An Outbreak of Carbapenem-Resistant Klebsiella Pneumoniae of K57 Capsular Serotype in an Emergency Intensive Care Unit of a Teaching Hospital in China

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Research

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Abstract

Background: *Klebsiella pneumoniae* is a common causative pathogen of nosocomial infections. The emergence of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) strains has further increased the threat posed by this bacterium. Here, we described an outbreak of 32 CR-hvKP isolates from the emergency intensive care unit (EICU) of a teaching hospital in China.

Methods: From January 29, 2019 to March 11, 2019, 32 CRKp isolates were collected from 6 patients and their surrounding environment in EICU. Patient information including age, gender, length of EICU stay, diagnosis, treatment, and outcomes were obtained from electronic medical records. The isolates were identified using Vitek-MS system. The hypermucoviscosity phenotype was determined by the “string test”. Antimicrobial susceptibility testing was performed using VITEK 2 compact system, E-test or the broth microdilution method. All isolates were serotyped for K1, K2, K5, K20, K54, and K57 serotypes, antimicrobial resistance genes and twelve virulence-associated genes were screened using PCR and DNA sequencing. Multilocus sequence typing (MLST) and pulse-field gel electrophoresis (PFGE) were employed to characterize the genetic relationships among the CPKP isolates. The virulence capability of 11 CRKp isolates from 6 patients was evaluated through *Galleria mellonella* larva infection assay.

Results: This outbreak involved 6 patients and lasted for 40 days. All 32 CR-hvKp isolates were obtained from 6 patients and their surrounding environment. PFGE showed that all 32 isolates belonged to one cluster, and MLST revealed that all belonged to ST11. All isolates exhibited high resistance to β-lactam antibiotics, quinolones, and aminoglycosides. They were susceptible to ceftazidime/avibatran, tigecycline, and colistin. All 32 isolates harbored multiple resistance determinants, including *bla*<sub>KPC-2</sub>, *bla*<sub>SHV-11</sub>, *bla*<sub>TEM-1</sub>, *rmtB*, and *qnrD*. The serotype of all 32 isolates was K57 that was rarely reported. In the virulence gene analysis, all 32 isolates contained 6 virulence genes, namely, *fimH*, *icuB*, *mrkD*, *mpA*, *uga*, and *wabG*. Infection assays demonstrated high mortality in the *Galleria mellonella* model. Following measures implemented by the hospital, the outbreak was controlled. The mortality rate was 83.3%.

Conclusions: The epidemiology of CR-hvKP should be monitored closely to detect early indications of this emerging public health threat.

Background

*Klebsiella pneumoniae* is a common causative pathogen of various nosocomial infections, including pneumonia, urinary tract infection, abdominal infection, and bacteraemia. *K. pneumoniae* is now recognized as an urgent threat to human health because of the emergence of multidrug-resistant strains associated with hospital outbreaks and hypervirulent strains associated with severe community-acquired infections [1]. Surveillance of antibiotic resistance by CHINET in China showed that 4.9% and 4.8% of *K. pneumoniae* strains were resistant to imipenem and meropenem, respectively, in 2009, compared with 25.3% and 26.8%, respectively, in 2019 ([http://chinets.com/Data/GermYear](http://chinets.com/Data/GermYear)). Infections caused by carbapenem-resistant *K. pneumoniae* (CRKp) have few treatment options and are associated with mortality rates upwards of 50% [2]. Several mechanisms are responsible for the carbapenem-resistance of *K. pneumoniae*, among which, carbapenemase production remains the most clinically relevant [3].

Over the past two decades, the newly emerged hypervirulent *K. pneumoniae* (hvKP) strain has caused great concern globally. hvKP is an evolving pathotype that is more virulent than classical *K. pneumoniae* (cKP). hvKP usually infects healthy individuals from the community. Infections are more common in the Asian Pacific Rim but are occurring globally [4]. Unlike cKP, hvKP is rarely resistant to commonly used antimicrobial agents, except for an intrinsic resistance to ampicillin. However, along with the global dissemination of mobile genetic elements conferring antibiotic resistance, antibiotic-resistant hvKP isolates, especially carbapenem-resistant hvKP (CR-hvKP), have attracted increasingly more attention and have been increasingly reported worldwide [5]. In China, several outbreaks of CR-hvKP have been reported [6–8].

