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ORIGINAL RESEARCH

Clinical and Molecular Characteristics of Carbapenem-Resistant Hypervirulent *Klebsiella pneumoniae* Isolates in a Tertiary Hospital in Shanghai, China

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Background: The convergence of carbapenem-resistance and hypervirulence in *Klebsiella pneumoniae* has led to a significant public health challenge. In recent years, there have been more and more reports on carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) isolates.

Materials and Methods: Clinical data of patients infected with CR-hvKP from January 2019 to December 2020 in a tertiary hospital were retrospectively evaluated. The number of isolates of *Klebsiella pneumoniae*, hypermucoviscous *Klebsiella pneumoniae* (hmKP), carbapenem-resistant hypermucoviscous *Klebsiella pneumoniae* (CR-hmKP) and carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) collected during the period of 2 years was calculated. The antimicrobial resistance gene, virulence-associated gene, capsular serotype gene and multilocus sequence typing (MLST) of CR-hvKP isolates were detected by PCR.

Results: During the study period, a total of 1081 isolates of non-repeat *Klebsiella pneumoniae* were isolated, including 392 isolates of hypermucoviscous *Klebsiella pneumoniae* (36.3%), 39 isolates of CR-hmKP (3.6%), and 16 isolates of CR-hvKP (1.5%). About 31.2% (5/16) of CR-hvKP were isolated from 2019, and 68.8% (11/16) of CR-hvKP were isolated from 2020. Among the 16 isolates of CR-hvKP, 13 isolates were ST11 and serotype K64, 1 isolate was ST11 and serotype K47, 1 isolate was ST23 and serotype K1, and 1 isolate was ST86 and serotype K2. The virulence-associated genes *entB, fimH, rmpA2, iutA, iucA* were present in all of 16 CR-hvKP isolates, followed by *mrkD* (n=14), rmpA (n=13), aerobactin (n=2), allS (n=1). Sixteen CR-hvKP isolates all carry carbapenemase gene bla_{KPC-2} and extended-spectrum β-lactamase gene bla_{SHV}. ERIC-PCR DNA fingerprinting results showed that 16 CR-hvKP isolates were highly polymorphic, and there were significant differences in bands among the isolates, presenting a sporadic state.

Conclusion: Although CR-hvKP was sporadically distributed, it showed an increasing trend year by year. Therefore, clinical attention should be paid, and necessary measures should be taken to avoid the cloning and transmission of superbacterium CR-hvKP.

Keywords: *Klebsiella pneumoniae*, carbapenem-resistant, hypervirulent, hypermucoviscous, epidemiology

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Introduction

Klebsiella pneumoniae is an opportunistic pathogen that causes a wide range of infections, including pneumonia, urinary tract infections, bacteremia, and meningitis.[1] In the past three decades, unlike classical *Klebsiella pneumoniae*

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(cKP), a new hypervirulent *Klebsiella pneumoniae* (hvKP) with hypermucoviscosity has emerged as a clinically important pathogen that can cause highly invasive infections such as liver abscess in healthy and immunocompromised individuals.[2] It is important to note that these infections are often accompanied by destructive disseminated infections, including endophthalmitis and meningitis.[3] The hypermucoviscous phenotype of hvKP is usually due to increased production of capsular polysaccharides and the presence of specific virulence genes, such as *rmpA* and *rmpA2*.[4] The hypermucoviscous phenotype is generally determined by the "string test". *K. pneumoniae* colonies grown on blood agar plates overnight were stretched with a loop, and the "string test" is positive when a viscous string >5mm in length is formed.[5] A recent study showed that *peg-344, iroB, iucA, rmpA* and *rmpA2* are biomarkers that can accurately identify hvkp.[6] In this study, hypervirulent *K. pneumoniae* is defined as having a hypermucoviscous phenotype (a positive string test result) and carrying *K. pneumoniae* virulence plasmid-associated loci (*rmpA2, iutA, iucA)*. In the 1980s, case reports from Taiwan first described community-acquired liver abscesses caused by hvKP with severe terminal organ damage, such as meningitis and endophthalmitis.[7,8] The sporadic spread of hvKP has occurred in many countries in Asia, Europe and the Americas. Although several cases of hvKP have been reported in Europe and the Americas, the prevalence of hvKP mainly occurs in Asian countries, especially in China.[9]

