Infection and Drug Resistance

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ORIGINAL RESEARCH

# Genomic Analysis Of A KPC-2-Producing *Klebsiella Pneumoniae* ST11 Outbreak From A Teaching Hospital In Shandong Province, China

This article was published in the following Dove Press journal:
*Infection and Drug Resistance*

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**Purpose:** *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* bacteria causes nosocomial infections worldwide. However, KPC-producing *K. pneumoniae* outbreak has never been reported in Shandong Province, China. The purpose of our study was to elucidate the epidemiological and drug resistance mechanisms of KPC-producing *K. pneumoniae* strains collected from a large teaching hospital in Shandong during the outbreak. Moreover, we attempted to characterize the genetic environment and phylogenetic analysis of $bla_{KPC-2}$ in outbreak isolates.

**Methods:** We monitored a 64-day outbreak of infection in a general hospital in Shandong Province, and the bacteria causing the infection were all ST11-type *K. pneumoniae*. The genotype correlation of KPC-producing *K. pneumoniae* isolates was assessed by whole-genome sequencing (WGS) phylogenetic analysis. Subsequent studies included antibiotic susceptibility testing, multilocus sequence typing (MLST) and S1-pulsed-field gel electrophoresis (S1-PFGE), Southern blot hybridization.

**Results:** From February 1, 2018 to April 5, 2018, 14 KPC-producing *K. pneumoniae* isolates from different wards were collected. All 14 isolates were resistant to carbapenems and carried the extended-spectrum β-lactamase (ESBL) gene as well as *fosA*, and *sul* genes. Whole-genome analysis showed that all 14 the outbreak isolates were all ST11 type. The $bla_{KPC-2}$ carrying plasmids were all belong to $IncFII_{K2}$ type, and the size ranged from 94 kb to 368 kb.

**Conclusion:** As far as we know, this report first describes the genomics characterization of KPC-2-producing *K. pneumoniae* outbreak isolates from Shandong Province, China. In our study, these isolates appeared to be cloned, and ST11 *K. pneumoniae* was the major clone caused the outbreak. Therefore, routine surveillance of such strains in this region is urgently warranted.

**Keywords:** *Klebsiella pneumoniae*, ST11, whole-genome sequencing, SNP, outbreak, $IncFII_{K2}$

## Introduction

Bacterial resistance can reduce the effectiveness of antibiotics and increase the difficulty of treating infectious diseases, becoming a major problem affecting global public health.[1] Carbapenems can be used to treat infections caused by various extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae isolates. However, due to the irrational use of carbapenems, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) has caused difficulties in clinical work.[2] KPC enzymes hydrolyze carbapenems, not only that, but it is also the most important

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http://doi.org/10.2147/IDR.S221788



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enzyme among class A carbapenemases.[3] Infections caused by KPC-producing organisms are associated with high mortality rates up to 51%, which poses a huge challenge for clinical diagnosis and treatment.[4,5]

*Klebsiellapneumoniae* is a gram-negative pathogen and is the most common cause of hospital-acquired and community-acquired infections.[6,7] It has been reported that the widespread of carbapenem-resistant *K. pneumoniae* (CRKP) is caused by horizontal transfer of mobile elements such as plasmids and insertion sequences.[8] According to the Carbapenem-Resistant Enterobacteriaceae Network, pneumonia and bloodstream infections caused by carbapenem-resistant *K. pneumoniae* have a higher mortality rate. Carbapenem-resistant *K. pneumoniae* nonbacteremic infections can result in a 24.3% mortality rate.[9]

KPC-producing *K. pneumoniae*, which can lead to outbreaks of serious diseases globally,[10,11] are rarely reported in Shandong Province, China. In this study, we identified 14 clinical *K. pneumoniae* strains carrying $bla_{KPC-2}$, all of which belong to ST11. The aim of our study was to elucidate the epidemiological and drug resistance mechanisms of KPC-producing *K. pneumoniae* strains collected from a large hospital in Shandong during the outbreak. Moreover, we attempted to characterize the genetic environment and phylogenetic analysis of $bla_{KPC-2}$ in outbreak isolates.

