Clinical and Microbiological Prognostic Factors of In-Hospital Mortality Caused by Hypervirulent Klebsiella Pneumoniae Infections: A Retrospective Study in a Tertiary Hospital of Southwestern China

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Research

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Abstract

Background Hypervirulent klebsiella pneumoniae (hvKP) is responsible for various invasive diseases and is associated with high mortality. However, the clinical and microbiological factors of hvKP infection to affect prognosis are not well studied. The purpose of this study is to evaluate prognostic factors of in-hospital mortality of hvKP infection, mainly focusing on clinical and microbiological characteristics.

Methods A retrospective study was conducted in southwestern China from February 2018 to June 2019 and strains positive for aerobactin and string test were defined as hvKP. According to the clinical outcomes during hospitalization, hvKP infected patients were divided into non-survivor group and survivor group. The clinical characteristics, capsule serotypes, multi-locus sequence types, virulence genes and antimicrobial susceptibility were compared between the two groups.

Results A total of 135 patients were classified as hvKP infection, with a prevalence rate of 22% and an in-hospital mortality rate of 11.9%. Univariate analysis exhibited that admission to intensive care unit (ICU) (p=0.008) and antimicrobial resistance of hvKP such as ampicillin/sulbactam(p=0.028), cefepime(p=0.033), aztreonam(p=0.049) and harboring iroN gene(p=0.023) were associated with higher in-hospital mortality. On the contrary, the rmpA gene showed an inverse association with in-hospital mortality(p=0.017). Multivariate logistic regression analysis revealed that admission to ICU (odds ratio [OR]=3.452, 95% confidence interval [CI]=1.052-11.329; P=0.041) and presence of iroN (OR=9.278, 95% CI=1.654-52.035; P=0.011) was considered to be the independent prognostic factors for in-hospital mortality of hvKP infection.

Conclusion Emerging hvKP infection may lead to relatively high in-hospital mortality. Therefore, early surveillance and better management are necessary for patients admitted to ICU and infected with hvKP harboring iroN gene.

Backgound

Klebsiella pneumoniae as an opportunistic pathogen are usually responsible for various infections such as pneumonia, intra-abdominal infection, urinary tract infection and bacteremia[1]. The elderly and immunocompromised population are prone to be caught by this type of classic *Klebsiella pneumoniae*(cKP)[2]. Unlike cKP, the emerging hypervirulent *Klebsiella pneumonia* (hvKP) can cause community-acquired infections in patients without major comorbidities or even young healthy individuals[3]. Although there is still no unified classification to capture all the hvKP strains, strategies for iron-acquisition systems and hypermucoviscous classification have clarified the trend of hvKP, which is helpful for their identification[4, 5]. In particular, the hvKP strain, generally considered normal intestinal flora, has the ability to invade and infect distant organs and tissues of the host, resulting in irreversible severe damage and higher clinical mortality[6]. However, the death-rate of infected hvKP patients varies greatly in different countries and regions, with the death-rate in Europe up to 17% and in China up to 41.2%[7, 8]. In addition to the patient's comorbidities, the site of the initial infection, bacterial pathogenic

factors including virulence factors and antimicrobial resistance are also potential factors affecting the clinical outcomes.

In previous studies of risk factors, patients with comorbidities of diabetes and malignancy are more vulnerable to hvKP infection[9]. A common feature of these types of comorbidities is the suppression of bacterial defense. Diabetics have impaired neutrophil responses and phagocytic capabilities, while the amounts of immune cells decreased due to the side effect of cytotoxic therapies in malignant tumor patient[10, 11]. These defects arise combine with other factors may lead to an increase in deaths from hvKP infection. Referring to the virulence factors of hvKP, which can be divided into capsule serotypes, multi-locus sequence types (MLST), siderophores, lipopolysaccharide (LPS), and other virulence genes[1]. The abundant capsule leads to increased anti-phagocytosis of bacteria, attenuated early inflammatory response in host and enhanced antagonistic complement - mediated effects, among which K1 and K2 serotypes expressed greater phagocytic resistance and virulence than other serotypes[12–14]. ST11 Carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) strains should be especially worrisome as the occurrence of the strains causes fatal outcomes[15]. Enterobactin, salmochelin, yersiniabactin and aerobactin as the 4 kinds of siderophores secreted by hvKP, have long been appreciated as important virulence factors for hvKP. Remarkably, a large virulence plasmid termed as pLVPK (a pK2044-like plasmid) encoding aerobactin, salmochelin and rmpA is unique to hvKP strains, and it may lead to fatal consequences once combined with carbapenem-resistant *K. pneumoniae* as a mobile genetic element[16, 17]. Another concern is the emerging population of incorporation with hypervirulence and antimicrobial resistance of *K. pneumoniae*. HvKP strains producing extended-spectrum–lactamases (ESBLs) and carbapenemases deserve particular attention because these bacteria are indestructible and as such, they have been proposed to be a potential prognostic factor in mortality[9, 18].

