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High Prevalence of Hypervirulent *Klebsiella pneumoniae* Infection in China: Geographic Distribution, Clinical Characteristics, and Antimicrobial Resistance

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Hypervirulent *Klebsiella pneumoniae* (hvKP) is traditionally defined by hypermucoviscosity, but data based on genetic background are limited. Antimicrobial-resistant hvKP has been increasingly reported but has not yet been systematically studied. *K. pneumoniae* isolates from bloodstream infections, hospital-acquired pneumonia, and intra-abdominal infections were collected from 10 cities in China during February to July 2013. Clinical data were collected from medical records. All *K. pneumoniae* isolates were investigated by antimicrobial susceptibility testing, string test, extended-spectrum β-lactamase (ESBL) gene detection, capsular serotypes, virulence gene profiles, and multilocus sequence typing. hvKP was defined by aerobactin detection. Of 230 *K. pneumoniae* isolates, 37.8% were hvKP. The prevalence of hvKP varied among different cities, with the highest rate in Wuhan (73.9%) and the lowest in Zhejiang (8.3%). Hypermucoviscosity and the presence of K1, K2, K20, and *rmpA* genes were strongly associated with hvKP ($P < 0.001$). A significantly higher incidence of liver abscess ($P = 0.026$), sepsis ($P = 0.038$), and invasive infections ($P = 0.043$) was caused by hvKP. Cancer (odds ratio [OR], 2.285) and diabetes mellitus (OR, 2.256) appeared to be independent variables associated with hvKP infections by multivariate analysis. Importantly, 12.6% of hvKP isolates produced ESBLs, and most of them carried bla_{CTX-M} genes. Patients with neutropenia (37.5% versus 5.6%; $P = 0.020$), history of systemic steroid therapy (37.5% versus 5.6%; $P = 0.020$), and combination therapy (62.5% versus 16.7%; $P = 0.009$) were more likely to be infected with ESBL-producing hvKP. The prevalence of hvKP is high in China and has a varied geographic distribution. ESBL-producing hvKP is emerging, suggesting an urgent need to enhance clinical awareness, especially for immunocompromised patients receiving combination therapy.

Over the past few decades, increasing rates of hypervirulent *Klebsiella pneumoniae* (hvKP) infection have been reported worldwide (1–4). Such strains are notorious for their capacity to cause serious and metastatic infections in young and healthy individuals, such as pyogenic liver abscesses and endophthalmitis (5). Hypermucoviscosity is an important *in vitro* parameter for identification of hvKP (6, 7). However, several controversies have arisen regarding the association of hypermucoviscosity phenotype and virulence (8, 9). Hypermucoviscosity-negative strains are more prone to cause severe infections and have a higher mortality rate in diabetic mice than hypermucoviscous *K. pneumoniae* (8). Our previous study also demonstrated that one of the five hypermucoviscous *K. pneumoniae* isolates showed high virulence in both *in vitro* and *in vivo* assays (9). Therefore, it is apparent that hvKP cannot be defined by string test alone (10). Aerobactin accounts for increased siderophore production and is a major virulence determinant and new defining trait for hvKP based on genetic background (11). Some advances have been made recently in the epidemiology of hvKP isolates in China (6, 7, 12), although most of the studies defined hvKP by hypermucoviscosity phenotype rather than genotype, which may have led to biased results. In the present study, hvKP strains were defined on the basis of aerobactin detection.

Although hypervirulent and antimicrobial-resistant populations of *K. pneumoniae* were largely nonoverlapping (13), some isolates with combined virulence and resistance were detected (9, 12, 14–16). More importantly, hypervirulent carbapenem-resistant *K. pneumoniae* isolates have emerged in clinical settings (9, 12, 16). The confluence of multidrug resistance and enhanced virulence has the potential to be the next clinical crisis (5). So far, research on antimicrobial-resistant hvKP strains has been limited and mainly based on case reports (9, 12). A systematic study on the molecular and clinical characteristics of antimicrobial-resistant hvKP infections is still lacking.

To reduce the bias caused by subjective identification methods and to investigate the recent pattern of hvKP in China, we conducted a nationwide study and systematically analyzed the clinical and molecular characteristics and antimicrobial resistance of hvKP isolates.

