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ORIGINAL ARTICLE

Hypervirulent *Klebsiella pneumoniae* induced ventilator-associated pneumonia in mechanically ventilated patients in China

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Abstract The purpose of this study was to investigate the clinical characteristics of hypervirulent *K. pneumoniae* (hvKP) induced ventilator-associated pneumonia (VAP) and the microbiological characteristics and epidemiology of the hvKP strains. A retrospective study of 49 mechanically ventilated patients with *K. pneumoniae* induced VAP was conducted at a university hospital in China from January 2014 to December 2014. Clinical characteristics and *K. pneumoniae* antimicrobial susceptibility and biofilm formation were analyzed. Genes of capsular serotypes K1, K2, K5, K20, K54 and K57 and virulence factors plasmid *rmpA*(p-*rmpA*), *iroB*, *iucA*, *mrkD*, *entB*, *iutA*, *ybtS*, *kfu* and *allS* were also evaluated. Multilocus sequence typing (MLST) and random amplified polymorphic DNA (RAPD) analyses were used to study the clonal relationship of the *K. pneumoniae* strains. Strains possessed p-*rmpA* and *iroB* and *iucA* were defined as hvKP. Of 49 patients, 14 patients (28.6 %) were infected by hvKP. Antimicrobial resistant rate was significantly higher in cKP than that in hvKP. One ST29 K54 extended-spectrum-beta-lactamase (ESBL) producing hvKP strain was detected. The prevalence of K1 and K2 in hvKP was 42.9 % and 21.4 %, respectively. The incidences of K1, K2, K20, p-*rmpA*, *iroB*, *iucA*, *iutA*, *Kfu* and *alls* were significantly higher in hvKP than those in cKP. ST23 was dominant among hvKP strains, and all the ST23 strains had identical RAPD pattern. hvKP has become a common pathogen of VAP in mechanically ventilated patients in China. Clinicians should increase awareness of hvKP induced VAP and enhance epidemiologic surveillance.

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Introduction

Klebsiella pneumoniae is one of the most common gram-negative bacteria causing hospital-acquired pneumonia (HAP) [1]. *K. pneumoniae* has been divided into two distinct groups. One is termed "classic" *K. pneumoniae* (cKP) which was considered the pathogen causing most *K. pneumoniae* induced hospital-acquired infection [2]. cKP has been notorious for acquiring antimicrobial resistance. The other is a new variant of *K. pneumoniae* and termed hypervirulent *K. pneumoniae* (hvKP). hvKP has appeared in the past three decades as the cause of community-acquired liver abscess in Taiwan, South Korea, and other Asian countries [3–5]. Recently, reports on hvKP-induced infection from countries outside Asia have increased [6–8]. hvKP is characterized by causing severe invasive community-acquired infection with metastatic spread in immunocompetent individuals [8]. A specific marker with high sensitivity for detecting hvKP strains is now lacking. A hypermucoviscous phenotype which can be determined by "string test" has been used to differentiate hvKP from cKP in previous studies [9–11]; however, it is unclear whether all hvKP strains are hypermucoviscous [8]. Possessing a large virulent plasmid with virulence factors encoded genes, including regulator of the mucoid phenotype gene (*rmpA*) and iron-acquisition systems aerobactin biosynthetic gene (*iucABCD*) and slmochelin biosynthetic gene (*iroBCD*), has been reported being associated with *K. pneumoniae* virulence [12–15]. In contrast to cKP, the majority of hvKP strains are susceptible to most antimicrobial with the exception of ampicillin. However, extended-spectrum-beta-lactamase (ESBL)-producing and

carbapenem-resistant hvKP strains have already occurred [10–14]. The occurrence of antimicrobial-resistant hvKP isolates has generated the concern that hvKP combining hypervirulence with antimicrobial resistance will become the next "superbug" [16].

Research on hvKP has identified several virulence factors, including capsular serotypes K1 and K2 [17, 18], mucoviscosity associated gene A (*magA*) [9], regulator of mucoid phenotype A (*rmpA*) [19], and iron acquisition factors [20]. However, most studies have focused on community-acquired infection caused by hvKP. Study on hvKP induced hospital-acquired infection has been limited. HAP is the second most common hospital-acquired infection after urinary tract infection and is the leading cause of mortality due to hospital-acquired infection [21]. Ventilator-associated pneumonia(VAP) is an important form of HAP [22], and the mortality rate of VAP was reported ranging from 30 to 70 % [23]. Yet, little is known about the clinical and microbiological characteristics of VAP caused by hvKP. The purpose of this study was to investigate the clinical characteristics of hvKP induced VAP in mechanically ventilated patients and the microbiological characteristics and epidemiology of the hvKP strains, using the cKP induced VAP as reference.

Material and methods

Patient information

A retrospective study was conducted at Xiangya Hospital (Hunan, China) from January 2014 to December 2014. Xiangya hospital is a 3500-bed tertiary university hospital with an annual admission of more than 100,000 inpatients. *K. pneumoniae* strains were isolated from lower respiratory tract secretion of hospitalized patients and stored at –80 °C before use. One *K. pneumoniae* strain per patient was collected and the initial strain isolated was used for collection. Medical records of patients from whom the collected strains were isolated were reviewed. Cases of VAP that met the following criteria were included: (1) receiving mechanical ventilation and presence of VAP. VAP was defined as pneumonia occurring 48 h or more after endotracheal intubation [16]. Patients had a new or progressive radiographic infiltrate, along with at least two of the following clinical findings: fever greater than 38 °C, a leukocyte count of more than 10,000/μL or less than 4000/μL, and purulent sputum [22]. (2) They must have *K. pneumoniae* isolated from lower respiratory tract. Patients without these conditions were excluded.

