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Original Article

Clinical and microbiological characteristics of hypervirulent *Klebsiella pneumoniae* (hvKp) in a hospital from North China

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Abstract

Introduction: The clinical and molecular characteristics of hypervirulent *Klebsiella pneumoniae* (hvKp) in various provinces of China have been reported, however, there have been few reports in Hebei Province, North China.

Methodology: The hvKp was identified by PCR amplification of hypervirulence-related genes, the hypermucoviscous phenotype was determined by the "string test", the drug susceptibility analysis was performed using the VITEK® 2 Compact Bacterial Identification and Monitoring System. Logistic regression was used to identify risk factors for hvKp infection. The molecular epidemiological characteristics of the strains were analyzed by pulsed-field gel electrophoresis (PFGE), and the capsular serotype of hvKp strain was detected by PCR.

Results: Overall, 52.21% (59/113) of *K. pneumoniae* isolates were hvKp, and the ratios of patients with older ages or a higher PMN cell count among hvKp infection were higher than those among classical *Klebsiella pneumoniae* (cKp) infection. hvKp are more susceptible to antibacterial drugs than cKp, and one ESBLs-producing hvKp strain was detected. The main capsular serotype of hvKp were K2, K57 and K1. PFGE indicated that the 59 strains of hvKp could be classified into 51 PFGE band types, forming 6 PFGE clusters.

Conclusions: In this study, the detection rate of hvKp was 52.21% (59/113) identified by virulence genes. People with older ages or a higher PMN cell count are more likely to gain hvKp infection. ESBLs-producing hvKp is emerging, indicating the importance of epidemiologic surveillance and clinical awareness of this pathogen in this region.

Key words: Hypervirulent *Klebsiella pneumoniae;* drug-resistance; clinical characteristics; capsular serotype.

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Introduction

Klebsiella pneumoniae is a well-known opportunistic pathogen that often causes infection such as pneumonia, sepsis, urinary tract infections and soft tissue infections in immunocompromised people. However, in 1986, Liu reported seven cases of pyogenic liver abscess associated with septic endophthalmitis caused by community-acquired *K. pneumoniae* infections, and some patients were accompanied by other infection syndromes such as pulmonary embolization, purulent meningitis or suspicious prostatic abscess [1]. Subsequently, Nassif reported seven K1, K2 serotypes of *K. pneumoniae* showed high virulence ($LD_{50} < 10^3$ CFU) in the mouse model, and found that four selected strains harbored a large plasmid with size of ~180 kb which was absent in the low virulence ($LD_{50}>10^6$ CFU) strains, further studies revealed that the plasmid contains gene cluster encoding aerobactin and its receptor [2]. In 2004, Fang [3] reported a liver abscess associated with meningitis and endophthalmitis caused by *K. pneumoniae*. The clinical features of the strains reported above differed from classical *K. pneumoniae* such as causing liver abscesses and severe metastatic infections (endophthalmitis, meningitis etc.) even in healthy people. These strains are more virulent than classical *K. pneumoniae* (cKp), so they are designated as hypervirulent *Klebsiella pneumoniae* (hvKp). Initially, the infections caused by hvKp were primarily reported in Asian countries, like China, Singapore, Korea and Vietnam, now, more and more cases are being reported in other parts of the world, and a few hvKp strains even acquired resistance genes [4]. The clinical and molecular characteristics of hvKp in various provinces

of China have been reported, however, there have been few reports in Hebei Province. The aim of this study was to analyze the clinical and microbiological characteristics of hvKp in a hospital from Hebei Province, and to provide insights for the development of effective therapeutic strategies for controlling hvKp infections in clinic. In this study, hvKp strains were screened from isolates collected in 2015 from the hospital by PCR amplification of virulence related genes for that the hypervirulence of the hvKp strains is partly mediated by genes on large virulence plasmids or chromosomal islands.

Methodology

Collection and identification of K. pneumoniae clinical isolates

A total of 113 non-repetitive *K. pneumoniae* (meaning that each isolate was obtained from a particular patient and each patient was sampled only once) in 2015 were collected from The First Affiliated Hospital of Hebei North University, a Grade-III Class-A Hospitals in Hebei Province from north China, and stored in the Chinese Academy of Medical Sciences Collection Center of Pathogen Microorganisms (CAMS-CCPM-AP). *K. pneumoniae* isolates were identified by VITEK® 2 Compact Bacterial Identification and Monitoring System and 16S rRNA gene was amplified using the universal primers 27F and 1492R. Primers, product sizes, and PCR conditions are listed in Table S1 in the supplemental material.