MATERIALS AND METHODS

Clinical *K. pneumoniae* isolates. A total of 230 consecutive cases of *K. pneumoniae* infection were collected from a national prospective surveillance program (February to July 2013 from the Chinese Antimicrobial Resistance Surveillance of Nosocomial Infections, including Beijing [$n = 45$], Tianjin [$n = 22$], Shenyang [$n = 22$], Jinan [$n = 14$], Xi'an [$n = 15$],

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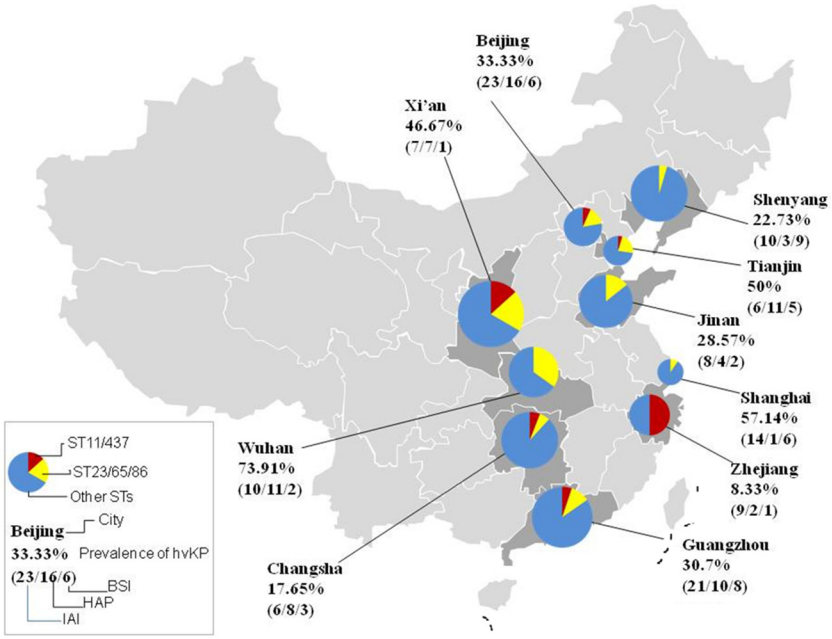

FIG 1 Geographic distribution of hvKP infection cases in China. The prevalence of hvKP infections ranged from 8.33% to 73.91%, with a varied geographic distribution. Virulence-associated ST types (including ST23, ST65, and ST86) and resistance-associated ST types (including ST11 and ST437) demonstrated different distributions among *K. pneumoniae* isolates from different cities.

Wuhan [$n = 23$], Changsha [$n = 17$], Zhejiang [$n = 12$], Shanghai [$n = 21$], and Guangzhou [$n = 39$]). Each center was asked to enroll the cases and collect strains isolated from hospital-acquired pneumonia, intra-abdominal infections, and bloodstream infections. Duplicate isolates from the same individual were excluded. All isolates were stored at −80°C until use. Confirmation of the *K. pneumoniae* isolates was performed by 16S rRNA sequencing.

Clinical data collection. Clinical data and patient information were obtained from medical records and included demographic characteristics, underlying medical conditions, clinical presentations, antimicrobial therapy administration, and outcomes. The study was approved by the Research Ethics Board at Peking University People's Hospital (Beijing, China).

Antimicrobial susceptibility testing and phenotypic confirmation of ESBL. Susceptibility testing was determined by the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (17). The following antimicrobial agents were tested: cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, imipenem, meropenem, amikacin, ciprofloxacin, minocycline, and tigecycline. The results were interpreted according to CLSI breakpoints (18). The interpretive criteria for tigecycline were based on the Food and Drug Administration (FDA) breakpoints (≤2 mg/liter was susceptible, 4 mg/liter was intermediate, and ≥8 mg/liter was resistant). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as controls for antimicrobial susceptibility testing. Extended-spectrum β-lactamase (ESBL) production was confirmed by agar dilution test using cefotaxime and ceftazidime along with clavulanate, in accordance with CLSI guidelines (17).

