Increasing Occurrence of Antimicrobial-Resistant Hypervirulent (Hypermucoviscous) *Klebsiella pneumoniae* Isolates in China

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Background. New hypervirulent variants of *Klebsiella pneumoniae* (hvKP) are emerging globally, most of which exhibit antimicrobial susceptibility.

Methods. A retrospective study was conducted in 88 patients with cultures positive for *K. pneumoniae* hospitalized in the Beijing You'an Hospital from April 2010 to June 2012. The clinical and molecular data of the hvKP isolates (defined as string test positive) were compared with those of the classic *K. pneumoniae* (cKP) isolates.

Results. Overall, 33.0% (29/88) of *K. pneumoniae* isolates were hvKP. Univariate analysis revealed the following risk factors for hvKP: virulence gene *rmpA* (odds ratio [OR], 16.92 [95% confidence interval {CI}, 4.842–59.145]), capsule antigens K1 (OR, 3.355 [95% CI, 1.153–9.768]) and K2 (OR, 9.280 [95% CI, 0.987–87.250]), alcoholic hepatitis (OR, 7.435 [95% CI, 1.397–39.572]), liver abscess (OR, 9.068 [95% CI, 1.747–47.061]), metastatic infection (OR, 2.752 [95% CI, 1.100–6.886]), community-acquired infection (OR, 10.432 [95% CI, 3.623–30.033]), sputum isolation (OR, 0.312 [95% CI, .095–1.021]), and HIV infection (<0.001 [not applicable]). Multivariate analysis implicated *rmpA* (OR, 17.398 [95% CI, 4.224–71.668]) and community-acquired infection (OR, 6.844 [95% CI, 1.905–24.585]) as independent risk factors. The proportion of hvKP isolates increased from April to December 2010, January to September 2011, and October 2011 to June 2012 (to 25.5%, 26.7%, and 54.5%, respectively). Resistance to 14 of 19 tested antimicrobials was found to be significantly greater in cKP compared to hvKP. Importantly, resistance to all the tested antimicrobials, except carbapenems and amikacin, was observed in a proportion of hvKP strains, 17% (5/29) of which expressed extended-spectrum β-lactamase. Furthermore, antimicrobial resistance in hvKP strains increased over time.

Conclusions. HvKP strains are being isolated from patients in China with increasing frequency and constitute an increasing proportion of *K. pneumoniae* strains, indicating an increasing propensity for the acquisition of antimicrobial resistance.

Keywords. *Klebsiella pneumoniae*; hypervirulent; antimicrobial resistance; epidemiology; clinical features.

The *Klebsiella pneumoniae* strains commonly recognized by clinicians and microbiologists are termed classic *K. pneumoniae* (cKP). Such strains are notorious for their capacity to cause nosocomial infections and acquire antimicrobial resistance [1–4]. A new variant of *K. pneumoniae*, designated as hypervirulent *K. pneumoniae* (hvKP), was first described in 1986 by a group of Taiwanese doctors reporting a clinical syndrome of community-acquired *K. pneumoniae* infections [5]. The hvKP strains exhibit unique features compared to cKP. First, compared to other pathogens that share features with hvKP, including the potential to cause nosocomial infections, the hvKP strains exhibit a striking capacity to cause serious infections in immunocompetent and young, healthy individuals [6–9]. For

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example, severe hvKP infections have been seen to cause community-acquired pyogenic liver abscesses in previously healthy adults [5, 10]. Second, unlike other enteric gram-negative bacilli, such as cKP, hvKP strains possess a propensity for causing metastatic infections [11]. Third, hvKP colonies grown on agar exhibit hypermucoviscosity. This phenotype has been used as a standard laboratory test to distinguish hvKP from cKP and is defined as a positive "string test." The mechanisms underlying the increased virulence and mucoviscosity of hvKP compared with cKP have been reviewed recently but remain to be fully elucidated [12].

After the original identification of hvKP isolates, additional reports followed, initially from Taiwan, Korea, Vietnam, and Japan, and more recently from South America [13], North America [8, 14–21], the Caribbean [22], the Middle East [23, 24], Europe [9, 25, 26], Africa [9, 23], and Australia [23]. Interestingly, despite its proximity to the Asian countries from which hvKP infections have been reported, to date, there have been no reports of hvKP infections from mainland China.

