Emergence of KPC-2-Producing ST11-K64 Hypervirulent *Klebsiella Pneumoniae* for the Elderly in Intensive Care Unit: Antimicrobial Resistance, Molecular and Clinical Characteristics

Tian Wei

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine <https://orcid.org/0000-0002-2398-0326>

Cheng-yun Zou

Shanghai University of Traditional Chinese Medicine

Jie Qin

Shanghai University of Traditional Chinese Medicine

Jian-min Tao

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine

Li Yan

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine

Jiang-jun Wang

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine

Hong Du

Second Affiliated Hospital of Soochow University

Fang Shen

Fifth People's Hospital of Shanghai Fudan University

Yan-qin Zhao

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine

Hai-ying Wang (✉ liang19961111@126.com)

Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine

Research

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Abstract

Background: In recent years, carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) has been increasingly reported and poses severe therapeutic challenge to global public health, but has not yet been systematically studied in elderly patients. This study aimed to investigate the clinical and molecular characteristics and risk factors of CR-hvKP infections in elderly patients.

Methods: We retrospectively investigated elderly patients with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infections in intensive care unit (ICU), between January 2020 and December 2020, the clinical data being collected from medical records, and microbiological data including antimicrobial susceptibility testing, phenotype experiment, carbapenemases production, string test, virulence gene, capsular serotype-specific (cps) genes and multilocus sequence typing, of the CR-hvKP group defined by the presence of some combination of *rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344* was compared with those of carbapenem-resistant non-hypervirulent *Klebsiella pneumoniae* (CR-non-hvKP) strains.

Results: Of 80 CRKP strains, 51 (63.8%) met the definition of CR-hvKP and 7 of them had all five of the virulence genes tested. The main mechanism of resistance to carbapenems found in CR-hvKP strains was the presence of the *bla_{KPC-2}* gene. There was no statistical significance in the resistance rates of antimicrobial agents between the CR-hvKP and CR-non-hvKP subgroups ($p \geq 0.05$). Compared with the minimum inhibitory concentration (MIC) 50 values of CR-non-hvKP group, those of antimicrobials, including ceftazidime, ceftazidime/avibactam, imipenem/avibactam, tigecycline, levofloxacin, cefoperazone-sulbactam, exhibited higher levels in the CR-hvKP group. Avibactam (4 µg/mL) significantly decreased the MIC₉₀ values more than sixteen-fold than that of ceftazidime and imipenem alone against KPC-2-producing *Klebsiella pneumoniae*. K64 and ST11 were highly prevalent and strongly associated with CR-hvKP ($P < 0.05$). Cardiovascular disease (odds ratio [OR] = 11.956) and ST11-K64 (OR = 8.385) appeared to be independent variables associated with CR-hvKP infection by multivariate analysis.

Conclusion: The CR-hvKP strains showed higher MIC₅₀ values than CR-non-hvKP strains. KPC-2-producing ST11-K64 CR-hvKP is emerging, which might become new significant “superbugs” and a threat to elderly patients.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*), an important community-acquired and nosocomial pathogen, especially in the intensive care unit (ICU), causes widespread infections including pneumonia, bacteremia, urinary tract infection, endophthalmitis, liver abscesses and sometimes even life-threatening septic shock. Two clinical pathotypes: hypervirulent *Klebsiella pneumoniae* (hvKP) and carbapenem resistant *Klebsiella pneumoniae* (CRKP) often cause life-threatening infections^[1]. In the conventional sense, hvKP is seldom resistant to antibiotics, however, because of virulence genes and antibiotic resistance genes transmitted by mobile genetic elements, increasing researches show that carbapenem-resistance hypervirulent *klebsilla pnemoniae* (CR-hvKP) strains are widely found^[2-4]. In China, the prevalence of CR-hvKP infections is 0% ~ 25.8%, with Henan and Shandong being the most serious^[5]. The emergence of CR-hvKP could lead to the next clinical crisis, has attracted worldwide attention^[2].

During the last decade, the global spread, of CRKP posed a severe challenge to public health, particularly in China. According to the China Antimicrobial Resistance Surveillance System report^[6], most CRKP strains were isolated from ICU, and were common in the elderly. The main resistant mechanism for CRKP in China is the production of KPC-2 carbapenemase, and ST11 is the dominant sequence type (ST)^[7]. A recent genomic analysis showed that ST11 classic CRKP strains can acquire the pLVPK-like plasmid and become CR-hvKP strains^[2].