String test

The hypermucoviscosity phenotype of *K. pneumoniae* was determined by string test described previously [4]. The string test is positive when a sterile inoculating loop being able to generate a viscous string of ≥ 5 mm in length by stretching a single colony of a strain on a 5% Columbia blood agar plate (Beijing SanYao Science & Technology Development Co., Beijing, China).

Identification of hvKp

According to the latest research results, hvKp strains were defined by PCR amplification of virulence related genes: plasmid-borne rmpA (*p-rmpA, p-rmpA2*), aerobactin synthase gene (*iucA*), salmochelin siderophore biosynthesis (*iroB*), putative transporter (*peg-344*) and putative carboxymuconolactone decarboxylase family (*peg-589*) together [5]. Specific primers, product sizes, and PCR conditions are listed in Table S1 in the supplemental material.

Patient information

To investigate the clinical characteristics of hvKp and cKp, the following data were collected by clinical laboratory: gender and age, *K. pneumoniae* infection acquisition, underlying disease (diabetes mellitus, hypertension, cardiovascular disease, neurologic disorder, cancer and digestive disease), infection type (pulmonary disease, liver abscess and urinary infection), catheter (drainage tube, stomach tube, urinary catheter and central intravenous catheter), host responsibility including white blood cell (WBC) count, polymorphonuclear (PMN) cell count. Additionally, the data of empirical antimicrobial therapy was also used to analyze risk factors of hvKp.

Antimicrobial susceptibility test

The susceptibility to antimicrobial agents and the identification of the ESBLs-producing *K. pneumoniae* clinical isolates was performed by VITEK® 2 Compact Bacterial Identification and Monitoring System in accordance with the manufactory's instructions. *Escherichia coli* ATCC® 25922™ was used as the quality control strain.

Capsular serotype gene detection by PCR

The K1, K2, K5, K16, K20, K54, K57 capsule serotype genes were detected by PCR as described previously [6,7], using primers listed in in Table S1 in the supplemental material, the PCR products were visualized by 1% agarose gel electrophoresis.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed on 59 hvKp isolates as follows: the genome DNAs of each strain were digested with restriction enzyme *Xba*I for overnight at 37 ºC and electrophoresed for 18.5h at 14 ºC, 120 degree angle, with switch times of 5 and 25 s at 6V/cm, and *Salmonella* serotype Braenderup strain (H9812) was used as the DNA size marker. The PFGE patterns were analyzed by BioNumerics software (version 7.6, Applied Maths) using the Dice Similarity coefficient. Strains with similarity coefficient of 100% were considered as the same PFGE type, while more than 85% of similarity was used as the threshold of a cluster.

Statistical analysis

Statistical analysis was performed using SPSS statistical software. The chi-square test was used to analyze categorical variables, and Student's *t* test was used to analyze continuous variables. Difference with $P < 0.05$ was considered to be statistically significant. Logistic regression was used to identify risk factors for

hvKp infection. All variables with P < 0.1 were included in the multivariate Logistic regression.

Results

Clinical strains

113 strains were isolated from different tissue including sputum (84, 74.34%), secretions (8, 7.08%), urine (8, 7.08%), throat swab (3, 2.65%), whole blood (3, 2.65%), joint fluid (2, 1.77%), cerebrospinal fluid (2, 1.77%), drainage fluid (1, 0.88%), ascites (1, 0.88%), and puncture fluid (1, 0.88%). Sample from hospital wards including neurology (24, 21.23%), ICU (19, 16.81%), endocrinology (10, 8.85%), neonatal (7, 6.19%), respiratory (7, 6.19%), cardiovascular (6, 5.31%), hematology (5, 4.42%), thoracic surgery (4, 3.54%), gastrointestinal tumor surgery (3, 2.65%), nephrology (3, 2.65%), geriatrics (3, 2.65%), gastroenterology (3, 2.65%), rehabilitation medicine (2, 1.77%), bone surgery (2, 1.77%), oncology (2, 1.77%), cardiology (2, 1.77%), cardiac surgery (2, 1.77%), urology (2, 1.77%), dermatology (1, 0.88%), traditional Chinese medicine (1, 0.88%), hepatobiliary surgery (1, 0.88%), stomatology (1, 0.88%), gynecology (1, 0.88%), otorhinolaryngology (1, 0.88%), and rheumatology and immunology (1, 0.88%).

HvKp and hmKp identification

Among 113 isolates, 59 isolates were identified to be hvKp by hypervirulent-associated gene detection, the remaining 54 isolates were considered to be cKp. String test was used to determine the hypermucoviscosity phenotype of *K. pneumoniae,* and the proportion of hypermucoviscous *K. pneumoniae* (hmKp) isolates among hvKp (40.68%, 24/59) was significantly higher than that among cKp (14.81%, 8/54), with p value lower than 0.01.