So far, the research on the prevalence and in-hospital mortality of hvKP infection in China is limited, and there is still a lack of systematic research on the prognostic factors affecting the in-hospital mortality of hvKP infection. Therefore, we conducted a comprehensive analysis of the prognostic factors with hvKP infection by a retrospective study, mainly focusing on clinical characteristics of patients and microbiological characteristics of strains including capsule serotypes, multi-locus sequence types, virulence genes and antimicrobial resistance.

Methods

Study design and definition

A retrospective study was conducted from February 2018 to June 2019 in the First Affiliated Hospital of Chongqing Medical University, a 3200-bed tertiary teaching hospital. First, a total of 613 consecutive cases of cKP infection were enrolled during the study period, of which 135 were defined as hvKP with positive for both string test and *aerobactin* gene, as has been described previously[19]. Second, according to the prognosis of hospitalization, 135 patients with hvKP were divided into survival group and death group to determine the prognostic factors related to in-hospital mortality of hvKP infection (Fig. 1). Only

the first strain isolated from patients at the time of the first diagnosis of *K. pneumoniae* infection was collected, and subsequent isolates from the same patient were not incorporated into the study. Patient information were obtained from medical records, including demographic characteristics, comorbidities, laboratory results, clinical manifestations, primary infection sites of hvKP, antimicrobial therapy administration and clinical outcomes during hospitalization. Patients were excluded with incomplete information above.

Nosocomial infections were defined as the appearance of infection-related symptoms and positive culture of *K. pneumoniae* after 48 hours of hospitalization. Immunosuppression included acceptance of chemotherapy or corticosteroids for at least 7 days within one month before the onset of hvKP, or severe anemia, hypoproteinemia, and neutropenia at hvKP onset. The bacterial colony can be extended to a viscous string at least 5 millimeters from the surface of the agar plate, which can be interpreted as positive for string test[20].

Strain typing

Polymerase chain reaction (PCR) was used to detect the capsular serotypes of K1, K2, K5, K20, K54 and K57. Multi-locus sequence typing was tested by amplifying and sequencing the partial sequences of seven standard housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB*) and comparing them with KP MLST database to determine the allele types and STs of hvKP isolates (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

Virulence genes

The 16 virulence related genes for allantoin metabolism (*allS*), adhesin (*fimH* and *mrkD*), biosynthesis of lipopolysaccharide (*ycfM* and *wabG*), Fucose synthesis (*wcaG*), mucoviscosity (*rmpA*,*rmpA2* and *magA*), siderophore (*entB, kfu, iroN, irp-1, irp-2*, *iutA, ybtS*,) were assessed by PCR. All the above primer sequences can be found in Additional file 1.

Antimicrobial susceptibility testing and resistance mechanism

The VITEK2 compact or VITEK MS automated system (BioMérieux, Marcy-l'Étoile, France) was used to identify *K. pneumoniae* and conduct routine antimicrobial susceptibility test. All automatic-identified carbapenem resistant strains were manually verified by standard broth microdilution on the basis of the institute of clinical and laboratory standards (CLSI) guidelines. For Tigecycline, the susceptibility test was detected manually by standard broth microdilution methods and the results were judged according to the breakpoint of FDA Recognized Susceptibility Test Interpretive Criteria that minimum inhibitory concentration (MIC) ≥ 8.0 mg/L were defined as resistant. The Escherichia coli ATCC 25922 strain was

applied as quality control. Genes encoding ESBLs ($bla_{CTX-M-1}$, bla_{SHV} and bla_{TEM}) and carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, *and* bla_{OXA-48}) were tested when the phenotypic test was positive. All positive PCR-amplified products were sequenced to validate their identity through BLAST website (https://blast.ncbi.nlm.nih.gov/blast.cgi).