Here, we have described an outbreak of CR-hvKP with 32 isolates from the emergency intensive care unit (EICU) of Taian City Central Hospital, Shandong, China. We sought to investigate the molecular and epidemiological features of these isolates during the outbreak, especially the characteristics of antimicrobial resistance determinants and virulence factors. This report confirmed that CR-hvKP can be transmitted nosocomially through the surrounding environment of the hospital. Hence, monitoring the nosocomial dissemination of CR-hvKP is essential for its prevention and outbreak management.

Methods

Collection of carbapenem-resistant *K. pneumoniae* from EICU

From January 29, 2019 to March 11, 2019, 32 CRKp isolates were collected from 6 patients and their surrounding environment in EICU. Patient information including age, gender, length of EICU stay, diagnosis, treatment, and outcomes were obtained from electronic medical records. The methods used in this study were approved by the Ethics Committee of Taian City Central Hospital and were carried out in accordance with the approved guidelines. The isolates were identified as *K. pneumoniae* using Vitek-MS system (BioMérieux, France). Phenotypic detection of carbapenemases was performed using carbapenem inactivation method (CIM) and EDTA-modified CIM (eCIM) test according to the approved standard of the Clinical and Laboratory Standards Institute 2020 guidelines [9].

Determination of hypermucoviscosity phenotype

The hypermucoviscosity phenotype was determined by the “string test”. Samples were cultured on agar plates overnight at 37°C. Then, a colony from the plate was stretched with a bacteriology loop. If a viscous string over 5 mm in length forms, it is considered to be a positive result.

Antibiotic susceptibility assay
Antimicrobial susceptibility testing was performed using VITEK 2 compact system (BioMérieux, France). The minimum inhibitory concentrations (MICs) of imipenem, meropenem, and etampenem were determined through an E-test (BioMérieux, France). The MICs of tigecycline and colistin were determined using the broth microdilution method (Bio-kont, China). The antimicrobial susceptibility of ceftazidime/avibactam was determined using Kirby-Bauer test (Oxide, America). Escherichia coli ATCC25922 and K. pneumoniae ATCC700603 served as the quality controls. All antibiotics were administered according to the approved standard of the 2020 European Committee on Antimicrobial Susceptibility Testing breakpoint (www.eucast.org/clinical-breakpoint).

PCR and DNA sequence analysis of drug resistance genes, serotype, and virulence genes

As described previously, a variety of antimicrobial resistance genes were screened using PCR and DNA sequencing [10]. These genes included carbapenem resistance genes, extended-spectrum β-lactamase genes (ESBLs), AmpC β-lactamase genes, plasmid-mediated quinolone resistance genes, and 16S-RMTase aminoglycoside resistance genes (mrtB, mrtC, amrA). The positive PCR products were sequenced by Beijing Genomics Institute Technology Co. Ltd. (Shanghai, China). Sequence alignments were completed by running BLAST at NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the isolates were serotyped for K1, K2, K5, K20, K54, and K57 serotypes, and twelve virulence-associated genes were screened using PCR, as previously described [11].

Multilocus sequence typing (MLST)

MLST of K. pneumoniae was performed according to protocols available on the MLST Pasteur website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). Seven conserved housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) were amplified, sequenced, and compared with those in the MLST databases.

Pulse-field gel electrophoresis (PFGE)

An overnight bacterial culture was suspended in cell suspension buffer [100 mM EDTA, 100 mM Tris-HCl (pH 8.0)] and adjusted to an optical density of 4.0 at the wavelength of 600 nm. The suspension was mixed with equal volume of 2% solution of low-melting agarose in Tris-EDTA [TE: 1 mM EDTA, 10 mM Tris-HCl (pH 8.0)]. After cooling, the agarose sections were incubated for 4 h at 54 °C in cell lysis buffer [50 mM Tris-HCl, 50 mM EDTA (pH 8.0), 0.01 g/mL N-lauroyl-sarcosine, sodium salt, 0.1 mg/mL proteinase K]. They were then washed thoroughly with TE buffer and digested overnight with XbaI restriction endonuclease (Takara Bio, Inc., Otsu, Japan). Genomic DNA separation was performed in 0.5 X Tris/borate/EDTA (TBE) buffer in a PFGE system (CHEF Mapper; Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 14 °C, using a voltage of 6 V/cm, a switch angle of 120° and a switch ramp of 636 s for 19 h. The Salmonella enterica serotype Braenderup H9812 was used as a marker for PFGE.

