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ORIGINAL RESEARCH

High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China

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Purpose: Aerobactin is a critical factor for hypervirulent *Klebsiella pneumoniae* (hvKp) in genetic backgrounds, but data based on the genotype for the elderly is limited.

Materials and methods: A retrospective study was conducted on elderly patients from June 2008 to July 2017 in 2 teaching hospitals. The clinical and microbiological data, including antimicrobial susceptibility testing, string test, extended-spectrum β-lactamase (ESBL) production, virulence gene, and multilocus sequence typing, of the hvKp group defined as aerobactin positive were compared with those of classic *K. pneumoniae* isolates.

Results: A total of 45.7% of 202 *K. pneumoniae* isolates were hvKp.ST23, which were predominant in 2 hospitals, but they were not highly associated with hvKp in different hospitals. Hypermucoviscosity, K1, K2, magA, and *rmpA/A2* genes were highly related to hvKp (P=0.000). With regard to the host, invasive infections (P=0.000), liver abscess (P=0.000), abdominal infection (P=0.000), pneumonia (P=0.037), and septic shock (P=0.045) were significantly higher in the elderly with hvKp. In the hvKp group, patients with better nutritional status were associated with a more severe sequential organ failure assessment score and a more serious inflammation reaction. Patients with diabetes (odds ratio [OR]=2.566) are more likely to be infected with hvKp. Previous hvKp is associated with hypermucoviscosity (OR=15.249) are often paralleled with hvKp. Importantly, 26% of hvKp isolates produced ESBLs, and most of them showed a carbapenems-resistant (CR) phenotype. Multivariate analysis implied that patients with a history of surgery within the last 1 month (OR=15.999) is an independent risk factor for CR-hvKp infection.

Conclusion: The prevalence of hvKP is high in the elderly. ESBL-hvKp, especially CR-hvKp, is emerging, which is a sign that clinical awareness and infection monitoring needs to improve.

Keywords: *Klebsiella pneumoniae*, hypervirulent, aerobactin, risk factor, ESBL-hvKp, CR-hvKp

Introduction

Klebsiella pneumoniae is a Gram-negative bacterium, causing various fatal infections. There are 2 pathotypes: hypervirulent (hvKp) and classical (cKp), which are detrimental to our health. Initially, a string test with a length >5 mm was defined as hypermucoviscosity, which is a traditional unique hvKp trait, triggering aggressive invasive infection, such as bloodstream infection and pyogenic liver abscesses (PLAs) for immunocompetent ambulatory younger individuals with non-underlying diseases.[1–4] However, many studies do not agree with the definition of hvKp defined by hypermucoviscosity phenotype.[5,6] The reason is that few hypermucoviscous *K. pneumoniae* (hmvKp) strains are associated with high virulence with in vitro and in vivo assays.[5,6] Thus, using hvKp by hypermucoviscosity phenotype as the sole indicator of hvKp is not appropriate.[7,8]

Until recently, aerobactin, the dominant siderophore, was regarded as a critical virulence factor for hvKp genetic background.[1,8,9] A multicenter study, focusing on middle-aged patients, first demonstrated the clinical and molecular characteristic of hvKp (defined as aerobactin positive) infection.[8] But data are limited on the elderly who may have various underlying diseases, nutrition status, and atypical manifestations, along with being infected with genotype hvKp.

Many previous studies have illustrated that hvKp is sensitive to most antibiotics, which is not frequently present in infection with cKp strains. But emerging multidrug resistance (MDR) hvKp, especially resistant to colistin and carbapenems, has been reported in China.[10–12] However, there are not enough adapted data on the elderly and the characteristics of antimicrobial-resistant hvKp infection.

Thus, for further investigation of the prevalence and antibiotic resistance of hvKp, we conducted a retrospective study in 2 teaching hospitals based on the genotype of hvKp, which was defined as aerobactin positive.

Materials and methods
Patients
A retrospective study was conducted on 202 *K. pneumoniae* culture-positive patients diagnosed at Beijing Tsinghua Changgung Hospital and Chinese PLA General Hospital from June 2008 to July 2017. The definition of elderly was if the patient was ≥65 years. Duplicate isolates from the same patient were excluded. The clinical characteristics, including underlying disease, infection type, nutritional status, mortality in 30 days, and sequential organ failure assessment (SOFA), were collected. To recognize the host responsibility and nutritional status between the 2 pathotypes, white blood cell count (WBC) and neutrophil percentage (NEU%) were used as primary and rough inflammatory factors. To evaluate the nutrition status, we used total protein (TP) and albumin (ALB) as markers. The study was approved by the Chinese PLA General Hospital Ethics Committee, and the Guidelines for Human Experimentation (China) were followed through the whole study. Informed consent was not needed due to the retrospective nature of the study; additionally, the patient data accessed in this research was anonymous. Therefore, the Chinese PLA General Hospital Ethics Committee waived the need for consent.