Patient characteristics and risk factors for hvKp infection

The patient characteristics of hvKp and cKp are shown in Table 1. The mean age of hvKp patients was older than the cKp patients (65.64 ± 1.805 *vs* 56.59 ±

Table 1. Clinical characteristics of *K. pneumonia.*

Characteristics	hvKp (n = 59)	cKp (n = 54)	P value
Age, years, mean ± SD	65.64 ± 1.805	56.59 ± 3.359	0.0167[a]
Male sex	43 (72.88%)	36 (66.67%)	0.4718
Community-acquired	39 (66.10%)	30 (55.56%)	0.2508
Underlying conditions			
Diabetes mellitus	14 (23.73%)	9 (16.67%)	0.3571
Hypertension	20 (33.90%)	20 (37.04%)	0.7275
Cardiovascular disease	6 (10.17%)	10 (18.52%)	0.2035
Neurologic disorder	18 (30.51%)	17 (31.48%)	0.9110
Cancer	11 (18.64%)	6 11.11%)	0.2632
Digestive disease	4 (6.78%)	5 (9.26%)	0.6268
Infection type			
Pulmonary disease	37 (62.71%)	12 (22.22%)	< 0.0001[a]
Liver abscess	2 (3.39%)	0 (0.00%)	0.4965
Urinary infection	2 (3.39%)	2 (3.64%)	0.9430
Catheter			
Drainage tube	14 (23.73%)	9 (16.67%)	0.3517
Stomach tube	13 (22.3%)	6 (11.11%)	0.1210
Urinary catheter	22 (37.29%)	11 (20.37%)	0.0482[a]
Central intravenous catheter	13 (22.03%)	10 (18.52%)	0.8290
Surgery	15 (25.42%)	12 (22.22%)	0.6429
Host responsibility			
WBC (10^9/L)	15.98 ± 4.588	9.411 ± 0.234	0.1768
PMN cell count	16.47 ± 4.623	6.859 ± 0.6932	0.0514[a]
Empirical therapy			
β-Lactam/β-lactamase inhibitor combination	29 (49.15%)	29 (53.70%)	0.6287
Aminoglycosides	1 (1.69%)	2 (3.70%)	0.5070
Carbapenem	6 (10.17%)	6 (11.11%)	0.8711
Glycopeptides	0 (0.00%)	1 (1.85%)	0.2938
Fluoroquinolone	7 (11.86%)	7 (12.96%)	0.8595
Cephalosporin	2 (3.39%)	2 (3.70%)	0.9281
Penicillin	1 (1.69%)	0 (0.00%)	0.3366

[a]: A P value of < 0.05 was considered to be statistically significant; WBC: white blood cell; PMN: polymorphonuclear.

Table 2. Regression analysis of variables associated with hvKp infections.

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.025 (1.003-1.047)	0.024	1.019 (0.995-1.044)	0.119
PMN cell count	1.045 (0.993-1.100)	0.091	1.042 (0.986-1.101)	0.146
Urinary catheter	1.875 (0.828-4.245)	0.132		

OR: odds ratio; CI: confidence interval; PMN: polymorphonuclear.

3.359 years, P = 0.0167). Compared with cKp patients, a higher number of patients with the hvKp infection presented with pulmonary disease (62.71% *vs* 22.22%, P < 0.0001). Urinary catheter was more frequently to be the source of hvKp infection than cKp infection (37.29% *vs* 20.37%, P = 0.0482). We also found that patients with hvKp infection had a higher PMN cell count than cKp infected patients (16.47 ± 4.623 *vs* 6.859 ± 0.6932, P = 0.0514). Univariate analysis showed that the age [odds ratio (OR) = 1.025], or a PMN cell count (OR = 1.045) were positive correlated with hvKp infection (Table 2).

Antimicrobial resistance among hvKp and cKp isolates.

K. pneumoniae is naturally resistant to ampicillin, in consistent with this, all the *K. pneumoniae* demonstrated resistance to ampicillin (Table 3). Generally, hvKp isolates are more susceptible to antimicrobial agents compared to cKp (Table 3), examples are ampicillin/sulbactam, cefazolin, ceftriaxone, gentamicin, tobramycin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole (P < 0.05). The ESBL positive rate in cKp isolates was significantly higher than that in hvKp isolates (P < 0.01). only one ESBL-producing hvKp was detected.