Galleria mellonella larva infection assay

The virulence capability of 11 CRKp isolates from 6 patients was evaluated through a commonly used in vivo infection model, the wax moth G. mellonella [6]. In the present study, each infection group included 10 larvae. Larvae were injected with 10 µL of bacterial suspension at a concentration of 10⁶ CFU per larva. As a control group, 10 µL PBS was injected in parallel. All larvae were placed in petri-dishes and kept at 37°C in the dark. At 24 h, 48 h, 72 h, and 96 h after injection, the number of dead larvae was recorded with notes on any melanization and lack of motility. All experiments were performed with biological triplicates and results were not considered if two or more larvae in any of the control groups died.

Results

Outbreak description

From January 29 to March 11, 2019, 6 patients admitted to EICU were included in this analysis. The age of patients ranged from 54 to 87 years and the patients were admitted to EICU due to respiratory failure, severe pneumonia, and septic shock. They all showed the typical symptoms of pulmonary edema, excessive sputum, pleural effusion, and shortness of breath. All 6 patients received traumatic and antibiotic treatment during their hospitalization in the EICU (Fig. 1). The antibiotics used mainly included carbapenems, cefoperazone/sulbactam, piperacillin/tazobactam, quinolones, and tigecycline. In addition, patient 2 was treated with voriconazole. The first CRKp was isolated from the sputum of patient 4, the first patient to be admitted to EICU. He had the longest survival time after CRKp isolation. The survival time of patient 3 was the shortest after CRKp isolation, which was only 3 days. Finally, only one patient improved and was discharged, four patients died during treatment in hospital, one gave up treatment and finally died on March 4, 2019 at home. The mortality rate was 83.3%.

In this study, 32 CRKp isolates were obtained, of which 11 isolates were from clinical samples of the 6 patients, including sputum, bronchoalveolar lavage fluid, and blood (Fig. 1). During the period of this study, the hospital environment was sampled on February 10 and February 20, 2019. 21 isolates were obtained from the patients’ environment in the hospital, including bed sheets, pillows, sphygmomanometers, ventilators, tables, mice, keyboards, and the hands of medical staff.

In order to control the outbreak, each patient was placed in a single room. The infected patients’ rooms were thoroughly disinfected with glutaraldehyde. The staffs of EICU were trained and educated in environmental disinfection and carrying out hand hygiene in a timely and effective manner. Eventually, the epidemic situation was effectively controlled. Except for these 6 patients, no other infected patients have been found in EICU to date.
Drug resistance and resistance genes of CRKp isolates

The antimicrobial susceptibility profiles of the 32 CRKp isolates are listed in Table 1. All isolates exhibited high resistance to aztreonam, cephalosporin, β-lactam/β-lactamase inhibitor combinations, carbapenems, quinolones, and aminoglycosides. Seven isolates (21.88%, 7/32) were resistant to trimethoprim-sulfamethoxazole. All isolates were susceptible to colistin, tigecycline, and ceftazidime/avibatran (100%). All strains were positive for MCIM test and negative for ECIM test.
| isolate No. | KZ | CXM | CRO | CAZ | FEP | FOX | ATN | SAM | TZP | ETP | IPM | MEM | CN | AK | CIP | LEV | SXT | TGC | COL |
|------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|-----|-----|-----|-----|-----|
| Kp1        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 0.5 |
| Kp2        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp3        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp4        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 0.5 |
| Kp5        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp6        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 1   |
| Kp7        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp8        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp9        | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 1   |
| Kp10       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |
| Kp11       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |
| Kp12       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |
| Kp13       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp14       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp15       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 1   |
| Kp16       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp17       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp18       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 0.5 |
| Kp19       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp20       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.25 | 0.5 |
| Kp21       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 1   |
| Kp22       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp23       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 0.5  | 0.5 |
| Kp24       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |
| Kp25       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |
| Kp26       | > 64 | > 16 | > 32 | > 16 | > 64 | > 16 | > 16 | > 16 | > 64 | > 32 | > 32 | > 8 | > 32 | > 2 | > 4 | <=2/38 | 1    | 0.5 |

