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ORIGINAL RESEARCH

Epidemiological Features of *Klebsiella pneumoniae* Infection in the Hepatobiliary System of Patients in Yantai, China, Based on Clinical and Genetic Analyses

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Purpose: To investigate the epidemiological features of *Klebsiella pneumoniae* infection of the hepatobiliary system of patients in Yantai, China.

Methods: This retrospective study was conducted from January to December 2019 in Yantai Yuhuangding Hospital. Patients for whom *K. pneumoniae* was isolated from the hepatobiliary system were considered for inclusion. The clinical features and genetic analyses were conducted to explore the epidemiological characteristics.

Results: A total of 88 cases were enrolled, including 69 cases of hypervirulent *K. pneumoniae* (hvKP) and 19 cases of classical *K. pneumoniae* (cKP). Community-acquired infections, fever, liver abscess, and C-reactive protein (CRP) and procalcitonin (PCT) levels were significantly higher, while biliary tract disease was lower in the hvKP group compared with the cKP group. Among the 69 hvKP infections, 61 developed a liver abscess. Community-acquired infections, fever, and CRP and PCT levels were higher, whereas biliary tract disease and malignancy were lower in the liver abscess group compared with the non-liver abscess group. All strains were susceptible to the majority of antibiotics tested. All hvKP strains possessed the bla_{SHV}, *oqxA, oqxB* and *fosA* resistance genes. K1 and K2 accounted for 78% of hvKP strains. K1 strains belonged to sequence types ST23 and ST700, whereas K2 strains belonged to ST65, ST86 and ST5212. K1 isolates possessed the most virulence determinants, followed by K2 and non-K1/K2 isolates. K2 isolates lacked the *allS* gene, which was rare in non K1/K2 isolates, but present in most K1 isolates. The *mceG* gene was only detected in K1 isolates. AllS and virulence determinants were significantly more prevalent in the liver abscess group than in the non-liver abscess group.

Conclusion: The prevalence of hvKP among *K. pneumoniae* infections of the hepatobiliary system is high in Yantai, China. Greater vigilance of hvKP infection is required in clinical and microbiological laboratories.

Keywords: liver abscess, *Klebsiella pneumoniae*, genome sequencing, virulence determinant, serotype

Introduction

Klebsiella pneumoniae is an increasingly prevalent bacterial pathogen capable of causing severe organ damage and life-threatening disease.[1] An important consequence of the continuous evolution of *K. pneumoniae* is the ability to obtain new genetic phenotypes. There are two recognized pathological types of *K. pneumoniae*, known as classical *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKP), which are currently prevalent,[2,3] each of which presents unique challenges for clinicians. In China, despite the research on *K. pneumoniae* infection reported to date, the antibiotic resistance, virulence determinants, genotype characteristics and clinical data of *K. pneumoniae* infection have not been extensively studied in Yantai, a coastal city of China.

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The aim of the present study was to systematically investigate the clinical and molecular characteristics of *K. pneumoniae* infection of the hepatobiliary system of patients in Yantai, so as to improve our understanding of this infection and increase the chance of early diagnosis and treatment.

Materials and Methods

Patients and Bacterial Isolates

This retrospective study was conducted from January to December 2019 in Yantai Yuhuangding Hospital of Shandong Province, a 3000-bed tertiary teaching hospital located in East China. Patients for whom *K. pneumoniae* was isolated from bile or pus of the hepatobiliary system were considered for inclusion in this study. All *K. pneumoniae* isolates collected for this study were cultured in blood agar in a 35°C incubator for 16–24 hours and then stored in skim milk at -80°C until use. Duplicate isolates collected from the same patient within 3 months were excluded. The study protocol, which included obtaining verbal informed consent, was approved by the Yantai Yuhuangding Hospital Ethics Committee.

Clinical Data Collection and Examination Methods

The clinical and demographic data for all study participants were retrospectively reviewed through medical records. The diagnostic criteria for a bacterial liver abscess were as follows: (a) The patient had clinical manifestations such as fever, nausea, chills, liver discomfort, liver tenderness or percussion pain; (b) Abdominal ultrasound, computed tomography or magnetic resonance imaging and other imaging findings found a liver abscess; (c) Clinical bacteriological examination results were positive or antibiotic treatment was effective. A community-acquired infection was defined as an infection in a patient acquired in the community without any prior exposure to healthcare facilities.