K. pneumoniae strains
All isolates were stored at −80°C and previously identified by the API 20 NE system and the Vitek II system. Additionally, species identification was further confirmed by 16S rRNA gene sequencing. HvKp was defined as aerobactin positive.

Hypermucoviscous phenotype was confirmed by string test as described previously.[13]

Antimicrobial susceptibility testing and phenotypic detection of ESBLs
Antimicrobial susceptibility testing was conducted and the results were interpreted by 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics include amikacin, gentamicin, tobramycin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, ceftriaxone, ceftazidime, ciprofloxacin, levofloxacin, piperacillin/ tazobactam, and trimethoprim/sulfamethoxazole. ESBL was confirmed by agar dilution test using ceftazidime and cefotaxime combined with clavulanate according to the CLSI guidelines.[8] MDR strains were defined as resistant to ≥3 different antimicrobial categories.[14] Isolates that are resistant to both imipenem and meropenem are defined as carbapenems-resistant (CR) isolates.

Detection of virulence gene
Genomic DNA of all *K. pneumoniae* isolates was extracted. Virulence-associated genes (*rmpA, rmpA2, magA,* and *aerobactin*) and capsular serotype-specific (*cps*) genes (*K1, K2, K5, K20, K54,* and *K57*) were amplified by polymerase chain reaction (PCR).[8,15–17] The primers are listed in Table S1.

Multilocus sequence typing (MLST) for *K. pneumoniae*
Seven housekeeping genes (*gapA, mdh, phoE, tonB, infB, pgi,* and *rpoB*) were amplified by PCR following the protocol (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) (Table S1). Allelic profiling and sequence types (STs) determination were also confirmed on the aforementioned website. Moreover, to further analyze the relationship among different STs, phylogenetic analysis of spliced 7 housekeeping genes for frequency >1 isolates and strains contributing to invasive infection and mortality was performed by the neighbor-joining method (MEGA 7.0).

Statistical analysis
SPSS software (version 20.0; IBM Corporation, Armonk, NY, USA) was performed for data analysis. Measurement data were assessed as mean ± SD. The count data was analyzed as percentages. Continuous variables were analyzed by Student's *t*-tests and the Wilcoxon rank-sum tests. Categorical variables were analyzed by χ^2 or Fisher's exact test. Univariate logistic regression analyses were performed for risk factor. To further analyze independent risk factors, a multivariable logistic regression analysis was conducted. All variables with

P values of <0.05 were included in the multivariate model. All tests were 2-tailed. *P*-value <0.05 was considered significant.

Results

Patient characteristics

A total of 202 *K. pneumoniae* culture-positive patients were diagnosed at the 2 hospitals from June 2008 to July 2017. A total of 96 (47.5%) isolates were hvKp and 121 (59.9%) were hmvKp. All the PLA patients (10 cases) were infected with hvKp. The main infection type distribution in hospital was pneumonia (146, 72.3%), while other infection types included urinary infection (42, 20.8%), invasive infection (37, 18.3%), and abdominal infection (26, 12.9%). Moreover, almost half of the patients (98, 48.5%) presented with sepsis, and 24 (11.9%) were diagnosed as septic shock. A total of 181 (89.6%) were males and 21 (10.4%) were females; the mean age was 84.43±7.84 years.

Clinical features: hvKp vs. cKp

Clinical features are shown in Table 1. The mean age of the hvKp group was younger than the cKp group (83.24±7.35 vs. 85.5±8.14 years, *P*=0.039). Diabetes (72.9% vs. 48.1%; *P*=0.000) and digestive diseases (22.9% vs. 13.5%; *P*=0.046) were highly associated with the hvKp group as their underlying diseases. Compared with the cKp group, a significantly higher number of patients with the hvKp presented with invasive infections (30.2% vs. 7.5%; *P*=0.000), liver abscess (10.4% vs. 0%; *P*=0.000), other abscess (26.0% vs. 2.8%; *P*=0.035), septic shock (16.7% vs. 7.5%; *P*=0.045), pneumonia (79.2% vs. 66.0%; *P*=0.037), and abdominal infection (21.9% vs. 4.7%; *P*=0.000). However, the incidence rates of urinary infection (13.5% vs. 27.4%, *P*=0.016) and stomach tube indwelling (60.4% vs. 77.4%, *P*=0.009) were comparably lower in the hvKp group.