Detection of virulence-associated features and *cps* genotyping. Hypermucoviscosity was identified by the string test as previously described (5). The formation of a viscous string of >5 mm was considered positive. We conducted *cps* genotyping of K serotype-specific alleles for K1, K2, K5, K20, K54, and K57 by PCR as previously described (19). Virulence-associated factors, such as the exopolysaccharide synthesis regulator gene (*rmpA*) and an iron uptake system (aerobactin), were determined by PCR (20, 21). Previously reported primers used for PCR were the following: aerobactin forward, 5′-GCATAGGCGGATACGAACAT-3′; aerobactin reverse, 5′-CACAGGGCAATTGCTTACCT-3′ (21). The reaction mixture was kept at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min, 72°C for 1 min, and 72°C for 10 min. The PCR products were visualized and analyzed by agarose gel electrophoresis and sequencing.

β-Lactamase gene identification. PCR was used to determine the prevalence of β-lactamase genes, including CTX-M, SHV, and TEM β-lactamase, as previously described (22). The $bla_{\text{CTX-M}}$ gene was first identified with the PAN-CTX-M primer pair as previously described (forward, 5′-TTTGCGATGTGCAGTACCAGTAA-3′; reverse, 5′-CGATATCGTTGGTGGTGCCATA-3′), followed by the group-specific primers (22). The products were submitted for sequencing.

MLST. Multilocus sequence typing (MLST) was performed as described on the Pasteur Institute MLST website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html), including DNA sequencing analysis of the seven housekeeping genes. New alleles and STs were submitted to the MLST website and were approved.

Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics version 20.0 and Graphpad Prism version 5. We used the χ^2 or Fisher's exact test for categorical variables. $P < 0.05$ was considered statistically significant. Logistic regression was used to identify variables associated with hvKP and ESBL-producing hvKP infections. All variables with P values of <0.1 were included in the multivariate model.

RESULTS

Virulence-associated features of *K. pneumoniae* isolates. Aerobactin-positive strains were designated hvKP, which was determined by PCR. Eighty-seven of 230 (37.8%) isolates were hvKP. The prevalence of hvKP infection ranged from 8.33% to 73.9% in China, with the highest rate in Wuhan and the lowest rate in Zhejiang. The geographic distribution in China is shown in Fig. 1. Among all these strains, 74.7% (65/87) were hypermucoviscous, while six hypermucoviscous isolates were detected in the aerobactin-negative group. Hypermucoviscosity was strongly associated