The following data were collected: sex, age, underlying diseases (diabetes, hypertension, chronic pulmonary disease, neurologic disease, chronic liver disease), Acute Physiology and Chronic Health Evaluation II (APACHE II) score [24], empirical antibiotics received and surgery performed within 7 days prior to positive culture of *K. pneumoniae*, days of hospitalization and days on mechanical ventilation prior to *K. pneumoniae* cultured, route of ventilation (oroendotracheal, tracheostomy), concurrent *K. pneumoniae* bacteremia, metastatic *K. pneumoniae* infection, days of hospital stay and in-hospital mortality. Neurologic diseases encompassed stroke and head injury. Chronic lung diseases included chronic obstructive pulmonary diseases, bronchiectasis, and old pulmonary tuberculosis. Chest radiography reports and laboratory data (white blood cell [WBC] count, albumin, C-reactive protein [CRP], procalcitonin [PCT], total bilirubin) were also obtained. Laboratory data were taken on the day of the first *K. pneumoniae* strain isolated from the lower respiratory tract. This study was approved by the Xiangya Hospital Ethics Committee.

Microbiological characteristics

String test was performed by using a standard bacteriologic loop to stretch a mucoviscous string from the colony. Strains with a mucoviscous string >5 mm were defined as positive string test [9]. All *K. pneumoniae* strains underwent antimicrobial susceptibility testing to ampicillin, cefazolin, cefotan, ceftriaxone, cefepime, ceftazidime, ampicillin-sulbactam, piperacillin-tazobactam, aztreonam, amikacin, tobramycin, gentamicin, ertapenem, imipenem, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole by bioMerieux VITEK-2 (bioMerieux). The minimum inhibitory concentrations (MICs) of antimicrobial agents were interpreted according to the performance standards for antimicrobial susceptibility testing issued by the Clinical and Laboratory Standards Institute (CLSI) in 2013 [25]. ESBL was also determined by the bioMerieux VITEK-2 system. In addition, carba NP test was performed to carbapenem resistant strains [26]; moreover, polymerase chain reaction (PCR) was used to detect KPC, NDM, IMP, VIM and OXA48 genes in carba NP positive strains as previously reported [27].

Biofilm assay

A crystal violet assay was performed to measure the ability of forming biofilm among *K. pneumoniae* strains, which was done in 96-well microtitre plates as previously described with a minor modification [28]. Briefly, bacteria were incubated in Luria-Bertani broth (LB) with 180 rpm in 35 °C for 24 h and then suspended in LB to make a concentration of 1.5×10^8 CFU/mL, then 200ul was added to 96-well plates for incubating at 35 °C for 48 h. Unbound bacteria were removed by washing with sterile distilled water, and the remaining bacteria were stained with 200 μL 1 % (*w/v*) crystal violet

dye for 20 min. Then unbound dye was washed away. The bound crystal violet dye was solubilized by using 200 μL 95 % ethanol. Biofilm was quantified by measuring the optical density (OD) of the supernatant at 570 nm. The biofilm assay for each strain was performed in triplicate and the mean absorbance value was determined.

Polymerase chain reaction-mediated detection of capsular serotype and virulence genes

Crude genomic DNA was extracted from *K. pneumoniae* strains and genes for K1, K2, K5, K20, K54, and K57 capsular serotypes were amplified by a multiplex PCR as previously described [29]. Virulent plasmid-derived genes of p-*rmpA*, *iucA*, and *iroB* were identified using the primes as previously reported [20, 30], which was also used to determine hvKP. Strains positive for p-*rmpA* and *iroB* and *iucA* were designated as hvKP. Other virulent factor genes of *mrkD*, *entB*, *iutA*, *ybtS*, *kfu* and *allS* were amplified as previously reported [31]. The primers used are listed in Table 1.

Multilocus sequence typing (MLST)

MLST was performed for all *K. pneumoniae* strains by amplifying and sequencing seven housekeeping genes for *K. pneumoniae* (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) according to the protocol provided on the MLST website (www.pasteur.fr/mlst) [32]. Alleles and sequence types (STs) were determined according to the MLST database (www.pasteur.fr/mlst/Kpneumoniae.html).

Random amplified polymorphic DNA (RAPD)

All *K. pneumoniae* strains were analyzed by RAPD-PCR using primers RAPD4 (AAGACGCCGT) as previously described [33]. PCR products were electrophoresed on 2.0 % (w/v) agarose gels, and the banding pattern analysis was performed by NTSYS-pc software (version 2.0). The similarity of banding pattern was calculated using the Dice coefficient. Cluster analysis was performed using the unweighted pair-group method with arithmetic averages (UPGMA). Strains were considered as the same RAPD type if they possessed ≥80 % similarity.

Statistical analyses

Contingency variables were analyzed by 2-tailed Chi-square test or Fisher's exact test. Medians with 25th–75th percentiles were used for continuous variables, unless otherwise indicated. Continuous variables were analyzed by Student's *t* test or the Mann–Whitney *U* test. A *P* value of <0.05 was considered to be statistically significant, and all probabilities were two-tailed. All statistical analyses were performed by SPSS, version 20.0 for Windows.