In the past, the definition of hvKP relied on clinical manifestations and phenotypes, which proven to have poor specificity and sensitivity^[8]. The *rmpA*, *rmpA2*, *iucA*, *iroN* and *peg-344* genes on virulence plasmids can be used for the identification of hvKP and classic KP(cKP) strains with pinpoint accuracy^[8]. The emergence of CR-hvKP will be a great challenge for clinician and result in adverse outcome. So far, researches on CR-hvKP in elderly patients remain limited ^[5]. Therefore, a cognitive of the risk factors of infections, microbiological and molecular characteristics in CR-hvKP will provide important insights into the control and treatment for CR-hvKP infections among the elderly patients.

In response to this emerging problem, a retrospective study in elderly patients in a Chinese hospital was conducted, the aim was to advance our cognitive of the clinical characteristics, risk factors, hypervirulence and epidemiology of CR-hvKP infections based on newly recognized biomarkers.

Methods

Bacterial strains and identification

We collected clinical CRKP strains from patients over sixty-five years old between January 2020 and December 2020 from ICU in Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, and the capacity of the hospital is more than 1200 beds. All the strains were collected consecutively and non-duplicated, only the first strain was collected from the same patient. All the *K. pneumoniae* identification and the drug resistance of imipenem and ertapenem were detected by Vitek 2 system (bioMérieux, France) and further species identification was confirmed with MALDI-TOF MS system (Bruker Daltonics, USA), the drug resistance of meropenem was tested by using a Kirby-Bauer disk diffusion susceptibility test. CRKP was defined as that resistant to either imipenem, meropenem or ertapenem ^[9] in accordance with the breakpoints of 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines^[10]. We collected all clinical information for CRKP positive patients from electronic medical records, including basic demographics, underlying diseases, clinical presentations, exposure to hospital care, treatment process, and outcomes.

Antimicrobial Susceptibility Testing

The Antimicrobial Susceptibility Testing (AST) for the CRKP strains were further performed by the broth microdilution assay (BIO-NONT, China). The antibiotics included ceftazidime, ceftazidime/avibactam, cefepime, aztreonam, piperacillin/tazobactam, imipenem, imipenem/

avibactam, meropenem, amikacin, polymyxin B, tigecycline, levofloxacin, cefoperazone/sulbactam. The AST breakpoints of 2020 CLSI guideline were adopted^[10] with exception of tigecycline and polymyxin B. The

breakpoint of tigecycline was based on the US Food and Drug Administration (FDA) standard^[11], and the breakpoint of polymyxin B was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard^[12]. The breakpoints of imipenem/avibactam and cefoperazone/sulbactam were referred to imipenem and cefoperazone in CLSI, respectively^[13-14]. The standard strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC700603 were used as quality control strains in each experiment.

DNA extraction

The strains were inoculated into the blood agar plate and incubated overnight at 35°C. The colonies were suspended in 500ml sterile distilled water. The suspension was boiled at 100°C for 10 minutes, centrifuged at 10000rpm for 13 minutes. The supernatant was used as a template for DNA reaction. The DNA was stored at -35°C.

Phenotype experiment and detection of Carbapenemases

We used phenylboronic acid (PBA) and ethylenediamine tetra-acetic acid (EDTA)^[15] comparing with imipenem to detect the phenotypes of KPC β -lactamase and metal β -lactamase (MBLs), respectively. In both tests, compared with imipenem alone, the diameter of the inhibition zone has increased $>$ or $=$ 5 mm by PBA or EDTA, which is considered to be a positive combined-disc test result that shows the presence of KPC β -lactamase or MBL enzyme, respectively. The presence of carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP} and *bla*_{OXA-48}) were detected by PCR as previously described^[16-17]. The primers are listed in Supplementary table 1. The PCR amplified products were sequenced (Sangon Biotech, Shanghai) and the results were compared with the BLAST searches in the NCBI Genbank.

Detection of virulence-associated features and capsular serotype-specific (cps) genotyping

Hypermucoviscosity as a virulence factor was identified by the string test. If a viscous string longer than 5mm is generated by stretching a bacterial colony on blood agar plate with a bacteriology inoculation loop, the string test is positive^[18]. Five virulence genes including *rmpA*, *rmpA2*, *iucA*, *iroB*, *peg-344*^[8,19-20] and eight capsular serotype-specific (cps) genes (K1, K2, K5, K20, K47, K54, K57 and K64)^[20-23] were identified by polymerase chain reaction (PCR) as previously described. The primers are listed in Supplementary table 1. The CRKP strain which detected with any one of the five virulence genes was defined as CR-hvKP.

Multilocus Sequence Typing (MLST)

Seven housekeeping genes (*gapA*, *mdh*, *phoE*, *tonB*, *infB*, *pgi* and *rpoB*) were performed according to the protocol, which were described in online databases. (<https://bigsd.b.pasteur.fr/klebsiella/>

Primers_used.html). The PCR amplified products were sequenced (Sangon Biotech, China), the results were compared by the use of this website.

