Genetic factors related to the widespread dissemination of ST11 extensively drug-resistant carbapenemase-producing *Klebsiella pneumoniae* strains within hospital

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Abstract

**Background:** Carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) poses distinct clinical challenges due to extensively drug resistant (XDR) phenotype, and sequence type (ST) 11 is the most dominant *bla*KPC-2-bearing CP-Kp clone in China. The purpose of this current retrospective study was to explore the genetic factors associated with the success of XDR CP-Kp ST11 strains circulated in the intensive care unit (ICU) of a Chinese tertiary hospital.

**Methods:** Six ST11 XDR CP-Kp strains were identified between May and December 2014 and validated by minimum inhibitory concentration examination, polymerase chain reaction, and pyrosequencing. The six ST11 XDR CP-Kp, as well as three multi-drug resistant (MDR) and four susceptible strains, were sequenced using single-molecule real-time method. Comprehensive structural and functional analysis based on comparative genomics was performed to identify genomic characteristics of the XDR ST11 CP-Kp strains.

**Results:** We found that ST11 XDR *bla*KPC-2-bearing CP-Kp strains isolated from inpatients spread in the ICU of the hospital. Functionally, genes associated with information storage and processing of the ST11 XDR CP-Kp strains were more abundant than those of MDR and susceptible strains, especially genes correlative with mobile genetic elements (MGEs) such as transposons and prophages. Structurally, eleven large-scale genetic regions taken for the unique genome in these ST11 XDR CP-Kp strains were identified as MGEs including transposons, integrons, prophages, genomic islands, and integrative and conjugative elements. Three of them were located on plasmids and eight on chromosomes; five of them were with antimicrobial resistance genes and eight with adaptation associated genes. Notably, a new *bla*KPC-2-bearing ΔTn1721-*bla*KPC-2 transposon, probably transposed and truncated from ΔTn1721-*bla*KPC-2 by IS903D and ISKpn8, was identified in all six ST11 XDR CP-Kp strains.

**Conclusion:** Our findings suggested that together with clonal spread, MGEs identified uniquely in the ST11 XDR CP-Kp strains might contribute to their formidable adaptability, which facilitated their widespread dissemination in hospital.

**Keywords:** Whole genome sequencing; Carbapenemase-producing *Klebsiella pneumoniae*; Mobile genetic elements; Antimicrobial resistance genes; Adaptation associated genes

Introduction

The human pathogenic carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) bacterium poses distinct clinical challenges due to its extensively drug resistant (XDR) phenotype.¹⁰ CP-Kp causes hospital-acquired and long-term care-related infections that feature high morbidity and mortality.²⁻⁴ It is now known that enzymes like *Klebsiella pneumoniae* carbapenemase (KPC) are important products of CP-Kp.⁵ KPC-2 (with the identical protein sequence to KPC-1) was the first known variant of the KPC enzymes, and it was identified in a *K. pneumoniae* strain in North Carolina, USA in 1996.⁶ In China, the first report of a KPC-producing *K. pneumoniae* strain was from Zhejiang province in 2007.⁷ Since then, this pathogen has been identified in several Chinese provinces; and it has been demonstrated that sequence type (ST) 11 is the dominant clone amongst the CP-Kp strains in China.⁸⁻¹⁷

Many researchers have explored the potential molecular
factors that govern the success of the ST258 CP-Kp clone,\(^5\)\(^,\)\(^18\) which is threatening European and American countries; however, relatively little progress has been made in the ST11 strains; so it is urgent to identify which characteristics have granted ST11 its particularly strong ability to spread rapidly.

Mobile genetic elements (MGEs), including plasmids, insertion sequences (ISs), transposons, integrons, and prophages, as well as integrative and conjugative elements (ICEs) and genomic islands,\(^9\)\(^,\)\(^19\) are important carriers of antimicrobial resistance genes (ARGs) and adaptation associated genes.\(^20\) Previous pan-genome comparative analyses has shown that horizontal gene transfer of MGEs across much of the bacterial world can explain many of the key differences between core genomes (the genes present in all strains within a group).\(^21\) XDR CP-Kp strains of ST11 are common vehicle of MGEs, and thus, we speculate that the successful widespread of XDR CP-Kp strains of ST11 is related to certain MGEs, which are always closely related to the acquisition and spread of resistance and/or adaptation associated genes as well as the broader evolution of bacteria.

Single-molecule real-time (SMRT) based whole genome sequencing (WGS) has emerged as a powerful tool for deep dissection of bacterial genomes and their functions, and its ability to generate long reads (with an average size of 10 Kb) that can cross complex repeat regions has substantially facilitated functional genomics research about many species.\(^22\) In the current study, we report a dissemination of ST11 XDR CP-Kp strains in intensive care unit (ICU) of a Chinese hospital between May 1st and December 31st, 2014 retrospectively. Then we assembled the complete genomes and plasmids of 13 clinical \(K.\ pneumoniae\) isolates based on SMRT sequencing data, including six ST11 CP-Kp XDR group strains, three non-CP-Kp multi-drug resistant (MDR) group strains and four susceptible (S) group strains, and performed comparative genomics analysis to explore the genetic factors unique to the ST11 XDR CP-Kp strains. Our analyses revealed extensive MGEs carrying ARGs and adaptation associated genes that can help to explain the widespread dissemination of XDR CP-Kp strains of ST11, and might facilitate the development of novel targets to these unique genetic factors.

**Methods**

**Ethical approval**

The retrospective study was approved by the Ethics Committee of Peking University People’s Hospital (2015PHB037-01) and the requirement of written informed consent was waived.