Molecular characteristics of hvKp isolates

The distribution of capsular serotypes of the 59 hvKp was as follows: K2 (17, 28.81%), K57 (15, 25.42%), K1 (14, 23.73%), K20 (2, 3.39%), K5 (1, 1.69%), K16 (1, 1.69%), K54 (1, 1.69%), and 8 strains did not belong to any of the tested serotypes. PFGE results showed that the 59 hvKp could be classified into 51 band types, forming 6 PFGE clusters (A, B, C, D, E, and F clusters, at least three isolates with > 85% similarity for each cluster). PFGE cluster A included 10 K1 strains, cluster B included 5 K2 strains, cluster C included 3 K57 strains, cluster D included 4 K57 strains, cluster E included 8 K2 strains and cluster F included five K-non-typable strains (Figure 1).

Discussion

Although hvKp has been described for more than 30 years since its first report, the gold standard for hvKp laboratory detection has not yet been determined. The putative hvKp infection is primarily determined by clinical features and/or a positive string test (using a sterile inoculating loop being able to generate a viscous string of > 5 mm in length by stretching a single colony of a strain on a blood agar plate). However, the two criteria are becoming increasingly problematic. The clinical definition of hvKp infection is the development

Table 3. The antimicrobial resistance profiling of hvKp and cKp isolates.

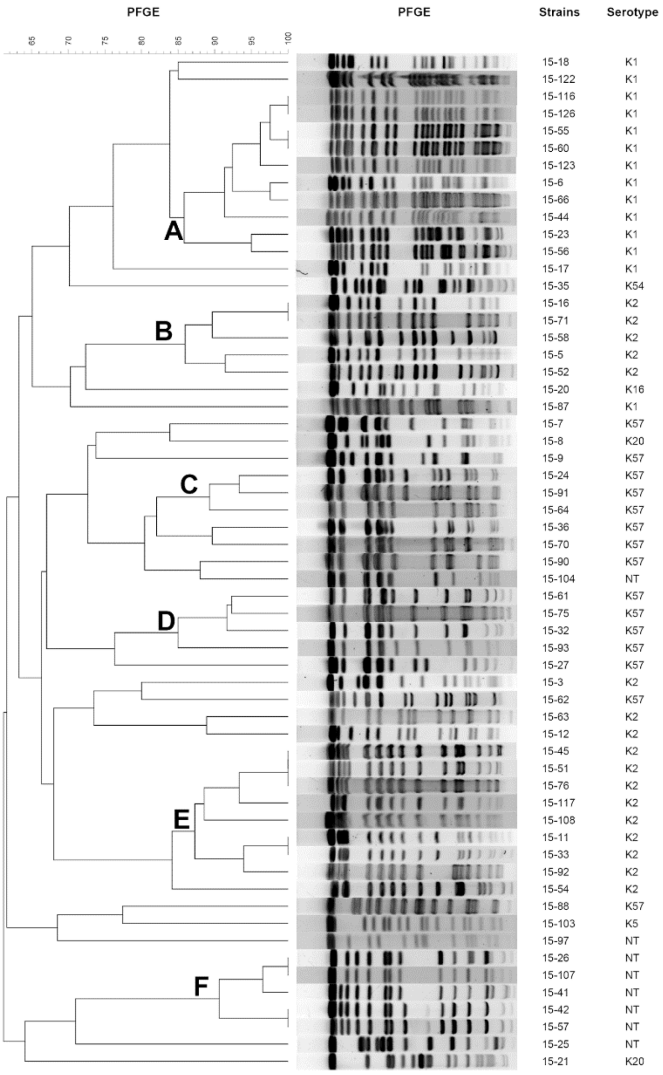
Compounds	Resistant bacteria n (%)			χ^2	P value
	Total (n = 113)	hvKp (n = 59)	cKp (n = 54)		
Ampicillin	113 (100)	59 (100.00)	54 (100.00)	NA	NA
Ampicillin/Sulbactam	11 (9.73)	1 (1.69)	10 (18.52)	9.082	0.0026 [a]
Cefazolin	13 (11.50)	1 (1.69)	12 (22.22)	11.67	0.0006 [a]
Cefuroxime	14 (12.39)	4 (6.78)	10 (18.52)	3.579	0.0585
Ceftazidime	5 (4.42)	1 (1.69)	4 (7.41)	2.176	0.1402
Ceftriaxone	11 (9.73)	1 (1.69)	10 (18.52)	9.082	0.0026[a]
Cefepime	4 (3.54)	1 (1.69)	3 (5.56)	1.231	0.2673
Aztreonam	6 (5.31)	1 (1.69)	5 (9.26)	3.209	0.0732
Gentamicin	6 (5.31)	0 (0.00)	6 (11.11)	6.923	0.009 [a]
Tobramycin	2 (1.77)	0 (0.00)	2 (3.70)	5.409	0.0200 [a]
Ciprofloxacin	7 (6.19)	0 (0.00)	7 (12.96)	8.153	0.0043 [a]
Levofloxacin	5 (4.42)	0 (0.00)	5 (9.26)	5.716	0.0168 [a]
Nitrofurantoin	43 (38.05)	19 (32.20)	24 (44.44)	1.792	0.1807
Trimethoprim/Sulfamethoxazole	6 (5.31)	0 (0.00)	6 (11.11)	6.923	0.009 [a]
ESBLs (+)	10 (8.85)	1 (1.69)	10 (18.52)	9.082	0.0026 [a]