## Materials And Methods

### Sample Collection

From February 1, 2018 to April 5, 2018, we have collected strains producing carbapenemase from the laboratory of a large teaching hospital in Jinan, Shandong Province. Bacterial identification was conducted with both matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany). Identification of carbapenemase genes ($bla_{KPC}$, $bla_{NDM}$, $bla_{OXA-48}$, $bla_{VIM}$ and $bla_{IMP}$) using PCR and Sanger sequencing.[5]

### Antibiotic Susceptibility Testing

Determine the minimum inhibitory concentrations (MICs) of antibiotics (Dalian Meilun Biotech Co., Ltd, Dalian, China) using agar dilution method: amikacin, aztreonam, cefotaxime, cefpirome, ciprofloxacin, ceftazidime, gentamicin, piperacillin-tazobactam, imipenem, meropenem, tobramycin, amoxicillin-clavulanic acid, chloramphenicol and fosfomycin.[12] Tigecycline and polymyxin were determined by the broth microdilution method. Results were interpreted using the CLSI standards (https://clsi.org). Controls were performed using *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

### Plasmid Characterization And Conjugation Assay

The plasmid was characterized by S1-PFGE, and the location of $bla_{KPC}$ was identified by Southern hybridization with digoxigenin-labelled $bla_{KPC}$ probe using the DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics). Plasmid conjugation experiments by mating with *E. coli* J53 as a recipient strain. Next, the transconjugants were cultured on agar (OXOID, Hampshire, UK) medium supplemented with 200 mg/L sodium azide and 2 mg/L meropenem. Finally, MALDI-TOFMS was identified for transconjugants, and $bla_{KPC}$ was tested by PCR to ensure that the plasmid was successfully transferred to the recipient strain.

### Whole-Genome Sequencing

Total DNA was obtained using an OMEGA Bacterial DNA Kit (Omega Bio-tek, Norcross, USA), followed by sequencing using Illumina HiSeq 4000-PE150 platform (Illumina, San Diego, CA, USA). We created genome sequence for 14 clinical *K. pneumoniae* isolates using SPAdes 3.11 by combining our Illumina sequencing reads. In addition, an online tool (http://www.genomicepidemiology.org/) was used to detect the acquired antimicrobial resistance genes in 14 isolates. The whole-genome sequences of the 14 isolates were deposited in GenBank under the following accession numbers: VJNW00000000-VJOJ00000000. The bacterial genome was annotated using the RAST server (http://rast.nmpdr.org/) and the transposon and IS elements were identified using the ISFinder database (https://www-is.biotoul.fr/). The genetic environment surrounding the carbe-genes were annotated using Easyfig 2.2.3. The presence of the virulence gene was identified by aligning the sequences of virulence factor from the database (http://www.mgc.ac.cn/VFs/). The gene sequence was uploaded to the PubMLST database (http://pubmlst.org/) to determine the ST type of the isolate.

### Phylogenetic Reconstruction And Analysis

Identification of core genomic single nucleotide polymorphisms SNPs on WGS data for 14 isolates was conducted by using the kSNP program.[13] kSNP is a program based on the k-mer analysis. Kchooser is used to evaluate the optimal value of k-mer before kSNP is run. After the run of kSNP program, the output file was used for further analysis.[14] The maximum likelihood tree of the core SNP matrix output of kSNP was generated by using iTOL (https://itol.embl.de/).

# Results

## Detection Of KPC-Producing K. Pneumoniae From Clinical Samples

The study population included all patients in the hospital, and the case patients were specimens producing carbapenem-resistant *K. pneumoniae*. Patients who were isolated from the first carbapenem-resistant *K. pneumoniae* were considered to be the source of transmission, and then carbapenem-resistant *K. pneumoniae* was continuously collected. The outbreak lasted for 64 days. A total of 14 carbapenem-resistant *K. pneumoniae* were collected from sputum (8/14), urine (5/14)and pus (1/14). After the bacteria were cultured and purified, single colonies were selected from the culture plates and identified with MALDI-TOF-MS. The isolates were recovered from the selected medium for PCR and sequencing, and the $bla_{KPC}$ gene was identified in all isolates, no other carbapenemase encoding genes were detected.

## Clinical Characteristics Of 14 Carbapenem-Resistant Isolates

Clinical characteristics of 14 carbapenem-resistant isolates were summarized in Table S1. In this study, isolates were defined as nonduplicated strains only if they were isolated from different patients. Among them, there are 5 females and 9 males, aged between 45 and 88 years old. The patients came from 4 different wards, including 10 from intensive care unit (ICU), 2 from respiratory ward, 1 from neurosurgical ward and 1 from recovery unit. Ten of them had a history of infectious diseases.