TABLE 1 Microbiological and clinical characteristics of hvKP isolates

Characteristic	Value (no. [%]; no. positive/total no.) for[a]: hvKP (n = 87)	cKP (n = 143)	P value
Microbiological features			
Hypermucoviscosity	65 (74.7)	6 (4.2)	**<0.001**
K serotype			
K1	29 (33.3)	8 (5.6)	**<0.001**
K2	15 (17.2)	4 (2.8)	**<0.001**
K5	2 (2.3)	0	0.142
K20	6 (6.9)	0	**0.003**
K57	4 (4.6)	3 (2.1)	0.431
rmpA	85 (97.7)	5 (3.5)	**<0.001**
Antimicrobial susceptibility			
Cefoxitin	78 (89.7)	103 (72.0)	**0.002**
Cefotaxime	75 (86.2)	70 (49.0)	**<0.001**
Ceftazidime	78 (89.7)	88 (61.5)	**<0.001**
Cefepime	82 (94.3)	91 (63.6)	**<0.001**
Piperacillin-tazobactam	86 (98.9)	111 (77.6)	**<0.001**
Imipenem	85 (97.7)	129 (90.2)	**0.033**
Meropenem	86 (98.9)	129 (90.2)	**0.011**
Amikacin	81 (93.1)	130 (90.9)	0.629
Ciprofloxacin	83 (95.4)	89 (62.2)	**<0.001**
ESBL production	11 (12.6)	61 (42.7)	**<0.001**
Clinical characteristics			
Male sex	62 (77.5; 62/80)	79 (67.5; 79/117)	0.149
Age (yr) (means ± SD)	55.9 ± 1.5	51.8 ± 1.7	0.230
Coexisting conditions			
Pulmonary disease	12 (15; 12/80)	11 (9.4; 11/117)	0.263
Cancer	**27 (33.8; 27/80)**	**22 (18.8; 22/117)**	**0.020**
Liver disease	8 (10; 8/80)	14 (12.0; 14/117)	0.819
Neurological disease	10 (12.5; 10/80)	21 (17.9; 21/117)	0.327
Diabetes mellitus	**22 (27.5; 22/80)**	**17 (14.7; 17/116)**	**0.030**
Neutropenia[b]	7 (8.8; 8/80)	13 (11.1; 13/117)	0.640
Splenectomy	0	2 (1.1; 2/117)	0.515
Surgery within 1 mo	22 (27.5; 22/80)	39 (33.3; 39/117)	0.434
Systemic steroid therapy	7 (8.8; 7/80)	16 (13.7; 16/117)	0.369
Use of immunosuppressant	7 (8.8; 7/80)	10 (8.6; 10/116)	1.000
Hospitalization within last 90 days	**20 (25; 20/80)**	**53 (45.3; 53/117)**	**0.004**
Transferred from another hospital	12 (15; 12/80)	17 (14.5; 17/117)	1.000
Receipt of antibiotics 30 days before infection	25 (31.3; 25/80)	52 (45.2; 52/115)	0.054
Use of invasive devices			
Artery cannula	4 (8.5; 4/47)	3 (3.6; 3/83)	0.253
Presence of central venous catheters	9 (19.1; 9/47)	12 (14.6; 12/82)	0.621
Tracheal cannula	9 (19.1; 9/47)	11 (13.3; 11/83)	0.450
Tracheotomy	3 (6.4; 3/47)	4 (4.8; 4/83)	0.703
Presence of Foley catheter	15 (31.9; 15/47)	23 (27.7; 23/83)	0.689
Clinical presentation			
Bacteremia	24 (30; 24/80)	35 (30.2; 35/116)	1.000
Abdominal infection	16 (20; 16/80)	27 (23.3; 27/116)	0.604
Hospital-acquired pneumonia	36 (45; 36/80)	43 (37.1; 43/116)	0.301
Liver abscess	**10 (11.5; 10/87)**	**5 (3.5; 5/143)**	**0.026**
Septic shock	6 (6.9; 6/87)	13 (9.1; 13/143)	0.629
Sepsis	**43 (49.4; 43/87)**	**50 (35.0; 50/143)**	**0.038**
Invasive infection[c]	**11 (12.6; 11/87)**	**7 (4.9; 7/143)**	**0.043**
Infection occurred in ICU	**11 (13.8; 11/80)**	**31 (26.9; 31/115)**	**0.033**
Surgical treatment	13 (18.3; 13/71)	24 (21.6; 24/111)	0.706
Outcomes			
Clinical improvement without modification of initial treatment	50 (70.4; 50/71)	86 (76.1; 86/113)	0.395
Change of initial antibiotics due to clinical worsening	17 (23.9; 17/71)	17 (15.0; 17/113)	0.172
Relapse	2 (2.8; 2/71)	1 (0.9; 1/113)	0.560
In-hospital mortality	2 (2.8; 2/71)	9 (8.0; 9/113)	0.208
Bacterial clearance after 72-h treatment	64 (83.1; 64/77)	95 (81.9; 95/116)	1.000
Persistent infection after 72-h treatment	7 (9.1; 7/77)	18 (15.5; 18/116)	0.274

[a] *K. pneumoniae* strains carrying aerobactin were designated hvKP, while cKP was defined by aerobactin negativity. Values in boldface are statistically significant.
[b] Absolute neutrophil count of <500 cells/μl.
[c] Invasive infections include liver abscess and meningitis.

TABLE 2 Regression analysis of variables associated with hvKP infections

Variable	Analysis result			
	Univariate		Multivariate	
	OR (95% CI[a])	*P* value	OR (95% CI)	*P* value
Cancer	1.963 (1.012–3.806)	0.046	2.285 (1.102–4.738)	0.026
Diabetes mellitus	2.086 (1.034–4.210)	0.040	2.256 (1.050–4.846)	0.037
Hospitalization within last 90 days	0.403 (0.216–0.751)	0.004	0.345 (0.171–0.695)	0.003
Receipt of antibiotics 30 days before infection	0.551 (0.303–1.002)	0.051	0.549 (0.279–1.078)	0.081
Infection occurred in ICU	0.432 (0.202–0.922)	0.030	0.412 (0.183–0.928)	0.032

[a] CI, confidence interval.

with hvKP (74.7% versus 4.2%; $P < 0.001$). A total of 71 (71/230; 30.9%) isolates were positive for K1, K2, K5, K20, and K57 serotypes, and the K54 serotype was not found in this study. K1, K2, and K20 were more likely to be identified in hvKP isolates (Table 1). The *rmpA* gene, a regulator of exopolysaccharide synthesis, had a strong relation to aerobactin (97.7% versus 3.5%; $P < 0.001$) (Table 1).