Statistical Analysis

All statistical data were analyzed by SPSS statistical software (version 25, IBM Corporation, Armonk, NY, USA). According to whether the statistical data conformed to the normal distribution, the categorical variables were tested by Chi-square test or Fisher's exact test, and the continuous variables were tested by two simple t-test or Mann-whitney U test. The goodness fit of logistic regression model was evaluated by Hosmer-Lemeshow test. All variables with P-value < 0.10 in the univariate analysis were included in the multivariate regression analysis to obtain the independent risk factors affecting the prognosis of patients. A 2-tailed P-value < 0.05 was considered to be statistically significant. The odds ratio (OR) and its 95% confidence interval (CI) were calculated to evaluate the strength of any association.

Ethical considerations

This retrospective study was approved by the Evaluation Committee and the Biomedical Ethics Committee of Chongqing Medical University. In light of the retrospective and anonymous nature of the study, the Ethics Committee did not require participants to provide written informed consent.

Results

Clinical characteristics of hvKP infected patients

In present study, a total of 135 patients were infected with hvKP, with a prevalence rate of 22% (135/613). Sixteen of these patients died during hospitalization, with an in-hospital mortality rate of 11.9% (16/135). The detailed characteristics of the 135 patients diagnosed with hvKP were summarized at Table 1. The non-survivor group consisted of 4 females and 12 males, with average age of 67.4 years. And the survivor group consisted of 44 females and 75 males, with average age of 59.5 years. Nosocomial infections were predominant in both the two groups(p = 0.188). The main underlying diseases in the non-survivor group included abdominal diseases (75%), respiratory diseases (56.3%) and hypertension (50%), while those in the survivor group included respiratory system diseases (63.9%), abdominal diseases (62.2%) and Chronic kidney diseases (38.7%). However, there was no statistical difference in underlying disease between the survivor group and non-survivor group. Respiratory tract infection, primary bacteremia and urinary tract infection were the main sources of infection in the two groups. The antimicrobial exposure within 4-week had a relatively small effect on mortality in both the surviving and non-surviving groups. The total length of hospital stays and post-culture of hospital stays were also similar in the two groups (p = 0.878, 0.107 respectively). However, univariate analysis expressed that the proportion of non-survivors admitted to ICU was much higher than that of survivors (68.8% versus 34.5%, p = 0.008).

Table 1
Clinical characteristics of survivor and non-survivor patients with hvKP.

Clinical characteristic	Total(n = 135)	Survivors (n = 119)	Non-survivors (n = 16)	p-value [a]
Age, mean ± SD, years	60.4 ± 17.1	59.5 ± 16.5	67.4 ± 20.7	0.082
Male gender	87(64.4)	75(63.0)	12(75.0)	0.508
Nosocomial infections	72(53.3)	61(51.3)	11(68.8)	0.188
Community-acquired infections	63(46.7)	58(48.8)	5(31.3)	0.188
Admission to ICU	52(38.5)	41(34.5)	11(68.8)	**0.008**
Post-culture length of hospital stay, median (IQR), days	4(1–13)	4(1–13)	10(2–26)	0.107
Total length of hospital stay, median (IQR), days	23(13–42)	23(13–37)	18(9-105)	0.878
Underlying diseases				
Hypertension	44(32.6)	36(30.3)	8(50.0)	0.114
Diabetes mellitus	48(35.6)	42(35.3)	6(37.5)	0.863
Malignancy	31(23.0)	25(21.0)	6(37.5)	0.141
Tuberculosis	4(3.0)	3(2.5)	1(6.3)	0.981
Cardiovascular disease	34(25.2)	28(23.5)	6(37.5)	0.227
Cerebral vascular disease	37(27.4)	30(25.2)	7(43.8)	0.119
Respiratory diseases	85(63.0)	76(63.9)	9(56.3)	0.554
Abdominal diseases	86(63.7)	74(62.2)	12(75.0)	0.469
Chronic kidney diseases	53(39.3)	46(38.7)	7(43.8)	0.695
Hematological diseases	9(6.7)	8(6.7)	1(6.3)	1
Endocrine diseases	47(34.8)	40(33.6)	7(43.8)	0.424
Peripheral vascular diseases	23(17.0)	20(16.8)	3(18.8)	1
Immunosuppression	44(32.6)	36(30.3)	8(50.0)	0.114

Notes: Data are expressed as no. (%) unless specified otherwise. SD: standard deviation; IQR, interquartile range;

ICU, intensive care unit.

a. Bold represents statistical differences ($p < 0.05$).