## Antibiotic Resistance Profiles Of 14 Carbapenem-Resistant Isolates

As shown in Figure 1, the antibiotic susceptibility test showed that all 14 isolates were resistant to piperacillin tazobactam, cefotaxime, ceftazidime, cefprozil, aztreonam, imipenem and meropenem, ciprofloxacin. Gentamicin (14%), tobramycin (14%) and amikacin (28%) have low sensitivity. Sensitivity to chloramphenicol (93%) and tigecycline (78%) was higher. Moreover, the experimental results showed that all 14 isolates were multi-drug resistant.

## Antimicrobial Resistance Genes

We present the data for the antimicrobial resistance genes in Table 1. The results of the analysis indicated that all isolates carried $bla_{KPC-2}$. Figure 2 shows the genetic environment surrounding the $bla_{KPC-2}$. There are four distinct genetic environments in the isolates carrying $bla_{KPC-2}$ in this study. Thirteen isolates (92%) carried $bla_{CTX-M-65}$, 12 isolates (85%) carried $bla_{SHV-11}$and 9 isolates carried $bla_{TEM-1B}$ (64%). All isolates carried *fosA*, and *sul*- genes, encoding fosfomycin- and sulphonamide-, resistance. Moreover, a high prevalence of tetracycline resistance gene (57% isolates) was also observed. In addition, 14 isolates also carry resistance genes such as *oqxA, oqxB, mphA, tetA* and *tetM* (Table 1).

## Molecular Characteristics Of 14 Clinical Isolates

MLST analysis found that 14 clinically derived carbapenemase-producing *K. pneumoniae* were all ST11. S1-PFGE and Southern blot analysis demonstrated that the plasmid size carrying $bla_{KPC-2}$ ranged from 94 kb to 368 kb (Table 1, Figure S1). By comparison of the plasmid sequences, 14 isolates belonged to the $IncFII_{K2}$ type plasmid. The results of the conjugation experiments showed that only the plasmid of the isolate 3C25 was successfully transferred to the *E. coli* J53. The virulence-related genes detected in 14 isolates included *ybtX* (100%, 14/14), *mrk* (93%, 13/14), *fim* (93%, 13/14), *entB* (93%, 13/). 14), *entC* (93%, 13/14), *entF* (93%, 13/14), *irp1* (93%, 13/14), *ybtE* (0.7%, 1/14), *ybtP* (0.7%, 1/14) and *entD* (0.7%, 1/14) (Figure S2). Phylogenetic analysis based on k-mer algorithm shows that 3C9 and 3C17, 3C18 and 3C29, 3C3 and 3C23 have certain phylogenetic relationships (Figure 3). Moreover, their $bla_{KPC-2}$ gene has the same genetic environment (Figure 2).

# Discussion

We reported the outbreak of ST11 KPC-producing *K. pneumoniae* for 2 months in a general hospital in Shandong Province, China. All 14 isolates belonged to ST11 and carried the KPC-2-encoded $IncFII_{K2}$ plasmid. ST11 KPC-producing *K. pneumoniae* isolates are very common in China.[15] In Shandong Province, there are few reports about KPC-producing *K. pneumoniae* causing outbreaks in the teaching hospital. Previously, we report the discovery of a KPC-2-producing *Raoultella ornithinolytica* isolate from well water in rural Shandong, but the