Statistical analysis

IBM SPSS Statistics version 19.0 was used for statistical analysis. The χ^2 or Fisher's exact test were used for categorical variables. $P < 0.05$ was considered statistically significant. To further identify variables associated

with CR- hvKP infections, Logistic regression was used. All variables with $P < 0.05$ were included in the multivariate analysis.

Results

CRKP strains and Virulence-associated features

A total of 80 CRKP strains isolated from 80 patients in ICU were included in the study. The percentages of 80 CRKP strains isolated from respiratory tract, urine, blood, pus and puncture fluid were 72.5%, 15.0%, 6.3%, 5.0% and 1.3%, respectively. CRKP strains were divided as CR-hvKP and carbapenem-resistant non-hypervirulent *Klebsiella pneumoniae* (CR-non-hvKP) based on whether they contained virulence genes. The detection of the presence of virulence genes showed that 51 (63.8%) of 80 CRKP strains were positive, suggesting carrying virulence plasmids, which were designated as CR-hvKP group, while the remaining 29 strains were designated as CR-non-hvKP group.

Among the five virulence-associated genes, *rmpA2*, *iucA*, *iroN*, *peg-344* and *rmpA* harboured by 51 strains (Figure 1), the most common virulence gene was *rmpA2*, the prevalence was 90.2% (46/51), followed by *iucA*, *iroN*, *peg-344* and *rmpA*, the detection rate were 88.2% (45/51), 27.5% (14/51), 21.6% (11/51) and 19.6% (10/51), respectively. Seven (13.7%) strains had all five of the virulence genes, which indicate that the full length of the virulence plasmid pLVPK could be harbored by these strains. The results of the string test showed that 41.2% (21/51) CR-hvKP strains showed negative results in string test, whereas the results of the 17 CR-non-hvKP strains were positive, accounting for 58.6% (17/29).

For the eight serotypes closely related to hvKP, 65 (81.3%) strains were positive for K47, K54, or K64 serotypes, additionally, there is no strain with K1, K2, K5, K20 or K57 serotypes in the present study. Serotype K64 was dominated serotype of CRKP strains [62 (77.5%) of 80 strains]. The frequency of K64 type (86.3% vs. 62.1%, $P = 0.013$) identified in CR-hvKP strains was significantly higher than that of CR-non-hvKP strains. K-non-typable (34.5% vs. 9.8%, $P = 0.007$) was strongly associated with CR-non-hvKP. Only two strains were identified as K47 or K54 (Table 1).

Susceptibility results and Carbapenemase resistance Genes

The antimicrobial susceptibility profiles of the CR-hvKP and CR-non-hvKP strains were listed in Table 2. According to the AST results, all strains were resistant to imipenem and meropenem with exception of one CR-hvKP strain being susceptible to meropenem, and showed high level resistance to cephalosporins, monobactam, β -lactams/ β -lactamase inhibitor combinations, fluoroquinolones, but were almost not resistant to ceftazidime/avibactam (2.0% vs. 3.5%), imipenem/avibactam (3.9% vs. 3.5%), polymyxin B (0.0% vs. 17.2%) and tigecycline (0.0% vs. 0.0%). Ceftazidime and imipenem by combining avibactam (4 μ g/mL) significantly decreased the MIC₉₀ values more than sixteen-fold than that of ceftazidime and imipenem alone against KPC-2-producing *Klebsiella pneumoniae*. Although we found there were no statistical significance in the resistance rates of antimicrobial agents between the two subgroups ($p \geq 0.05$), except polymyxin B ($P = 0.002$). The MIC₅₀ values of the following antibiotics of the CR- hvKP strains increased two-fold compared with those of CR-non-hvKP: ceftazidime (>128 vs. 128 μ g/mL), ceftazidime/avibactam 4/4 vs. 2/4 μ g/mL, imipenem/avibactam

(0.5/4 vs. 0.25/4 µg/mL), tigecycline (2 vs. 1µg/mL), levofloxacin (64 vs. 32µg/mL), cefoperazone/sulbactam (>256/128 vs. 256/128µg/mL).

According to phenotypic experiment results and detection of carbapenemase genes, all CR-hvKP and 96.6% (28/29) CR-non-hvKP strains showed only produced *bla*_{KPC-2} carbapenemase gene and the presence of KPC - lactamas. Only one CR-non-hvKP strains showed the presence of MBL enzyme, harbored *bla*_{IMP-4}.

