a prospective cohort study indicated that, in hospital, the prevalence of ST11 CR-hm-hvKp infection in hospitalized patients with diarrhoea was 8.01% (65/811) in Zhejiang Province, China,[62] which indicated the human gut of patients who were recently hospitalized could be the reservoir. The Centers for Disease Control and Prevention (CDC) recently reported the emergence of ST23 CR-hv*Kp* infection in the American community population,[63] which shared the same ST type in our study. Thus, the possible dissemination of CR-hvKp in the community cannot be ignored.

Our findings have several implications. First, there is a certain level of hm-hvKp infection in community cases, especially there is a high proportion of hm-hvKp infection in the elderly group. Thus, intensive surveillance of the community population remains extremely important and should be considered by public health authorities. Second, hm-hvKp in our study shared the same molecular type with those causing liver abscesses, bloodstream, and urinary tract infection, which provides a theoretical basis for exploring the hypotheses of the source of extraintestinal hm-hvKp infection.

Highlights of this study are as follows. In our study, WGS was used to characterize hm-hvKp isolates from community patients. In recent years, WGS has been widely used in the study of the genetic evolution, population migration, and epidemiology of pathogenic bacteria and exhibits higher resolution and better typing ability than traditional molecular typing methods. WGS can fully reveal the characteristics of antimicrobial resistance determinants, virulence determinants, ST types, and evolutionary relationship of isolates. Our study described the epidemiological and molecular characteristics of hm-hvKp in community cases in Shanghai. The cases in this study were from the community population inhabited in the largest district of Shanghai, and the results are highly representative.

Our study has a few limitations. First, the comparison with clinical isolates of hm-hvKp from local hospitals and health care facilities was lacking. Second, hypervirulence of the isolates has not been evaluated in in vivo models. Third, the number of hm-hvKp isolates was limited and may not yet be fully representative of isolates characteristic of the region, and more studies are needed in the future.

Conclusion

The hm-hvKp is carried by the community population of Shanghai. ST23/KL1 and ST86/KL2 isolates were predominant, and they were closely related to global hypervirulent isolates of different continents, different sources, and different virulence levels. Ongoing surveillance of hm-hvKp infection in the community population is critical. Our study not only deepened the understanding of this bacterium but also provided crucial clues for the future development of effective prevention and control strategies. However, it is essential to acknowledge some limitations in our study, such as the in vivo model experimentation. Future research could further explore the pathogenic mechanisms of hm-hvKp and develop more effective treatment and prevention strategies. These efforts hold the promise of making a greater contribution to protecting public health.

Ethics Statement

Ethical approval for this study was granted by the Ethical Review Board of Pudong New Area Center for Disease Control and Prevention (PDCDC). All procedures involving human participants complied with the ethical standards of the Declaration of Helsinki. Informed consent was obtained from all respondents or their parents/legal guardians. All potentially identifying factors were removed to prevent identification of individuals.

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Disclosure

The authors report no conflicts of interest in this work.

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