An increasing number of cKP stains are being shown to exhibit resistance to the currently available antibiotics, including carbapenemases [27, 28]. Fortunately, most of the hvKP strains identified to date are susceptible to antimicrobials with the exception of ampicillin [7, 29]. However, the enhanced virulence of hvKP strains and the possibility of acquiring antimicrobial resistance are a cause for concern. Combined with the increased risk to susceptible populations, these issues have attracted calls for preemptive intervention to mitigate the possibility of the globally damaging effects of hvKP infections.

We speculated that hvKP strains are currently present in mainland China. To test this hypothesis, we conducted a retrospective analysis of 88 patients with positive cultures for *K. pneumoniae* hospitalized in the Beijing You'an Hospital (People's Republic of China [PRC]) from April 2010 to June 2012. The presence of hvKP in Beijing was established. Analysis of the clinical and molecular characteristics of the isolates indicated that the proportion of hvKP strains among *K. pneumoniae* isolates appears to be increasing. Furthermore, the frequency of antimicrobial resistance among these hvKP isolates is increasing over time.

MATERIALS AND METHODS

Patient Information

A retrospective study was conducted on 88 consecutive *K. pneumoniae* culture–positive patients hospitalized at the Beijing You'an Hospital (the study center) from April 2010 to June 2012. More than half of the patients were referred directly from other hospitals. Clinical and laboratory data were gathered and comparisons were made between patients from which hvKP strains and cKP strains were isolated.

A hospital-acquired *K. pneumoniae* infection was defined as one that appeared in the first 3 days after a patient was admitted to the Beijing You'an Hospital or another healthcare facility before being transferred to the study center. Most of the identified cases had already been hospitalized elsewhere for >3 days with or without isolation of a *K. pneumoniae* strain. In such cases, infections were considered to be nosocomial if the patient was admitted to the study center within 24 hours of discharge from another hospital, even if a *K. pneumoniae*–positive culture was obtained from a sample isolated upon admission to the study center. Cases without these conditions were defined as community-acquired infections.

Rates of metastatic *K. pneumoniae* infections were compared between cKP and hvKP isolates. Microbiological evidence of secondary *K. pneumoniae* was not obtained in a number of patients; therefore, metastatic infection was defined on the basis of the clinical diagnosis of the presence of >1 infection site in a single patient. Specifically, cases of metastatic infection were diagnosed in patients with *K. pneumoniae*–positive culture obtained from 1 or more sites in addition to a clear infection site, such as a liver abscess, even if the abscess fluid culture was negative for *K. pneumoniae*.

The protocol for this study was approved by the Beijing You'an Hospital Ethics Committee, and the Guidelines for Human Experimentation (PRC) were followed throughout. All patients gave written informed consent upon admission for their information to be stored and used for research.

Clinical Microbiologic Characterization of the *K. pneumoniae* Strains

All *K. pneumoniae* isolates were frozen and stored at −80°C. Susceptibility testing (Phoenix 100 automated microbiology system, BD, Franklin Lakes, New Jersey) to amikacin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, aztreonam, ceftizoxime, cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, levofloxacin, piperacillin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, imipenem, meropenem, and piperacillin-tazobactam was conducted for every strain. Extended spectrum β-lactamase (ESBL) production was also determined using the Phoenix 100 system. A string test was performed to distinguish hvKP from cKP. A positive string test was defined as the formation of a mucoviscous string of >5 mm, when using a bacteriology inoculation loop to stretch a colony grown overnight on an agar plate at 37°C. *Klebsiella pneumoniae* strains with a positive string test were designated as hvKP.