In general, hvKP has a high sensitivity to antibiotics, while carbapenem-resistant *K. pneumoniae* (CRKP) has a low toxicity. However, with the spread of drug-resistant and virulence plasmids, CR-hvKP was first described by Zhang et al in 2015, and more and more reports have been described in China.[10] Because CR-hvKP can cause severe and hard-to-treat infections, it could become the next "superbug" if a pandemic clone emerges. So far, most CR-hvKP-induced infections have occurred in sporadic cases, and small outbreaks are rare.[11,12]

At present, the detection rate of CR-hvKP is low, and the relevant studies are few. The molecular epidemiology of CR-hvKP is different in different regions, so it is necessary to study the clinical distribution and molecular epidemiological characteristics of CR-hvKP in this region. In this study, antimicrobial resistance genes, virulence-associated genes and MLST of CR-hvKP were analyzed comprehensively. We attempted to investigate the prevalence and molecular epidemiological characteristics of CR-hvKP in a tertiary hospital in Shanghai, eastern China. This study is of significance for understanding the molecular epidemiology of CR-hvKP in Shanghai.

Materials and Methods

Collection and Identification of *K. pneumoniae* Isolates

A retrospective study was conducted to collect non-repeated *Klebsiella pneumoniae* isolates from Shanghai Fifth People's Hospital affiliated to Fudan University from January 2019 to December 2020. The percentage of hmKP, CRKP, CR-hmkp and CR-hvKP was calculated. All isolates were identified by VITEK-2 compact automated microbiology analyzer (Biomerieux, Marcy L'Etoile, France). The Maldi-Tof mass spectrometry (Bruker Daltonics, Billerica, MA, USA) was used to recheck the identification of the bacterial strain. The hypermucoviscous phenotype was determined by the "string test". Carbapenem resistance was determined when imipenem or meropenem was resistant by antimicrobial susceptibility test. Hypervirulent *Klebsiella pneumoniae* is defined as having a hypermucoviscous phenotype (a positive string test result) and carrying *K. pneumoniae* virulence plasmid-associated loci (*rmpA2, iutA, iucA*).[6]

String Test

A single *Klebsiella pneumoniae* colony was inoculated on 5% sheep blood agar plate. After overnight culture at 37°C, the bacterial colony was gently pulled up with the inoculation loop and repeated for 3 times. If a viscous string formed in all 3 times and the length was longer than 5mm, it was considered positive for the "string test", and the strain was hypermucoviscous phenotype.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility to several commonly used antibiotics was detected by broth microdilution in the VITEK-2 compact automated microbiology analyzer (Biomerieux, Marcy L'Etoile, France). The results were interpreted according to the guideline document established by Clinical and Laboratory Standards Institute (CLSI, 2019). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as controls for antimicrobial susceptibility testing.

PCR Detection of Antimicrobial Resistance Genes, Virulence-Associated Genes and Capsule Serotype (CPS) Gene

Genomic DNA of all *Klebsiella pneumoniae* isolates was extracted by TIANamp Bacteria Genomic DNA Kit (TiangenBiotechCo. Ltd., Beijing, China). The extended-spectrum β-lactamase genes (bla_{CTX-M}, bla_{SHV} and bla_{TEM}), carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}) and 9 representative virulence-associated genes including the pLVPK-like plasmid genetic loci (*allS, fimH, mrkD, entB, iutA, rmpA, rmpA2, iucA*, and *aerobactin*) were amplified by PCR as previously described.[13,14] Capsular serotype-specific genes (K1, K2, K5, K20, K54, and K57) were amplified by PCR as previously described.[14] If negative, *wzi* loci were amplified and sequenced to determine capsular serotype-specific genes.[15] The primers used in this study are listed in Table S1. The positive PCR products were sequenced by the NextSeq 500 sequencing platform (Illumina, San Diego, CA, USA). Nucleotide sequences were compared by running BLAST at NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST) was performed as described on the Pasteur Institute MLST website (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Seven housekeeping genes *gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB* were amplified by PCR and sequenced. The sequence types (STs) were determined by comparing the sequencing results with the MLST database.