[a]: A *P* value of < 0.05 was considered to be statistically significant; NA: not applicable.

of tissue-invasive, community-acquired infections in a healthy host, which excludes hvKp infections in patients with comorbidities, immunocompromization or in a health care environment [8]. Positive string test is used widely to identify hvKp strains currently, but data suggested that string test is not accurate enough to detect hvKp, because hypermucoviscous phenotype was also observed in cKp [5].

The hypervirulence of the hvKp strains is partly mediated by genes on large virulence plasmids (220-kb pK2044, 219-kb pLVPK, 230-kb pRJA166b etc.) [9-11] or chromosomal islands. Curing of hypervirulence-associated plasmids can significantly reduce the virulence of the strain [12]. And a recent study showed that cKp can exhibit a hypervirulent phenotype by acquiring a roughly 170-kb pLVPK-like plasmid and lead to a fatal outbreak of ventilator-associated pneumonia in a Chinese hospital [13]. The genetic structures of these virulence plasmids were characterized by whole genome sequencing (WGS), and the results suggested that these plasmids carry hypervirulent genes, such as siderophore-mediated iron-acquisition system genes, *iucABCDiutA* and *iroBCDN*; mucus phenotype-regulated gene, *p-rmpA/A2*; putative metabolite transporter-encoding gene, *peg-344*; putative carboxymuconate decarboxylase family-encoding gene, *peg-589*. Another way to mediate the hyper-virulent phenotype of hvKp is the integrative and conjugative element (ICE), for instance, 76-kb ICE (*ICEKp1*) in the hvKp strain NTUH-K2044 and *ICEKp10* in sublineage CG23-I [14,15]. However, the virulence genes carried on ICEs are not unique to hvKp, the cKp also contains genes encoding yersiniabactin and colibactin. Therefore, a recent study suggested that the genes on virulence plasmids, i.e *iroB*, *iucA*, *p-rmpA*, *p-rmpA2*, *peg-344* and *peg-589* can differentiate the hvKp-rich and cKp-rich strain cohorts with high accuracy, and these genes can also accurately predict mortality in a murine sepsis model [5].

Figure 1. PFGE analysis of the hvKp strains.



NT: non-typable serotype.

In this study, we identified hvKp by PCR checking of virulence genes, and found that 59 among 113 (52.21%) *K. pneumoniae* strains in the studied hospital were hvKp. The detection rate varied from region to region, however, it is difficult to made comparison with other report for these methodologies are not equivalent. The detection rate of hvKp defined by positive string test was 31.4% (22/70) from 2008 till 2012 in Beijing Chao-Yang Hospital [16]. Another study reported that 45.7% (92/202) *K. pneumoniae* isolates were hvKp defined by aerobactin detection at Beijing Tsinghua Changgung Hospital and Chinese PLA General Hospital from June 2008 to July 2017 [17]. In a multicenter study, *K. pneumoniae* isolates were collected from 10 cities in China during February to July 2013, and hvKp were designated as aerobactin-positive strains. The study found that 37.8% (87/230) isolates were hvKp, and the prevalence of hvKp varied among different cities, with the highest rate in Wuhan (73.9%) and the lowest in Zhejiang (8.3%) [18]. Based on being positive for either *iucA*, or *iroB* gene, a total of 65 *K. pneumoniae* isolated from 65 patients with hospital-acquired infections in the ICUs of Mansoura University Hospital in Egypt were subjected to hvKp identification, of which only four strains were identified as probable hvKp [19]. With *magA* and *rmpA* genes being amplified by end point PCR, only three (7.9 %) out of 38 *K. pneumoniae* isolates carried one or both

hvKp-associated genes in Houston, TX, USA [20]. Of 31 blood cultured *K. pneumoniae* isolates, 4 isolates were identified as hvKp by hypermucoviscous phenotype and *magA/rmpA* genotypes in South Australian hospitals [21].