MIC: Minimum inhibitory concentration; KZ: cefazolin; CXM: cefuroxime; CRO: ceftriaxone; CAZ: cefazidime; FEP: cefepime; FOX: cefoxitin; ATN: aztreonam; SAM: ampicillin sulbactam; TZP: piperacillin/tazobactam; ETP: ertapenem; IPM: imipenem; MEM: meropenem; CN: gentamicin; AK: amikacin; CIP: ciprofloxacin; LEV: levofloxacin; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; CO: colisoin; CZA: cefazidime/avibatan. *: diameter of bacteriostatic zone.
| Isolate No. | KZ    | CXM   | CRO   | CAZ   | FEP   | FOX   | ATM   | SAM   | TZP   | ETP   | IPM   | MEM   | CN    | AK    | CIP   | LEV   | SXT   | TGC   | COL   |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Kp27       | >64   | >16   | >32   | >16   | >16   | >16   | >16   | >64   | >32   | >32   | >8    | >32   | >2    | >4    | <2/38 | 0.5   | 0.5   | 1     |
| Kp28       | >64   | >16   | >32   | >16   | >16   | >16   | >16/8 | >64   | >32   | >32   | >8    | >32   | >2    | >4    | <2/38 | 0.5   | 1     | 1     |
| Kp29       | >64   | >16   | >32   | >16   | >16   | >16   | >16/8 | >64   | >32   | >32   | >8    | >32   | >2    | >4    | 2/38  | 0.25  | 0.5   | 1     |
| Kp30       | >64   | >16   | >32   | >16   | >16   | >16   | >16/8 | >64   | >32   | >32   | >8    | >32   | >2    | >4    | 2/38  | 0.5   | 0.5   | 1     |
| Kp31       | >64   | >16   | >32   | >16   | >16   | >16   | >16/8 | >64   | >32   | >32   | >8    | >32   | >2    | >4    | <2/38 | 0.5   | 0.5   | 1     |
| Kp32       | >64   | >16   | >32   | >16   | >16   | >16   | >16/8 | >64   | >32   | >32   | >8    | >32   | >2    | >4    | <2/38 | 0.25  | 0.5   | 1     |

MIC: Minimum inhibitory concentration; KZ: cefazolin; CXM: cefuroxime; CRO: ceftriaxone; CAZ: ceftazidime; FEP: cefepime; FOX: cefoxitin; ATM: aztreonam; SAM: ampicillin sulbactam; TZP: piperacillin/tazobactam; ETP: ertapenem; IPM: imipenem; MEM: meropenem; CN: gentamicin; AK: amikacin; CIP: ciprofloxacin; LEV: levofloxacin; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; CZA: ceftazidime/avibactam. *: diameter of bacteriostatic zone.

For the resistance to β-lactams, all 32 isolates carried the $\text{bla}_{\text{KPC}-2}$, $\text{bla}_{\text{SHV}-11}$, and $\text{bla}_{\text{TEM}-1}$ genes (Table 2). In addition, 27 isolates carried $\text{bla}_{\text{CTX}-14}$ gene, and 3 isolates (Kp15, Kp16, and Kp17) from the hospital environment of patient 3 carried $\text{bla}_{\text{CTX}-14}$ and $\text{bla}_{\text{CTX}-15}$ simultaneously. For aminoglycoside resistance, all isolates carried $\text{rmtB}$, and three isolates also carried $\text{armA}$. In addition, all 32 isolates carried $\text{qnrD}$, which may have led to quinolone resistance.
### Table 2
The drug-resistance genes and virulence genes of 32 CR-hvKP isolates