WBC (12.74±3.94 10^9/L vs. 10.59±3.48 10^9/L, *P*=0.000) and NEU% (78.70±8.02 vs. 75.60±8.50, *P*=0.003) of patients with hvKp, represented as host responsibility, were significantly higher than cKp group. However, patients infected with hvKp were more likely to have a poorer nutritional status in TP (64.74±5.42 vs. 62.83±6.32, *P*=0.023) and ALB (34.98±3.40 vs. 33.78±3.73, *P*=0.019). Moreover, although the mortality in 30 days (16.7% vs. 21.7%, *P*=0.366) was not significantly different, SOFA score in patients with hvKp was notably higher (6.79±2.88 vs. 4.93±2.59, *P*=0.000; Table 1).

Genetic and phenotype characteristics: hvKp vs. cKp

Previous studies confirmed that virulence-associated genes (*rmpA, rmpA2*, and *magA*) and *cps* genes (*K1, K2, K5,*

K20, K54, and *K57*) are clustered in the hvKp group.[18–20] A significant difference was that *K1, K2, rmpA, rmpA2,* and *magA* were highly clustered in hvKp (*P*=0.000, respectively), and *K5, K20, K54*, and *K57* were not associated with hvKp (*P*=0.106, 0.627, 0.894, and 0.211, respectively). There was no strain in the cKp group with K5 (Table 1). It was strongly noted that hypermucoviscosity was highly associated with hvKp (*P*=0.000).

Antimicrobial resistance and prevalence of ESBL-producing *K. pneumoniae* isolates

Most of the hvKp isolates were sensitive to most of the antibiotics, with the exception of ampicillin, imipenem, and meropenem (Table 2). All Kp strains were resistant to ampicillin. In the hvKp group, 24 strains (25.0%) were MDR. A total of 25 hvKp isolates were identified as ESBL-producing *K. pneumoniae* isolates, which were more common in the cKp group (53.8% vs. 26.0%, *P*=0.001). One CR-hvKp isolate existed in one of these 2 teaching hospitals. In another referral center, 10 CR-hvKp strains were detected. The detailed information for the 11 CR-hvKp strains is shown in Table S2.

Risk factors: hvKp vs. cKp

In this study, univariate regression analysis showed that diabetes (odds ratio [OR]=2.903), digestive diseases (OR=2.127), and hypermucoviscosity (OR=17.446) were notable risk factors for hvKp infection. However, indwelling stomach tube (OR=0.447) was a protective factor for hvKp infection. Moreover, multivariate analysis revealed that diabetes (OR=2.566) and hypermucoviscosity (OR=15.249) were independent risk factors for hvKp infections (Table 3).

Risk factors: CR-hvKp vs. non-CR-hvKp

Patients with surgery history within 1 month (OR=19.5) and catheterized tracheal catheter (OR=6.051) were closely associated with CR-hvKp. However, patients with diabetes (OR=0.256) were more likely to be infected with non-CR-hvKp. A history of surgery within the last 1 month is an independent risk factor for CR-hvKp infection (OR=15.999) (Table 4).

MLST genotypic analysis

Among the 202 *K. pneumoniae* isolates, no new ST was identified in MLST database. The most prevalent ST in this study was ST23 (n=28; 13.9%), followed by ST412 (n=10; 5.0%), ST37 (n=7; 3.5%), ST65 (n=6; 3.0%), ST11 (n=5; 2.5%), ST17 (n=5; 2.5%), ST2905 (n=5; 2.5%), and ST2906 (n=5; 2.5%). The aforementioned STs accounted for 35.1% (43/202) of the total strains. Among the primary