Clinical characteristic	Total(n = 135)	Survivors (n = 119)	Non-survivors (n = 16)	p-value [a]
Infection source				
Skin and soft tissue	10(7.4)	8(6.7)	2(12.5)	0.749
Respiratory tract	65(48.1)	58(48.8)	7(43.8)	0.708
Intra-abdomen	7(5.2)	6(5.0)	1(6.3)	0.595
Biliary tract	7(5.2)	7(5.9)	0	1
Urinary tract	15(11.1)	13(10.9)	2(12.5)	1
Catheter	5(3.7)	5(4.2)	0	1
Primary bacteremia	26(19.3)	22(18.5)	4(25.0)	0.777
Antimicrobial exposure within 4weeks				
Penicillin	27(20.0)	23(19.3)	4(25.0)	0.594
Cephalosporins	90(66.7)	78(65.5)	12(75.0)	0.451
Carbapenem	40(29.6)	33(27.7)	7(43.8)	0.188
Aminoglycosides	1(0.7)	1(0.8)	0	1
Fluoroquinolone	21(15.6)	17(14.3)	4(25.0)	0.458
Tetracycline	2(1.5)	1(0.8)	1(6.3)	0.119
Macrolides	1(0.7)	1(0.8)	0	1
Metronidazole	18(13.3)	16(13.4)	2(12.5)	1
Glycopeptide	10(7.4)	8(6.7)	2(12.5)	0.749
Tigecycline	4(3.0)	3(2.5)	1(6.3)	0.968
Notes: Data are expressed as no. (%) unless specified otherwise. SD: standard deviation; IQR, interquartile range;				
ICU, intensive care unit.				
a. Bold represents statistical differences ($p < 0.05$).				

Strain types

As shown in Table 2, K1 and K2 were the most prevalent capsular serotypes, accounted for 31.9% (43/135) and 25.2% (34/135). Moreover, there were 26.7% (36/135) of strains that do not belong to the

most common serotypes of K1, K2, K5, K20, K54, K57. However, there was no statistical difference in the proportion of above 6 serotypes between non-survivor group and survivor group.

Table 2
Bacterial characteristics of hvKP in survivor and non-survivor groups.

Variables	**total(n = 135)**	**Survivors (n = 119)**	**Non-survivors (n = 16)**	**p-value** [a]
Capsule serotypes				
K1	43(31.9)	40(33.6)	3(18.8)	0.231
K2	34(25.2)	31(26.1)	3(18.8)	0.745
K5	4(3.0)	4(3.4)	0	1
K20	5(3.7)	4(3.4)	1(6.3)	1
K54	2(1.5)	1(0.8)	1(6.3)	0.562
K57	11(8.1)	9(7.6)	2(12.5)	0.848
Multi-locus sequence types				
ST23	37(27.4)	35(29.4)	2(12.5)	0.26
ST86	10(7.4)	10(8.4)	0	0.607
ST65	7(5.2)	7(5.9)	0	1
ST412	6(4.4)	5(4.2)	1(6.3)	1
ST380	5(3.7)	5(4.2)	0	1
ST29	5(3.7)	4(3.4)	1(6.3)	1
Allantoin metabolism				
alls	48(35.6)	44(37.0)	4(25.0)	0.508
Adhesin				
fimH	125(92.6)	109(91.6)	16(100.0)	0.607
mrkD	127(94.1)	113(95.0)	14(87.5)	0.534
Biosynthesis of lipopolysaccharide				
ycfM	133(98.5)	117(98.3)	16(100.0)	1
wabG	105(77.8)	94(79.0)	11(68.8)	0.355
Fucose synthesis				

Notes: Data are expressed as no. (%).

a. Bold represents statistical differences ($p < 0.05$).