Polymerase Chain Reaction–Mediated Detection of *rmpA, rmpA2,* and Capsular Serotype-Specific Genes

Genomic DNA was extracted from all *K. pneumoniae* strains (Qiagen DNA extraction kit) and the *rmpA*, *rmpA2*, and

Table 1. Primers

Primer Name	Sequence
rmpA	
Forward	5-ACTGGGCTACCTCTGCTTCA-3
Reverse	5-CTTGCATGAGCCATCTTTCA-3
rmpA2	
Forward	5-CTTTATGTGCAATAAG-GATGTT-3
Reverse	5-CCTCCTGGAGAGTAAGCATT-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

serotype-specific genes for the K1, K2, K5, K20, K54, and K57 capsular serotypes were amplified by polymerase chain reaction (PCR; Bio-Rad real-time system C1000 Thermal Cycler) as described previously [7, 30]. The primers used are listed in Table 1.

Multilocus Sequence Typing

Multilocus sequence typing (MLST) of 7 housekeeping genes (*gapA*, *mdh*, *phoE*, *tonB*, *infB*, *pgi*, *and rpoB*) was performed according to the protocol described on the *K. pneumoniae* MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). Alleles and sequence types (STs) were assigned by using the MLST database (http://www.pasteur.fr/cgi-bin/genopole/PF8/mlstdbnet.pl?file=klebs_profiles.xml). Alleles and STs that had not been described previously were submitted to the database.

Statistical Analysis

SPSS software (version 15.0) was used for data analysis. Student *t* test and the Wilcoxon rank-sum test were used for analysis of continuous variables. Continuous variables were assessed for normality and presented as the mean ± SEM. Continuous variables were compared with the Spearman ρ correlation analysis. Categorical variables were compared using the χ^2 test or Fisher exact test. *P* values of < .05 were considered statistically significant based on the Bonferroni correction for multiple comparisons. A statistical trend was defined as a *P* value < .1 but > .05. Logistic regression was used to analyze risk factors for hvKP. All variables with a *P* value < .01 were included in the multivariate model. Forward selection with use of the likelihood-ratio test was used to select the final multivariate model for hvKP risk factors.

RESULTS

Patient Characteristics

From April 2010 to June 2012, a total of 88 patients admitted to Beijing You'an Hospital were identified as having cultures positive for *K. pneumoniae* at 1 or more sites. Sixty (68.2%) were males and 28 (31.8%) were females; the mean age was 51.4 ± 12.1 years. Isolates of hvKP and cKP were obtained from 29 (33.0%) and 59 (67.0%) of patients, respectively. Neither age nor sex was associated with positive string test (both *P* > .05). Community-acquired infections were identified in more hvKP patients (18/29 [62.1%]) than in cKP patients (8/59 [13.6%]) (*P* < .001). The strains were isolated from blood (cKP 34%, hvKP 28%), urine (cKP 10%, hvKP 0%), sputum (cKP 34%, hvKP 14%), ascites (cKP 14%, hvKP 28%), bile (cKP 7%, hvKP 14%), and abscess fluid (cKP 5%, hvKP 7%). A significantly higher number of patients with cKP in sputum (*P* = .038) was detected. Otherwise, no significant differences were detected in between the isolation of hvKP and cKP strains in any of the sample types.

Compared with patients with cKP infections, patients with hvKP infections were more likely to have liver abscesses (24% vs 3%, *P* = .005) and alcoholic hepatitis (21% vs 3%, *P* = .014) and were less likely to have human immunodeficiency virus (HIV; 0% vs 15%, *P* = .022) (Table 2). There were no differences in other comorbidities between the groups.

Rates of metastatic KP infections were compared between cKP and hvKP. Metastatic infection was more commonly seen for hvKP (18/29 [62%]) than cKP (22/59 [37%]) (*P* = .025). There were only 7 cases with *K. pneumoniae* culture–positive secondary infection sites, 5 (71.4%) of which were hvKP.

Fifteen (17.0%) patients died while in hospital; cKP and hvKP strains were isolated from 11 of 59 (18.6%) and 4 of 29 (13.8%) patients, respectively. There was no difference in mortality between these 2 groups.