Results

Clinical characteristics

From January 2014 to December 2014, a total of 49 mechanically ventilated patients were identified with *K. pneumoniae* induced VAP. The patients had mean ± standard deviation age of 56.0 ± 19.0 years. Thirty-nine (79.6 %) were male and 10 (20.4 %) were female. As determined by positive p-*rmpA* and *iroB* and *iucA*, isolates of hvKP were obtained from 14 (28.6 %) patients and cKP strains were from 35 (71.4 %) patients. The baseline characteristics of patients with *K. pneumoniae* induced VAP is presented in Table 2. Most patients (75.5 %) had neurologic disorder. There was no statistically significant difference in underlying disease between hvKP and cKP groups. The laboratory findings and clinical characteristics of the patients with VAP due to hvKP and cKP are compared in Table 3. Patients infected with hvKP induced VAP had a shorter duration of hospitalization prior to *K. pneumonia* cultured, the median length of stay was 11 vs. 18 days among cKP infected patients ($P = 0.054$), whereas the full hospital stay did not differ between them. There was no statistically significant difference in severity of disease as expressed by APACHE II score between patients with hvKP and cKP induced VAP. Metastatic *K. pneumoniae* infection to other sites of the body did not occur in either the hvKP or cKP infected group. No in-hospital death was found in any of the 49 cases.

Antimicrobial resistance analysis

All 49 *K. pneumoniae* strains showed uniform resistance to ampicillin. cKP showed significantly higher antimicrobial resistant rate for almost all antimicrobials than hvKP (Table 4). All but one hvKP strain were susceptible to all the tested anitimicrobials except ampicillin. This hvKP strain was an ESBL-producing strain which was resistant to ampicillin, ampicillin-sulbactam, cefazolin, ceftriaxone and aztreonam. The percentage of ESBL-producing strains among cKP was significantly higher than that among hvKP (62.9 % vs. 7.1 %, $P < 0.001$). Eleven cKP strains resistant to Imipenem and Ertapenem were carbaNP positive. Among them, ten cKP were KPC positive, and 1 cKP possessed NDM.

Microbiological characteristics

All but one hvKP strain (92.5 %) showed hypermucosicovity phenotype as evidenced by positive string test, whereas,

Table 1 Primers used for capsular serotypes and virulence factors of *Klebsiella pneumoniae*

Primer	Nucleotide sequence	Reference
K1		
Forward	5-GGTGCTCTTTACATCATTGC-3	[29]
Reverse	5-GCAATGGCCATTTGCGTTAG-3	
K2		
Forward	5-GACCCGATATTCATACTTGACAGAG-3	[29]
Reverse	5-CCTGAAGTAAAATCGTAAATAGATGGC-3	
K5		
Forward	5-TGGTAGTGATGCTCGCGA-3	[29]
Reverse	5-CCTGAACCCACCCCAATC-3	
K20		
Forward	5-CGGTGCTACAGTGCATCATT-3	[29]
Reverse	5-GTTATACGATGCTCAGTCGC-3	
K54		
Forward	5-CATTAGCTCAGTGGTTGGCT-3	[29]
Reverse	5-GCTTGACAAACACCATAGCAG-3	
K57		
Forward	5-CTCAGGGCTAGAAGTGTCAT-3	[29]
Reverse	5-CACTAACCCAGAAAGTCGAG-3	
p-r*mpA*		
Forward	5-ACGACTTTCAAGAGAAATGA-3	[30]
Reverse	5-CATAGATGTCATAATCACAC-3	
iucA		
Forward	5- ATAAGGCAGGCAATCCAG-3	[20]
Reverse	5- TAACGGCGATAAACCTCG-3	
iroB		
Forward	5- TGTGTGCTGTGGGTGAAAGC-3	[20]
Reverse	5- ATGTTCGGTGAGATTCGCCAGT-3	
mrkD		
Forward	5-AAGCTATCGCTGTACTTCCGGCA-3	[31]
Reverse	5-GGCGTTGGCGCTCAGATAGG-3	
entB		
Forward	5-GTCAACTGGGCCTTTGAGCCGTC-3	[31]
Reverse	5-TATGGGCGTAAACGCCGGTGAT-3	
iutA		
Forward	5-GGGAAAGGCTTCTCTGCCAT-3	[31]
Reverse	5-TTATTCGCCACCACGCTCTT-3	
ybtS		
Forward	5-GACGGAAACAGCACGGTAAA-3	[31]
Reverse	5-GAGCATAATAAGGCGAAAGA-3	
kfu		
Forward	5-GGCCTTTGTCCAGAGCTACG-3	[31]
Reverse	5-GGGTCTGGCGCAGAGTATGC-3	
allS		
Forward	5-CATTACGCACCTTTGTCAGC-3	[31]
Reverse	5-GAATGTGTCGGCGATCAGCTT-3	

only 1 cKP strain (2.9 %) possessed hypermucosicovity phenotype. The biofilm-forming abilities had no significant difference between hvKP and cKP strains (OD570 values (mean ± SD) 0.7367 ± 0.3770 vs. 0.8086 ± 0.375, $P = 0.411$). Capsular genotypes K1 and K2 comprised 14.3 % (7/49) and 6.1 % (3/49) of all *K. pneumoniae* strains. All *K. pneumoniae* strains had *entB* and *mrkD*. The majority of *K. pneumoniae* strains (83.7 %) possessed *ybtS*. The prevalence of K1 and K2 in hvKP strains were 42.9 % (6/14) and 21.4 % (3/14), respectively. All K1 strains had *allS* and *kfu* genes while all K2 strains did not. The incidences of K1, K2, K20, p-*rmpA*, *iroB*, *iucA*,*iutA*,