MLST genotyping

MLST analysis revealed that ST11 and ST15 were the most common STs in CRKP strains, and ST198 and ST438 were distributed in one strain of CR-hvKP strain and one strain of CR-non-hvKP strain. The frequency of ST11 type (88.2% vs. 69.0%, $P=0.034$) identified in CR-hvKP strains was significantly higher than that of CR-non-hvKP strains, however, the proportion of ST15(9.8% vs.27.6%, $P=0.039$) strains was lower (Table 1). The strains were divided into several clones by combination of capsular genotypes and STs. We found that ST11-K64 CRKP strains (86.3% vs.58.6%, $P=0.005$) were strongly associated with CR-hvKP, while ST15- K-non-typable (7.8%vs. 27.6%, $P=0.017$) strains were correlated with CR-non-hvKP (Table 1).

Clinical factors for CR-hvKP infection of elderly patients

Demographic and clinical factors of patients with CR-hvKP and CR-non-hvKP infections are summarized in Table 1. The age, gender, antibiotic exposure, hospitalization within the last 90 days, or length of stay from CRKP isolation to outcome (In-hospital mortality or discharge) didn't have significant difference between CR-hvKP and CR-non-hvKP ($P>0.05$). In addition, there was no statistical difference among most underlying conditions such as pulmonary disease, diabetes mellitus, hypertension, cerebrovascular diseases, malignancy, hypoproteinemia and liver abscess ($P>0.05$), except cardiovascular disease (60.8% vs.17.2%, $P<0.001$). Gastric tube was more prevalent in CR-non-hvKP group than in CR-hvKP group (96.6% vs.80.4%, $P=0.045$), whereas the other invasive procedures and devices had no significant difference. Most of the outcomes between the two groups ($P>0.05$) had no statistical difference, the frequency of change of initial antibiotics due to clinical worsening was higher in CR-hvKP group than CR-non-hvKP group (58.8% vs.34.5%, $P=0.036$) (Table 1).

Risk factors of CR-hvKP infection for the elderly patients

Nine variables with $P<0.05$ in univariate analysis were included in the multivariate analysis, including K64, K-non-typable, ST11, ST15, ST11-K64, ST15-K-non-typable, cardiovascular disease, gastric tube, change of initial antibiotics due to clinical worsening. The results showed that ST11-K64(odds ratio [OR] =8.385; $P=0.003$) and cardiovascular disease (OR=11.956; $P<0.001$) were independent predictors of CR-hvKP infections for elderly patients.(Table 3).

Discussion

More than 30 years ago, a report first described hvKP in China^[24], in recent years, reports on CR-hvKP have increased gradually, which poses a greater challenge to clinical treatment due to its high drug resistance and high virulence^[5]. So far a limited number of data based on the microbiological and molecular characteristics in

the elderly can be searched. According to the results of China's census in 2021, elderly patients over 65 years old account for 13.5% of the total population^[25], China has entered an aging society. As is known to all, ICU is a relatively popular setting for CRKP infections in hospitals and due to low immune function and cumulative basic diseases, the elderly patients are more prone to nosocomial infections. In this study, we focused on elderly patients hospitalized in ICU, reported antimicrobial resistance, molecular and clinical characteristics of CR-hvKP, and found the main CR-hvKP clone KPC-2-producing ST11-K64 among elderly patients.

It is worth noting that the main cause of liver abscess is hvKP infection^[26–27], however, we found that the main specimen type of both CR-hvKP strains and CR-non-hvKP strains isolated from was sputum, and only 7.8% (4/51) patients with CR-hvKP infections had symptoms of liver abscess, suggesting that CR-hvKP can also invade other parts of the body^[28]. The production of carbapenemases is the most common mechanism of drug resistant in CRKP strains^[29], KPC-2 was the most frequently identified among CR-hvKP strains^[2]. As reported in previous studies^[30], the CRKP strains were resistant to most antibiotics with exception of ceftazidime-avibactam, imipenem/ avibactam, polymyxin B and tigecycline, only one CR-non-hvKP strain which harbored *bla*IMP-4 displayed resistance to ceftazidime/avibactam, this is consistent with the fact that avibactam is inactive against MBL producing strains^[31]. Avibactam, as the most effective β -lactamase inhibitor^[32], significantly increased the activity of ceftazidime and imipenem against KPC-producing *K. pneumoniae* in vitro, which decreased the MIC₉₀ values of ceftazidime and imipenem more than sixteen-fold. We also identified one KPC-2-producing strain exhibited resistant to ceftazidime/ avibactam, with MIC value 16 ug/mL, it might due to OmpK35/36 defects, *bla*KPC-2 point mutation and high KPC expression^[33–34]. We found a phenomenon that deserves special attention, namely, compared with CR-non-hvKP strains, CR-hvKP strains exhibited high level MIC₅₀ values, especially ceftazidime/avibatam, imipenem/avibatam, tigecycline, which are our last line in treating CRKP-infected patients.