In the previous risk factor studies, patients with community-acquired infections and underlying disease like diabetes mellitus, cancer and hypertension are more likely to encounter hvKp infection, and males might be slightly more likely to be infected than females [22]. However, in our study, the ratios of patients with older ages or a higher PMN cell count among hvKp infection were higher than those among cKp infection, but only showed a statistical trend in univariate analysis. It suggested that hvKp strains were prone to infect older people with lower immunity than younger people. White blood cell (WBC) count and polymorphonuclear (PMN) cell count are important laboratory indicator of infectious disease, WBC count and PMN count in the hvKP group were higher than that in the cKp group, even though only PMN count show the difference was statistically significant. These features may be related to higher levels of infection in the hvKp group. Invasive operation like urinary catheter was more frequently to be the source of hvKp infection than cKp infection (37.29% *vs* 20.37%, P = 0.0482), clinicians should pay attention to hvKp become the cause of urinary catheter infection.

Capsule polysaccharide (CPS) is necessary for *K. pneumoniae* virulence, and *K. pneumoniae* has over 70 serotypes (K1 to K78) classified by CPS antigens (K antigens). In contrast to previous studies that the serotype in hvKp is mainly K1 and K2 [18,23], we found that the main capsular serotype of hvKp in this hospital is K2 (17, 28.81%), K57 (15, 25.42%) and K1 (14, 23.73%), and the rate of K57 type was even slightly higher than that of the K1 type. K57 has also been reported in other reports with different detection rates, such as 9.6% (8/84) [24], 13.6% (3/22) [16], 10.4% (10/96) [25] and 18.9% (7/37) [26], suggesting that the serotype distribution of hvKp varied in different regions. The 59 strains of hvKp were also subjected to PFGE molecular typing analysis, PFGE results demonstrated that there was no clonal dissemination among the 59 hvKp isolates and the results suggested that the same serotype strains can almost be classified into the same cluster, but the same cluster can be divided into different band types according to different genetic backgrounds, indicating that PFGE molecular typing technology can further differentiate various strains based on genetic characters.

In consistent with previous reports that hvKp was highly susceptible to commonly used antimicrobial agents compare to cKp, we found that hvKp strains were significantly less resistant than cKp to ampicillin/sulbactam, cefazolin, ceftriaxone, gentamicin, tobramycin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole. Antimicrobial resistance can be mediated by chromosomal mutations, or by mobile elements such as plasmids, insertion sequences, and transposons. The higher drug susceptibility of hvKp may be due to hvKp hyper-expression of capsule, which may provide a physical barrier against transformation and conjugation and also CRISPR/Cas systems, which defend against foreign DNA that does manage to penetrate or maintain in the cell [15]. However, in recent years, many multidrug-resistant hvKps have been reported, such as ESBLs-producing, carbapenem resistant, or colistin resistant hvKp [11,27-30]. In our study, we also identified an ESBL-producing hvKp with resistance to a broad spectrum of antibiotics. It indicates us that we need to pay attention to the detection and diagnosis of hvKp infection. At present, there is still a lack of controlled trials to evaluate the efficacy of drugs for the treatment of hvKp infection. As previous report, phage and phage-encoded proteins and novel therapeutic antibodies (Abs) targeting the K1-serotype capsules polysaccharide (CPS) of hvKp strains are potential tool to treat and prevent infection with hvKp [31,32]. However, in our study, serotype K1 was not the most prevalent serotype in this region, and this problem should be addressed in future research.

Conclusion

In this study, the detection rate of hvKp was 52.21% (59/113) identified by virulence genes. And people with older ages or a higher PMN cell count are more likely to gain hvKp infection. The result of serotype detection suggesting that the serotype distribution of hvKp varied in different regions. Although hvKp was highly susceptible to commonly used antimicrobial agents compare to cKp, the emerging of the ESBLs-producing hvKp indicating the importance of epidemiologic surveillance and clinical awareness of this pathogen in this region.