Table 2 Baseline characteristics of patients with *Klebsiella pneumoniae* induced ventilator-associated pneumonia

Characteristic	Patients with cKP VAP ($n=35$)	Patients with hvKP VAP ($n=14$)	*P* Value
Age, years	58.0 (44.0–72.0)	55.5 (42.8–62.8)	0.383
Male sex	29 (82.9)	10 (71.4)	0.614
Underlying disease			
Diabetes mellitus	3 (8.6)	2 (14.3)	0.941
Hypertension	11 (31.4)	2 (14.3)	0.384
Chronic pulmonary disease	5 (14.3)	1 (7.1)	0.836
Neurologic disorder	26 (74.3)	11 (78.6)	1.000
Chronic liver disease	4 (11.4)	0 (0.0)	0.458
Surgery within 7 days prior to *K. pneumonia* isolated	5 (14.3)	3 (21.4)	0.855
Route of ventilation			1.000
Oroendotracheal	8 (22.9)	3 (21.4)	
Tracheostomy	27 (77.1)	11 (78.6)	
Days on mechanical ventilation			0.110
Less than 3 days	7 (20.0)	7 (50.0)	
3 to 7 days	8 (22.9)	2 (14.3)	
More than 7 days	20 (57.1)	5 (35.7)	
Empirical antibiotics received			
Any antibiotic	29 (82.9)	11 (78.6)	1.000
Penicillin	2 (5.7)	1 (7.1)	1.000
Cephalosporin	9 (25.7)	1 (7.1)	0.287
Carbapenem	4 (11.4)	2 (14.3)	1.000
β-Lactam/β-lactamase inhibitor combinations	18 (51.4)	7 (50.0)	0.928
Aminoglycoside	5 (14.3)	0 (0.0)	0.332
Fluoroquinolone	1 (2.9)	1 (7.1)	0.494
Tetracycline	1 (2.9)	0 (0.0)	1.000
Sulfonamide	1 (2.9)	0 (0.0)	1.000

Values are presented as median (25th–75th percentile) or no. (%) of patients

VAP ventilator-associated pneumonia

Kfu and *alls* genes were significantly higher in hvKP than those in cKP ($P=0.002$, $P=0.020$, $P=0.020$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$ and $P=0.002$, respectively) (Table 5).

Table 3 Laboratory data and clinical characteristics of patients with *Klebsiella pneumoniae* induced ventilator-associated pneumonia

Characteristic	Patients with cKP VAP ($n=35$)	Patients with hvKP VAP ($n=14$)	*P* Value
WBC count, $\times 10^9$/L	11.4 (7.9–20.0)	9.7 (7.0–13.1)	0.806
Albumin, g/dL	33.0 (32.1–38.2)	30.7 (30.4–34.2)	0.658
PCT, ng/mL	0.7 (0.4–1.1)	0.4 (0.3–0.6)	0.993
CRP, mg/L	31.1 (14.2–90.5)	39.9 (37.6–67.1)	0.606
Total bilirubin, μmol/L	8.7 (6.2–16.3)	12 (9.8–14.5)	0.216
APACHE II score	19.0 (17.0–22.0)	18.0 (11.5–18.5)	0.110
Concurrent bacteremia	5 (14.3)	0 (0.0)	0.332
CXR-bilateral involvement	32 (91.4)	13 (92.9)	1.000
Hospital stay prior to *K. pneumonia* isolated, days	18 (10–30)	11 (3–17)	0.054
Full hospital stay, days	31 (20.5–39)	40 (24–65)	0.094

Values are presented as median (25th–75th percentile) or no. (%) of patients

VAP ventilator-associated pneumonia, *ICU* intensive care unit, *CXR* chest X radiology, *WBC* white blood cell, *PCT* procalcitonin, *CRP* C-reactive protein, *APACHE* acute physiologic and chronic health evaluation

Table 4 Antimicrobial resistance of *Klebsiella pneumoniae*

Antimicrobial agent	cKP ($n=35$)	hvKP ($n=14$)	*P* Value
Ampicillin	35 (100)	14 (100)	NA
Ampicillin-sulbactam	34 (97.1)	1 (7.1)	<0.001*
Piperacillin-tazobactam	13 (37.1)	0 (0)	0.021*
Cefazolin	34 (97.1)	1 (7.1)	<0.001*
Cefotan	11 (31.4)	0 (0.0)	0.045*
Ceftriaxone	34 (97.1)	1 (7.1)	<0.001*
Cefepime	24 (68.6)	0 (0.0)	<0.001*
Ceftazidime	21 (60.0)	0 (0)	<0.001*
Aztreonam	26 (74.3)	1 (7.1)	<0.001*
Imipenem	11 (31.4)	0 (0)	0.045*
Ertapenem	11(31.4)	0 (0)	0.045*
Tobramycin	13 (37.1)	0 (0)	0.021*
Gentamicin	20 (57.1)	0 (0)	<0.001*
Amikacin	10 (28.6)	0 (0)	0.064
Levofloxacin	11 (31.4)	0 (0)	0.045*
Ciprofloxacin	16 (45.7)	0 (0)	0.006*
Trimethoprim-sulfamethoxazole	18 (51.4)	0 (0)	0.001*
ESBL	22 (62.9)	1 (7.1)	<0.001*

Values are presented as no. (%) of patients

cKP classic *Klebsiella pneumoniae*, *hvKP* hypervirulent *Klebsiella pneumoniae*, *ESBL* extended spectrum β-lactamase, *NA* not applicable