Strain Identification, Antibiotic Susceptibility Tests and Hypermucoviscous Phenotype Tests

All isolates collected in this study were cultured in blood agar and MacConkey agar plates in a 35°C incubator for 16–24 hours and identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Karlsruhe, Germany). Antibiotic susceptibility tests were carried out via the VITEK®2 compact system (Biomérieux, Marcy l'Etoile, France). The sensitivity of the strains to cefazolin, cefuroxime, ceftriaxone, cefotetan, ceftazidime, cefepime, aztreonam, ampicillin/sulbactam, piperacillin/tazobactam, cefoperazone/sulbactam, ertapenem, meropenem, imipenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin, amikacin and trimethoprim/sulfamethoxazole was tested. All procedures were performed in accordance with the manufacturer's instructions. The minimum inhibitory concentration breakpoints were interpreted according to CLSI M100-S31. *Escherichia coli* ATCC 25922 was used as a quality control. The hypermucoviscous (HMV) phenotype was defined by the formation of viscous strings >5 mm in length when an inoculation loop was used to stretch the colony on an agar plate, also known as a positive string test.[4]

Genome Sequencing

Genomic DNA was extracted using a Genomic DNA kit (Tiangen, DP305, Beijing, China) and the DNA concentration was quantified using a NanoDrop™ 2000 (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer and verified by agarose gel electrophoresis. For library preparation, >50 ng of extracted DNA was required. Libraries were prepared using the TruePrep™ DNA Library Prep Kit V2 for Illumina (Vazyme). Using a single "transposase" enzymatic reaction, sample DNA was simultaneously fragmented and tagged with adapters. An optimized, limited-cycle polymerase chain reaction (PCR) protocol amplified tagged DNA and added sequencing indexes. Individual libraries were assessed on an QIAxcel Advanced Automatic nucleic acid analyzer, and then quantitated through quantitative real-time PCR (qPCR) by the use of KAPA SYBR® FAST qPCR kits. Finally, the library was sequenced on an Illumina HiSeq 2500 sequencing platform (Illumina Inc., San Diego, CA, USA) and 150 bp paired-end reads were generated. Raw data were filtered to remove low-quality reads, then clean data were assembled via SPAdes v3.13. The capsular serotype was annotated by comparison with Kaptive. Sequence typing was performed using MLST (https://cge.cbs.dtu.dk/services/MLST/). The antimicrobial resistance genes and virulence genes were identified by BLAST using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/https://cge.cbs.dtu.dk/services/ResFinder/) and VirulenceFinder (https://cge.cbs.dtu.dk/ser

vices/VirulenceFinder/) with thresholds of 90% identity and a minimum length coverage of 80%. The hvKP strains in this study were considered by the presence of the *peg-344* or *iucA* gene, otherwise strains were considered to be cKP.

Statistical Analyses

SPSS Statistics 26 (IBM Corporation, NY, USA) was used for data entry and coding. Categorical data were summarized using numbers (percentages). For comparison of categorical variables, the chi-square test or Fisher's exact test was performed. Continuous variables were presented as means ± standard deviations and compared using the Student's *t*-test or the Mann–Whitney *U*-test, as appropriate. $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics of the Patients

According to the inclusion criteria, a total of 88 cases of non-repetitive *K. pneumoniae* infection were enrolled, including 69 (78%) cases of hvKP and 19 (22%) cases of cKP. The age of the patients ranged from 27 to 88 years, with an average of 61.74±12.36 years, of which 72% (63/88) were male and 28% (25/88) were female. Among the 69 patients with hvKP infection, 61 (88.4%) developed a liver abscess. All patients with a liver abscess underwent surgical drainage, except for two cases with a non-liquefied abscess. All patients in this study received effective antibiotic treatment (mainly piperacillin/ tazobactam, followed by ceftizoxime, cefoperazone/sulbactam and imipenem). The demographic characteristics, symptoms, underlying diseases, laboratory findings and prognoses of patients with *K. pneumoniae* and hvKP infection are listed in Tables 1 and 2.