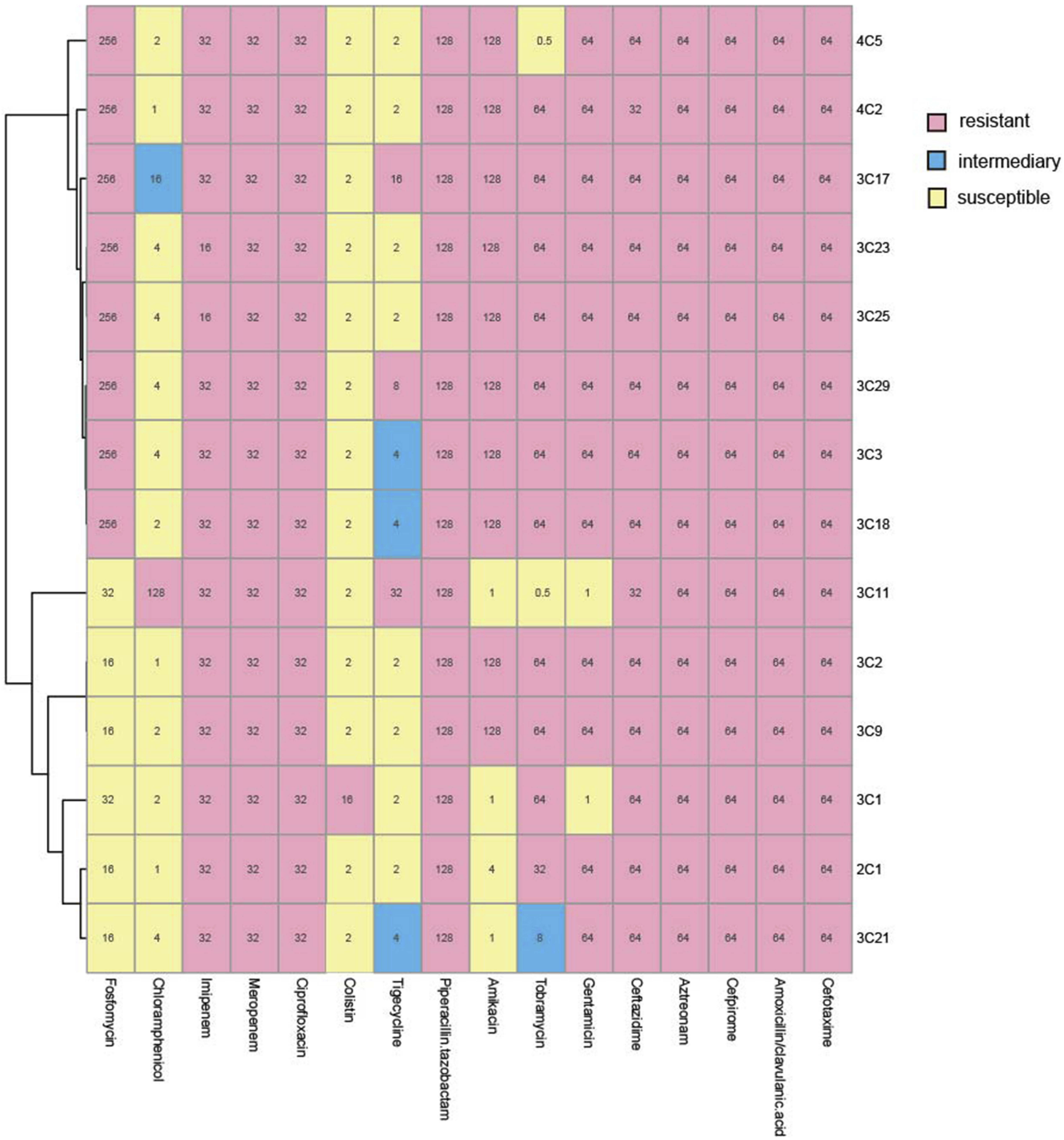


**Figure 1** MICs were determined by agar dilution methods for all antibiotics except for colistin and tigecycline, for which broth microdilution was used. Results were interpreted using the CLSI guidelines. Pink indicates resistance, blue indicates mediation, and light yellow indicates sensitivity.

gene characteristics were not described in detail.[16] In this study, we describe the genetic characteristics of *bla*$_{\text{KPC-2}}$, similar to other parts of China, and can be mediated through different molecular mechanisms.[17,18] Recent reports have shown a clear correlation between the *K. pneumoniae* ST11 and IncFII-like plasmid. This result indicates that the IncFII-like plasmid may promote the spread of the *bla*$_{\text{KPC}}$ gene in *K. pneumoniae* ST11 in China.[19] In this work, 14 CRKP strains were isolated from a large teaching hospital in Shandong Province in two months. MIC results (Figure 1) demonstrated that 14 isolates exhibited multidrug resistance.[20] All the 14