Genetic Characteristics of hvKP vs cKP

Previous reports have implied that the virulence genes *rmpA* and *rmpA2* and genes for capsule K antigens are associated with hvKP [24, 31–33]. The isolated strains were tested for the presence of *rmpA* and *rmpA2* by PCR. No *rmpA2*-positive strains were identified. However, HvKP strains were strongly

Table 2. Clinical Disorders Found in the 88 *Klebsiella pneumoniae*–Infected Patients

Disorder	cKP, No.	hvKP, No.	*P* Value
Liver disorders	44/59 (75%)	28/29 (97%)	.052
Cirrhosis	25/59 (42%)	15/29 (52%)	.274
Viral hepatitis	26/59 (44%)	14/29 (48%)	.233
Hepatitis B virus	18/59 (31%)	12/29 (41%)	.219
Hepatitis C virus	8/59 (14%)	2/29 (7%)	.294
Alcoholic hepatitis	2/59 (3%)	6/29 (21%)*	.014
Cholecystitis	11/59 (19%)	2/29 (7%)	.125
Drug hepatitis	4/59 (7%)	0/29 (0%)	.139
Liver cancer	14/59 (24%)	4/29 (14%)	.213
Hepatoma	2/59 (3%)	2/29 (7%)	.637
Fatty liver	1/59 (2%)	0/29 (0%)	.345
Post–liver transplant	3/59 (5%)	2/29 (7%)	.328
Gallbladder stone	5/59 (9%)	0/29 (0%)	.108
Autoimmune hepatitis	1/59 (2%)	0/29 (0%)	.670
Liver abscess	2/59 (3%)	7/29 (24%)*	.005
Respiratory tract disorders	21/59 (36%)	8/29 (28%)	.308
Cardiovascular diseases	9/59 (15%)	4/29 (14%)	.565
Diabetes	7/59 (12%)	8/29 (28%)	.064
Tuberculosis	5/59 (9%)	0/29 (0%)	.128
Syphilis	2/59 (3%)	0/29 (0%)	.504
Pancreatitis	2/59 (3%)	0/29 (0%)	.504
Nephritis	4/59 (7%)	1/29 (3%)	.465
HIV infection	9/59 (15%)	0/29 (0%)*	.022
Nonhepatic malignancies[a]	3/59 (5%)	0/29 (0%)	.447
Nonhepatic abscess[b]	0/59 (0%)	1/29 (3%)	.330
Thyroid disorders	3/59 (5%)	2/29 (7%)	.237

Abbreviations: cKP, classic *Klebsiella pneumoniae*; HIV, human immunodeficiency virus; hvKP, hypervirulent *Klebsiella pneumoniae*.

[a] Pancreatic, duodenal ampulla, and esophageal carcinomas.

[b] Neck abscess.

* $P < .05$ compared with cKP (string test–negative strains).

associated with *rmpA* (16/29 [55%], $P < .001$), whereas only 7% (4/59) of cKP strains were *rmpA*-positive.

Genes encoding the capsule K antigens were tested in all *K. pneumoniae* strains. K1 and K2 were associated positively with hvKP ($P = .024$ and $P = .039$, respectively), and K-nontypeable was associated negatively ($P = .002$). K5, K20, K54, and K57 were not associated with hvKP ($P = .670$, .670, .308, and .252, respectively). A single strain expressing K20 was identified as a cKP strain (Table 3).

MLST Genotypic Analysis

MLST analysis revealed a total of 72 genotypes among the 88 *K. pneumoniae* strains. Among the identified STs were ST23, ST29, ST37, and ST65 (n = 4 each) and ST380, ST412, ST660, and ST860 (n = 2 each). There were 25 genotypes that did not belong to any known ST; these sequences have been submitted to the database.

Table 3. Characteristics of *Klebsiella pneumoniae* Strains

Characteristic	cKP, No.	hvKP, No.	*P* Value
K serotype			
K1	8/59 (14%)	10/29 (34%)*	.024
K2	1/59 (2%)	4/29 (14%)*	.039
K5	1/59 (2%)	0/29 (0%)	.670
K20	1/59 (2%)	0/29 (0%)	.670
K54	3/59 (5%)	3/29 (10%)	.308
K57	1/59 (2%)	2/29 (7%)	.252
K-nontypable	46/59 (78%)	12/29 (41%)*	.002
Virulence gene			
rmpA	4/59 (7%)	16/29 (55%)*	<.001
rmpA2	0/59 (0%)	0/29 (0%)	NA

Abbreviations: cKP, classic *Klebsiella pneumoniae*; hvKP, hypervirulent *Klebsiella pneumoniae*; NA, not applicable.