Table 1 Demographic Characteristics, Symptoms, Underlying Diseases, Laboratory Findings and 30-Day Survival of Patients with *K. pneumoniae* Infection of the Hepatobiliary System, and the Correlation with a Hypermucoviscous Phenotype

Variates	hvKP (n=69)	cKP (n=19)	*p* value
Demographics			
Age (years)	60.84±12.36	65.00±12.45	0.906
Sex (male)	47(68.1%)	16(84.2%)	0.276
Community acquired	63(91.3%)	8(42.1%)	0.000
Symptoms			
Fever (T>38.5°C)	58(84.1%)	6(31.6%)	0.000
Chills	16(23.2%)	1(5.3%)	0.154
Abdominal pain	26(37.7%)	11(57.9%)	0.114
Nausea or vomiting	14(20.3%)	3(15.8%)	0.911
Underlying conditions			
Liver abscess	61(88.4%)	0(0.0%)	0.000
Diabetes	38(55.1%)	7(36.8%)	0.159
Hypertension	19(27.5%)	5(26.3%)	0.916
Biliary tract disease	29(42.0%)	17(89.5%)	0.000
Malignancy	10(14.5%)	5(26.3%)	0.225
Laboratory findings			
White blood cell (*10^9/L)	12.86±4.97	13.16±7.04	0.829
Percentage of neutrophil (%)	84.74±10.09	83.96±12.01	0.776
CRP (mg/L)	190.18±116.95	79.86±64.81	0.001
PCT (ng/mL)	15.93±11.25	8.38±5.56	0.019
30-day survival	66(95.7%)	19(100.0%)	1.000
Positive string test	59(85.5%)	3(15.8%)	0.000

Table 2 Demographic Characteristics, Symptoms, Underlying Diseases, Laboratory Findings and 30-Day Survival of Patients with hvKP Infection of the Hepatobiliary System

Variates	Liver Abscess (n=61)	Non-Liver Abscess (n=8)	*p* value
Demographics			
Age (years)	60.49±12.32	63.50±13.20	0.522
Sex (male)	42(68.8%)	5(62.5%)	1.000
Community acquired	61(100.0%)	2(25.0%)	0.000
Symptoms			
Fever (T>38.5°C)	56(91.8%)	2(25.0%)	0.000
Chills	15(24.6%)	1(12.5%)	0.752
Abdominal pain	23(37.7%)	3(37.5%)	1.000
Nausea or vomiting	14(22.9%)	0(0.0%)	0.193
Underlying conditions			
Diabetes	35(57.4%)	3(37.5%)	0.494
Hypertension	17(27.9%)	2(25.0%)	1.000
Biliary tract disease	22(36.1%)	7(87.5%)	0.017
Malignancy	6(9.8%)	4(50.0%)	0.012
Laboratory findings			
White blood cell (*10^9/L)	13.01±4.97	10.47±4.85	0.178
Percentage of neutrophil (%)	85.44±8.05	75.03±20.01	0.187
CRP (mg/L)	194.08±111.86	84.59±113.38	0.046
PCT (ng/mL)	17.59±22.41	0.58±1.04	0.000
30-day survival	59(96.7%)	7(87.5%)	0.779

As shown in Table 1, the two groups (hvKP and cKP) had a similar sex ratio and mean age ($p > 0.05$). The number of community-acquired infections was higher in the hvKP group compared with the cKP group ($p = 0.000$). All symptoms and underlying conditions were similar between the groups, except that there was a higher occurrence of fever and liver abscess, and a lower occurrence of biliary tract disease in the hvKP group compared with the cKP group ($p = 0.000$). In addition, laboratory findings revealed that CRP and PCT levels were significantly higher in the hvKP group ($p < 0.05$). After antibiotic treatment and/or surgical drainage, the patients generally had a good prognosis, with the exception of three patients with hvKP infection that died.

The baseline characteristics of patients with hvKP infection are listed in Table 2. There were no significant differences in sex ratio or mean age ($p > 0.05$). The occurrence of community-acquired infections and fever was higher in the liver abscess group compared with the non-liver abscess group ($p = 0.000$), whereas the occurrence of biliary tract disease and malignancy was lower in the liver abscess group ($p < 0.05$). Moreover, CRP and PCT levels were significantly higher in the liver abscess group than in the non-liver abscess group ($p < 0.05$). There was no significant difference in 30-day survival between the two groups.

Among the 61 patients with a liver abscess, 55 (90.2%) occurred in the right lobe, 6 (9.8%) in the left lobe and 2 (3.3%) in the caudate lobe. A single abscess occurred in 54 (88.5%) patients and multiple abscesses occurred in 7 (11.5%) patients. In addition, the diameter of the abscess was less than 5 cm in 18 (29.5%) cases, 5–10 cm in 34 (55.7%) cases and more than 10 cm in 9 (14.8%) cases. Three patients developed endophthalmitis.