Clinical characteristics of hvKP infection. Table 1 shows the baseline characteristics of patients with hvKP infections. Compared with the classic *K. pneumoniae* (cKP) group, more patients with hvKP infections had cancer (33.8% versus 18.8%; $P = 0.02$) and diabetes mellitus (27.5% versus 14.7%; $P = 0.03$) as their underlying diseases. A significantly higher number of patients presenting with liver abscess (11.5% versus 3.5%; $P = 0.026$), sepsis (49.4% versus 35%; $P = 0.038$), and invasive infections (12.6% versus 4.9%; $P = 0.043$) were infected by hvKP. It was also noted that patients with a history of hospitalization within the previous 90 days (25% versus 45.3%; $P = 0.004$) and intensive care unit (ICU) stay (13.8% versus 26.9%; $P = 0.033$) were more likely to have a cKP infection.

Variables associated with hvKP infection. Univariate regression analysis revealed that cancer (odds ratio [OR], 1.963) and diabetes mellitus (OR, 2.086) were statistically significant variables associated with hvKP infections (Table 2). In addition, cancer (OR, 2.285) and diabetes mellitus (OR, 2.256) were independent variables associated with hvKP infections, while hospitalization within the previous 90 days (OR, 0.345) and infection in the ICU (OR, 0.412) were independent protective factors for hvKP by multivariate analysis.

Antimicrobial susceptibility and prevalence of ESBL genes among *K. pneumoniae* isolates. The antimicrobial resistance rate of cKP was significantly higher than that of hvKP strains (Table 1). ESBL was identified in 72 isolates (72/230; 31.3%) and was less common in the hvKP group (12.6% versus 42.7%; $P < 0.001$). Among 87 hvKP isolates, 12.6% (11/87) produced ESBLs, and one of them exhibited resistance to carbapenem.

The designation of the 11 ESBL-producing hvKP strains was by patient number. The demographics, virulence-associated features, and clinical characteristics of the 11 patients are summarized in Table 3. A total of six patients developed septicemia or bacteremia, and most of the strains were isolated from blood samples. All of these isolates harbored CTX-M, SHV, and TEM β-lactamase, and most of them had one or two genes that encoded ESBLs.

Patient characteristics and variables associated with hvKP isolates with and without ESBL production. Patients with neutropenia (37.5% versus 5.6%; $P = 0.020$), history of systemic steroid therapy (37.5% versus 5.6%; $P = 0.020$), and combination therapy (use of multiple antimicrobials) (62.5% versus 16.7%; $P = 0.009$) were more likely to be infected with ESBL-producing hvKP.

MLST genotyping. MLST identified 100 STs among 230 *K. pneumoniae* isolates. The most prevalent ST in *K. pneumoniae* isolates was ST23 (10.9%), followed by ST11 (5.2%). ST23, ST268, ST375, ST412, and ST660 were strongly associated with hvKP, while ST11, ST15, and ST37 were more common in the cKP group. ST17, ST23, ST35, ST65, ST268, ST367, ST420, and ST1658 were identified in hvKP isolates with ESBL production. In addition, STs showed different distributions among different cities (Fig. 1). In Wuhan, ST23 was the most dominant, with a prevalence of 21.7%, while ST11 was the most common in Zhejiang, with a prevalence of 25%.

DISCUSSION

To our knowledge, this is the first systematic multicenter study on hvKP in China, and it provides the most comprehensive understanding of hvKP defined by genetic background. According to previous designation criteria for hvKP, 30.9% of *K. pneumoniae* isolates were identified as string test positive in our study. The proportion was lower than that reported in previous studies conducted in a single center in China, with a prevalence of 33% in Beijing (7). However, it remains unclear whether all hvKP isolates had this phenotype. Serotypes, STs, and other genomic background data should be taken into consideration when defining hvKP. Therefore, the prevalence of hvKP might be underestimated due to the lack of objective diagnostic methods.