Previously, a positive string test was used as screening test for hvKP, the definition of hvKp is controversial. so we employed five virulence genes, including two regulator genes which have been associated with hypermucoviscous phenotype (*rmpA* and *rmpA2*), siderophore aerobactin (*iucA*) and salmochelin (*iroB*)^[5] and the putative metabolite transporter *peg344*^[35] attribute to the hypervirulent phenotype of *K. pneumoniae*. We found 51 strains which defined as CR-hvKP harbored a part of combination of these markers, *rmpA2* and *icuA* were the most common virulence genes in our study^[36]. In term of the thick polysaccharide capsules encoded by the *cps*^[1], unlike carbapenem-sensitive hypervirulent *K. pneumoniae* which belong to serotypes K1, K2, or K57^[37], the most popular CR-hvKP clone was K64 in our study^[38]. The most predominant MLST type of the 80 strains was ST11^[39]. Notably 86.3% (44/51) CR-hvKP strains belonged to ST11-K64 clone, which was an independent predictor factor for CR-hvKP infections, being more resistant and exhibited elevated virulence, leading to a high mortality and transmissibility in infected patients^[5,40–41]. Since all the strains were collected from the same ward ICU, we speculated clonal dissemination of KPC-2-producing ST11-K64 *K. pneumoniae* existed, and during the process of transmission, KPC-2-producing ST11-K64 *K. pneumoniae* acquire of a PLVKP-like virulence plasmid, leading to KPC-2-producing ST11-K64 CR-hvKP. ZHANG et al^[42] also provided evidence that ST11-K64 *K.pneumoniae* may be a competent host strain for a hypervirulent plasmid. The emergence of KPC-2-producing ST11-K64 CR-hvKP might pose a serious challenge to the management of elderly patients infected with these strains in the future, as these strains contain both drug resistance and virulence genes and show higher resistance than CR-non-hvKP strains^[32].

In order to take appropriate intervention measures correctly, a better understanding of risk factors is essential. Univariate analysis showed that change of initial antibiotics due to clinical worsening was strongly associated with CR-hvKP. This reminds the individualized therapy must be used to treat CR-hvKP infections based on in vitro antimicrobial susceptibility profiles, molecular type, and the patient's health status^[43]. Cardiovascular disease was independent predictor to CR-hvKP infections. Patients with underlying diseases are more likely to develop hvKP infections^[44], such patients typically receive inappropriate empiric therapy, more frequent hospital exposure associated with severe comorbidities may result in exposure to CR-hvKP and possible infections^[45]. Meanwhile, older age and serious complications could reduce immunity and thus increase the risk of infection/settlement and even death^[46]. Clinicians should pay close attention to these risk factors in clinical practice to reduce emergence of CR-hvKP strains.

There were some limitations in our study. First, this is a one-year retrospective study conducted in a single center, not a multi-center, longitudinal molecular epidemiological study on CR-hvKP. Second, Pulsed field gel electrophoresis was not done in this study, we speculated nosocomial transmission based on MLST typing, capsule serotype and drug resistance genotype of the clinically strains.

Conclusions

CR-hvKP is emerging as a common pathogen in the elderly in China. Cardiovascular disease and ST11-K64 appeared to be independent variables associated with CR-hvKP infections. We firstly found the CR-hvKP strains showed higher MIC50 values than CR-non-hvKP strains. KPC-2-producing ST11-K64 CR-hvKP might become new significant “superbugs” and a threat to elderly patients.

Abbreviations

AST: antimicrobials susceptibility testing; BLAST: Basic Local Alignment Search Tool; cKP: classic *K. pneumoniae*; CLSI: Clinical and Laboratory Standards Institute; cps: capsular serotype-specific; CRKP: Carbapenem-resistant *Klebsiella pneumoniae*; CR-hvKP: carbapenem-resistant hypervirulent *Klebsiella pneumoniae*; CR-non-hvKP: carbapenem-resistant non-hypervirulent *Klebsiella pneumoniae*; PBA: Phenyloronic acid; EDTA: ethylenediamine tetra-acetic acid; *E. coli*: Escherichia coli. EUCAST: European Committee on Antimicrobial Susceptibility Testing; FDA: Food and Drug Administration; hvKP: hypervirulent *Klebsiella pneumoniae*; *K. pneumoniae*: *Klebsiella pneumoniae*; ICU: intensive care unit; KPC: *Klebsiella pneumoniae* carbapenemase; MBL: metal β -lactamase; MIC: Minimal Inhibitory Concentration; MLST: Multilocus sequence type; NCBI: National Center for Biotechnology Information; OR: odds ratio; PCR: Polymerase Chain Reaction; PFGE: Pulsed-field gel electrophoresis; SPSS: Statistical Package for the Social Sciences; ST: Sequence Type;

Declarations

Ethics approval and consent to participate

Informed consent was not needed due to retrospective nature of the study. The study was approved by the by the research Ethics Committee of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine Shanghai University of Traditional Chinese Medicine, Shanghai, China (approval number (2021)060).