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) DNA Fingerprint

The homology of *Klebsiella pneumoniae* was analyzed. Genomic DNA of *Klebsiella pneumoniae* was extracted as template, and ERIC primer was listed in Table S1. Genomic DNA was amplified by PCR, and the fingerprint of genomic DNA was constructed.[16] The PCR products were detected by 2% agarose gel electrophoresis. The DNA fingerprinting results were identified by band identification using QuantityOne software, and genetic analysis was performed by unweighted pair group method with arithmetic mean (UPGMA). The isolates with similarity > 75% was regarded as the same genotype, and those with similarity < 75% as different genotypes.

Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows version 22.0. Data were described as means ±standard deviation (SD). Categorical variables were assessed by Chi-square test or Fisher's exact test. All statistical tests were 2 tailed and a P-value <0.05 was considered to be statistically significant.

Results

Prevalence of Carbapenem-Resistant hvKP Isolates

A total of 1081 *Klebsiella pneumoniae* isolates were collected from Shanghai Fifth People's Hospital affiliated to Fudan University from January 1, 2019 to December 31, 2020, and the duplicate isolates of the same patient were excluded. Among them, 392 isolates (36.3%) were hmKP, 341 isolates (31.5%) were CRKP, 39 isolates (3.6%) were CR-hmKP and 16 isolates (1.5%) were CR-hvKP. Notably, 33.3% (13/39) of CR-hmKP and 31.2% (5/16) of CR-hvKP were isolated from 2019, 66.7% (26/39) of CR-hmKP and 68.8% (11/16) of CR-hvKP were isolated from 2020. Thirty-nine CR-hmKP were recovered from sputum (17 isolates), urine (12 isolates), drainage fluid (4 isolates), blood (2 isolates), pus (2 isolates), bile (1 isolate) and pleural fluid (1 isolate), respectively. Sixteen CR-hvKP were recovered from sputum (9 isolates), urine (5 isolates), blood (1 isolate) and pleural fluid (1 isolate).

Clinical Characteristics of 16 Patients Infected with CR-hvKP Isolates

Sixteen isolates of CR-hvKP were screened out through strain identification, antimicrobial susceptibility testing, string test and virulence-associated gene detection. Clinical characteristics of 16 patients infected with CR-hvKP isolates are summarized in Table 1. Thirteen (81.3%) of the 16 patients were males, and all patients were older than 62 years (the mean age: 83.1±10.5 y). They came from 8 wards, more than half from the central ICU (9 cases). Underlying conditions included cerebrovascular diseases (75%, 12/16), hypertension (50%, 8/16), chronic obstructive pulmonary disease (50%, 8/16), etc. Invasive procedures included mechanical ventilation (62.5%, 10/16), urinary catheter (37.5%, 6/16), gastrictube (18.8%, 3/16), surgery (12.5%, 2/16) and venous catheter (6.3%, 1/16). Nine of the 16 patients died, and seven patients improved and were discharged.