*$P<0.05$ compared with cKP

Table 5 Microbiological characteristics of *Klebsiella pneumoniae*

Variable	cKP ($n=35$)	hvKP ($n=14$)	*P* value
Capsular serotype			
K1	1 (2.9)	6 (42.9)	0.002*
K2	0 (0)	3 (21.4)	0.020*
K20	0 (0)	3 (21.4)	0.020*
K54	0 (0)	1 (7.1)	0.286
K57	0 (0)	0 (0)	NA
K5	0 (0)	0 (0)	NA
Virulence factors			
p-*rmpA*	1 (2.9)	14 (100)	<0.001*
iroB	0 (0)	14 (100)	<0.001*
iucA	1 (2.9)	14 (100)	<0.001*
iutA	1 (2.9)	13 (92.9)	<0.001*
ybtS	31 (88.6)	10 (71.6)	0.202
Kfu	1(2.9)	7 (50.0)	<0.001*
allS	1 (2.9)	6 (42.9)	0.002*
mrkD	35 (100)	14 (100)	NA
entB	35 (100)	14 (100)	NA

Values are presented as no. (%) of patients

cKP classic *Klebsiella pneumoniae*, *hvKP* hypervirulent *Klebsiella pneumoniae*, *NA* not applicable

*$P<0.05$ compared with cKP

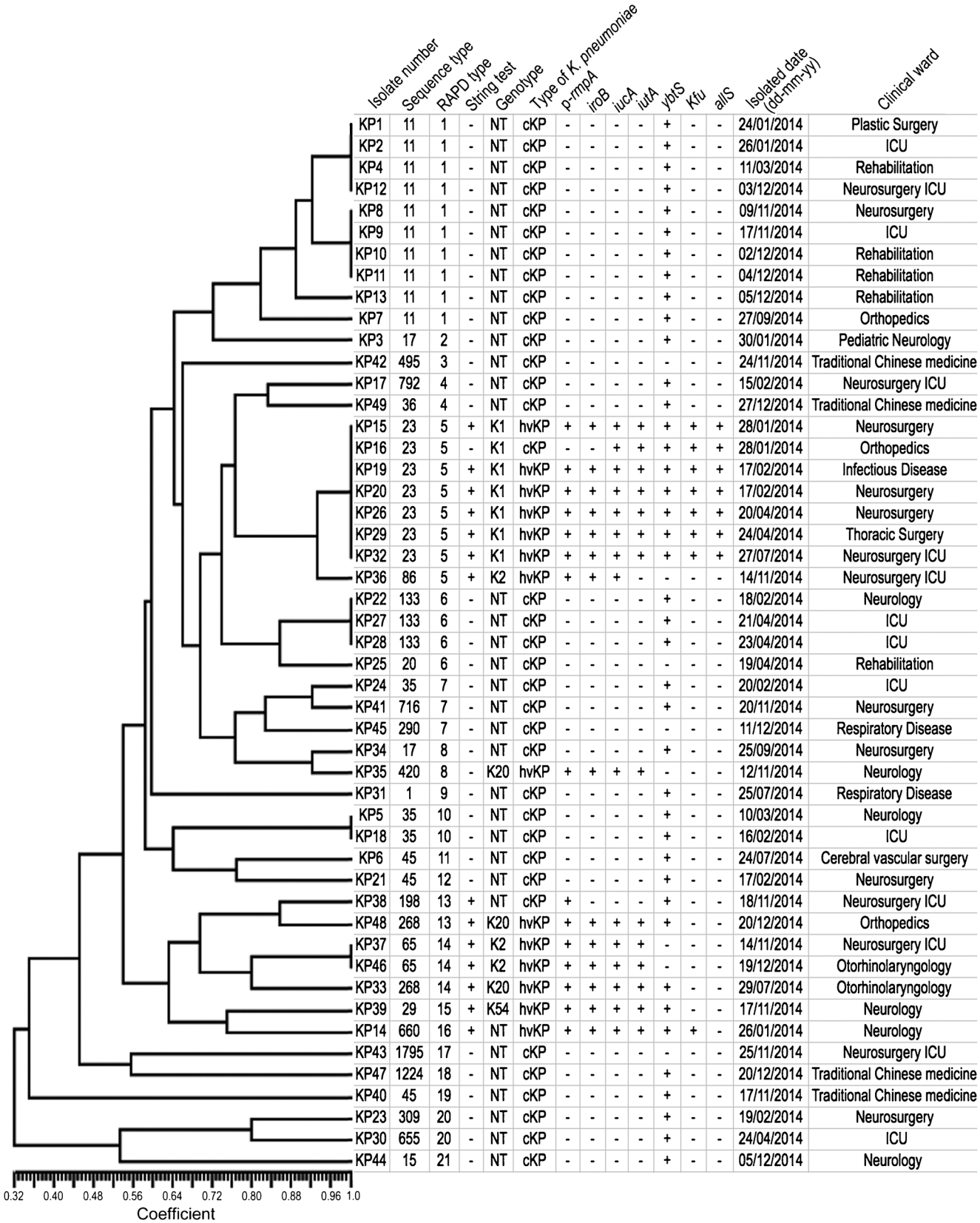
MLST and RAPD analyses

MLST analysis identified 25 sequence types (STs) among the 49 *K. pneumoniae* strains (Fig. 1). The most prevalent ST in hvKP strains was ST23 (42.8 %), followed by ST65 (14.3 %). All K1 *K. pneumoniae* belonged to ST23. Two K2 *K. pneumoniae* belonged to ST65 and the other K2 belonged to ST86. Two K20 *K. pneumoniae* belonged to ST268 and the other K20 belonged to ST420. The K54 *K. pneumoniae* belonged to ST29. The most prevalent ST in cKP was ST11 (28.5 %) and they were all KPC positive *K. pneumoniae*, followed by ST35, ST45 and ST133 (8.6 % each). RAPD analysis showed that 49 *K. pneumoniae* strains revealed 21 different types. The banding patterns were designated RAPD types 1 to 21 (Fig. 1). Fourteen hvKP strains had five different RAPD patterns. Seven ST23 *K. pneumoniae* strains showed the identical RAPD banding pattern. Among them, three were isolated from the neurosugery unit in January, February and April 2014, respectively, the other four ST23 were from different clinical wards. Thirty-five cKP strains showed 16 different RAPD types. Ten ST11 *K. pneumoniae* strains showed the same RAPD type. Dendrogram based on RAPD of 49 *K. pneumoniae* strains is showed in Fig. 1.

Discussion

This retrospective study was conducted in 49 mechanically ventilated patients with VAP caused by *K. pneumoniae* from January 2014 to December 2014 in Xiangya Hospital. It was the first systematic study focusing on the clinical characteristics of VAP due to hvKP and its microbiological characteristics in mechanically ventilated patients. We found hvKP accounted for nearly one-third of *K. pneumoniae* induced VAP cases among mechanically ventilated patients. There was no statistically significant difference in severity of disease between cKP and hvKP groups. The prevalence of genotypes K1, K2, p-*rmpA*, *iroB*, *iucA*, *iutA*, *Kfu* and *alls* in hvKP strains were statistically significantly higher than those in cKP strains. The most prevalent ST in hvKP strains was ST23 with identical RAPD pattern. One ST29 with K54 serotype ESBL-producing *K. pneumoniae* was found.

In this study, hvKP accounted for 28.6 % (14/49) *K. pneumoniae* induced VAP in mechanically ventilated patients. This is the highest rate reported in hvKP induced hospital-acquired infection. Liu et al. reported 4.5 % hvKP infection in hospital-acquired *K. pneumonia* bacteremia in China [34]. In two previous studies in Taiwan, the prevalence of hvKP among hospital-acquired *K. pneumoniae* bacteremia were reported 14.8 % and 15.2 %, respectively [19, 35]. Li et al. reported 33.0 % hvKP of *K. pneumoniae* isolated from various specimens; however, most hvKP strains were from community-acquired infection [10]. It has been known that