Table 1 Clinical and microbiological features of hvKp

Characteristic	hvKp (N=96)	cKp (N=106)	*P*-value
K serotype			
K1	**33 (34.4%)**	**2 (1.9%)**	**0.000**
K2	**20 (20.8%)**	**2 (1.9%)**	**0.000**
K5	3 (3.1%)	0 (0%)	0.106
K20	6 (6.3%)	4 (3.8%)	0.627
K54	4 (3.8%)	3 (2.8%)	0.894
K57	10 (10.4%)	6 (6.3%)	0.211
rmpA	**76 (79.2%)**	**12 (11.3%)**	**0.000**
rmpA2	**68 (70.8%)**	**15 (14.2%)**	**0.000**
magA	**77 (80.2%)**	**50 (47.2%)**	**0.000**
Hypermucoviscosity	**86 (89.6%)**	**35 (33.0%)**	**0.000**
Basic demographics			
Age	**83.24±7.35**	**85.5±8.14**	**0.039**
Male	93 (96.9%)	88 (83.0%)	0.361
Underlying diseases			
Pulmonary disease	86 (89.6%)	85 (80.2%)	0.064
Diabetes	**70 (72.9%)**	**51 (48.1%)**	**0.000**
Cardiovascular disease	45 (46.9%)	60 (56.6%)	0.167
Cerebrovascular disease	15 (15.6%)	27 (25.5%)	0.085
Cancer	28 (29.2%)	27 (25.5%)	0.556
Surgery within 1 month	14 (14.6%)	11 (10.4%)	0.365
Digestive disease	**22 (22.9%)**	**13 (13.5%)**	**0.046**
Catheter			
Central intravenous catheter	65 (67.7%)	66 (62.3%)	0. 418
Urinary catheter	73 (76.0%)	87 (82.1%)	0.291
Tracheal catheter	34 (35.4%)	39 (36.8%)	0.839
Stomach tube	**58 (60.4%)**	**82 (77.4%)**	**0.009**
Infection type			
Pneumonia	**76 (79.2%)**	**70 (66.0%)**	**0.037**
Urinary infection	**13 (13.5%)**	**29 (27.4%)**	**0.016**
Invasive infection	**29 (30.2%)**	**8 (7.5%)**	**0.000**
Bacteremia	8 (8.3%)	4 (3.8%)	0.171
Liver abscess	**10 (10.4%)**	**0 (0%)**	**0.000**
Other abscess	**25 (26.0%)**	**3 (2.8%)**	**0.000**
Abdominal infection	**21 (21.9%)**	**5 (4.7%)**	**0.000**
Sepsis	48 (50.0%)	50 (47.2%)	0.688
Septic shock	**16 (16.7%)**	**8 (7.5%)**	**0.045**
Host responsibility			
WBC (10⁹/L)	**12.74±3.94**	**10.59±3.48**	**0.000**
NEU%	**78.70±8.02**	**75.6±8.50**	**0.003**
Nutrition status			
TP	**64.74±5.42**	**62.83±6.32**	**0.023**
ALB	**34.98±3.40**	**33.78±3.73**	**0.019**
SOFA score	**6.79±2.88**	**4.91±2.61**	**0.000**
Infection occurred in ICU	19 (19.8%)	16 (15.1%)	0.378
Mortality in 30 days	16 (16.7%)	23 (21.7%)	0.366

Notes: Data presented as mean ± standard deviation, unless otherwise stated. Bold values indicate *P*<0.05.
Abbreviations: ALB, albumin; cKp, classic *Klebsiella pneumoniae*; ESBLs, extended-spectrum β-lactamases; hvKp, hypervirulent *Klebsiella pneumoniae*; ICU, intensive care unit; NEU%, neutrophil percentage; SOFA, sequential organ failure assessment; TP, total protein; WBC, white blood cell count.

STs, ST23 (24/28), ST412 (6/9), ST17(5/5), and ST65(3/6) were strongly associated with hvKp, while ST11(0/5), ST2905(1/5), ST2906(1/5), and ST37(2/7) were more common in the cKp group. The more common clone complexes (CCs) of the CR-hvKp group were CC23 (n=3) and CC17 (n=3). There is an important phenomenon in the phylogenetic tree that a branch clustered with ST347, ST595, ST1469, ST2905, and ST2906 contributed to poor prognosis death in 30 days, which should be paid more attention (Figure 1).

Discussion

To our knowledge, this is the first and biggest systematic study focusing on the elderly infected with hvKp in China. In this study, 59.9% of hvKp were identified as hypermucoviscous

Table 2 Antibiotic resistance: hvKp vs. cKp

Antibiotic agent	hvKp (N=96)	cKp (N=106)	P-value
ESBLs	25 (16.3%)	57 (40.0%)	0.000
Amikacin	10 (2.5%)	22 (11.6%)	0.044
Gentamicin	16 (8.8%)	41 (29.5%)	0.001
Ampicillin/sulbactam	28 (20.0%)	60 (44.2%)	0.000
Aztreonam	18 (8.8%)	41 (23.2%)	0.002
Cefazolin	28 (18.8%)	59 (44.2%)	0.000
Cefotetan	14 (14.6%)	31 (29.2%)	0.012
Cefepime	15 (5.0%)	34 (14.7%)	0.006
Ceftriaxone	25 (16.3%)	51 (34.7%)	0.001
Ceftazidime	17 (7.5%)	43 (25.3%)	0.000
Ciprofloxacin	18 (10.0%)	43 (25.3%)	0.001
Levofloxacin	14 (6.3%)	40 (22.1%)	0.000
Trimethoprim/sulfamethoxazole	15 (8.8%)	44 (34.7%)	0.000
Piperacillin/tazobactam	13 (3.8%)	33 (13.7%)	0.003
Imipenem	11 (1.3%)	21 (2.1%)	0.104
Meropenem	12 (2.5%)	23 (2.1%)	0.085
Tobramycin	17 (8.8%)	39 (27.4%)	0.002

Abbreviations: cKp, classic *Klebsiella pneumoniae*; ESBLs, extended spectrum β-lactamase; hvKp, hypervirulent *Klebsiella pneumoniae*.