**Table 1** Antibiotic Resistance Genes, STs, Plasmid Type And Plasmid Size Of KPC-Producing *K. Pneumoniae* Isolates

| Isolate | Source | Antibiotic Resistance Genes | MLST | Plasmid Type | Plasmid Size |
|---|---|---|---|---|---|
| 2C1 | Sputum | *bla*$_{\text{CTX-M-65}}$, *fosA6, sul2, aph(3")-Ib, aph(6)-Id, bla*$_{\text{SHV}}$, *bla*$_{\text{CTX-M-3}}$, *qnrS1, sul1, aph(3')-Ia, qnrB2, aac(3)-Iid, mph(A), aadA16, dfrA27, ARR-3, aac (6')Ib-cr* | ST11 | IncF II$_{\text{K2}}$ | 94 kb |
| 3C1 | Sputum | *dfrA1, ant(3")-Ia, erm(42), ant(3")-Ia, sul1, sul2, bla*$_{\text{SHV-11}}$, *fosA6* | ST11 | IncF II$_{\text{K2}}$ | 94 kb |
| 3C2 | Sputum | *rmtB, bla*$_{\text{TEM-1B}}$, *bla*$_{\text{SHV-11}}$, *bla*$_{\text{CTX-M-65}}$, *fosA6, ant(3")-Ia, sul1* | ST11 | IncF II$_{\text{K2}}$ | 140 kb |
| 3C3 | Sputum | *bla*$_{\text{CTX-M-65}}$, *oqxA, oqxB, bla*$_{\text{TEM-1B}}$, *rmtB, bla*$_{\text{SHV-11}}$, *fosA3, fosA6, sul1, ant(3")-Ia, bla*$_{\text{CTX-M-15}}$ | ST11 | IncF II$_{\text{K2}}$ | 140 kb |
| 3C9 | Urine | *bla*$_{\text{SHV-11}}$, *fosA6, ant(3")-Ia, sul1, rmtB, bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CTX-M-65}}$ | ST11 | IncF II$_{\text{K2}}$ | 138.9 kb |
| 3C11 | Sputum | *mph(A), aph(6)-Id, aph(3")-Ib, sul2, tet(A), fosA6, catA2, bla*$_{\text{CTX-M-65}}$, *dfrA12, aadA2, sul1, bla*$_{\text{SHV-11}}$, *bla*$_{\text{TEM-1B}}$, *rmtB, aph(3')-Ia* | ST11 | IncF II$_{\text{K2}}$ | 336.5 kb |
| 3C17 | Urine | *erm(42), ant(3")-Ia, sul1, bla*$_{\text{CMY-2}}$, *fosA3, bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{TEM-1B}}$, *rmtB, dfrA1, sul2, fosA6* | ST11 | IncF II$_{\text{K2}}$ | 160 kb |
| 3C18 | Sputum | *bla*$_{\text{SHV-12}}$, *ant(3")-Ia, sul1, fosA6, rmtB, bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CTX-M-65}}$ | ST11 | IncF II$_{\text{K2}}$ | 135 kb |
| 3C21 | Urine | *sul1, aadA2, dfrA12, bla*$_{\text{SHV-11}}$, *bla*$_{\text{CTX-M-65}}$, *aac(3)-Iid, fosA6* | ST11 | IncF II$_{\text{K2}}$ | 140 kb |
| 3C23 | Sputum | *fosA3, sul1, aac(6')Ib-cr, ARR-3, dfrA27, aadA16, aph(3")-Ib, aph(6)-Id, bla*$_{\text{CTX-M-65}}$, *rmtB, sul2, aadA2, bla*$_{\text{TEM-141}}$, *bla*$_{\text{SHV-11}}$, *fosA6, oqxB, oqxA* | ST11 | IncF II$_{\text{K2}}$ | 368 kb |
| 3C25 | Sputum | *aadA2, bla*$_{\text{TEM-141}}$, *sul1, bla*$_{\text{CTX-M-15}}$, *aac(6')Ib-cr, ARR-3, dfrA27, aadA16, aph(6)-Id, aph(3")-Ib, rmtB, sul2, bla*$_{\text{CTX-M-65}}$, *oqxA, oqxB, bla*$_{\text{SHV-11}}$, *fosA3* | ST11 | IncF II$_{\text{K2}}$ | 140 kb |
| 3C29 | Urine | *bla*$_{\text{TEM-1B}}$, *rmtB, bla*$_{\text{CTX-M-65}}$, *ant(3")-Ia, sul1, bla*$_{\text{SHV-11}}$, *fosA6* | ST11 | IncF II$_{\text{K2}}$ | 150 kb |
| 4C2 | Urine | *fosA3, fosA6, dfrA14, sul2, aph(3")-Ib, aph(6)-Id, qnrS1, sul1, aadA2, bla*$_{\text{CTX-M-65}}$, *rmtB, bla*$_{\text{TEM-1B}}$, *bla*$_{\text{SHV-11}}$ | ST11 | IncF II$_{\text{K2}}$ | 94 kb |
| 4C5 | Pus | *bla*$_{\text{SHV-11}}$, *rmtB, bla*$_{\text{TEM-1B}}$, *sul1, ant(3")-Ia, bla*$_{\text{CTX-M-65}}$, *fosA6, fosA3* | ST11 | IncF II$_{\text{K2}}$ | 150 kb |