Table 1 Clinical Characteristics of 16 Patients Infected with CR-hvKP Isolates

Isolates	Age (Years)	Sex	Ward	Underlying Conditions	Treatment	Invasive Procedures	Outcome
KP3	83	F	Neurology Department	Cerebrovascular disease, type 2 diabetes mellitus, ventilator-associated pneumonia, epilepsy	SCF, TZP	Mechanical ventilation	Died
KP58	86	F	Central ICU	Cerebrovascular disease, coronary heart disease, hypertension, urinary tract infection	TZP, TGC	Urinary catheter, Gastric tube	Survived
KP63	89	M	Central ICU	Cerebrovascular disease, hypertension, Parkinson's disease, renal malignancy, pulmonary infection	TGC	Mechanical ventilation	Died
KP104	74	M	Traditional Chinese Medicine Department	Cerebrovascular disease, hypertension, urinary tract infection, pulmonary embolism	TZP, CRO	Mechanical ventilation, Urinary catheter	Survived
KP129	67	M	Central ICU	Cerebrovascular disease, type 2 diabetes, hypertension, coronary heart disease, colon tumors	CAZ	Mechanical ventilation, Urinary catheter, Gastric tube	Died
KP178	96	M	Geriatric Ward	Cerebrovascular disease, COPD, coronary heart disease	TZP, SCF, MER	Mechanical ventilation	Died
KP188	91	M	Respiratory Intensive Care Unit	Chronic obstructive pulmonary disease, coronary heart disease, hypertension	TZP	Mechanical ventilation	Survived
KP194	87	M	Central ICU	Pulmonary infection,hypertension, cerebrovascular disease	TZP, SCF, MER, CMZ	Venous catheter	Died
KP284	90	M	Central ICU	Cerebrovascular disease,Chronic obstructive pulmonary disease	SCF	Mechanical ventilation	Died
KP290	63	M	Central ICU	Cerebrovascular disease,hypertension,type 2 diabetes	SCF, TGC, CAZ-AVI	Mechanical ventilation, Urinary catheter	Died
KP298	83	M	Central ICU	Cerebrovascular disease,hypertension, respiratory failure	IPM, AN	Mechanical ventilation, Urinary catheter	Died
KP302	87	M	Gastroenterology Department	Chronic obstructive pulmonary disease,Sigmoid colon cancer	CMZ	Surgery	Survived
KP358	75	M	General Surgery Department	Coronary heart disease, gallstone with cholecystitis, COPD	SCF, CMZ, SXT	Gastric tube	Died
KP359	85	F	Central ICU	Cerebrovascular disease,hypertension	SCF, AN	Mechanical ventilation	Survived
KP374	102	M	Endocrinology Department	Cerebrovascular disease, Parkinson's disease, coronary heart disease	TZP	Urinary catheter	Survived
KP393	71	M	Central ICU	Rectum cancer, COPD, cerebrovascular disease, hypertension	CMZ	Surgery	Survived

Abbreviations: M, male; F, female; COPD, chronic obstructive pulmonary disease; SCF, cefoperazone/sulbactam; TZP, piperacillin/tazobactam; AN, amikacin; CMZ, cefmetazole; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; CRO, ceftriaxone; CAZ, ceftazidime; MER, meropenem; IPM, imipenem; CAZ-AVI, ceftazidime/avibactam.

Table 2 Virulence-Associated Gene Positive Rate of 39 CR-hmKP Isolates

Virulence-Associated Gene	Viscous String Length ≤25mm (n=20)	Viscous String Length>25mm (n=19)	P value
rmpA	6(30%)	7(36.8%)	0.651
rmpA2	8(40%)	8(42.1%)	0.894
iutA	8(40%)	8(42.1%)	0.894
iucA	8(40%)	8(42.1%)	0.894

Virulence-Associated Gene Positive Rate of 39 CR-hmKP Isolates

Thirty-nine CR-hmKP isolates were divided into two groups according to the length of viscous string. Among them, 20 CR-hmKP isolates with viscous string length ≤25mm were divided into one group, and 19 CR-hmKP isolates with viscous string length >25mm were divided into the other group. The positive rates of virulence-associated genes *rmpA, rmpA2, iutA* and *iucA* were detected by PCR method. The positive rates of virulence-associated genes of CR-hmKP in the two groups are shown in Table 2. The positive rate of virulence-associated genes in CR-hmKP strain showed no statistical difference between the two groups.

The Antimicrobial Resistance Profile of 16 CR-hvKP Isolates

The detailed antimicrobial resistance profile of the 16 drugs is listed in Table 3. Sixteen isolates of CR-hvKP showed multiple drug resistance. All isolates were resistant to ampicillin, ampicillin/sulbactam, cefoperazone/sulbactam, piperacillin/tazobactam, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, imipenem, meropenem. The antimicrobial resistance rate of Trimethoprim-sulfamethoxazole was the lowest (43.8%), followed by amikacin (62.5%), gentamicin (68.8%) and ciprofloxacin (87.5%).

Virulence-Associated Genes, Antimicrobial Resistance Genes, Capsule Serotype Genes and MLST

The distribution of virulence-associated genes, antimicrobial resistance genes, capsular serotype genes and MLST of 16 CR-hvKP isolates is shown in Figure 1. Agarose gel electrophoresis results of some virulence-associated genes, antimicrobial resistance genes and capsule serotype genes are shown in Figure 2. MLST analysis revealed a total of 3 STs, ST11 was

Table 3 The Antimicrobial Resistance Profile of 16 CR-hvKP Isolates [n(%)]