Isolate number	Sequence type	RAPD type	String test	Genotype	Type of *K. pneumoniae*	*p-rmpA*	*iroB*	*iucA*	*iutA*	*ybtS*	*Kfu*	*allS*	Isolated date (dd-mm-yy)	Clinical ward
KP1	11	1	-	NT	cKP	-	-	-	-	+	-	-	24/01/2014	Plastic Surgery
KP2	11	1	-	NT	cKP	-	-	-	-	+	-	-	26/01/2014	ICU
KP4	11	1	-	NT	cKP	-	-	-	-	+	-	-	11/03/2014	Rehabilitation
KP12	11	1	-	NT	cKP	-	-	-	-	+	-	-	03/12/2014	Neurosurgery ICU
KP8	11	1	-	NT	cKP	-	-	-	-	+	-	-	09/11/2014	Neurosurgery
KP9	11	1	-	NT	cKP	-	-	-	-	+	-	-	17/11/2014	ICU
KP10	11	1	-	NT	cKP	-	-	-	-	+	-	-	02/12/2014	Rehabilitation
KP11	11	1	-	NT	cKP	-	-	-	-	+	-	-	04/12/2014	Rehabilitation
KP13	11	1	-	NT	cKP	-	-	-	-	+	-	-	05/12/2014	Rehabilitation
KP7	11	1	-	NT	cKP	-	-	-	-	+	-	-	27/09/2014	Orthopedics
KP3	17	2	-	NT	cKP	-	-	-	-	+	-	-	30/01/2014	Pediatric Neurology
KP42	495	3	-	NT	cKP	-	-	-	-	-	-	-	24/11/2014	Traditional Chinese medicine
KP17	792	4	-	NT	cKP	-	-	-	-	+	-	-	15/02/2014	Neurosurgery ICU
KP49	36	4	-	NT	cKP	-	-	-	-	+	-	-	27/12/2014	Traditional Chinese medicine
KP15	23	5	+	K1	hvKP	+	+	+	+	+	+	+	28/01/2014	Neurosurgery
KP16	23	5	-	K1	cKP	-	-	+	+	+	+	+	28/01/2014	Orthopedics
KP19	23	5	+	K1	hvKP	+	+	+	+	+	+	+	17/02/2014	Infectious Disease
KP20	23	5	+	K1	hvKP	+	+	+	+	+	+	+	17/02/2014	Neurosurgery
KP26	23	5	+	K1	hvKP	+	+	+	+	+	+	+	20/04/2014	Neurosurgery
KP29	23	5	+	K1	hvKP	+	+	+	+	+	+	+	24/04/2014	Thoracic Surgery
KP32	23	5	+	K1	hvKP	+	+	+	+	+	+	+	27/07/2014	Neurosurgery ICU
KP36	86	5	+	K2	hvKP	+	+	+	-	-	-	-	14/11/2014	Neurosurgery ICU
KP22	133	6	-	NT	cKP	-	-	-	-	+	-	-	18/02/2014	Neurology
KP27	133	6	-	NT	cKP	-	-	-	-	+	-	-	21/04/2014	ICU
KP28	133	6	-	NT	cKP	-	-	-	-	+	-	-	23/04/2014	ICU
KP25	20	6	-	NT	cKP	-	-	-	-	-	-	-	19/04/2014	Rehabilitation
KP24	35	7	-	NT	cKP	-	-	-	-	+	-	-	20/02/2014	ICU
KP41	716	7	-	NT	cKP	-	-	-	-	+	-	-	20/11/2014	Neurosurgery
KP45	290	7	-	NT	cKP	-	-	-	-	-	-	-	11/12/2014	Respiratory Disease
KP34	17	8	-	NT	cKP	-	-	-	-	+	-	-	25/09/2014	Neurosurgery
KP35	420	8	-	K20	hvKP	+	+	+	+	-	-	-	12/11/2014	Neurology
KP31	1	9	-	NT	cKP	-	-	-	-	+	-	-	25/07/2014	Respiratory Disease
KP5	35	10	-	NT	cKP	-	-	-	-	+	-	-	10/03/2014	Neurology
KP18	35	10	-	NT	cKP	-	-	-	-	+	-	-	16/02/2014	ICU
KP6	45	11	-	NT	cKP	-	-	-	-	+	-	-	24/07/2014	Cerebral vascular surgery
KP21	45	12	-	NT	cKP	-	-	-	-	+	-	-	17/02/2014	Neurosurgery
KP38	198	13	+	NT	cKP	+	-	-	-	+	-	-	18/11/2014	Neurosurgery ICU
KP48	268	13	+	K20	hvKP	+	+	+	+	+	-	-	20/12/2014	Orthopedics
KP37	65	14	+	K2	hvKP	+	+	+	+	-	-	-	14/11/2014	Neurosurgery ICU
KP46	65	14	+	K2	hvKP	+	+	+	+	-	-	-	19/12/2014	Otorhinolaryngology
KP33	268	14	+	K20	hvKP	+	+	+	+	+	-	-	29/07/2014	Otorhinolaryngology
KP39	29	15	+	K54	hvKP	+	+	+	+	+	-	-	17/11/2014	Neurology
KP14	660	16	+	NT	hvKP	+	+	+	+	+	+	-	26/01/2014	Neurology
KP43	1795	17	-	NT	cKP	-	-	-	-	-	-	-	25/11/2014	Neurosurgery ICU
KP47	1224	18	-	NT	cKP	-	-	-	-	+	-	-	20/12/2014	Traditional Chinese medicine
KP40	45	19	-	NT	cKP	-	-	-	-	+	-	-	17/11/2014	Traditional Chinese medicine
KP23	309	20	-	NT	cKP	-	-	-	-	+	-	-	19/02/2014	Neurosurgery
KP30	655	20	-	NT	cKP	-	-	-	-	+	-	-	24/04/2014	ICU
KP44	15	21	-	NT	cKP	-	-	-	-	+	-	-	05/12/2014	Neurology