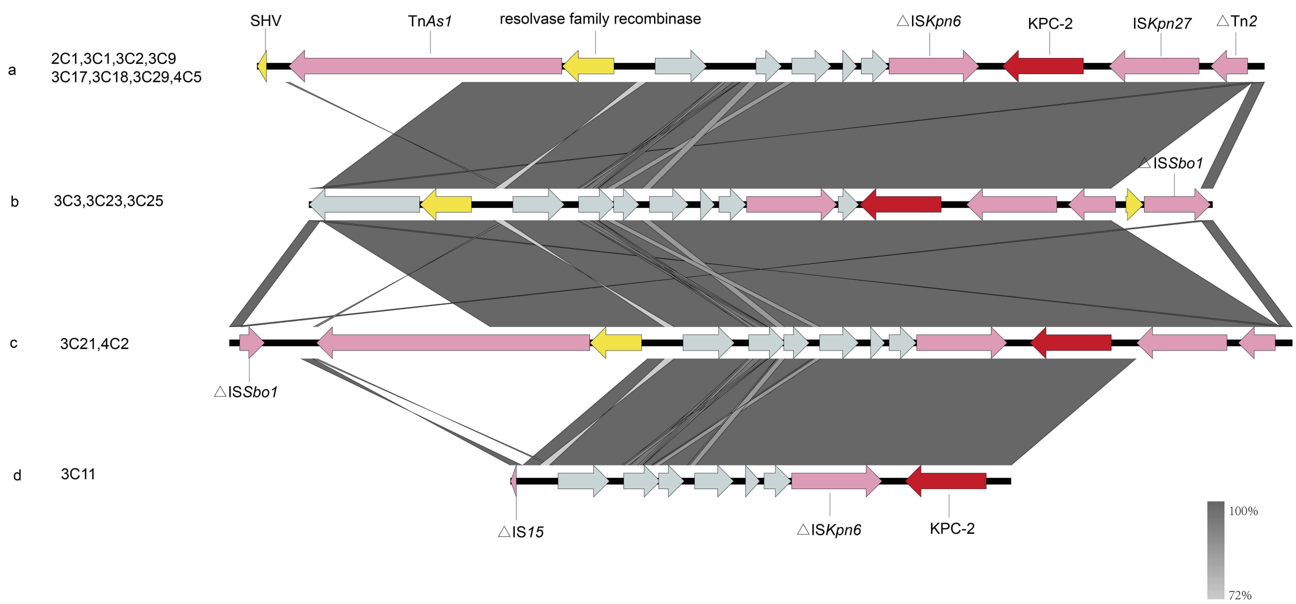


**Figure 2** The genetic environment of the $bla_{KPC-2}$ gene in *K. pneumoniae* was isolated from clinical sources. The arrows represent the direction of transcription. The red open reading frame (ORF) indicates the $bla_{KPC-2}$ gene, the pink ORF indicates the mobile element, the yellow ORF indicates other resistance genes or enzymes and the gray ORF indicates other genes or genes of unknown function. (**A**) The genetic environment of $bla_{KPC-2}$ is similar in isolates 2C1, 3C1, 3C2, 3C9, 3C17, 3C18, 3C29 and 4C5. (**B**) The isolates 3C3, 3C23 and 3C25 all carry $bla_{KPC-2}$ and share the identical genetic environment surrounding the same gene. (**C**) The isolate 3C21 and the isolate 4C2 have the same genetic environment. (**D**) The genetic environment of the isolate 3C11 carrying the $bla_{KPC-2}$ gene.