| Isolates no. | Drug-resistance genes | Virulence genes |
|--------------|-----------------------|-----------------|
|              | β-lactamases                      | Aminoglycoside | Quinolone | Capsule | Fimbriae | Inteplact | Immuclact |
|              | blaKPC-2 | blaSHV-11 | blaTEM-1 | blaCTX-M14 | blaCTX-M15 | mttB | armA | qnrD | mpa | wabG | uge | fimH | mrd | lu |
| Kp1          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp2          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp3          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp4          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp5          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp6          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp7          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp8          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp9          |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp10         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp11         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp12         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp13         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp14         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp15         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp16         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp17         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp18         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp19         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp20         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp21         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp22         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp23         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp24         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp25         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp26         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp27         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp28         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp29         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp30         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp31         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |
| Kp32         |          |          |          |          |          |      |      |      |      |      |      |      |      |      |      |

*: positive; blank: negative

### Serotype and virulence of CRKp isolates

The string test of 32 isolates showed negative results. The serotype of all 32 isolates was K57. In the virulence gene analysis, all 32 isolates were found to contain 6 virulence genes, including fimH, iucB, mrkD, mpa, uge, and wabG (Table 2).
We infected *G. mellonella* larvae with 11 CRKp isolates obtained from clinical specimens of the 6 patients. Kp1 and Kp11 from patient 1 were the least toxic, with 20% of larvae surviving after 96 hours. All larvae infected by the other 9 strains died within 96 hours. Kp3 from patient 3 was the most toxic. The 24-hour and 48-hour survival rates of larvae infected with Kp3 were 40% and 20% respectively, and all larvae died within 72 hours. The results are shown in Fig. 2.

**MLST and PFGE analysis of CRKp isolates**

MLST analysis revealed that all 32 CRKp isolates were of the ST11 type, which is the most common type of CRKp found in China. The results of PFGE showed that the homology of 32 isolates was more than 80%, indicating that they were a cluster. With the exception of Kp3, Kp21, Kp22, and Kp30, the homology of other 28 isolates was higher than 91.8%. Furthermore, three isolates (Kp5, Kp21, and Kp22) from patient 5 and four isolates (Kp1, Kp12, Kp13, and Kp14) from patient 1 showed complete homology (Fig. 3).

**Discussion**

*K. pneumoniae* is widely recognized as a pathogen with a propensity for acquiring antibiotic resistance. It is capable of causing a range of hospital-acquired infections and community-acquired invasive infections [12]. The appearance of CR-hvKP makes this bacterium more threatening. Here we described a nosocomial outbreak of CR-hvKP in the EICU of a teaching hospital. This outbreak involved 6 patients and lasted for 40 days. Thirty-two CR-hvKP were isolated from 6 patients and their surrounding environment. These 6 patients did not share one ward. However, PFGE showed that all 32 CR-hvKP isolates belonged to one cluster. The hospital took positive measures and the outbreak was controlled. Hospital monitoring showed that this outbreak of CRKP might have been caused due to the contaminated hands of cleaners. Therefore, in order to prevent the spread and prevalence of multidrug-resistant bacteria, early detection of cases, timely submission of specimens, and detection of multi-drug resistant bacteria are very important in addition to disinfection, isolation, and treatment [13].

The capsule polysaccharide of *K. pneumoniae* has long been viewed as an important virulence factor that promotes resistance to phagocytosis and serum bactericidal activity. To date, more than 100 capsular (K) serotypes of *K. pneumoniae* have been identified [14]. Notably, many reports have shown that K1 and K2 serotypes are strongly associated with hvKP [15, 16]. Nevertheless, serotype K57 has also been considered as one of the pyogenic liver abscess-associated capsular types that are rarely reported [17, 18]. In 2020, Wei *et al.* studied 39 K57 *K. pneumoniae* isolates. Among them, ST412 was the most prevalent, and all isolates harbored *bla*KPC-2 [18]. In the present study, we identified that all 32 CR-hvKP isolates belonged to K57 and ST11. To our knowledge, this is the first report of K57 and ST11 *K. pneumoniae* with carbapenem resistance associated with clinical infection.