Fig. 1 Dendrogram of RAPD patterns and molecular characteristics of 49 *Klebsiella pneumoniae*

the prevalence of hvKP in invasive *Klebsiellae* liver abscess was high, ranging from 53 to 98 % [9, 14, 36, 37], and *Klebsiellae* liver abscess were mostly community-acquired infection.

hvKP is characterized by causing severe community-acquired liver abscess and other community-acquired infections in young healthy people, with the ability to cause metastatic spread [8]. However, in this study, the hvKP infected VAP patients had a mean age of 51 years, and there was no metastatic infection which occurred in hvKP infected patients. The reason for no metastatic spread could be due to prompt effective antibiotic treatment. Compared with patients infected by cKP caused VAP, hvKP infected patients had shorter hospitalization prior to isolated *K. pneumoiae* (median 11 vs 18 days; $P=0.054$) even though the difference was not statistically significant. Based on this, clinicians should be alert to hvKP induced VAP in the early stage of hospitalization for mechanically ventilated patients. The reason for this difference could be that hvKP appears to colonize in the community and patients with VAP due to hvKP were colonized on admission, whereas cKP strains were more likely to be acquired during hospital stay. A previous study showed that 4 % and 2 % of Chinese adults who live in Taiwan, Hong Kong and China or other Asian countries were colonized with a K1 or K2 serotype of *K. pneumoiae* [38].

In accordance with previous studies [10, 11], we found hvKP were less resistant to most antimicrobials than cKP. ESBL-producing hvKP was found in 7.1 % (1/14) of cases of hvKP. The ESBL-producing hvKP was resistant to ampicillin, cefazolin, ceftriaxone, ampicillin sulbactam and aztreonam. This strain was isolated from a 60-year-old female with type 2 diabetes 3 days after she was hospitalized for stroke. This patient had no history of antibiotic use before being infected by this hvKP strain. ESBL-producing hvKP strains have been reported, with the trend of increasing occurrence [10, 11, 39]. Moreover, two hvKP strains were found resistant to carbapenem and polymyxin B while harboring some virulence genes in Brazil [40]. Recently, hypervirulent carbapenem-resistant *K. pneumoniae* has emerged in China [34]. Based on whole-genome sequencing, genomic analysis has detected *K. pneumoniae* strains combining virulence and resistance features [41]. Siu et al. successfully transferred KPC-producing plasmid into a hvKP strain which was originally only resistant to ampicillin and streptomycin, then it became resistant to all beta-lactams after conjugating KPC without losing its virulence [42]. Taken together, these data strongly support the convergence of virulence, and antimicrobial resistance genes could lead to untreatable *K. pneumoniae* infections [16]. This has generated great interest of developing antivirulence agents to target the virulence factors of hvKP strains instead of using antimicrobial to kill them [14, 20].