Aerobactin-positive isolates are associated with more serious infections (11, 23). Recently, aerobactin was considered a defining genetic trait and potential antivirulence target for hvKP (24). In our study, 37.8% of *K. pneumoniae* isolates were defined as hvKP by aerobactin. The prevalence showed a varied geographic distribution, with the highest rate in Wuhan and the lowest in Zhejiang. In Wuhan, 34.8% of the isolates belonged to ST23, ST65, and ST86, which are closely related to high virulence (9, 25). However, resistance-associated STs, including ST11 and ST437, were prevalent in Zhejiang, with a prevalence of 50%, and no isolate belonged to ST23 (26).

The prevalence of resistance in hvKP was still lower than that in cKP in the present study, particularly with regard to ESBLs. Contrary to the previous view that the prevalence of antimicrobial-resistant hvKP is low, a high percentage (12.6%) of ESBL production was found among hvKP in our study. Most of them carried $bla_{\text{CTX-M}}$ genes, indicating the compatibility of plasmids containing $bla_{\text{CTX-M}}$ genes with hvKP strains. Even more disturbing is that

CTX-M-type ESBL is the most common genotype in China (27). The widespread dissemination of the CTX-M plasmid into hypervirulent variants from different STs is a frightening prospect in the future. Such isolates could produce various broad β-lactamases and ESBLs with a high level of resistance to multiple antimicrobials. Recently, carbapenemase-producing hvKP isolates have been identified in clinical settings, causing fatal infections (12, 16). *K. pneumoniae* carbapenemase-producing plasmid could be transferred into hvKP strains successfully (28). The situation calls for an immediate response to multidrug-resistant hvKP infections.

hvKP is clinically important because it can cause severe infections, and it should be given priority in clinical settings. Our results also demonstrate that hvKP is more likely to cause liver abscess, sepsis, and invasive infections than cKP, especially for patients in non-ICU departments and with no hospitalization prior to infection. The risk of ESBL-producing hvKP infection increased with neutropenia, systemic steroid therapy, and combination therapy. Therefore, compromised immunity is an important factor for antimicrobial-resistant hvKP infections.

There were some limitations to our study. Genotypic identification did not necessarily imply the expression of aerobactin; thus, further studies are needed to better define the hvKP strains. In addition, *K. pneumoniae* isolates were collected only in China, and whole-genome framework was not investigated. Therefore, little is known about the lineage diversification in our isolates, and it is unclear that our results can be generalized to hvKP outside China (29, 30). A study that includes more isolates from other regions, especially for antimicrobial-resistant strains, is needed.

In conclusion, the prevalence of hvKP was high in China, with a varied geographic distribution. Such strains were more likely to cause serious infections, such as liver abscess and sepsis in clinical settings. Our study highlights the urgent need to enhance clinical awareness and management of hvKP infections. Given the high prevalence of CTX-M ESBLs in China, the propensity for the acquisition of resistance genes among hvKP isolates from different STs, especially for immunocompromised patients with multiple antimicrobials treatment, should increase our concern.

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REFERENCES

1. **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*

TABLE 3 Clinical and microbiological characteristics of ESBL-producing hvKP isolates

Clinical characteristic	Value for patient isolate:										
	1	2	3	4	5	6	7	8	9	10	11
Age (yr)	76	76	49	43	60	53	20	53	62	48	1 mo
Gender	Male	Male	Male	Female	Female	Male	Female	Male	Female	Female	Male
City	Shanghai	Guangzhou	Guangzhou	Shenyang	Shenyang	Shenyang	Tianjin	Xi'an	Beijing	Jinan	Wuhan
Specimen type	Blood	Sputum	Sputum	Bile	Bile	Blood	Blood	Blood	Blood	Blood	Blood
Date of specimen collection (yr/mo/day)	2013/4/13	2013/4/2	2013/3/6	2013/1/31	2013/2/18	2013/1/29	2013/3/17	2013/2/25	2013/7/23	2013/7/26	2013/3/26
Infection type	Septicemia	Pneumonia	Pneumonia	Liver abscess	Abdominal infection	Liver abscess	Septicemia	Bacteremia	Septicemia	Bacteremia	Septicemia
Clinical outcome	Survived	Survived	Survived	Unknown	Unknown	Unknown	Survived	Survived	Survived	Survived	Survived
β-Lactamase(s)	SHV-75, CTX-M-55	SHV-11, TEM-1, CTX-M-55	SHV-11, TEM-1, CTX-M-55	SHV-11, TEM-1, CTX-M-like	SHV-11, TEM-1, CTX-M-like	SHV-11, TEM-1, CTX-M-like	SHV-148	CTX-M-14	SHV-11, CTX-M-14	CTX-M-14	SHV-11, TEM-53
Virulence-associated features											
String test	+	−	+	+	+	+	+	+	+	−	+
cps genotyping	K20	K1	K1	K1	K20	K20	K2	K1	K nontypeable	K nontypeable	K2
rmpA gene	+	+	+	+	+	+	+	+	+	+	+
MLST	ST420	ST23	ST23	ST268	ST268	ST268	ST1658	ST367	ST17	ST35	ST65