Several virulence factors have been suggested to contribute to the pathogenesis of hvKP strains. To date, *rmpA* and *magA* are the most frequently reported factors that have direct correlation with hvKP virulence [19]. *RmpA* is located either on the chromosome or on a large virulence plasmid. The correlation between *rmpA* and hypermucoviscosity is very high, and the presence of *rmpA* among a set of genes has been proposed as biomarker to identify potential hvKP strains [20]. However, in this study, all 32 hypermucoviscosity-negative isolates were positive for *rmpA* and a previous study reported similar results [18]. The underlying reason may be that *rmpA* cannot work alone, or there may be a mutation of *rmpA*. In addition, a correlation between the presence of *rmpA* and aerobactin has been described, wherein 96% of *mpA* positive isolates coproduced aerobactin [21]. In this study, all 32 isolates were aerobactin producers. The ability to acquire iron is essential for bacterial growth and the progression of infection. *iusABC*, which encodes for aerobactin and its cognate receptor, is more prevalent in hvKP than cKP [22].

In addition, the 32 isolates expressed four other virulence genes, including *fimH, mrkD, uge*, and *wabG*. *FimH* and *mrkD* encode type I and type III fimbriae, respectively. Fimbriae allow bacteria to attach to the host cells to establish infection. A previous report confirmed observations that type III fimbriae contribute to biofilm formation, and demonstrated that the expression of type III fimbriae is positively correlated with iron concentration [22]. In this study, capsule-associated genes *uge* and *wabG* were detected in 32 isolates simultaneously. A previous study showed that the deletion of genes for capsule synthesis decreased the virulence of *K. pneumoniae* to varying degrees [23]. In the absence of the gene *uge*, *K. pneumoniae* is less capable of causing urinary tract infections, pneumonia, and sepsis [21]. In general, the serotype of the 32 isolates was K57, and their virulence genes were the same. However, the virulence test performed by infecting *G. mellonella* larvae showed that the virulence of 11 isolates from 6 patients was different, and their virulence was closely related to the survival time of the patients.

In China, *bla*KPC-2 is the predominant carbapenemase in both CRKp and CR-hvKP [24, 25]. ST11 is the major sequence type of CR-hvKP from Asia, especially China [21]. All CR-hvKP isolates in the present study were *bla*KPC-2 producing ST11 *K. pneumoniae*. In addition to *bla*KPC-2, all 32 isolates harbored *bla*SHV-11 and *bla*TEM-1. This is similar to previous reports [6, 8]. Moreover, 32 isolates except two carried *bla*CTX-M. These resistance genes lead to the resistance to β-lactams in the isolates. Furthermore, all isolates were resistant to aminoglycosides and quinolones, which severely limited the clinical choice of antibiotics. Fortunately, all strains were sensitive to tigecycline, colistin, and ceftazidime/avobactan. However, only one patient recovered and was discharged, and the mortality rate was 83.3%. This is higher than that described in previous reports [5, 26].

**Conclusions**

We described an outbreak of ST11 *K. pneumoniae*, which exhibited hypervirulence and carbapenem resistance, in EICU of a teaching hospital in China. The serotype of all 32 isolates was K57. The epidemiology of CR-hvKP should be monitored closely to detect early indications of this emerging threat to public health.
Abbreviations

cKP, classical K. pneumoniae; CRKp, carbapenem-resistant K. pneumoniae; hvKP, hypervirulent K. pneumoniae; CR-hvKP, carbapenem-resistant hypervirulent K. pneumoniae; EICU, emergency intensive care unit; MLST, Multilocus sequence typing; PCR: Polymerase chain reaction; PFGE: Pulse-field gel electrophoresis.

Declarations

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Authors’ contributions

CHS carried out the experiments and wrote the paper. YJ performed the results analysis. SL collected clinical information of patients. MJJ and SPZ designed the experiments and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The methods used in this study were approved by the Ethics Committee of Taian City Central Hospital and were carried out in accordance with the approved guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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