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TW and HYW were responsible for study design and article writing. CYZ and JQ performed PCR and statistical analyses. JMT, LY and JJW collected the laboratory and clinical data. TW, HD, FS, YQZ and HYW performed critical data review. All authors read and approved the final version of the manuscript.

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Author details

*Correspondence: liang19961111@126.com

¹Department of Clinical Laboratory, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China.

² Shanghai University of Traditional Chinese Medicine, Shanghai, China.

³Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, China.

⁴Department of Clinical Laboratory, Shanghai Fifth People's Hospital, Fudan University, Shanghai, China.

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Tables

Table1. Microbiological and clinical characteristics of CR-hvKP strains

Characteristic	CR-hvKP(N=51)	CR-non-hvKP(N=29)	P value
Microbiological characteristics			
Capsular serotype			
k64	44(86.3)	18(62.1)	0.013
k47	1(2.0)	1(3.4)	0.684
K54	1(2.0)	0(0.0)	0.451
K-non-typable	5(9.8)	10(34.5)	0.007
MLST			
ST11	45(88.2)	20(69.0)	0.034
ST15	5(9.8)	8(27.6)	0.039
ST198	1(2.0)	0(0.0)	0.451
ST438	0(0.0)	1(3.4)	0.185
ST-capsular serotype			
ST11-K64	44(86.3)	17(58.6)	0.005
ST11-K47	1(2.0)	1(3.4)	0.684
ST11- K-non-typable	0(0.0)	2(6.9)	0.256
ST15-K54	1(2.0)	0	0.451
ST15- K-non-typable	4(7.8)	8(27.6)	0.017
ST198- K-non-typable	1(2.0)	0	0.451
ST438-K64	0	1(3.4)	0.185
Basic demographics			
Age—median	85.0 (75.0~88.0)	78.0(69.5~86.0)	0.085
Male	40(78.4)	20(69.0)	0.347
Underlying diseases			
Pulmonary disease	47(92.2)	28(96.6)	0.435
diabetes mellitus	26(51.0)	16(55.2)	0.718
Hypertension	30(58.8)	17(58.6)	0.986
Cerebrovascular Diseases	25(49.0)	16(55.2)	0.597
Cardiovascular disease	31(60.8)	5(17.2)	<0.001
Malignancy	4(7.8)	7(24.1)	0.087

Hypoproteinemia	10(19.6)	6(20.7)	0.907
liver abscess	4(7.8)	1(3.4)	0.648
Invasive procedures and devices			
Surgery within 1 month	4(7.8)	6(20.7)	0.097
Mechanical ventilation	41(80.4)	23(79.3)	0.907
Central venous catheter	35(68.6)	25(86.2)	0.081
Urinary catheter	42(82.4)	27(93.1)	0.907
Gastric tube	41(80.4)	28(96.6)	0.045
Antibiotic exposure			
Cephamycins	7(13.7)	5(17.2)	0.674
Penicillin	7(13.7)	6(20.7)	0.420
Cephalosporins	27(52.9)	16(55.2)	0.847
β -lactam/ β -lactamase inhibitors	33(64.7)	17(58.6)	0.589
Fluoroquinolones	28(54.9)	18(62.1)	0.533
Carbapenems	33(64.7)	22(75.9)	0.301
Tigecycline	27(52.9)	13(44.8)	0.485
Polymyxin B	3(5.9)	1(3.4)	0.633
Glycopeptides	12(23.5)	2(6.9)	0.06
Aminoglycosides	6(11.8)	4(13.8)	0.793
Fosfomycin	4(7.8)	3(10.3)	0.705
Hospitalization within last 90 days	22(43.1)	7(24.1)	0.089
Outcomes			
Clinical improvement without modification of initial treatment	8(15.7)	4(13.8)	0.821
Change of initial antibiotics due to clinical worsening	30(58.8)	10(34.5)	0.036
Bacterial clearance after 72h treatment	2(3.9)	2(6.9)	0.56
Persistent infection after 72h treatment	40(78.4)	17(58.6)	0.06
stable,discharged	25(49.0)	14(48.3)	0.949
In-hospital mortality	21(41.2)	15(51.7)	0.362
length of stay median	24(15~47)	22(16.5~34.5)	0.417