by string test, which was significantly different from a previous retrospective study conducted in a single center in China, with a prevalence of 33% in Beijing.[13] In addition, the incidence of hvKp (47.5%) in genetic background is also higher than the figure in the previous multicenter studies (37.8%).[8] It can be concluded that hvKp is emerging as the major pathotype for the elderly in the 2 hospitals, which should be paid more attention. The prevalent ST in 2 hospitals is the same: ST23. However, different hospitals isolated with ST23 are not highly associated with hvKp, which indicates that fully relying on STs to identify hvKp may be unreliable. Although the definition of hvKp is controversial, it is an objective marker, like plasmid type, biofilm producing, serotypes and the ability of trigger inflammatory factors that may be needed for further study to complement the real hypervirulence. Therefore, the prevalence of hvKp may be incorrectly estimated due to the lack of definite and objective diagnostic methods.

Table 3 Risk factors for hvKp vs. cKp

Variable	Univariate OR (95% CI)	P-value	Multivariate OR (95% CI)	P-value
Infection occurred in ICU	1.388 (0.668–2.884)	0.380		
Male	1.538 (0.608–3.888)	0.363		
Hypermucoviscosity	**17.446 (8.079–37.673)**	**0.000**	**15.249 (6.905–33.875)**	**0.000**
Pulmonary diseases	2.125 (0.945–4.779)	0.068		
Diabetes	**2.903 (1.610–5.236)**	**0.000**	**2.566 (1.258–5.235)**	**0.010**
Cardiovascular disease	0.676 (0.388–1.179)	0.168		
Cerebrovascular disease	0.542 (0.268–1.095)	0.088		
Cancer	1.205 (0.648–2.240)	0.556		
Surgery within 1 month	1.475 (0.635–3.426)	0.367		
Digestive diseases	**2.127 (1.004–4.505)**	**0.046**		
Central intravenous catheter	1.271 (0.711–2.271)	0.419		
Urinary catheter	0.693 (0.350–1.372)	0.293		
Tracheal catheter	0.942 (0.530–1.673)	0.839		
Stomach tube	**0.447 (0.242–0.824)**	**0.010**		

Note: Bold values indicate *P*<0.05.
Abbreviations: cKp, classic *Klebsiella pneumoniae*; hvKp, hypervirulent *Klebsiella pneumoniae*; ICU, intensive care unit; OR, odds ratio.

Table 4 Risk factor for CR-hvKp vs. non-CR-hvKp

Variable	Univariate OR (95% CI)	P-value	Multivariate OR (95% CI)	P-value
Hypermucoviscous	0.468 (0.086–2.550)	0.380		
Infection occurred in ICU	2.667 (0.692–10.277)	0.154		
Pulmonary diseases	3.879 (0.052–1.112)	0.162		
Cancer	2.246 (0.625–8.077)	0.215		
Diabetes	**0.256 (0.071–0.930)**	**0.038**		
Cardiovascular disease	0.613 (0.167–2.250)	0.461		
Cerebrovascular disease	3.884 (0.965–15.313)	0.056		
Surgery within 1 month	**19.5 (4.567–83.265)**	**0.000**	**15.999 (3.412–75.026)**	**0.000**
Digestive diseases	2.386 (0.662–8.602)	0.184		
Central intravenous catheter	2.330 (0.472–11.501)	0.299		
Tracheal catheter	**6.051 (1.485–24.658)**	**0.012**		
Stomach tube	1.867 (0.462–7.535)	0.381		
Urinary catheter	3.492 (0.422–28.865)	0.246		