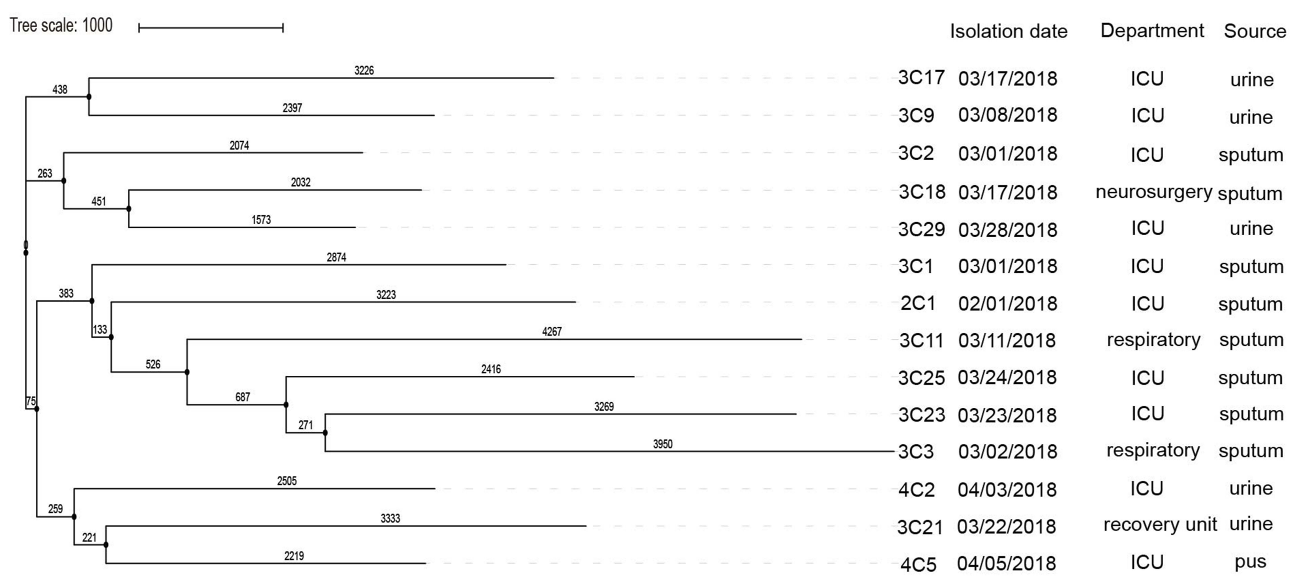


**Figure 3** SNP analysis of KPC-2-producing *K. pneumoniae* isolates, performed using kSNP. The maximum likelihood analysis of the core SNP matrix output for kSNP is performed in iTOL.

isolates were resistant to meropenem and imipenem, and the whole-genome sequencing results showed that all isolates carried $bla_{KPC-2}$, indicating that the drug-resistant phenotype was consistent with the genotype. Moreover, we have also found other drug resistance genes, such as $bla_{CTX-M-65}$ and $bla_{TEM-1}$ $bla_{SHV-11}$ encoding β-lactam resistance; *mphA* encoding macrolide resistance; *aac(6')Ib-cr* and *aph(3')- Ia* encodes aminoglycoside resistance; *sul1, sul2* and *sul3* encode sulfonamide resistance; *oqxA* and *oqxB* encode fluoroquinolone resistance; *fosA* encodes fosfomycin resistance; *tetA* and *tetM* encode tetracycline resistance; and *dfrA1* encodes trimethoprim resistance.[21] These findings illustrate the multi-drug resistant phenotype of these *K. pneumoniae* isolates.

It is very difficult to clarify the transmission events between patients based only on epidemiological data.[22] Combining genetic information with clinical epidemiological information can explain the spread of outbreak strains. Based on phylogenetic analysis and SNP differences, we can divide the 14 isolates into three branches, each representing a single transmission event (Figure 3). Combined with patient inpatient department and SNP differences, the transmission of pathogens from one patient to another was demonstrated. For example, 3C3 and 3C23, 3C18 and 3C29 have a certain affinity with each other, but from different departments, not only that, they also have the same genetic environment. The 14 isolates in this study had shorter sampling intervals and the same plasmid carrying *bla*$_{KPC-2}$, belonging to the same clone, and having a certain relationship with each other, indicating that the outbreak occurred in a short period of time.[23] When an outbreak occurs, the patient and the environment should be disinfected immediately, and the patient should be given appropriate treatment, and if necessary, the patient should be isolated, which can effectively contain the outbreak.