isolates. Infection **41:**559–563. http://dx.doi.org/10.1007/s15010-012-0374-y.
2. **Pomakova DK, Hsiao CB, Beanan JM, Olson R, MacDonald U, Keynan Y, Russo TA.** 2012. Clinical and phenotypic differences between classic and hypervirulent *Klebsiella pneumonia*: an emerging and under-recognized pathogenic variant. Eur J Clin Microbiol Infect Dis **31:**981–989. http://dx.doi.org/10.1007/s10096-011-1396-6.
3. **Decré D, Verdet C, Emirian A, Le Gourrierec T, Petit JC, Offenstadt G, Maury E, Brisse S, Arlet G.** 2011. Emerging severe and fatal infections due to *Klebsiella pneumoniae* in two university hospitals in France. J Clin Microbiol **49:**3012–3014. http://dx.doi.org/10.1128/JCM.00676-11.
4. **Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH, Ip M.** 2011. Molecular typing and virulence analysis of serotype K1 *Klebsiella pneumoniae* strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. J Clin Microbiol **49:**3761–3765. http://dx.doi.org/10.1128/JCM.00977-11.
5. **Shon AS, Bajwa RP, Russo TA.** 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence **4:**107–118. http://dx.doi.org/10.4161/viru.22718.
6. **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. http://dx.doi.org/10.1128/AAC.02523-14.
7. **Li W, Sun GZ, Yu YH, Li N, Chen M, Jin R, Jiao Y, Wu H.** 2014. Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in China. Clin Infect Dis **58:**225–232. http://dx.doi.org/10.1093/cid/cit675.
8. **Lin YC, Lu MC, Tang HL, Liu HC, Chen CH, Liu KS, Lin C, Chiou CS, Chiang MK, Chen CM, Lai YC.** 2011. Assessment of hypermucoviscosity as a virulence factor for experimental *Klebsiella pneumoniae* infections: comparative virulence analysis with hypermucoviscosity-negative strain. BMC Microbiol **11:**50. http://dx.doi.org/10.1186/1471-2180-11-50.
9. **Zhang YW, Zeng J, Liu WE, Zhao F, Hu Z, Zhao C, Wang Q, Wang X, Chen H, Li H, Zhang F, Li S, Cao B, Wang H.** 2015. Emergence of a hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolate from clinical infections in China. J Infect **71:**553–560. http://dx.doi.org/10.1016/j.jinf.2015.07.010.
10. **Alcántar-Curiel MD, Girón JA.** 2015. *Klebsiella pneumoniae* and the pyogenic liver abscess: implications and association of the presence of *rmpA* genes and expression of hypermucoviscosity. Virulence **6:**407–409. http://dx.doi.org/10.1080/21505594.2015.1030101.
11. **Russo TA, Olson R, Macdonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM.** 2014. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. Infect Immun **82:**2356–2367. http://dx.doi.org/10.1128/IAI.01667-13.
12. **Zhang R, Lin D, Chan EW, Gu D, Chen GX, Chen S.** 2016. Emergence of carbapenem-resistant serotype K1 hypervirulent Klebsiella pneumoniae (hvKP) strains in China. Antimicrob Agents Chemother **60:**709–711. http://dx.doi.org/10.1128/AAC.02173-15.
13. **Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decré D, Brisse S.** 2014. Genomic definition of hypervirulent and multidrug-resistant Klebsiella pneumoniae clonal groups. Emerg Infect Dis **20:**1812–1820. http://dx.doi.org/10.3201/eid2011.140206.
14. **Su SC, Siu LK, Ma L, Yeh KM, Fung CP, Lin JC, Chang FY.** 2008. Community-acquired liver abscess caused by serotype K1 *Klebsiella pneumoniae* with CTX-M-15-type extended-spectrum beta-lactamase. Antimicrob Agents Chemother **52:**804–805. http://dx.doi.org/10.1128/AAC.01269-07.