Table 2. Susceptibility of CR-hvKP and CR-non-hvKP strains against different antimicrobial agents

Antibiotic agents	CR-hvKP(n=51)			CR-non-hvKP(n=29)			P Value
	MIC50 (µg/mL)	MIC90 (µg/mL)	%R	MIC50 (µg/mL)	MIC90 (µg/mL)	%R	
Ceftazidime	>128	>128	100.0%	128	>128	100.0%	/
Ceftazidime/avibactam	4/4	8	2.0%	2/4	8	3.5%	0.684
Cefepime	>128	>128	96.1%	>128	>128	100.0%	0.283
Aztreonam	>128	>128	100.0%	>128	>128	100.0%	/
Piperacillin/Tazobactam	>512/4	>512/4	100.0%	>512/4	>512/4	100.0%	/
Imipenem	>32	>32	100.0%	>32	>32	100.0%	/
Meropenem	>32	>32	98.0%	>32	>32	100.0%	0.451
Imipenem/avibactam ^a	0.5/4	2/4	3.9%	0.25/4	1/4	3.5%	0.915
Amikacin	>512	>512	86.3%	>512	>512	58.6%	0.050
Polymyxin B ^b	0.5	1	0.0%	0.5	16	17.2%	0.002
Tigecycline	2	2	0.0%	1	2	0.0%	/
Levofloxacin	64	>64	100.0%	32	64	100.0%	/
Cefoperazone-Sulbactam ^a	256/128	>256/128	100.0%	>256/128	>256/128	100.0%	/

a: Breakpoints for imipenem/avibactam and cefoperazone/sulbactam have not yet been determined, therefore breakpoints for imipenem and cefoperazone have been applied.

b: Colistin MIC results can predict the sensitivity of polymyxin B, therefore breakpoints for Colistin have been applied.

Table 3. Univariate and Multivariate Logistic Regression Analysis of CR-hvKP infections

Variables	Univariate Analysis		Multivariable Analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
ST11-K64	4.437(1.496-13.161)	0.005	8.385[2.017-34.858]	0.003
Cardiovascular disease	7.440[2.438-22.700]	<0.001	11.956[3.072-46.538]	<0.001