Resistance Gene Detection and Antibiotic Susceptibility Tests

The antimicrobial resistance genes of 69 hvKP strains were detected. The results showed that all strains possessed the bla_{SHV} gene, among which $bla_{SHV\text{-}190}$ was the most prevalent (52.2%, n = 36), followed by $bla_{SHV\text{-}11}$ (11.6%, n = 8), $bla_{SHV\text{-}67}$ (10.1%, n = 7), $bla_{SHV\text{-}185}$ (8.7%, n = 6) and $bla_{SHV\text{-}28}$ (7.2%, n = 5). The *oqxA* and *oqxB* genes encoding

quinolone resistance, and the *fosA* gene encoding fosfomycin resistance, were present in all strains. Furthermore, 13 strains (18.8%) possessed the tetracycline resistance gene *tetA*, 10 strains (14.5%) possessed the sulfonamide resistance genes *sul1, sul2* or *dfrA*, and 8 strains (11.6%) possessed the aminoglycoside resistance genes *aph(6)-Id, aph(3")-Ib* or *aac(3)-I*. The $bla_{\text{CTX-M}}$, bla_{TEM} and $bla_{\text{LAP-2}}$ genes were found in six, six and five strains (8.7%, 8.7% and 7.2%), respectively. The azithromycin resistance gene *mph(A)* was found in five strains (7.2%).

The results of antibiotic susceptibility tests are shown in Figure 1. Amikacin, tobramycin and imipenem showed excellent activity against all *K. pneumoniae* strains with no resistant cases detected. The resistance rates of cefotetan, ceftazidime, cefepime, piperacillin/tazobactam, cefoperazone/sulbactam, meropenem, ertapenem and levofloxacin were lower than 10%, while the resistance rates were higher than 10% for cefazolin, cefuroxime, ceftriaxone, aztreonam, ampicillin/sulbactam, ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole. With the exception of meropenem and ertapenem, the antibiotic resistance rates of strains in the cKP group were higher than those in the hvKP group.

Capsular Serotypes and Sequence Types

The capsular serotype (K) and sequence type (ST) of hvKP strains are shown in Figure 2. Regarding capsular serotype, K1 accounted for 60.9% (n = 42) of hvKP cases, followed by K2 (17.4%, n = 12). Other capsular serotypes included K5 (4.3%, n = 3), K20 (4.3%, n = 3), K47 (2.9%, n = 2), K14 (1.4%, n = 1), K31 (1.4%, n = 1), K54 (1.4%, n = 1), K57 (1.4%, n = 1), K63 (1.4%, n = 1) and an unknown capsular serotype (2.9%, n = 2). Regarding sequence type, K1 hvKP strains belonged to ST23 (85.7%) and ST700 (14.3%), whereas K2 hvKP strains belonged to ST65 (50.0%) and ST86 (41.7%). Significantly, one K2 strain belonged to the novel sequence type ST5212, which had not been reported previously in hvKP. The proportions of K1 and K2 were significantly higher in the liver abscess group than in the non-liver abscess group ($p < 0.05$, Table 3).

Hypermucoviscous (HMV) Phenotype and Virulence Determinants

The HMV phenotype of 88 *K. pneumoniae* isolates is shown in Table 1. The HMV phenotype and the virulence determinants of 69 hvKP isolates are listed in Figure 3 and Table 3. The number of isolates showing a positive string test was higher in the hvKP group compared with the cKP group (59 [85.5%] vs 3 [15.8%], $p = 0.000$), but there was no significant difference between the liver abscess group and the non-liver abscess group ($p > 0.05$). Regarding biomarker genes, 69 (100%) strains of hvKP possessed *peg-344*, 60 (87.0%) strains possessed *iucA*, 57 (82.6%) strains possessed *iroN* and *rmpA*, 56 (81.2%) strains possessed *rmpA2*, 37 (53.6%) strains possessed *allS*, 63 (91.3%) strains possessed *ybtP*, 68 (98.6%) strains possessed *iroNB*, 42 (60.9%) strains possessed *clbH* and 35 (50.7%) strains possessed *mceG*. The prevalence of allantoin metabolism gene *allS* and the number of virulence determinants were significantly higher in the liver abscess group than in the non-liver abscess group ($p < 0.05$).