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References

1. Liu YC, Cheng DL, Lin CL (1986) *Klebsiella pneumoniae* liver abscess associated with septic endophthalmitis. Arch Intern Med 146: 1913-1916.
2. Nassif X, Sansonetti PJ (1986) Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plasmid encoding aerobactin. Infect Immun 54: 603-608.
3. Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT (2004) A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. J Exp Med 199: 697-705.
4. S Shon A, Bajwa R, A Russo T (2013) Hypervirulent (hypermucoviscous) *Klebsiella Pneumoniae*: A new and dangerous breed, Virulence 4(2):107-18.
5. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR (2018) Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. J Clin Microbiol 56: e00776-18.
6. Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC (2007) *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis 45: 284-293.
7. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, Wu MC, Wang JT (2013) Capsular types of *Klebsiella pneumoniae* revisited by wzc sequencing. PloS one 8: e80670.
8. Russo TA, Marr CM (2019) Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev 32: e00001-19.
9. Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, Fung CP, Chen HJ, Liu YM, Wang JT, Fang CT, Chang SC, Shu HY, Liu TT, Chen YT, Shiau YR, Lauderdale TL, Su IJ, Kirby R, Tsai SF (2009) Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. J Bacteriol 191: 4492-4501.
10. Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL (2004) Sequencing and analysis of the large virulence plasmid pLVPK of *Klebsiella pneumoniae* CG43. Gene 337: 189-198.
11. Xie Y, Tian L, Li G, Qu H, Sun J, Liang W, Li X, Wang X, Deng Z, Liu J, Ou HY (2018) Emergence of the third-generation cephalosporin-resistant hypervirulent *Klebsiella pneumoniae* due to the acquisition of a self-transferable bla_{DHA-1}-carrying plasmid by an ST23 strain. Virulence 9: 838-844.
12. Tang HL, Chiang MK, Liou WJ, Chen YT, Peng HL, Chiou CS, Liu KS, Lu MC, Tung KC, Lai YC (2010) Correlation between *Klebsiella pneumoniae* carrying pLVPK-derived loci and abscess formation. Eur J Clin Microbiol Infect Dis 29: 689-698.
13. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, Chan EW, Shu L, Yu J, Zhang R, Chen S (2018) A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis 18: 37-46.
14. Lin TL, Lee CZ, Hsieh PF, Tsai SF, Wang JT (2008) Characterization of integrative and conjugative element *ICEKp1*-associated genomic heterogeneity in a *Klebsiella pneumoniae* strain isolated from a primary liver abscess. J Bacteriol 190: 515-526.
15. Lam MMC, Wyres KL, Duchene S, Wick RR, Judd LM, Gan YH, Hoh CH, Archuleta S, Molton JS, Kalimuddin S, Koh TH, Passet V, Brisse S, Holt KE (2018) Population genomics of hypervirulent *Klebsiella pneumoniae* clonal-group 23 reveals early emergence and rapid global dissemination. Nat Commun 9: 2703.
16. Liu YM, Li BB, Zhang YY, Zhang W, Shen H, Li H, Cao B (2014) Clinical and molecular characteristics of emerging hypervirulent *Klebsiella pneumoniae* bloodstream infections in mainland China. Antimicrob Agents Chemother 58: 5379-5385.
17. Liu C, Shi J, Guo J (2018) High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China. Infect Drug Resist 11: 1031-1041.
18. Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Li H, Zhang F, Li S, Wang R, Wang H (2016) High prevalence of hypervirulent *Klebsiella pneumoniae* infection in China: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob Agents Chemother 60: 6115-6120.
19. El-Mahdy R, El-Kannishy G, Salama H (2018) Hypervirulent *Klebsiella pneumoniae* as a hospital-acquired pathogen in the intensive care unit in Mansoura, Egypt. Germs 8: 140-146.
20. Chou A, Nuila RE, Franco LM, Stager CE, Atmar RL, Zechiedrich L (2016) Prevalence of hypervirulent *Klebsiella pneumoniae*-associated genes *rmpA* and *magA* in two tertiary hospitals in Houston, TX, USA. J Med Microbiol 65: 1047-1048.
21. Chang L, Bastian I, Warner M (2013) Survey of *Klebsiella pneumoniae* bacteraemia in two South Australian hospitals and detection of hypermucoviscous phenotype and *magA/rmpA* genotypes in K . pneumoniae isolates. Infection 41: 559-563.
22. Li J, Ren J, Wang W, Wang G, Gu G, Wu X, Wang Y, Huang M, Li J (2018) Risk factors and clinical outcomes of hypervirulent *Klebsiella pneumoniae* induced bloodstream infections. Eur J Clin Microbiol Infect Dis 37: 679-689.
23. Yan Q, Zhou M, Zou M, Liu WE (2016) Hypervirulent *Klebsiella pneumoniae* induced ventilator-associated pneumonia in mechanically ventilated patients in China. Eur J Clin Microbiol Infect Dis 35: 387-396.
24. Guo Y, Wang S, Zhan L, Jin Y, Duan J, Hao Z, Lv J, Qi X, Chen L, Kreiswirth BN, Wang L, Yu F (2017) Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. Front Cell Infect Microbiol 7: 24.
25. Liu C, Shi J, Guo J (2018) High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China. Infect Drug Resist 11: 1031-1041.
26. Sun Y, Wu H, Shen D (2016) Clinical and molecular analysis of *Klebsiella pneumoniae* causing liver abscess in China. J Mol Microbiol Biotechnol 26: 245- 251.
27. Shen D, Ma G, Li C, Jia X, Qin C, Yang T, Wang L, Jiang X, Ding N, Zhang X, Yue L, Yin Z, Zeng L, Zhao Y, Zhou D, Chen F (2019) Emergence of a multidrug-resistant hypervirulent *Klebsiella pneumoniae* sequence type 23 strain with a rare $bla_{CTX-M-24}$-harboring virulence plasmid. Antimicrob Agents Chemother 63: e02273-18.