Figures

ID	MLST	Resistant phenotype	Carbapenemase genes					Virulence gene																			
			KPC	NDM	IMP	OXA-48	rmpA	rmpA2	hlyA	fliC	fliD	prg-344	serotype	String test	Cefazolin	Cefazolin/ceftriaxone	Cefepime	Ampicillin	Piperacillin/Tazobactam	Imipenem	Imipenem/ceftriaxone	Meropenem	Amikacin	Polymyxin B	Tigecycline	Levofloxacin	Cefepime/Sulbactam
10	11	KPC-lactamase											K64	+	>128	8/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	>128/4
15	11	KPC-lactamase											K64	+	>128	1/4	>128	>128	>512/4	32	0.35/4	>32	>512	0.5	2	64	>512/128
16	11	KPC-lactamase											K64	+	>128	4/1	128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	>512/128
21	11	KPC-lactamase											K64	+	>128	2/4	64	>128	>512/4	32	0.35/4	>32	>512	0.5	0.5	64	>512/128
34	11	KPC-lactamase											K64	+	>128	2/4	64	>128	>512/4	32	0.35/4	>32	4	0.75	1	64	>512/128
32	11	KPC-lactamase											K64	+	>128	2/1	128	>128	>512/4	16	0.35/4	>32	>512	0.25	0.5	64	128/4
51	11	KPC-lactamase											K64	+	>128	4/1	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	>512/128
64	11	KPC-lactamase											K64	+	>128	2/4	>128	>128	>512/4	>32	0.35/4	>32	4	0.5	1	>64	>512/128
65	11	KPC-lactamase											K64	+	>128	4/1	64	>128	>512/4	32	0.35/4	>32	>512	0.5	1	64	>512/128
55	11	KPC-lactamase											K64	+	>128	2/1	128	>128	>512/4	>32	0.35/4	>32	>512	0.25	1	64	>512/128
59	11	KPC-lactamase											K64	+	64	2/4	32	>128	>512/4	32	0.35/4	>32	>512	0.5	1	12	>512/128
62	11	KPC-lactamase											K64	+	32	8/1	64	>128	>512/4	4	0.5/4	0.5	>512	2	1	64	>512/128
62	11	KPC-lactamase											K64	+	>128	2/4	>128	>128	>512/4	>32	0.5/1	>32	>512	0.5	1	32	>512/128
64	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.35/4	>32	>512	0.5	1	32	>512/128
65	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.35/4	>32	>512	0.5	2	64	>512/128
66	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.35/4	>32	>512	0.5	2	>64	>512/128
67	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.35/4	>32	>512	0.5	2	64	>512/128
69	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	1	2	64	128/4
70	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.4	>32	>512	0.5	1	64	>512/128
71	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	1/4	>32	>512	2	2	64	>512/128
72	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	1/4	>32	>512	0.5	2	64	>512/128
74	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	2/4	>32	>512	0.5	1	>64	>512/128
75	11	KPC-lactamase											K17	+	>128	2/4	>128	>128	>512/4	>32	0.5/1	>32	4	0.5	0.5	16	>512/128
76	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	>64	>512/128
79	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	>512/128
81	11	KPC-lactamase											K64	+	>128	4/1	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	>512/128
82	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	2	64	128/4
83	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	1/4	>32	>512	1	2	64	>512/128
85	11	KPC-lactamase											K64	+	>128	4/1	>128	>128	>512/4	32	0.5/4	>32	>512	0.5	2	64	>512/128
86	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	1/4	>32	>512	0.5	1	64	>512/128
88	11	KPC-lactamase											K64	+	32	1/4	32	128	>512/4	16	0.35/4	>32	>512	1	1	32	>512/128
89	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.5	1	>64	>512/128
92	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	1/4	>32	>512	0.5	1	64	>512/128
93	11	KPC-lactamase											K64	+	64	2/4	32	>128	>512/4	>32	0.35/4	>32	8	0.5	1	32	>512/128
94	11	KPC-lactamase											K64	+	75	1/1	>128	>128	>512/4	32	0.35/4	>32	>512	0.25	0.5	32	>512/128
96	11	KPC-lactamase											K64	+	32	1/4	64	128	>512/4	16	0.35/4	>32	>512	0.25	1	>64	>512/128
97	11	KPC-lactamase											K64	+	>128	1/4	>128	>128	>512/4	>32	1/4	>32	>512	1	2	64	>512/128
98	11	KPC-lactamase											K64	+	64	1/4	>128	>128	>512/4	>32	2/4	>32	>512	0.25	1	32	>512/128
100	11	KPC-lactamase											K64	+	>128	2/4	>128	>128	>512/4	>32	>512	>32	>512	2	1	64	>512/128
101	11	KPC-lactamase											K64	+	>128	2/4	>128	>128	>512/4	>32	0.35/4	>32	>512	4	1	32	>512/128
104	11	KPC-lactamase											K64	+	64	1/4	128	>128	>512/4	>32	0.35/4	>32	>512	0.5	1	32	>512/128
106	11	KPC-lactamase											K64	+	>128	8/4	>128	>128	>512/4	>32	0.35/4	>32	>512	2	1	64	>512/128
107	11	KPC-lactamase											K64	+	>128	8/4	>128	>128	>512/4	>32	1/4	>32	>512	0.5	1	64	>512/128
108	11	KPC-lactamase											K64	+	>128	8/4	>128	>128	>512/4	>32	1/4	>32	>512	1	1	64	64/4
109	11	KPC-lactamase											K64	+	>128	4/4	>128	>128	>512/4	>32	0.5/4	>32	>512	0.4	2	>64	>512/128
39	15	KPC-lactamase											K-iso-rydable	+	16	2/4	16	128	>512/4	32	0.5/4	>32	4	0.5	0.5	32	>512/128
47	15	KPC-lactamase											K-iso-rydable	+	128	1/4	128	>128	>512/4	32	0.4	>32	4	1	2	16	>512/128
49	15	KPC-lactamase											K-iso-rydable	+	>128	1/4	4	128	>512/4	8	0.35/4	>32	>512	0.25	0.5	8	>512/128
78	15	KPC-lactamase											K-iso-rydable	+	>128	2/1	>128	>128	>512/4	16	0.35/4	>32	>512	0.5	1	64	>512/128
103	15	KPC-lactamase											K-iso-rydable	+	128	1/4	8	>128	>512/4	8	0.35/4	8	>512	0.5	1	>64	128/4
45	158	KPC-lactamase											K-iso-rydable	+	>128	1/4	32	>128	>512/4	8	0.35/4	>32	>512	0.5	1	>64	>512/128

Figure 1

MLST, resistance and virulence factors, and MIC values of 51 CR-hvKP strains. MLST, resistance and virulence factors and MICs are shown on the left, middle and right, respectively. The presence of genes in a specific genome is represented by the light yellow box and the absence of genes is represented by a green box. Each row of the heatmap (middle) indicates a strain, and each column represents a gene that belongs to the indicated functional clusters shown at the top.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Primers6.xlsx](#)