Note: Bold values indicate *P*<0.05.
Abbreviations: cKp, classic *Klebsiella pneumoniae*; CR, carbapenems-resistant; hvKp, hypervirulent *Klebsiella pneumoniae*; ICU, intensive care unit; OR, odds ratio.
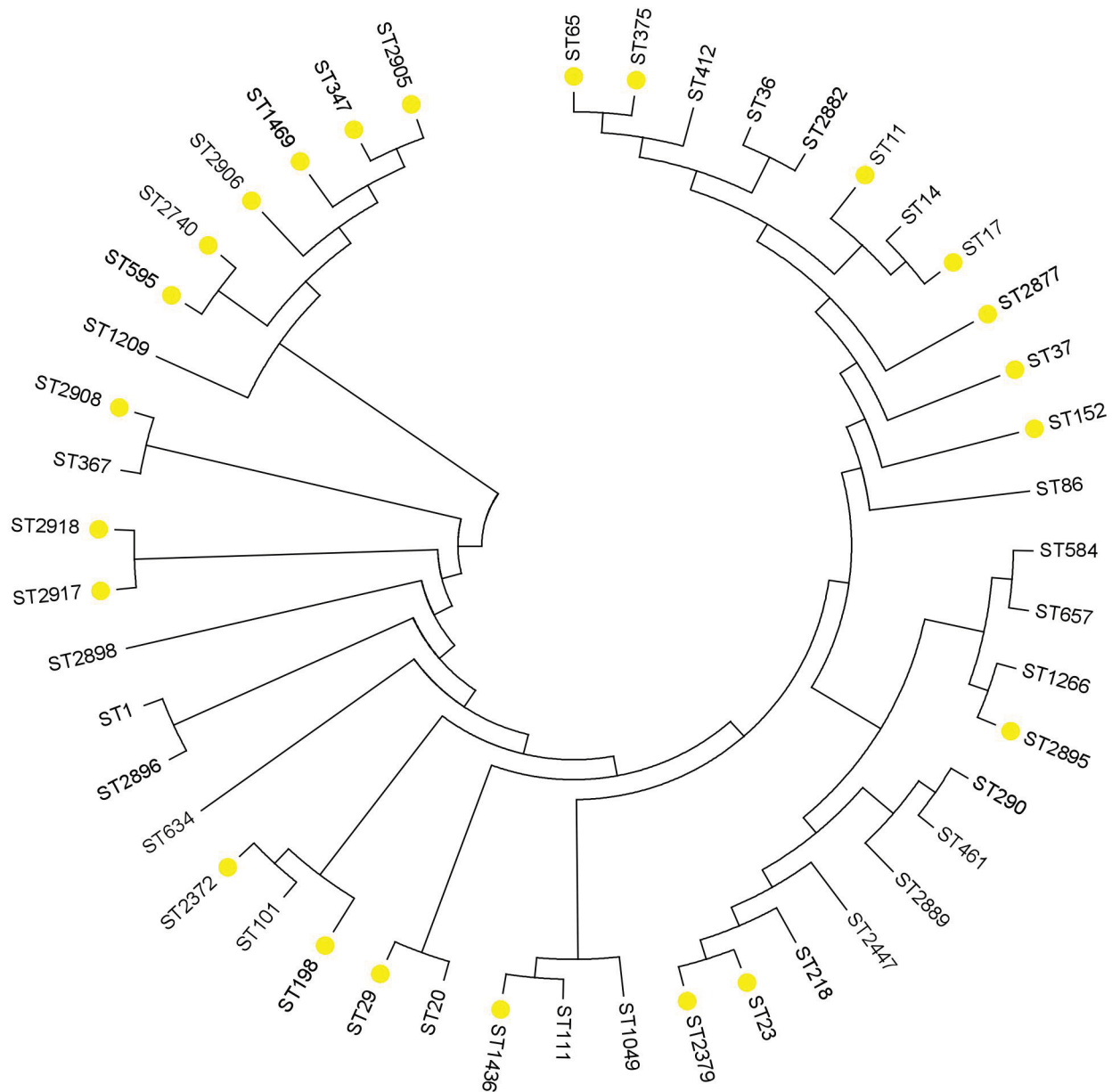
Figure 1 Neighbor-joining dendrogram showing concatenated sequences of 7 housekeeping genes from the MLST database for the frequency >1 STs and isolates contributing to invasive infection and mortality. Yellow solid rim represents death.
Abbreviations: MLST, multilocus sequence typing; ST, sequence type.

HvKp causes various severe infections, posing a serious threat to health. Various types of K-antigen have been reported,[17,21,22] with the more important elements in Asia being K1 and K2, which are the reason for severe infection. But they are not the unique trait for hvKp.[23,24] MagA is not a specific virulence gene for hvKp isolates causing PLA,[25] but it is highly associated with *cps* K1.[22,26] Moreover, the mutant isolate (knockout magA) could not show hmv phenotype.[27] RmpA/RmpA2 was proposed as a virulent factor

in addition to magA and cps K1/K2.[16] Although *rmpA* is not an independent factor contributing to pyogenic liver abscess, it promotes capsule synthesis, which is associated with hypermucoviscous.[3,16] Our results are consistent with a previous study: invasive infection (especially liver abscess), hypermucoviscosity, and mainly virulence factors (*K1, K2, rmpA*, and *magA* genes) are highly presented in hvKp group.[8] So, a better understanding of risk factors is essential to make interventions. Our results show that patients with diabetes are

more likely to be infected with hvKp. Additionally, hypermucoviscosity is strongly associated with hvKp. In our study, patients with surgery history of <1 month are an independent risk factor for CR-hvKp infection, which should be focused more on how to prevent infection. A previous study concluded that major histocompatibility complex variants, nutritional status, and gut microbiota are essential host factors to improve the understanding of the hypervirulence phenomenon.[7] Our results demonstrated that in the hvKp group, patients with better nutritional status are associated with a more severe SOFA score and more serious inflammation reaction. All aforementioned characteristics may be a potential marker for early and precise empirical interventions for the elderly with hvKp.