Antimicrobials	CR-hvKP(n=16)	
	Susceptible	Resistant
Ampicillin	0	16(100)
Ampicillin/sulbactam	0	16(100)
Cefoperazone/sulbactam	0	16(100)
Piperacillin/tazobactam	0	16(100)
Cefazolin	0	16(100)
Cefuroxime	0	16(100)
Ceftazidime	0	16(100)
Ceftriaxone	0	16(100)
Cefepime	0	16(100)
Cefoxitin	0	16(100)
Imipenem	0	16(100)
Meropenem	0	16(100)
Amikacin	6(37.5)	10(62.5)
Gentamicin	5(31.2)	11(68.8)
Ciprofloxacin	2(12.5)	14(87.5)
Trimethoprim-sulfamethoxazole	9(56.2)	7(43.8)

the most predominant ST (87.5%, 14/16), followed by ST23 (6.25%, 1/16) and ST86 (6.25%, 1/16). According to the results of the wzi typing, a total of 4 different capsular serotypes were identified (Figure 1). Among the 16 Carbapenem-Resistant hvKP isolates, K64 was the most common serotype (n=13), followed by K1 (n=1), K2 (n=1), and K47 (n=1). In addition, the strain of capsular serotype K1 is ST23, the strain of capsular serotype K2 is ST86, and the remaining 13 isolates of K64 and 1 strain of K47 were all ST11. The positive rates of the 9 virulence genes in 16 CR-hvKP isolates are shown in Figure 1, the virulence-associated genes *entB, fimH, rmpA2, iutA, iucA* were present in all of 16 CR-hvKP isolates, followed by *mrkD* (n=14), *rmpA* (n=13), *aerobactin* (n=2), *allS* (n=1). Sixteen CR-hvKP isolates all carried carbapenemase gene $bla_{\text{KPC-2}}$ and extended-spectrum β-lactamase gene bla_{SHV}. Sixteen CR-hvKP isolates did not carry carbapenem genes bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{\text{OXA-48}}$ and extended-spectrum β-lactamase genes bla_{TEM}, $bla_{\text{CTX-M-2}}$ group, $bla_{\text{CTX-M-8}}$ group. Among 16 CR-hvKP isolates, 5 isolates carried extended-spectrum β-lactamase gene $bla_{\text{CTX-M-1}}$ group, and 6 isolates carried extended-spectrum β-lactamase gene $bla_{\text{CTX-M-9}}$ group.

ERIC-PCR DNA Fingerprint Analysis of *Klebsiella pneumoniae* Isolates

ERIC-PCR was used to analyze the homology of 16 CR-hvKP isolates. After PCR amplification and agarose gel