* $P < .05$ compared with cKP (string test–negative strains).

Risk Factors: hvKP vs cKP

Univariate analysis revealed that *rmpA* (odds ratio [OR] = 16.923), K1 (OR = 3.355), K2 (OR = 9.280), alcoholic hepatitis (OR = 7.435), liver abscess (OR = 9.068), metastatic infection (OR = 2.752), and community-acquired infection (OR = 10.432) were statistically significant risk factors for hvKP. Sputum isolation (OR = 0.312) and HIV infection (OR < 0.001) appeared to be protective factors for hvKP. Multivariate regression analysis showed that *rmpA* (OR = 17.398) and community-acquired infection (OR = 6.844) were independent risk factors for hvKP (Table 4).

Proportion of hvKP Strains Identified Among *K. pneumoniae* Isolates Tested From 2010 to 2012

The proportion of hvKP strains among all *K. pneumoniae* isolates increased over the 27 months evaluated (Figure 1). During the periods from April to December 2010, January to September 2011, and October 2011 to June 2012, 25.5%, 26.7%, and 54.5% of the total *K. pneumoniae* isolates identified, respectively, were hvKP.

Antimicrobial Resistance Among hvKP Isolates

The number of cKP strains exhibiting resistance to the tested antimicrobials was significantly higher than that of the hvKP strains, with the exception of ampicillin, piperacillin-tazobactam, chloramphenicol, and carbapenems (Table 5). All of the 5 strains determined to be resistant to carbapenems (identified as *K. pneumoniae* carbapenemase [KPC] producing) were cKP. All hvKP strains were resistant to ampicillin, which is consistent with previous studies [7, 29, 31]. However, disturbingly, resistance to all the tested antimicrobials, except carbapenems and amikacin, was observed in a proportion of hvKP strains, 17% (5/29) of which expressed ESBL (Table 5).

Table 4. Risk Factors for Hypervirulent vs Classic *Klebsiella pneumoniae*

	Univariate Analysis		Multivariate Analysis	
Risk Factor	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Virulence gene *rmpA*	16.923 (4.842–59.145)	<.001	17.398 (4.224–71.668)	<.001
Community-acquired infection	10.432 (3.623–30.033)	<.001	6.844 (1.905–24.585)	.003
Liver abscess	9.068 (1.747–47.061)	.005	6.548 (.881–48.645)	.066
Capsule antigen K2	9.280 (0.987–87.250)	.039		
Alcoholic hepatitis	7.435 (1.397–39.572)	.014		
Capsule antigen K1	3.355 (1.153–9.768)	.024		
Metastatic infection	2.752 (1.100–6.886)	.025		
Sputum isolation	0.312 (.095–1.021)	.038		
HIV infection	<0.001 (NA)	.022		

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; NA, not applicable; OR, odds ratio.

The degree of antimicrobial resistance over time was also investigated. During the periods from April to December 2010, January to September 2011, and October 2011 to June 2012, 15.4%, 16.7%, and 25.0% of hvKP isolates, respectively, were found to be resistant to at least 3 antimicrobials other than ampicillin. Furthermore, an increase in the number of ESBL-producing strains was observed during the periods from April to December 2010 (n = 1), January to September 2011 (n = 1), and October 2011 to June 2012 (n = 3). A significant reduction in the difference between the number of antibiotics for which the cKP and hvKP isolates exhibited resistance was identified during the periods from April to December 2010 (n = 13), January to September 2011 (n = 1), and October 2011 to June 2012 (n = 2) (Figure 2). Taken together, these data support the concept that antimicrobial resistance is increasing among hvKP strains. However, these data are preliminary and definitive conclusions regarding this issue require further investigation in studies conducted in larger populations.