Previous studies have revealed that most hvKp and antimicrobial-resistant patterns were non-overlapping.[8,13] In this study, most hvKp were sensitive to most of the aforementioned antibiotics. In the hvKp group, the number of MDR-hvKp (25.0%) and ESBL-hvKp (26.0%) is significantly higher compared to the previous study performed in the multicenter study, with a prevalence of 12.6%.[8] It is alarming that the number of elderly with MDR-hvKp infection is increasing. Moreover, 1 CR-hvKp isolate was detected in 1 hospital, where long-term patients were hospitalized. However, 10 CR-hvKp strains were detected in the other hospital, which was a referral center receiving patients from other hospitals and the community. Therefore, the incidence of CR-hvKp may be underestimated in this region. Thus, these data revealed that MDR-hvKp is emerging among the elderly. However, to confirm this conclusion, further investigation using a larger population is needed.

The CR-hvKp was not detected in nosocomial environment by routine nosocomial infection surveillance. In addition, the 2 hospitals did not apply the use of anal swab for monitoring nosocomial infection. It is unclear whether gut microbiota composed of hvKp contributed to the infection. Thus, it is essential to enhance hospital infection surveillance for the elderly. A previous study suggested that wards previously infected with CR-hvKp should be disinfected and left unoccupied for >2 weeks.[10] Otherwise it may be a good site for a fatal outbreak of the organism.

There are some limitations in our study. First, it was a retrospective study in 2 teaching hospitals for over 10 years. Most key inflammatory and nutrition status marker were not achieved. Second, in vitro and in vivo experiments as objective evidence, such as *Galleria mellonella*, mouse, or human neutrophil assay, may be needed to identify the real virulent Kp. Third, to further explore the pathogen genomic characteristics, especially for virulent and antibiotic-resistant environment, whole genome sequencing, transcriptomics, and proteomics may be needed. A larger prospective multicenter study, focusing on host, pathogen, and host–pathogen interaction (inflammatory factor), is needed to better defining the hvKp strains.

Conclusion

The prevalence of hvKp may be higher than expected in the elderly. The epidemiology for hvKp in different hospitals is different. Although the definition of hvKp is still controversial, hvKp (aerobactin positive) strains were more likely to cause serious infections, such as liver abscess and septic shock, and more severe inflammatory reaction in the host. To further understand hvKp, host, pathogen, and host–pathogen interaction may be taken into consideration. The emerging MDR-hvKp, especially CR-hvKp, will be a great challenge for treatment. It is essential to enhance clinical awareness and management for the different types of hvKp infections.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Primers

Name	Sequence
rmpA	
Forward	5-ACTGGGCTACCTCTGCTTCA-3
Reverse	5-CTTGCATGAGCCATCTTTCA-3
rmpA2	
Forward	5-CTTTATGTGCAATAAG-GATGTT-3
Reverse	5-CCTCCTGGAGAGTAAGCATT-3
magA	
Forward	5-GGTGCTCTTTACATCATTGC-3
Reverse	5-GCAATGGCCATTTGCGTTAG-3
aerobactin	
Forward	5-GCATAGGCGGATACGAACAT-3
Reverse	5-CACAGGGCAATTGCTTACCT-3
K1	
Forward	5-GTAGGTATTGCAAGCCATGC-3
Reverse	5-GCCCAGGTTAATGAATCCGT-3
K2	
Forward	5-GGAGCCATTTGAATTCGGTG-3
Reverse	5-TCCCTAGCACTGGCTTAAGT-3
K5	
Forward	5-GCCACCTCTAAGCATATAGC-3
Reverse	5-CGCACCAGTAATTCCAACAG-3
K20	
Forward	5-CCGATTCGGTCAACTAGCTT-3
Reverse	5-GCACCTCTATGAACTTTCAG-3
K54	
Forward	5-CATTAGCTCAGTGGTTGGCT-3
Reverse	5-GCTTGACAAACACCATAGCAG-3
K57	
Forward	5-CGACAAATCTCTCCTGACGA-3
Reverse	5-CGCGACAAACATAACACTCG-3
rpoB	
Forward	5-GGCGAAATGGCWGAGAACCA-3
Reverse	5-GAGTCTTCGAAGTTGTAACC-3
gapA	
Forward	5-TGAAATATGACTCCACTCACGG-3
Reverse	5-CTTCAGAAGCGGCTTTGATGGCTT-3
mdh	
Forward	5-TGAAATATGACTCCACTCACGG-3
Reverse	5-CTTCAGAAGCGGCTTTGATGGCTT-3
pgi	
Forward	5-GAGAAAAACCTGCCTGTACTGCTGGC-3
Reverse	5-CGCGCCACGCTTTATAGCGGTTAAT-3
phoE	
Forward	5-ACCTACCGCAACACCGACTTCTTCGG-3
Reverse	5-TGATCAGAACTGGTAGGTGAT-3
infB	
Forward	5-CTCGCTGCTGGACTATATTCG-3
Reverse	5-CGCTTTCAGCTCAAGAACTTC-3
tonB	
Forward	5-CTTTATACCTCGGTACATCAGGTT-3
Reverse	5-ATTCGCCGGCTGRGCRGAGAG-3