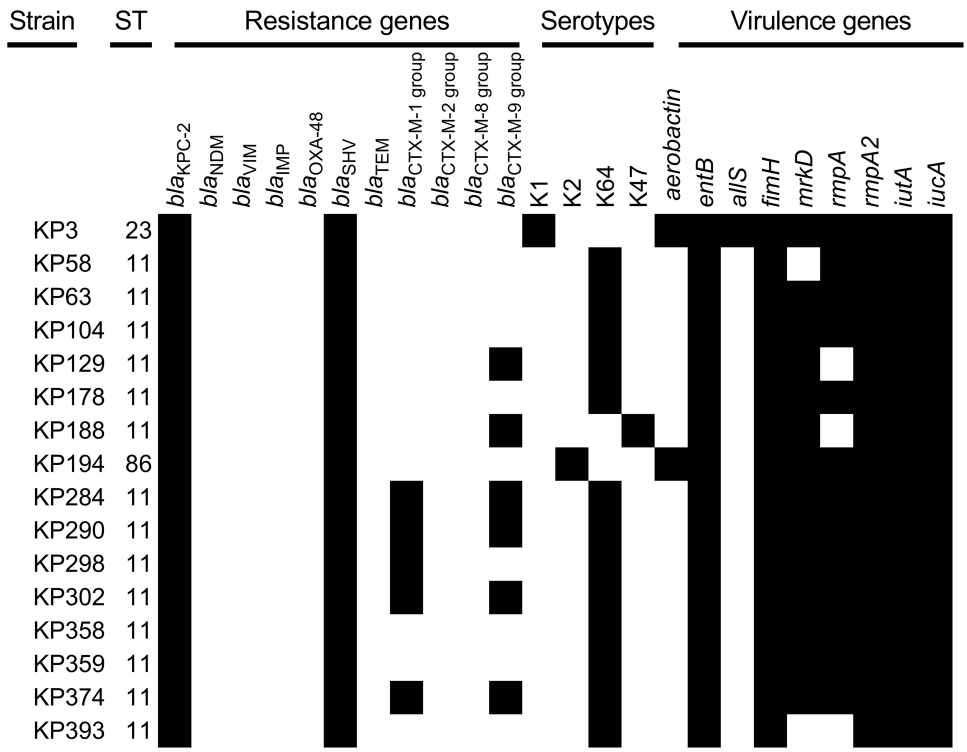

Figure 1 Virulence-associated genes, antimicrobial resistance genes, capsular serotype genes and MLST of 16 CR-hvKP isolates.
Note: The black color indicated the presence of a gene in the corresponding strain.

electrophoresis, there were 3–9 DNA fragments. The fingerprint results showed that 16 CR-hvKP isolates were highly polymorphic, and there were obvious differences among isolates (Figure 3).

Discussion

In recent years, there have been more and more reports on CR-hvKP isolates. The emergence of CR-hvKP isolates poses a significant threat to public health because they can cause serious, hard-to-treat infections in healthy populations. In this study, the prevalence and molecular epidemiological characteristics of CR-hvKP in a tertiary hospital in Shanghai area from 2019 to 2020 were studied to evaluate whether there is a risk of CR-hvKP outbreak and its development trend in this area. At the same time, this study can provide a more comprehensive clinical assessment of infectivity, which is of great importance to prevent the further spread of such isolates.

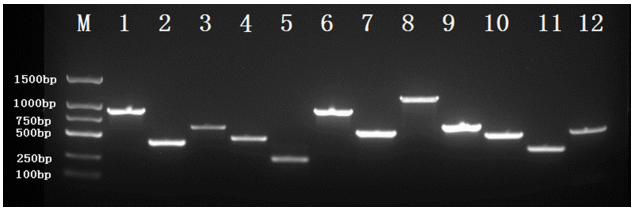

Figure 2 Agarose gel electrophoresis of some virulence-associated genes, antimicrobial resistance genes and capsule serotype genes.
Notes: M, DNA marker; 1, bla_{KPC}(893bp); 2, *entB*(400bp); 3, *rmpA2*(609bp); 4, *rmpA*(429bp); 5, *iucA*(239bp); 6, *iutA*(880bp); 7, *aerobactin*(556bp); 8, K1(1283bp); 9, K2(641bp); 10, *allS*(508bp); 11, *mrkD*(340bp); 12, *fimH*(609bp).

This study retrospectively analyzed the clinical distribution and change trend of CR-hvKP from 2019 to 2020. The CR-hvKP isolates showed an increasing trend from 2019 to 2020. About 31.2% (5/16) of CR-hvKP were isolated in 2019, and 68.8% (11/16) of CR-hvKP were isolated in 2020. This is consistent with the increasing trend of CR-hvKP literature reports. Since the first description of CR-hvKP by Zhang et al in 2015,[10] more and more CR-hvKP literature has been reported,[17–20] mainly in the Asia-Pacific region, especially in China. CR-hvKP is a super bacterium with hypervirulence and multidrug resistance, which has great harm to people's health and high mortality rate. Therefore, attention should be paid to it, and measures should be taken to prevent its spread.

Antimicrobial resistance analysis of 16 isolates of CR-hvKP showed high resistance rate to antibiotics. All isolates were resistant to ampicillin, ampicillin/sulbactam, cefoperazone/sulbactam, piperacillin/tazobactam, cefazolin,