15. **Cheng NC, Yu YC, Tai HC, Hsueh PR, Chang SC, Lai SY, Yi WC, Fang CT.** 2012. Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial *Klebsiella pneumoniae* necrotizing fasciitis. Clin Infect Dis **55:**930–939. http://dx.doi.org/10.1093/cid/cis565.
16. **Liu Y, Li XY, Wan LG, Jiang WY, Yang JH, Li FQ.** 2014. Virulence and transferability of resistance determinants in a novel *Klebsiella pneumoniae* sequence type 1137 in China. Microb Drug Resist **20:**150–155. http://dx.doi.org/10.1089/mdr.2013.0107.
17. **Clinical and Laboratory Standards Institute.** 2015. M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 10th ed. CLSI, Wayne, PA.
18. **Clinical and Laboratory Standards Institute.** 2016. Performance standards for antimicrobial susceptibility testing, 26th ed. Approved standard M100S. CLSI, Wayne, PA.
19. **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. http://dx.doi.org/10.1086/519262.
20. **Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, Kassis-Chikhani N, Arlet G, Decré D.** 2014. Multiplex PCR for the detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. J Clin Microbiol **52:**4377–4380. http://dx.doi.org/10.1128/JCM.02316-14.
21. **Lin JC, Koh TH, Lee N, Fung CP, Chang FY, Tsai YK, Ip M, Siu LK.** 2014. Genotypes and virulence in serotype K2 *Klebsiella pneumoniae* from liver abscess and non-infectious carriers in Hong Kong, Singapore and Taiwan. Gut Pathog **6:**21. http://dx.doi.org/10.1186/1757-4749-6-21.
22. **Lewis JS, II, Herrera M, Wickes B, Patterson JE, Jorgensen JH.** 2007. First report of the emergence of CTX-M-type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother **51:**4015–4021. http://dx.doi.org/10.1128/AAC.00576-07.
23. **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.
24. **Russo TA, Olson R, MacDonald U, Beanan J, Davidson BA.** 2015. Aerobactin, but not yersiniabactin, salmochelin, or enterobactin, enables the growth/survival of hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* ex vivo and in vivo. Infect Immun **83:**3325–3333. http://dx.doi.org/10.1128/IAI.00430-15.
25. **Struve C, Roe CC, Stegger M, Stahlhut SG, Hansen DS, Engelthaler DM, Andersen PS, Driebe EM, Keim P, Krogfelt KA.** 2015. Mapping the evolution of hypervirulent *Klebsiella pneumoniae*. mBio **6:**e00630.
26. **Andrade LN, Curiao T, Ferreira JC, Longo JM, Clímaco EC, Martinez R, Bellissimo-Rodrigues F, Basile-Filho A, Evaristo MA, Del Peloso PF, Ribeiro VB, Barth AL, Paula MC, Baquero F, Cantón R, Darini AL, Coque TM.** 2011. Dissemination of blaKPC-2 by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil. Antimicrob Agents Chemother **55:**3579–3583. http://dx.doi.org/10.1128/AAC.01783-10.
27. **Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, Ma Y.** 2007. Resistance of strains producing extended-spectrum beta-lactamases and genotype distribution in China. J Infect **54:**53–57. http://dx.doi.org/10.1016/j.jinf.2006.01.014.
28. **Siu LK, Huang DB, Chiang T.** 2014. Plasmid transferability of KPC into a virulent K2 serotype *Klebsiella pneumoniae*. BMC Infect Dis **14:**176. http://dx.doi.org/10.1186/1471-2334-14-176.
29. **Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR.** 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci U S A **112:**E3574–E3581. http://dx.doi.org/10.1073/pnas.1501049112.
30. **Hennequin C, Robin F.** 2016. Correlation between antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Eur J Clin Microbiol Infect Dis **35:**333–341. http://dx.doi.org/10.1007/s10096-015-2559-7.