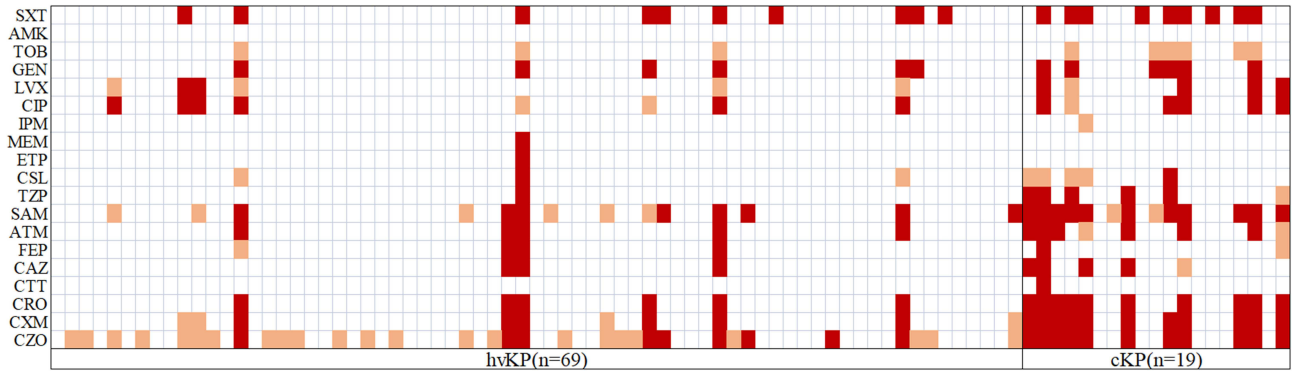

Figure 1 Antibiotic susceptibility tests of 88 *K. pneumoniae* strains.
Notes: Red: resistant, orange: intermediate.
Abbreviations: CZO, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; CTT, cefotetan; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; CSL, cefoperazone/sulbactam; ETP, ertapenem; MEM, meropenem; IPM, imipenem; CIP, ciprofloxacin; LVX, levofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole.