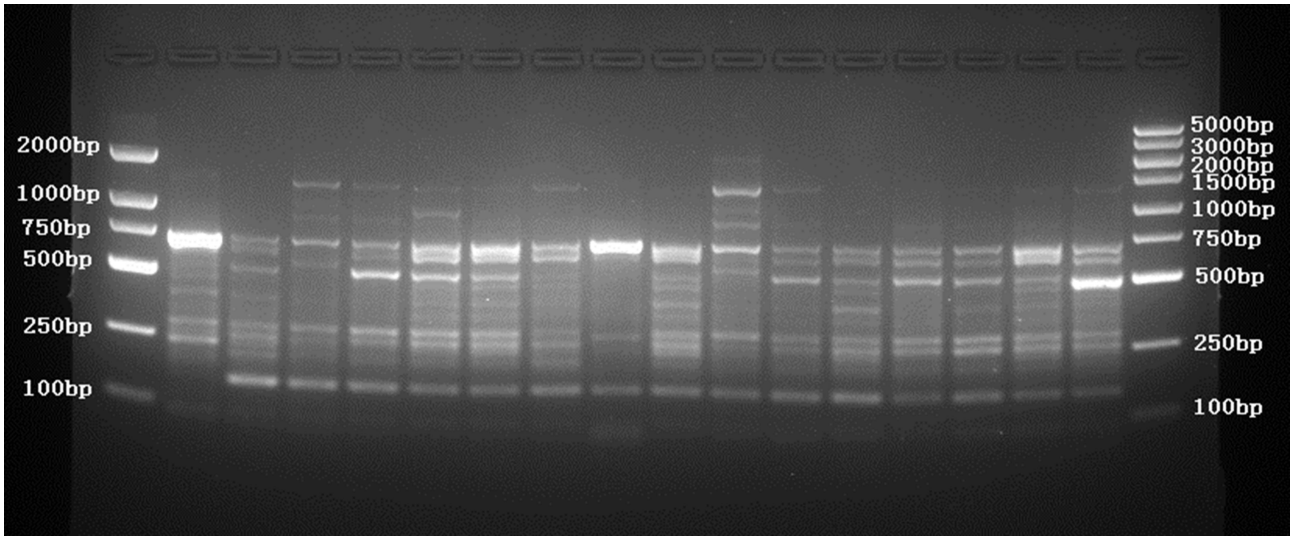

Figure 3 ERIC-PCR DNA fingerprint of 16 CR-hvKP isolates.

cefuroxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, imipenem, meropenem. The antimicrobial resistance rate of Trimethoprim-sulfamethoxazole was the lowest (43.8%), followed by amikacin (62.5%), gentamicin (68.8%) and ciprofloxacin (87.5%). The antimicrobial resistance rate of CR-hmkp studied by Lingling Zhan et al is similar to this study.[12] Patients infected with CR-hvKP have many underlying diseases, low immunity and weak ability of independent bacteria elimination. Therefore, timely treatment according to the results of antimicrobial sensitivity test is very important. If necessary, infection site can be found and treated by means of drainage and debridement.

Thirty-nine CR-hmKP isolates were divided into two groups according to the length of viscous string. Among them, 20 CR-hmKP isolates with viscous string length ≤25mm were divided into one group, and 19 CR-hmKP isolates with viscous string length >25mm were divided into the other group. The positive rate of virulence-associated gene of CR-hmKP was compared between the two groups, and there was no statistical significance in the positive rate of virulence gene between the two groups. The study of Lin Z et al revealed that the virulence gene positive rate of hypermucoviscous *Klebsiella pneumoniae* was significantly higher than that of classical *Klebsiella pneumoniae*.[21] However, it is not clear whether the virulence gene positive rate is positively correlated with the length of viscous string. Other studies have shown that classical *Klebsiella pneumoniae* may also be hypervirulent *Klebsiella pneumoniae*, with a high virulence gene positive rate.[22] In this study, it was found that the virulence gene positive rate of CR-hmKP was not positively correlated with the length of viscous string (or did not increase with the length of viscous string).

In this study, the ERIC PCR fingerprint was pleomorphic and there was no clinical intersection between patients, so 16 patients infected with CR-hvKP were sporadic cases. In the past, the majority of CR-hvKP-induced infections have been reported as isolated or sporadic cases,[23,24] and small outbreaks of CR-hvKP are rare in the literature.[11,25] ST11 is the most prevalent ST among CRKP and CR-hvKP isolates in China.[26,27] Although ST11 CR-hvKP accounted for 87.5% (14/16) of the 16 CR-hvKP isolates in this study, it could not be assumed that the 14 ST11 CR-hvKP were from the same clone strain, so it was necessary to conduct homology analysis by ERIC PCR fingerprint.