28. Liu Y, Long D, Xiang TX, Du FL, Wei DD, Wan LG, Deng Q, Cao XW, Zhang W (2019) Whole genome assembly and functional portrait of hypervirulent extensively drug-resistant *NDM-1* and *KPC-2* co-producing *Klebsiella pneumoniae* of capsular serotype K2 and ST86. J Antimicrob Chemother 74: 1233-1240.
29. Dong N, Lin D, Zhang R, Chan EW, Chen S (2018) Carriage of bla_{KPC-2} by a virulence plasmid in hypervirulent *Klebsiella pneumoniae*. J Antimicrob Chemother 73: 3317-3321.
30. Lu Y, Feng Y, McNally A, Zong Z (2018) The occurence of colistin-resistant hypervirulent *Klebsiella pneumoniae* in China. Front Microbiol 9: 2568.
31. Diago-Navarro E, Calatayud-Baselga I, Sun D, Khairallah C, Mann I, Ulacia-Hernando A, Sheridan B, Shi M, Fries BC (2017) Antibody-based immunotherapy to treat and prevent infection with hypervirulent *Klebsiella pneumoniae*. Clin Vaccine Immunol 24: e00456-16.
32. Lin TL, Hsieh PF, Huang YT, Lee WC, Tsai YT, Su PA, Pan YJ, Hsu CR, Wu MC, Wang JT (2014) Isolation of a bacteriophage and its depolymerase specific for K1 capsule of *Klebsiella pneumoniae*: implication in typing and treatment. The J Infect Dis 210: 1734-1744.

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Annex – Supplementary Items

Supplementary Table 1. Primers, product sizes, and PCR conditions used for this study.

Gene	Primer sequence	Amplicon size	Annealing temperature
16S rRNA	27F: 5'-AGAGTTTGATCCTGGCTCAG-3' 1492R: 5'-GGTTACCTTGTTACGACTT-3'	1250 bp	60°C
iroB	F: 5'- ATCTCATCATCTACCCTCCGCTC-3' R: 5'-GGTTCGCCGTCGTTTTCAA-3'	235 bp	59°C
iucA	F: 5'- AATCAATGGCTATTCCCGCTG-3' R: 5'-CGCTTCACTTCTTTCACTGACAGG -3'	239 bp	59°C
p-rmpA	F: 5'-GAGTAGTTAATAAATCAATAGCAAT-3' R: 5'-CAGTAGGCATTGCAGCA-3'	332 bp	50°C
p-rmpA2	F: 5'- GTGCAATAAGGATGTTACATTA-3' R: 5'-GGATGCCCTCCTCCTG-3'	430 bp	50°C
peg-344	F: 5'- CTTGAAACTATCCCTCCAGTC-3' R: 5'-CCAGCGAAAGAATAACCCC -3'	508 bp	53°C
peg-589	F: 5'- TGAACCCCTGAAGGTCTATC-3' R: 5'-GTGATGAATAAACTACTGCGGC -3'	236 bp	55°C
K1	F: 5'- GGTGCTCTTTACATCATTGC -3' R: 5'-GCAATGGCCATTTGCGTTAG -3'	1283 bp	50°C
K2	F: 5'-GACCCGATATTCATACTTGACAGAG -3' R: 5'-CCTGAAGTAAAATCGTAAATAGATGGC -3'	641 bp	52°C
K5	F: 5'-TGGTAGTGATGCTCGCGA -3' R: 5'-CCTGAACCCACCCCAATC -3'	280 bp	53°C
K16	F: 5'-GTGCTTAACGGAGAACTGAAC -3' R: 5'-CCTCACCTGGAAGAAGTGTA -3'	922 bp	53°C
K20	F: 5'-CGGTGCTACAGTGCATCATT -3' R: 5'-GTTATACGATGCTCAGTCGC -3'	741 bp	54°C
K54	F: 5'-CATTAGCTCAGTGGTTGGCT -3' R: 5'-GCTTGACAAACACCATAGCAG -3'	881 bp	52°C
K57	F: 5'-CTCAGGGCTAGAAGTGTCAT -3' R: 5'-CACTAACCCAGAAAGTCGAG -3'	1037bp	54°C