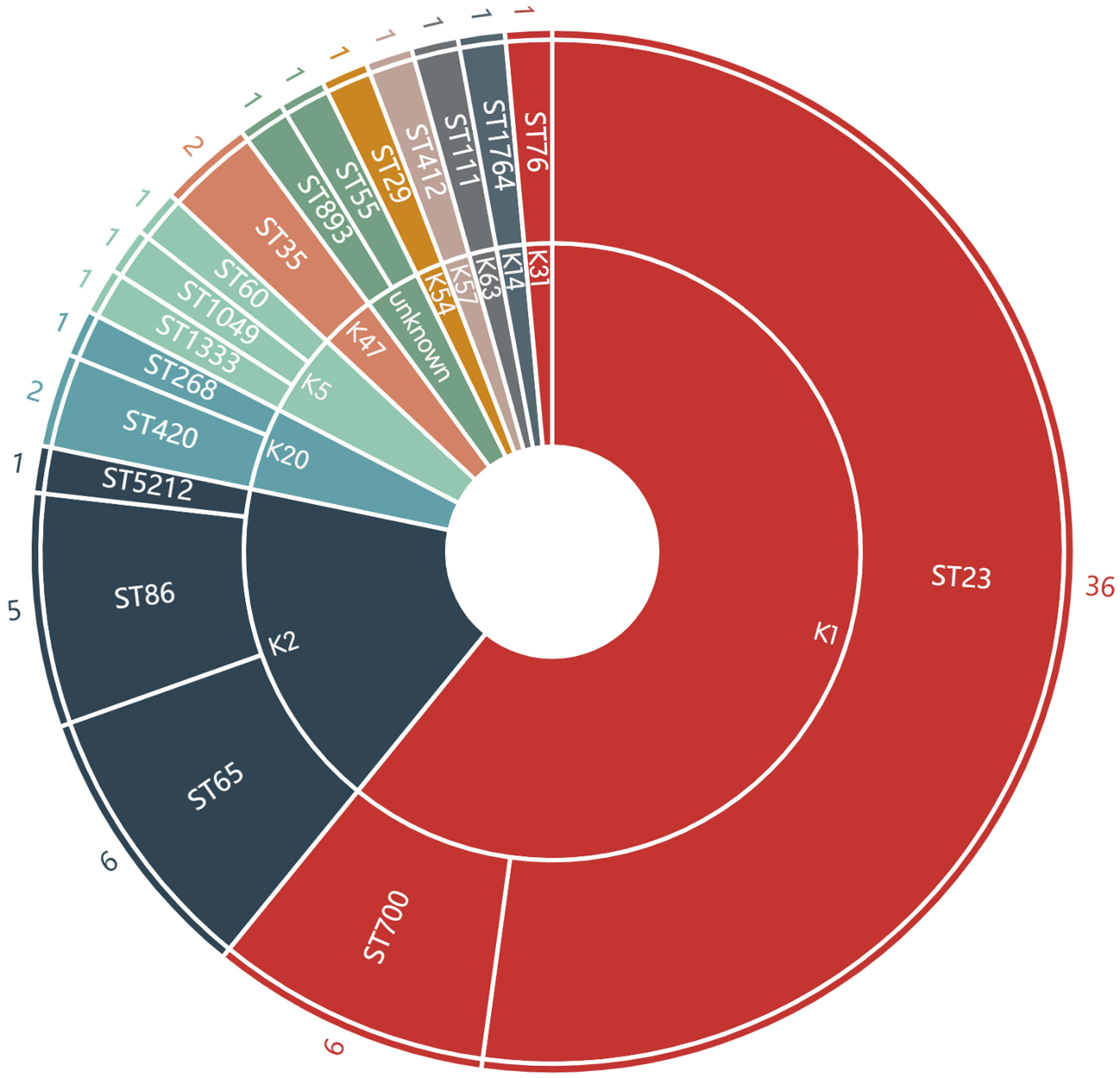

Figure 2 The capsular serotype (K) and sequence type (ST) of hvKP strains.

There were no significant differences in the other virulence determinants between the two groups (all $p > 0.05$). By further comparing the number of virulence determinants of different K types, it was found that the average number of virulence determinants of K1 type was 8.98±2.30, K2 type was 7.5±0.67 and all other K types was 5.13±1.96. Comparisons between each of the groups were all statistically significant ($p < 0.05$).

Discussion

CKP infection is a widespread infection that is frequently encountered by clinicians, particularly in healthcare settings. As an opportunistic pathogen, cKP mainly causes infection in hosts who are immunocompromised, or who have existing barrier breakdown (eg, intravascular devices, an endotracheal tube or surgical wounds).[5] HvKP is best described as a virulent pathogen. Distinguishing characteristics of hvKP include community-acquired infections in healthy individuals of any age, multiple sites of infection and a tendency for metastatic dissemination to various sites (eye, lung, central

Table 3 Hypermucoviscous Phenotype and Virulence Determinants of hvKP

Variates	Liver Abscess (n=61)	Non-Liver Abscess (n=8)	*p* value
Positive string test	53(86.9%)	6(75.0%)	0.716
K1 and K2	51(83.6%)	3(37.5%)	0.012
peg-344	61(100.0%)	8(100.0%)	
iucA	53(86.9%)	7(87.5%)	1.000
iroN	52(85.2%)	5(62.5%)	0.271
rmpA	52(85.2%)	5(62.5%)	0.271
rmpA2	51(83.6%)	5(62.5%)	0.340
allS	36(59.0%)	1(12.5%)	0.035
ybtP	57(93.4%)	6(75.0%)	0.283
iroNB	61(100.0%)	7(87.5%)	0.227
clbH	39(63.9%)	3(37.5%)	0.291
mceG	34(55.7%)	1(12.5%)	0.054
Number of virulence determinants	8.13±2.43	6.00±2.78	0.025

nervous system).[6,7] Complications such as meningitis and endophthalmitis are associated with poor outcomes and high mortality rates.[8]

The prevalence of hvKP among *K. pneumoniae* cases varies, but can be high in hvKP-endemic areas.[7,9,10] The high prevalence of hvKP among patients with infections of the hepatobiliary system in Yantai, China, indicates that early diagnosis of hvKP infection is essential to control hepatobiliary infection. This study found that compared with cKP, patients with hvKP infection often displayed a higher body temperature, higher levels of CRP and PCT, and liver abscesses. Furthermore, cases of hvKP infection were more likely community-acquired, and patients often had no biliary tract disease. Taken together, these findings suggest that hvKP infection should be considered in patients with hepatobiliary infection if they have high CRP and PCT levels, a high body temperature and the infection was community-acquired. In addition, patients with hvKP infection are more likely to develop a liver abscess if the infection was community-acquired or CRP and PCT levels, and body temperature are elevated, whereas patients with biliary tract disease and malignancy are more likely to develop a biliary infection other than a liver abscess. Early antibiotic treatment and abscess drainage have proven to be crucial to improve the prognosis of patients with a liver abscess.