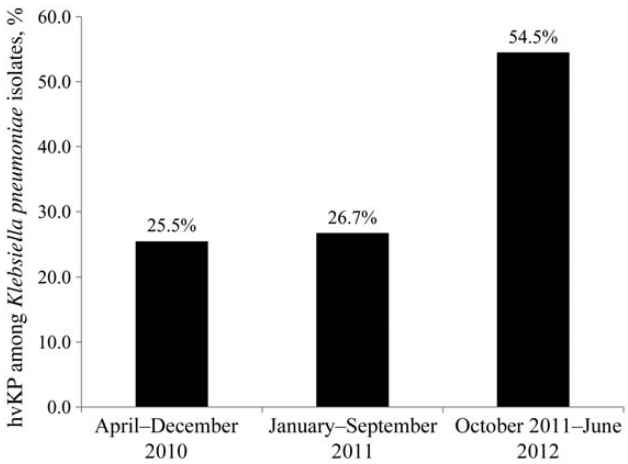

Figure 1. Percentage of hypervirulent *Klebsiella pneumoniae* (hvKP) strains among identified *K. pneumoniae* strains from April 2010 to June 2012.

DISCUSSION

This retrospective study was conducted in 88 *K. pneumoniae* culture–positive patients hospitalized during the period from

Table 5. Percentage of Antimicrobial Resistance of *Klebsiella pneumoniae* Strains

	cKP (%)	hvKP (%)	*P* Value
Ampicillin	50/59 (85%)	29/29 (100%)	.637
Ampicillin-sulbactam	34/59 (58%)	10/29 (34%)	.014*
Amoxicillin-clavulanate	25/59 (42%)	6/29 (21%)	.015*
Piperacillin	34/59 (58%)	9/29 (31%)	.006*
Piperacillin-tazobactam	16/59 (27%)	4/29 (14%)	.081
Ceftizoxime	31/59 (53%)	7/29 (24%)	.003*
Ceftazidime	18/59 (31%)	3/29 (10%)	.013*
Cefotaxime	27/59 (46%)	6/29 (21%)	.010*
Cefepime	16/59 (27%)	3/29 (10%)	.005*
Aztreonam	27/59 (46%)	6/29 (21%)	.010*
Imipenem	5/59 (9%)	0/29 (0%)	.105
Meropenem	4/59 (7%)	0/29 (0%)	.167
Gentamicin	18/59 (31%)	2/29 (7%)	.005*
Amikacin	7/59 (12%)	0/29 (0%)	.043*
Ciprofloxacin	19/59 (32%)	3/29 (10%)	.010*
Levofloxacin	16/59 (27%)	2/29 (7%)	.012*
Tetracycline	29/59 (49%)	6/29 (21%)	.003*
Chloramphenicol	27/59 (46%)	10/29 (34%)	.115
Trimethoprim-sulfamethoxazole	31/59 (53%)	4/29 (14%)	<.001*
ESBL	33/59 (56%)	5/29 (17%)	.001*

Abbreviations: cKP, classic *Klebsiella pneumoniae*; ESBL, extended spectrum β-lactamase; hvKP, hypervirulent *Klebsiella pneumoniae*.

* $P < .05$ compared with cKP (string test–negative strains).

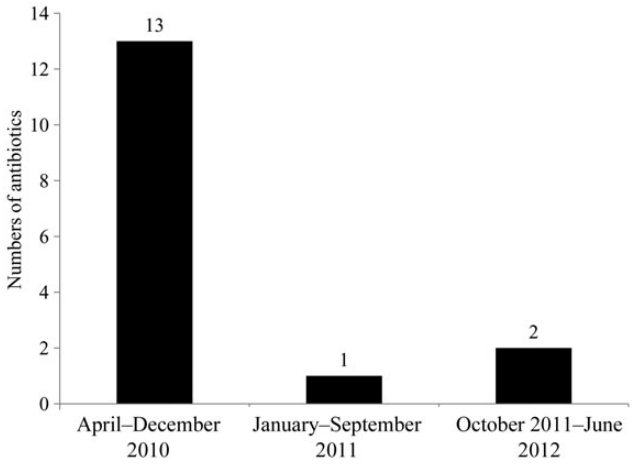

Figure 2. Hypervirulent *Klebsiella pneumoniae* (hvKP) strains have become more resistant to antibiotics if compared with classic *K. pneumoniae* (cKP). Y-axis is for the numbers of antibiotics that are less effective to cKP than to hvKP.