In this study, all 16 patients infected with CR-hvKP underwent invasive procedures. An outbreak of fatal ventilator-associated pneumonia caused by CR-hvKP has been reported,[11] suggesting that invasive procedures may increase the risk of CR-hvKP infection. Meanwhile, all 16 patients infected with CR-hvKP had underlying conditions, among which cerebrovascular disease was the most common. A previous study showed that cerebrovascular disease is a significant independent risk factor for CR-hvKP infection.[28] The reason for this phenomenon may be that patients with cerebrovascular diseases have low immunity and cannot independently exclude pathogenic bacteria, and only rely on the bactericidal effect of antibiotics, which will lead to the phenomenon of multiple drug resistance combined with hypervirulence in the long run. Of the 16 patients, 9 patients died, with a mortality rate of 56.3% (9/16). The

mortality rate was higher than that reported by previous studies[10,12] and lower than that reported by previous studies.[11,21] The mean age of 16 patients was 83.1±10.5 y, which showed that the elderly were more susceptible to CR-hvKP infection. Previous study has showed that young people are susceptible to hypervirulent *Klebsiella pneumoniae*.[29] However, other studies have showed that the elderly are susceptible to hypervirulent *Klebsiella pneumoniae*,[24,28] and the present study is consistent with that.

Among the 16 isolates of CR-hvKP, except 1 isolate of ST23 CR-hvKP and 1 isolate of ST86 CR-hvKP, the other 14 isolates were all ST11 CR-hvKP. The capsular serotype corresponding to ST23 CR-hvKP is K1, and the capsular serotype corresponding to ST86 CR-HVKP is K2, which is similar to previous studies.[30–32] Patients infected with ST23 (K1) CR-hvKP or infected with ST86 (K2) CR-hvKP died, with a mortality rate (100%) significantly higher than that of patients infected with ST11 CR-hvKP (50%). As shown in Figure 1, the virulence-associated gene positive rate of ST23 (K1) or ST86 (K2) isolates were higher than that of ST11 (K64) isolates, and the mortality rate might be related to the virulence-associated gene positive rate. In this study, 16 isolates of CR-hvKP all carried the carbapenemase gene $bla_{\text{KPC-2}}$ and extended-spectrum β-lactamase gene bla_{SHV}. $bla_{\text{KPC-2}}$ is the most prevalent carbapenemase gene in CR-hvKP in China.[33] In the study of Zhao, Y.et al,[25] bla_{SHV} is the extended-spectrum β-lactamase gene with the highest positive rate. The virulence genes *entB, fimH, rmpA2, iutA, iucA* were present in all of 16 CR-hvKP isolates, followed by *mrkD* (n=14), *rmpA* (n=13), *aerobactin* (n=2), *allS* (n=1), which were similar to previous study.[34] Some studies have shown that *rmpA* and *rmpA2* (regulator of the mucoid phenotype gene) can promote capsule polysaccharide secretion, resulting in the hypermucoviscosity phenotype and increase in virulence.[35] *Aerobactin* is encoded by the *iucABCD* gene, and its cognate receptor is encoded by the *iutA* gene, they had higher virulence levels in *G. mellonella* infection assays. allS is a marker for K1-ST23 and is not in pLVPK, which is a virulence plasmid from a K2 hypervirulent type. allS is a HTH-type transcriptional activator. These virulence genes were known to contribute to virulence and are responsible for colonization, invasion, and pathogenicity.[36]

Conclusion

This study described the prevalence and molecular epidemiological characteristics of CR-hvKP in Shanghai, China. Although the infection caused by CR-hvKP was sporadic, it showed an increasing trend year by year. The results support previous studies indicating that the ST11 CR-hvKP is the most prevalent CR-hvKP in China. ST23 and ST86 CR-hvKP showed higher virulence than ST11 CR-hvKP, despite both being hypervirulent Klebsiella pneumoniae. With the increase of the percentage of hypervirulent *Klebsiella pneumoniae*, the antimicrobial resistance rate of *Klebsiella pneumoniae* may decrease, which will lead to blind optimism in clinical practice. Therefore, it is necessary to study the virulence of *Klebsiella pneumoniae* as well as its antimicrobial resistance.

Ethics Approval and Informed Consent

The medical ethics council of Shanghai Fifth People's Hospital approved this study (Approval No. 104, 2020). The clinical samples were part of the routine hospital laboratory procedure.

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Disclosure

The authors report no conflicts of interest in this work.

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