Our results indicated that men are more likely to develop hvKP infection than women (72% vs 28%), and the average age of patients was 61 years, which is consistent with previous studies.[11] Diabetes is generally considered to be a significant risk factor for acquiring a hvKP infection, but not cKP infection.[12,13] The reason for this may be that increased serum glucose levels can increase capsule production. Moreover, hyperglycemia may inhibit the chemotaxis, adhesion and phagocytosis of white blood cells, as well as impairing intestinal barrier function and promoting the spread

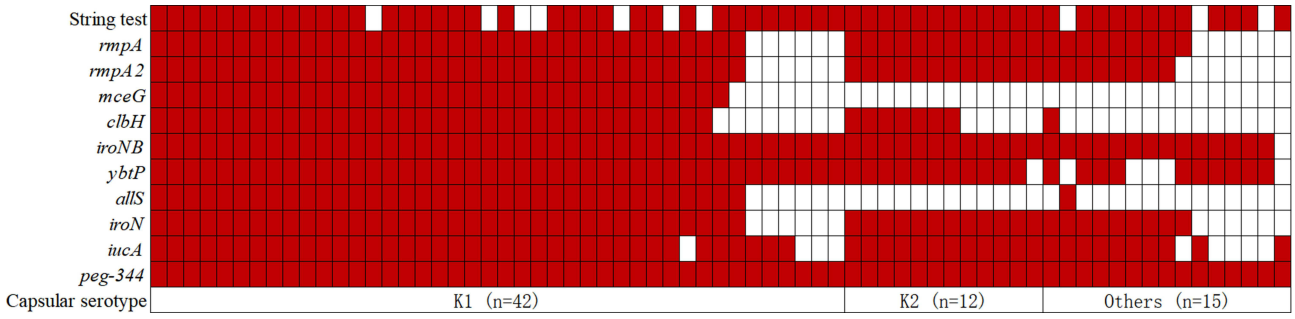

Figure 3 The hypermucoviscous phenotype and virulence determinants of hvKP strains.
Note: Red: positive.

of *K. pneumoniae*. However, not all studies have reported this association,[14,15] and our results did not confirm this correlation, suggesting that the connection between diabetes and hvKP infection may vary by region or clinical presentation.

Antibiotic susceptibility tests showed that all *K. pneumoniae* strains in our study were susceptible to the majority of the antibiotics tested, but vigilance is still needed regarding the emergence of multidrug-resistant hvKP. One hvKP strain was resistant to meropenem and ertapenem, but no carbapenemase gene was found, only $bla_{\text{CTX-M}}$ and $bla_{\text{TEM-1B}}$ were detected. The mechanism of resistance may be related to the high expression of $bla_{\text{CTX-M}}$ and $bla_{\text{TEM-1B}}$ and the loss of membrane porins, which needs to be further confirmed. Although the quinolone resistance genes *oqxA* and *oqxB* were detected in all hvKP strains, the resistance rates of hvKP to ciprofloxacin and levofloxacin were only 8.7% and 2.9%, respectively, indicating that antibiotic resistance was not completely related to resistance genes in this case, but may also be affected by other factors such as gene expression levels.

First recognized in Asia, hvKP has emerged as a pathogen of concern globally.[16] Research has focused on the identification of a suitable method or genetic determinant to distinguish hvKP and cKP. A trait that was initially believed to be specific to hvKP strains was the HMV phenotype,[1] which can be defined by a positive string test.[17] This has since been shown not be the case,[18] not all hvKP strains are HMV, and not all strains with a HMV phenotype are hvKP. In our study, 85% of hvKP strains and 15% of cKP strains showed a HMV phenotype, indicating that it was not advisable to define hvKP by HMV phenotype alone. Subsequently, the capsular serotype, the sequence type and the presence of virulence-associated genes were all used to differentiate hvKP from cKP strains.