April 2010 to June 2012 in the Beijing You'an Hospital, which is a major medical center specializing in infectious diseases and liver disorders. An hvKP strain was defined on the basis of a positive string test. Analysis of the clinical and molecular characteristics of the isolates indicated that the proportion of hvKP strains among *K. pneumoniae* isolates appears to be increasing. Furthermore, the frequency of antimicrobial resistance among these hvKP isolates is increasing over time.

Although *K. pneumoniae* is known to be a common pathogen responsible for hospital-acquired pneumonia as well as blood and urinary tract infections [2, 29], our data demonstrated a negative association between sputum and hvKP ($P = .038$). This implies that cKP strains predominantly are associated with respiratory infections.

Previous studies have consistently confirmed that *K. pneumoniae* strains with a positive string test, namely hvKP, are more invasive and associated with serious infections in healthy young adults [8, 29, 32, 34]. In this study, 29 hvKP strains among 88 *K. pneumoniae* isolates (33.0%) were identified by positive string tests. This percentage is higher than that reported in urinary tract infection samples (27.8%) by Lin et al [31]. Furthermore, the percentage of hvKP among the *K. pneumoniae* isolates investigated increased consistently from 2010 to 2012 (Figure 1), indicating an elevated risk of hvKP infection among the general population.

Among all diseases listed, only alcoholic hepatitis and liver abscesses were associated positively with hvKP ($P = .014$ and $P = .005$, respectively). These features are consistent with previous reports, which suggest that hvKP infects immunocompetent subjects, causing liver abscesses [8, 31].

In accordance with a number of previous reports, metastatic infections were associated more positively with hvKP than with cKP ($P = .025$) [9, 11, 26, 33]. However, definitive evidence of metastatic infections is based on positive cultures from >1 infection site. These details were confirmed in the medical records of only 7 (<10%) of the patients in this study; therefore, further investigations are required to confirm this conclusion.

Genetic characterization of the hvKP and cKP strains was performed. In contrast to a previous study conducted in a Korean population, none of the strains analyzed here were *rmpA2* positive. It can be speculated that this is due to racial variation [32]. However, the hvKP strains were strongly associated with *rmpA* ($P = .025$) [31, 32] and the capsular antigens (K, K1, and K2) ($P = .024$ and .039, respectively), whereas K antigen–negative isolates were strongly associated with the nonhypervirulent cKP strains ($P = .002$; Table 3). These data are consistent with previous reports, which describe an association between hvKP and K1 and K2 expression [24, 31–33]. An association between hvKP strains and K1 and *rmpA* expression was also identified ($P = .049$), which is consistent with previous reports [35].

In this study, we compared the drug resistance characteristics of hvKP and cKP isolates. Previous studies have indicated that K1 subtype *K. pneumoniae* isolated from liver abscesses is less resistant to antibiotics if compared with other subtypes [7, 29], whereas more recent studies have indicated that such strains are strongly associated with antibiotic resistance [36, 37]. Consequently, these data are inconclusive. In the present study, hvKP strains were less resistant than cKP strains to 14 of 19 antimicrobial drugs tested. The reason for this difference remains unknown. It can be speculated that hvKP strains cannot acquire resistance-related plasmids, or that some drug-resistant genes are lost when they become hypervirulent. Further investigations are required to confirm these speculations. However, the results of our study indicate that this difference in the occurrence of antimicrobial resistance between hvKP and cKP strains is diminishing over time (Figure 2). KPC-producing bacteria are particularly difficult to control. Among the 88 strains, we found 5 (5.7%) KPC-producing strains, 1 of which was sensitive to chloramphenicol only, 1 to amoxicillin-clavulanate, and 1 to amikacin, chloramphenicol, and tetracycline. The other 2 were resistant to all the tested antimicrobials (Table 5). These results differ to some extent from previous reports, in which polymyxins, tigecycline, and aminoglycoside antibiotics were used to treat KPC infections [38].

In conclusion, the proportion of hvKP isolates among *K. pneumoniae*–positive cultures increased during the period of this study. Furthermore, although the hvKP strains were more susceptible to the antibiotics tested than the cKP strains, the degree of antimicrobial resistance among hvKP strains increased over time. These data indicate that this group of life-threatening

pathogens warrants further investigation to avert potential damage that may be caused in the future.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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