Variables	total(n = 135)	Survivors (n = 119)	Non-survivors (n = 16)	p-value [a]
wcaG	100(74.1)	92(75.6)	8(50.0)	0.26
Mucoviscosity				
magA	44(32.6)	41(34.5)	3(18.8)	0.33
rmpA	118(87.4)	107(89.9)	11(68.8)	**0.017**
rmpA2	99(73.3)	90(75.6)	9(56.3)	0.1
Siderophore				
entB	134(99.3)	118(99.2)	16(100.0)	1
kfu	68(50.4)	60(50.4)	8(50.0)	0.975
iroN	83(61.5)	69(57.9)	14(87.5)	**0.023**
irp-1	116(85.9)	103(86.6)	13(81.3)	0.849
irp-2	116(85.9)	103(86.6)	13(81.3)	0.849
iutA	104(77.0)	93(78.2)	11(68.8)	0.401
ybtS	93(68.9)	82(68.9)	11(68.8)	0.99
β-Lactamase production				
$bla_{CTX-M-1}$	26(19.3)	22(18.5)	4(25.0)	0.777
bla_{SHV}	18(13.3)	16(13.4)	2(12.5)	1
bla_{TEM}	15(11.1)	13(10.9)	2(12.5)	1
bla_{KPC}	5(3.7)	4(3.4)	1(6.3)	1
Notes: Data are expressed as no. (%).				
a. Bold represents statistical differences ($p < 0.05$).				

MLST analysis were implemented on the all 135 hvKP strains and 41 different STs were detected. Overall, ST23 was the most prevalent ST in the study(27.4%, 37/135), followed by ST86(7.4%, 10/135), ST65□5.2%, 7/135□, ST412□4.4%, 6/135□, ST380(3.7%, 5/135), ST29(3.7%, 5/135). The above 6 STs accounted for 51.9% (70/135). Univariate analysis showed no statistical difference in the proportion of the above 6 STs between the dead and the survivors.

Virulence factors

Over 90 percent of strains harbored *ycfM*, *fimH*, *mrkD*, *entB*, accounting for 98.5%, 92.6%, 94.1%, 99.3% respectively. Notably, the incidence of *iroN* in hospitalized deaths was much higher than in survivors (87.5% versus 57.9%, p = 0.023). In contrast, the incidence of *rmpA* was higher in survivors than the dead (68.8% versus 89.9%, p = 0.017). whereas, our results did not find significant differences in the frequency of other virulence genes between the two groups (Table 2).

Antimicrobial susceptibility and resistance mechanism

Univariate analyses of prognostic factors for mortality related to antimicrobial resistance are presented in Table 3. We found that the hvKP strains isolated from non-survivor group exhibited higher rates of antimicrobial resistance for all antibiotics than the hvKP isolated from survivor group. Among them, the resistance rates for ampicillin/sulbactam, cefepime and aztreonam in non-survivor group were significantly higher from those in survivor group (37.5%versus15.1%, p = 0.028; 25%versus5.9%, p = 0.033; 25%versus7.6%, p = 0.049 respectively). Surprisingly, the frequency of β-Lactamase production exhibited no significant difference between the two groups. However, our study has identified CR-hvKP from 5 patients and all these strains harbored *Klebsiella pneumoniae* carbapenemase (KPC) gene. It is worth noting that a patient infected with KPC-producing CR-hvKP died of septic shock during hospitalization.

Table 3
Antimicrobial resistance of hvKP in survivor and non-survivor groups.

Antimicrobial agents	total(n = 135)	Survivors (n = 119)	Non-survivors (n = 16)	p-value [a]
Piperacillin / tazobactam	5(3.7)	3(2.5)	2(12.5)	0.201
Ampicillin/sulbactam	24(17.8)	18(15.1)	6(37.5)	**0.028**
Cefotetan	4(3.0)	3(2.5)	1(6.3)	0.4
Cefuroxime	16(11.9)	14(11.8)	2(12.5)	1
Ceftriaxone	19(14.1)	15(12.6)	4(25.0)	0.339
Ceftazidime	11(8.1)	8(6.7)	3(18.8)	0.244
Cefoperazone / sulbactam	4(3.0)	3(2.5)	1(6.3)	0.968
Cefepime	11(8.1)	7(5.9)	4(25.0)	**0.033**
Cefoxitin	11(8.1)	9(7.6)	2(12.5)	0.848
Ertapenem	5(3.7)	3(2.5)	2(12.5)	0.201
Imipenem	5(3.7)	3(2.5)	2(12.5)	0.201
Meropenem	4(3.0)	3(2.5)	1(6.3)	0.968
Tobramycin	5(3.7)	3(2.5)	2(12.5)	0.201
Amikacin	4(3.0)	2(1.7)	2(12.5)	0.107
Levofloxacin	9(6.7)	6(5.0)	3(18.8)	0.126
Aztreonam	13(9.6)	9((7.6)	4(25.0)	**0.049**
Sulfamethoxazole	19(14.1)	15(12.6)	4(25.0)	0.339
Minocycline	15(11.1)	13(10.9)	2(12.5)	1
Furazolidone	5(3.7)	4(3.4)	1(6.3)	0.473
Tigecycline	6(4.4)	5(4.2)	1(6.3)	0.538
Notes: Data are expressed as no. (%).				
a. Bold represents statistical differences ($p < 0.05$).				