Table S2 Detailed clinical and microbiological features of CR-hvKp strains

Clinical features	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Age (years)	87	87	87	88	79	71	65	65	77	84	94
Gender	F	M	M	F	M	F	F	M	M	M	M
Clinical department	Internal medicine	Emergency	General surgery	ICU	General surgery	ICU	ICU	Thoracic surgery	ICU	Thoracic surgery	Respiratory
Main underlying diseases	Bone fracture; Cardiovascular diseases	Diabetes	Surgery with in 1 month	Cerebrovascular disease	Cancer; Surgery with in 1 month	Cancer; Surgery with in 1 month	Diabetes; Cerebrovascular disease	Cancer	Cancer; Surgery with in 1 month	Surgery with in 1 month	Cancer; Cerebrovascular disease; diabetes
Tube	CVC; Ureter	Ureter; Stomach tube	None	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter	CVC; Ureter; Stomach tube; Tracheal catheter
Specimen type	Sputum	Sputum	Pyogenic fluids	Bile	Sputum +Blood +Wound	Bile+Blood	Sputum+Urine +Feces	Sputum	Pyogenic fluids	Sputum+Urine	Drainage liquid
Infection type	Pneumonia	Pneumonia	Abscess	PLA	Sepsis	Septic shock	Septic shock	Sepsis	Septic shock	Septic shock	Sepsis
WBC(10⁹/L)	12.21	10.85	13.76	16.44	8.80	15.49	10.0	18.32	11.66	9.51	4.64
NEU (%)	75.1	86.6	92.5	82.2	56.8	83.4	69.9	87.4	88.0	65.2	68.3

(*Continued*)

Table S2 (*Continued*)

Clinical features	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
TP (g/L)	79.4	60.5	69.4	52.8	54.7	49.6	59	70.9	62	67.4	61.2
ALB (g/L)	40.3	27.7	34.6	28.2	20.6	30.0	32.6	36.4	27.4	31.7	35.0
Sensitive antibiotics	N	N	N	N	GEN; LEV; TOB	N	SMZ; TOB; AMK	SMZ	N	N	SMZ; AMK
SOFA score	6	10	2	13	7	9	7	4	5	6	7
Empiric therapy	CIP+CAZ	CIP+CAZ	IPM	IPM+ISE	IPM+MXF	CIP+CAZ	IPM+ISE	IPM	IPM	MXF	MEM
Switched therapy	MEM+TGC	MEM+TGC	TGC	TGC	GEN+LEV	TGC+MEM	TGC+MEM+FOS	TGC+MEM	TGC+MEM	TGC+MEM+FOS	TGC
Clinical outcome	Survived	Survived	Survived	Survived	Survived	Survived	Survived	Died	Survived	Survived	Survived
String test	+	+	+	+	+	+	+	-	-	+	+
Virulence-associated genes											
rmpA	+	+	+	+	+	+	+	-	-	+	+
rmpA2	+	+	+	+	+	+	+	-	-	+	+
magA	+	+	+	+	+	+	+	-	+	+	+
cps genes											
K1	+	-	-	+	+	+	+	-	+	+	-
K2	+	+	+	+	+	+	+	-	+	+	-
K5	-	-	-	-	-	-	-	+	+	-	-
K20	-	+	-	-	+	-	-	-	+	-	-
K54	-	-	-	-	-	-	+	-	-	-	-
K57	+	-	-	-	-	+	-	-	-	-	-
MLST genotyping	23	23	17	17	347	17	2905	2906	23	412	2874

Abbreviations: ALB, albumin; AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CMZ, cefmetazole; CVC, central venous catheter; F, female; FOS, fosfomycin; GEN, gentamicin; ICU, intensive care unit; IPM, imipenem; ISE, isepamicin; LEV, levofloxacin; MLST, multilocus sequence typing; M, male; MEM, meropenem; Mo, month; MXF, moxifloxacin; NEU, neutrophils; N, no; PLA; pyogenic liver abscess; SMZ, sulfamethoxazole; SOFA, sequential organ failure assessment; TGC, tigecycline; TOB, tobramycin; TP, total protein; TZP, piperacillin tazobactam; WBC, white blood cell; Y, yes; +, positive; −, negative.