In this study, the abilities of biofilm-forming had no significant difference between cKP and hvKP groups. However, Wu et al. reported the biofilm-forming abilities of the pyogenic liver abscess-associated *K. pneumoniae* were significantly higher than those of the non-tissue invasive *K. pneumoniae* [43]. The reason for this discrepancy may be due to the fact that those *K. pneumoniae* strains were isolated from blood, but the *K. pneumoniae* strains in our study were isolated from respiratory tracts of mechanically ventilated patients. The presence of intubation tube among mechanically ventilated patients favored biofilm formation by providing an insert surface for the attachment of bacteria. *MrkD* involves type 3 fimbrial adhesin and mediates binding to the extracellular matrix [44]. In this study, all *K. pneumoniae* strains (100 %) possessed *mrkD* gene which could also explain the high abilities of biofilm-forming among them.

Capsular serotypes are important virulence factors for *K. pneumoniae*, especially K1 and K2 serotypes [17, 18]. In this study, the prevalence of K1 and K2 among hvKP was 42.9 and 21.4 %, respectively. A previous study reported 53.8 % hvKP strains isolated from liver abscess were K1 [37], while another study showed 98 % *K. pneumoniae* from invasive liver abscess possessed K1 [9]. The prevalence of K1 in hvKP from bacteremia has ranged from 23 to 42 % [11, 18, 31]. K2 distribution among hvKP has been reported as 10–46 % [10, 11, 37]. However, only 3.1 % of *K. pneumoniae* from hospital-acquired bacteremia was reported possessing K1 [11]. In addition to K1 and K2, we have detected three K20 strains, and one K54 strain which was an ESBL-producing ST29 hvKP. To our knowledge, this is the first report of ESBL-producing *K. pneumoniae* ST29 with K54 serotype. Chuang et al. reported a case of mycotic aneurysm caused by ST29 K54 hvKP which was susceptible to most antimicrobials except ampicillin, and stated it was difficult to treat [45]. The combination of antimicrobial resistant with hvKP might be even worse.

rmpA regulates the synthesis of extracellular polysaccharide capsule, which is responsible for the clinical manifestation of hypermucoviscous phenotype [19]. Three genes have been reported to encode for this factor: two large-plasmid-carried genes, p-*rmpA* and p-*rmpA2*, and one chromosomal gene c-*rmpA*; and it showed 78/79 *rmpA*/*A2*-positive *K. pneumoniae* strains possessed p-*rmpA* gene, which occurred more commonly than p-*rmpA2* (62/79) and c-*rmpA* (10/79) [46]. In this study, we found one hvKP strain which possessed p-*rmpA* did not show hypermucoviscous phenotype. This indicated that there may be other regulatory mechanisms for hypermucoviscous phenotype expression [19].

Iron acquisition factors, siderophore enterobactin, aerobactin, salmochelin and yersiniabactin and *Kfu*, which mediate uptake of ferric iron have been reported as virulence factors in *K. pneumoniae* infection [20, 30, 47]. In this study, all the *K. pneumoniae* strains (100 %) had *entB*. This agreed with a previous study [31]. The majority of *K. pneumoniae* strains (83.6 %) possessed *ybtS* and the prevalence had no

significant difference between hvKP and cKP strains (71.6 vs. 88.6 %; $P=0.202$). The high prevalence of *ybtS* in the respiratory tract confirmed the point that yersiniabactin is a virulence factor for *K. pneumoniae* during pulmonary infection [47]. The *iutA* gene is responsible for encoding a receptor for aerobactin [30]. In this study, strains possessing *iutA* gene were more prevalent among hvKP than that among cKP (92.9 vs. 2.9 %; $P<0.001$) and most p-*rmpA* positive *K. pneumoniae* strains (86.6 %) had *iutA*. This high coexistence of *iutA* and *rmpA* agreed with a previous study that *rmpA*-borne plasmid pLVPK with other *rmpA*-related genetic loci including *iutA* was essential to *K. pneumonia* virulence, not *rmpA* gene alone [30]. In accordance with previous studies of *K. pneumoniae* isolated from blood and liver abscess [14, 19], we found all K1 strains had *allS* and *kfu* genes while all K2 strains did not; whereas, Lin et al. reported 11.5 % *kfu* gene was found in K2 strains [18].

In previous studies, ST23 *K. pneumoniae* was the most common ST in liver abscesses [36, 48] and it has been reported clonally related in Taiwan by Lau et al. [49], while Cheng et al. reported *K. pneumoniae* liver abscess in Taiwan is not caused by a clonal spread strain [50]. In this study, ST23 was dominant among hvKP strains, and all ST23 strains had the identical RAPD pattern, suggesting they were epidemiologically related, as three of them were isolated from the neurosurgery unit.

In conclusion, contrary to the traditional view that hvKp is an important pathogen mainly causing community-acquired infection, this study demonstrates hvKP has become a common pathogen of VAP in mechanically ventilated patients in China. Our finding of a ST29 with K54 serotype ESBL-producing hvKp strain highlights the need for clinicians to increase awareness of hvKP induced VAP and enhance the epidemiologic surveillance of this pathogen.

Compliance with ethical standards

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Conflict of interest None declared.

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