Prognostic factors

Based on the study of the clinical characteristics of patients and microbiological characteristics of the strains, the variables in univariate analysis including age, admission to ICU, drug resistance to cefepime,

aztreonam, ampicillin / sulbactam, and harboring *iroN*, *rmpA* gene were enrolled into the multivariate logistic regression analysis. The results displayed in Table 4 that admission to ICU and harboring *iroN* gene were independent prognostic factors for in-hospital mortality of hvKP infection (Hosmer Lemeshow test, P = 0.799).

Table 4
Prognostic factors associated with in-hospital mortality of hvKP infection.

	Univariable analysis		**Multivariable analysis**	
Variables	OR (95%CI)	P-value	OR (95%CI)	P-value [a]
Age	NA	0.082		
Admission to ICU	4.185(1.362–12.862)	0.008	3.452(1.052–11.329)	**0.041**
Resistant to Cefepime	5.333(1.362–20.886)	0.033		
Resistant to Aztreonam	4.074(1.088–15.250)	0.049		
Resistant to Ampicillin/sulbactam	3.367(1.088–10.417)	0.028		
iroN	5.072(1.103–23.324)	0.023	9.278(1.654–52.035)	**0.011**
rmpA	0.247(0.073–0.831)	0.017		
Notes: OR, odds ratio; CI, confidence interval; NA, not available; ICU, intensive care unit.				
a. Bold represents values that are significant (P < 0.05).				

Discussion

Considering that hvKP tend to cause severe invasive diseases and thus elevate mortality, it is necessary to investigate the prevalence and mortality of hvKP and further reveal the predictive factors affecting prognosis. It is not difficult to seen from Table 5 that the prevalence of hvKP in Asia is much higher than other regions. The prevalence and mortality rates of hvKP vary greatly depending on the different region and definition criteria. The prevalence of hvKP infection in our study is 22%, which is slightly lower than the 24.5–47.5% previously reported in China, but much higher than the 6–21% reported in other countries except China[7, 21–23]. Our consecutive study revealed that the in-hospital mortality rate for hvKP infection was 11.9%, slightly lower than the 17.5% reported in a previous retrospective study in Beijing that also defined hvKP in terms of string test and *aerobactin*, but far exceeds the in-hospital mortality of 2.3% reported in another study conducted in 10 cities of China[9, 19].

Table 5
The prevalence and mortality of hvKP in different region.

Region	Time period of collection	Infection source	Definition of hvKP	Prevalence of hvKP	Mortality rate	Reference
Beijing, China	2008.6-2012.4	Blood	string test	31.4% (22/70)	4.5% [a] (1/22)	[3]
Beijing, China	2010.4-2012.6	Blood, urine, sputum, ascites, bile, abscess fluid	string test	33% (29/88)	13.8% [a] (4/29)	[33]
Beijing, China	2008.6-2017.7	Sputum, abscess fluid, blood, urine	*aerobactin*	47.5% (96/202)	16.7% [b] (16/96)	[23]
Beijing, China	2008.1-2014.1	Sputum, abscess fluid, blood, urine	*aerobactin* and string test	45.7% (80/175)	17.5% [c] (14/80)	[19]
Beijing, China	2008.11-2017.12	sputum	*aerobactin*	46.6% (34/73)	41.2% [b] (14/34)	[8]
Nanjing, China	2015.9-2016.12	Blood	*rmpA* and *icuA*	24.5% (35/143)	37.1% [b] (13/35)	[22]
10 cities of China	2013.2-2013.7	Sputum, abscess fluid, blood	*aerobactin*	37.8% (87/230)	2.3% [a] (2/87)	[9]
Chongqing, China	2018.2-2019.6	Blood, urine, sputum, ascites, bile, abscess fluid	*aerobactin* and string test	22% (135/613)	11.9% [a] (16/135)	This study
Taiwan, China	1999.1-2001.6	Sputum, abscess fluid, blood, urine, CSF	string test	41.5% (83/200)	26.9% [d] (21/83)	[34]
Canada	2001.1-2007.12	Sputum, abscess fluid, blood, urine, bile and others	string test	7.5% (10/134)	NA	[35]
Japan	2012.1-2018.4	Sputum, bile, abscess fluid, urine	string test	21% (24/114)	29.2% [a] (7/24)	[21]

CSF: cerebrospinal fluid; NA: not available.

a.in-hospital mortality; b. 30-day mortality; c. 28-day mortality; d.14-day mortality.