By analyzing virulence genes, all isolates carry the *mrk* operon, encoding the genes for yersiniabactin (*irp1, irp2, fyuA and ybtAEPQSTUX*), however, the *rmpA* or *rmpA2* genes were absent from all isolates (Figure S2), both of which encode a high mucus phenotype and serve as high mark of virulence. The *mrk* operon encodes type 3 fimbriae, a virulence factor prevalent in *Streptococcus pneumoniae*.[24] Type 3 fimbriae can not only mediate biofilm formation but also enhance bacterial adhesion to medical devices. Moreover, type 3 fimbriae may be an important factor in the formation of biofilm-associated infections, which can enter the host and persist in the clinical environment.[25,26] Yersiniabactin is an iron carrier that helps bacteria gain the ability to chelate iron from infected host cells.[27] In this study, all isolates carried the yersiniabactin genes pose challenges for clinical treatment.

Previous investigations have documented the diversity of *bla*$_{KPC}$-harboring plasmids, which include IncFII, IncN, IncL/M, IncR and ColE1 groups, ranging in size from 10 to 300 kb.[28–31] IncF replicons can be divided into FIA, FIB, FIC and FII groups, wherein the IncFII plasmid family exists in various Enterobacter species and plays an important role in the spread of antibacterial resistance genes such as *bla*$_{KPC}$.[32,33] In addition, the FII replicons can be divided into different subtypes, including FIIY, FIIK and FIIS, generating a number of compatible variants for overcoming the incompatibility barrier with obtaining plasmids.[34] In our study, all 14 isolates belonged to the IncFII$_{K2}$ type plasmid. IncFII$_{K2}$ was a common *bla*$_{KPC}$-harboring plasmids, reported in the United States, Israel, the United Kingdom, Italy and Colombia.[32] Unlike previous studies, plasmid sizes in this study ranged from 94 kb to 368 kb, which was relatively large. The results of the conjugation assay showed that the plasmid binding in this study was difficult and the binding efficiency was less than 10%. The results correlated not only with plasmid size, but also with the presence of mobile genetic elements.[35]Although the 14 isolates were all in the IncFII$_{K2}$ type plasmid, the size of the plasmid carrying the *bla*$_{KPC-2}$ was different, indicating that the *bla*$_{KPC-2}$ in the hospital may be of various origins and spread in hospitals for many years. It is necessary to conduct a long-term retrospective genomic study of KPC-producing *K. pneumoniae* throughout the hospital to elucidate the evolution of KPC-producing *K. pneumoniae* in the hospital.

In addition, we investigated the genetic environment surrounding *bla*$_{KPC-2}$ (Figure 2) and the results suggest that mobile genetic elements may promote the transmission of the *bla*$_{KPC-2}$ gene. Although the genetic structure of these bacteria is different, the genetic background of *bla*$_{KPC-2}$ is relatively similar in all plasmids. The *bla*$_{KPC-2}$ genes were located in the same genetic context, the insertion sequence IS*Kpn27* is located upstream, and IS*Kpn6* is located downstream, except for 3C11, which is the same as previously reported.[36]

## Conclusion

We first reported an outbreak of ST11-type KPC-2-producing *K. pneumoniae* in a large hospital in Shandong Province in a short period of time, although it is prevalent in China. We presented the genomic characteristics of *bla*$_{KPC-2}$ positive *K. pneumoniae* isolates through whole-genome sequencing. All 14 isolates carrying the *bla*$_{KPC-2}$ gene and have four different types of genetic environments. All isolates carried the virulence genes pose challenges for clinical treatment. It is now necessary to carry out routine genomic monitoring of such plasmids to effectively curb the spread and spread of resistant bacteria in this area.

## Acknowledgments

The authors would like to thank the participants, coordinators and administrators for their support during the study. This project was supported by the National Natural Science Foundation of China (41771499 and 81741098) and the Fundamental Research Funds of Shandong University (2018JC102).

## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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