Capsule is a polysaccharide synthesized by all *K. pneumoniae* strains that acts as a protective layer on the exterior of the bacterium, inhibiting phagocytosis, antimicrobial peptides, complement and the induction of the host inflammatory response.[19] The most common hvKP capsular serotypes (K) are K1, K2, K5, K20 and K47, with K1 and K2 accounting for 78% of hvKP strains in our study. The proportions of K1 and K2 among the hvKP strains were significantly higher in the liver abscess group compared with the non-liver abscess group. Sequence type (ST) is another identifier of hvKP. It has been reported that ST23 is the dominant ST among hvKP isolates, and is strongly associated with the K1 capsule type, while ST65 and ST86 are related to the K2 capsule type.[20] Our study further confirmed this conclusion. In addition, our study also found that ST700 was closely related to the K1 capsule type, accounting for 14.3% of K1 capsule-type strains. Moreover, a novel ST, ST5212, which belonged to the K2 capsule type, was detected that had not been reported previously.

Iron acquisition is critical for bacterial growth.[8] HvKP strains have the capability to produce four different siderophores: enterobactin, salmochelin (*iro*), yersiniabactin (*ybt*) and aerobactin (*iuc*).[15] Aerobactin has been proven to be the primary virulence determinant that enables systemic infection, and salmochelin and aerobactin are hvKP-specific.[21] *Peg-344* is a metabolic transporter of unknown function that has hvKP specificity.[21] The regulator of mucoid phenotype *rmpA/rmpA2, allS* (involved in allantoin metabolism) and *mceG* (an energy-related gene) are also used to distinguish between hvKP and cKP strains.[22,23] A combination of these biomarkers (eg, *peg-344, iroB, iucA* and *rmpA/rmpA2*) showed higher accuracy in the diagnosis of hvKP than a single phenotype or single virulence-associated gene alone.

Among 69 hvKP strains, more virulence determinants were detected in the liver abscess group compared with the non-liver abscess group, which may partly explain the higher body temperature, and higher levels of CRP and PCT in this group. The comparison of different K types of hvKP showed that K1 carried the most virulence determinants, followed by K2, and non K1/K2 isolates carried the least virulence determinants, suggesting that K1 may have the strongest virulence, followed by K2 and non K1/K2 isolates. It was also found that all K2 isolates lacked the allantoin metabolism gene *allS*, which was also rare in non K1/K2 isolates, but present in most K1 isolates. The *mceG* gene was only present in K1 isolates. The correlation between the *allS* and *mceG* genes and different K capsule types has rarely been reported, and the mechanism therefore requires further study.

A critical feature of hvKP is the ability to produce increased amounts of capsular polysaccharide. This is mediated, at least in part, by the *rmpA* and/or *rmpA2* genes.[24] In this study, 10 hvKP strains were detected that tested negative for the *rmpA/rmpA2* genes but gave a positive string test, indicating that capsule synthesis was not necessarily required for the HMV phenotype. Indeed, early reports of *rmpA* proposed that capsule overexpression did not necessarily lead to a HMV

phenotype.[25,26] Eight hvKP strains that tested positive for the *rmpA/rmpA2* genes but gave a negative string test confirmed this conclusion.

This report presents the epidemiological features of *K. pneumoniae* infection of the hepatobiliary system. Some limitations of this study need to be taken into account. First, this was a retrospective, single-center study, on a small sample of strains, which may not be fully representative. Second, the location of virulence determinants (chromosomes or plasmids) was not determined, and antimicrobial resistance genes and virulence genes other than *peg-344* and *iucA* of cKP strains were not analyzed. Finally, whether there is any epidemiologic evidence for the relationship between virulence determinants and clinical manifestations remains to be investigated. However, our results do contribute towards effective identification of *K. pneumoniae*-infecting strains and provide insight into the molecular characteristics of hvKP strains.

Conclusions

The prevalence of hvKP among *K. pneumoniae* infections of the hepatobiliary system of patients is high in Yantai, China. Antibiotic treatment and surgical drainage are effective therapeutic measures for hvKP infection. Our findings highlight that hvKP infection warrants careful surveillance in clinical and microbiological laboratories.

Ethics Approval and Informed Consent

Approval and verbal informed consent was obtained for experimentation with human subjects due to the retrospective nature of the study. The study protocol, including the verbally informed consent procedure, was approved by the Yantai Yuhuangding Hospital Ethics Committee. This study complied with the Declaration of Helsinki.

Consent for Publication

All of the images, tables and recordings can be published.

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Disclosure

The authors have no relevant financial or non-financial competing interests to disclose.

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