Region	Time period of collection	Infection source	Definition of hvKP	Prevalence of hvKP	Mortality rate	Reference
Japan	2013.12-2014.3	Blood	One of *rmpA, rmpA2, iroN, icuA, iutA*	18.6% (26/140)	7.7% [a] (2/26)	[36]
Spain	2007–2013	Blood	string test	6% (53/878)	17% [c] (9/53)	[7]
CSF: cerebrospinal fluid; NA: not available.						
a.in-hospital mortality; b. 30-day mortality; c. 28-day mortality; d.14-day mortality.						

To our best knowledge, this is the first study to explore the prognostic factors for in-hospital mortality of hvKP infection which was defined by positive for *aerobactin* and string test. siderophores as a secondary metabolite of bacteria, breaks the host restriction on the acquisition of free iron by bacteria[9, 24]. In addition to this traditional role, siderophores also play an crucial role in promoting the dissemination of bacteria, defensing against the host's immune function of neutrophils and regulating production of virulence genes[25–27]. HvKP secrets 4 kinds of siderophores, which are enterobactin, salmochelin, yersiniabactin and aerobactin. Salmochelin was first identified as a C-glucosylated enterobactin produced by Salmonella enterica and uropathogenic *Escherichia coli* strains and other *Enterobacteriaceae*, including *K. pneumoniae*[28]. Importantly, this modification prevents salmochelin from being bound by lipocalin-2, which is capable of specifically sequestering the siderophore enterobactin with the highest affinity for iron, allowing salmochelin to suck more iron from its host[29]. In a recent analysis of 2733 genomes of the *K. pneumoniae* complex, it was found that the trend of simultaneous existence of *iro* and *iuc* loci encoding aerobactin and salmochelin synthesis was obvious, indicating the importance of salmochelin to *K. pneumoniae*[30]. The *iroN* gene encodes the outer-membrane receptor of salmochelin[28]. Max et al reported that *iroN* contributed to the formation of biofilm of extraintestinal pathogenic *Escherichia coli*[31]. Moreover, the *iroN* was located on a large plasmid termed as pLVPK, which was demonstrated crucial to the pathogenicity of hvKP[16, 32]. Taken together, the above findings suggest that *iroN* is independent prognostic factor for increased in-hospital mortality of hvKP infection.

The increased in-hospital mortality associated with admission to ICU could be explained by the following aspects. First, the *iroN* gene showed a higher positive rate in patients with a history of ICU hospitalization (OR = 3.101, 95% CI = 1.428–6.734; P = 0.004). Second, patients admitted to ICU preferred to exhibit multiple resistance to ampicillin / sulbactam, ceftazidime, cefepime, amikacin, aztreonam, which is bound to lead to inappropriate empirical treatment. In addition, more severe underlying illness and more fragile immune defenses in ICU patients, as well as the increase in invasive procedures, can be elements with fatal outcomes. Previous study has reported mortal outcomes in patients with an outbreak of hvKP infection admitted to the ICU[17]. To sum up, Patients admitted to ICU intend to have more devastating infection, coupled with inappropriate empirical treatment, so it is not surprising for the enhanced in-hospital mortality rate.

Unexpectedly, the resistance mechanism of β-lactamases and carbapenem did not exhibit a significant association with mortality, which possibly due to the limited number of the deceased or the existence of other resistance genes that were not detected in this study. However, our findings reported a case that infected with KPC-producing CR-hvKP died of septic shock during hospitalization. More worryingly, it shares the KPC-2 gene with four other strains of CR-hvKP. This must alert medical staffs to the possibility of horizontal transmission of resistant plasmids in hvKP.

The generalizability of these results is subject to certain limitations. First, in light of hvKP was defined as positive for string test and aerobactin in this study and the sample size was relatively small, the results of this study may not be applicable to other set of studies. Second, the results of this study were based on a retrospective analysis and data statistics, prospective studies and animal experiments were urgently needed to verify the prognostic factors of hvKP infection. Nevertheless, our findings reveal the prevalence and in-hospital mortality of hvKP in southwestern China, and systematically analyze the prognostic factors of death during hospitalization, which provides some predictive factors for clinicians to identify patients with poor prognosis in advance.

Conclusions

The prevalence and in-hospital mortality attributable to hvKP infection defined by positive for aerobactin and string test was 22% and 11.9%, respectively. ICU admission and harboring *iroN* may be independent predictors of in-hospital mortality of hvKP. Given the relatively high in-hospital mortality of hvKP infection, early surveillance and better management are necessary for patients admitted to ICU and infected with hvKP harboring *iroN* gene.

Abbreviations

ICU
Intensive Care Unit; cKP:classic *Klebsiella pneumoniae*; hvKp:hypervirulent *Klebsiella pneumoniae*; CR-hvKP:Carbapenem-resistant hypervirulent *Klebsiella pneumoniae*; pLVPK:pK2044-like plasmid; KPC:*Klebsiella pneumoniae* carbapenemase; OR:odds ratio; CI:confidence interval.

Declaration

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Authors' contributions

YT and HL participated in the study design and writing of the manuscript. YT conducted experiments. JXZ, MY and YLY collected the data. JQZ, YZC and DWZ analyzed the data. YX revised the manuscript

critically for important intellectual content. All authors contributed to the interpretation of the data, critically reviewed the manuscript, and approved the final submission.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Evaluation Committee and the Biomedical Ethics Committee of Chongqing Medical University. No written informed consent was acquired due to the retrospective and anonymous nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

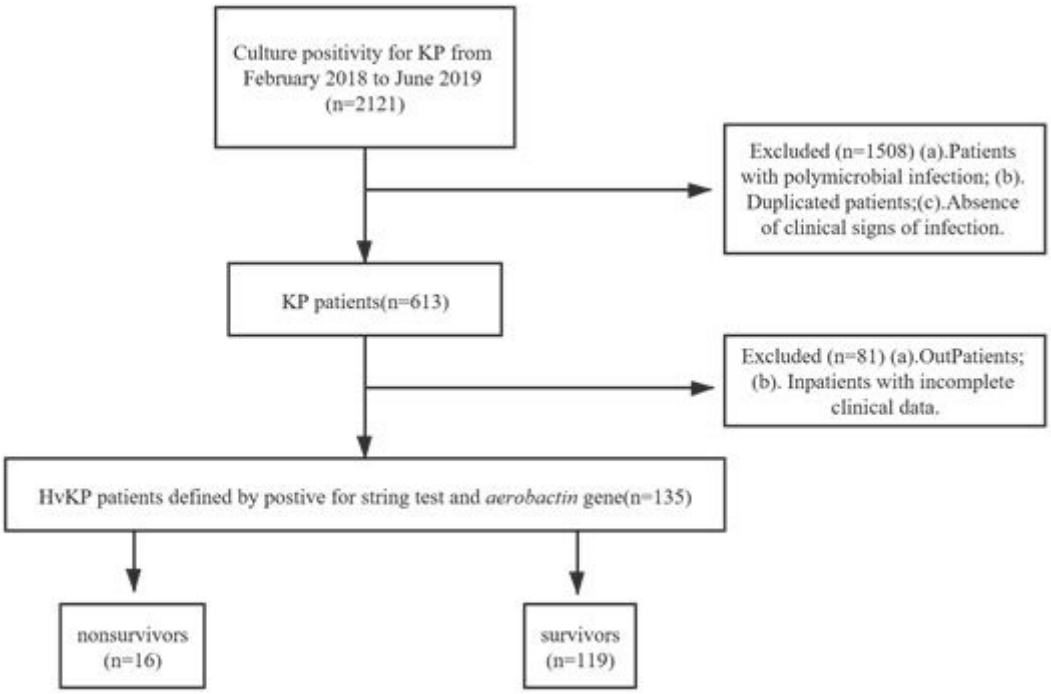

Figure 1

. Flow chart for study inclusion. KP: Klebsiella pneumoniae; HvKP: hypervirulent Klebsiella pneumoniae;

Supplementary Files

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