**Klebsiella pneumoniae strains and bacterial experiments**

We analyzed six patients with CK-Kp infection who were admitted to a five-ward ICU of a Fujian tertiary hospital with 2400 beds in China between May 1st and December 31st, 2014. Six \(K.\ pneumoniae\) strains were isolated from urine, sputum, bile, ascites, end of catheter, and blood. Multi-locus sequence typing, ARGs including \(bla_{KPC-2}\), and later gene confirmation was determined by polymerase chain reaction (PCR) and pyrosequencing.\(^23\) Minimum inhibitory concentrations were determined using a VITEK2 automated system (Biomerieux Vitek, Inc., France), and antibiotic susceptibilities were assessed according to the Clinical and Laboratory Standards Institute.\(^24\) According to the Clinical and Laboratory Standards Institute, the isolates were defined as XDR ST11 CP-Kp strains. Besides, another seven non-CP-Kp strains were isolated in the same hospital during the same period to compare with XDR CP-Kp strains, and three of these non-CP-Kp strains were defined as MDR and four were defined as susceptible (S) strains.

**Genomic DNA extraction, genome sequencing, and assembly**

Bacterial cells of a total 13 \(K.\ pneumoniae\) strains were collected at the stationary phase by centrifugation (12,000 \(xg\) for 10 min at 4°C. Total DNA was extracted using a Qiagen DNA Mini Kit (Qiagen, Valencia, CA, USA) according to its protocol. The integrity of extracted DNA was assessed by gel electrophoresis using 0.7% agarose gel, and the quantification was performed using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Libraries were constructed according to the large SMRT Bell gDNA protocol (Pacific Biosciences, Menlo Park, CA, USA), and the whole genomes of isolates were sequenced via SMRT sequencing with the PacBio RS II platform. Genomes were assembled by using the Short Oligonucleotide Analysis Package (SOAPdenovo 2.3.0). The final contigs were checked for circularization, and the overlapping ends were trimmed.

**Pan-genome analysis**

The protein coding sequences (CDSs) were predicted using GeneMarkS software (http://topaz.gatech.edu/genemark/genemarks.cgi), and annotated via BLAST analysis against the NCBI non-redundant (NR) protein sequence database. The core genome for XDR, MDR, or S group strains was determined as homologous proteins (as defined by 70% coverage and 50% sequence identity using cd-hit\(^25\)\(^,\)\(^26\)) presenting in all of the isolates inside the group. Each of the three intra-group sets of core genes were created by extracting one sequence for every core gene in each group. Three intra-group sets of core genes were then compared and clustered by cd-hit\(^23\) (using the same coverage and sequence identity above) and the “supercore” genes (core genes that were present in all three groups) were determined. The dispensable genome of XDR, MDR, or S group, designated as DI, DII, or DIII, was created by subtracting the “supercore” genes from each group’s core genome. A Venn diagram was generated to depict the number of core genes in each of the three groups as well as the “supercore” genes and the dispensable genes.

**Single-nucleotide polymorphisms (SNPs) and phylogenetic analysis**

The 13 \(K.\ pneumoniae\) genomes we sequenced were used for phylogenetic analysis along with 12 \(K.\ pneumoniae\) and two \(Klebsiella variicola\) genomes from GenBank. The core
SNPs inside each group strains and across the 27 examined genomes were called from the de novo-assembled sequences using the kSNP with k-mers size of 21. The core SNPs across the 27 examined genomes were used to construct a phylogenetic tree using the maximum-likelihood based approach in FastTreeMP with 1000 bootstraps. K. variicola X39 and K. variicola DSM 15968 set as the out-group sequences. Local support values for each node were calculated. The tree was visualized in iTOL (https://itol.embl.de/).

Structural and functional analyses of bacterial genomes

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Results

Dissemination of six ST11 XDR CP-Kp strains

The six CP-Kp strains, which were resistant to imipenem and meropenem, were isolated from different wards of the ICU in the hospital within 8 months (between May 1st and December 31st, 2014) [Table 1]. They were all identified as the ST11 type, and the capsular type K47. The six CP-Kp strains. Phylogenetic analysis was performed and visualized using EasyFig v2.3.

WGS data were then used to analyze the dissemination of the six ST11 XDR CP-Kp strains. Phylogenetic analysis was employed to assess the relationships between the 13 SMRT sequenced strains along with 12 K. pneumoniae and two K. variicola strains from GenBank [Supplementary Table 4, http://links.lww.com/CM9/A320]. The six ST11 XDR CP-Kp strains (F1, F5, F77, F127, F132, and F138) were significantly supported as a single clade that was clearly separate from other groups [Figure 1A]. There were fewer core SNPs (68) among the six ST11 XDR CP-Kp strains, while there were much more core SNPs among the three MDR strains (26,131), or the four susceptible strains (32,724). The numbers of core SNPs between each pair of the six ST11 CP-Kp XDR strains ranged from 4 to 55 [Supplementary Figure 2, http://links.lww.com/CM9/A319].

Statistical analysis

The patients’ ages, genome sizes, and numbers of predicted CDSs and MGEs, as well as positive genes percentages of the PCR confirmation test, were compared using the Kruskal-Wallis and Mann-Whitney test. Categorical variables of predicted functional genes were compared by the Chi-square test. Using Prism GraphPad software version 6.01 (GraphPad software Inc.; La, Jolla, CA, USA), P ≤ 0.05 was considered significant.

Sequence data accession numbers

The whole genomes we sequenced in this study are available in the NCBI repository under the accession numbers CP026130-CP026131 (F1), CP026132-CP026135 (F5), CP026136-CP026139 (F77), CP026140-CP026142 (F127), CP026145-CP026148 (F132), CP026149-CP026152 (F138), CP026153-CP026154 (F10(AN)), CP026155-CP026156 (B12(AN)), CP026157-CP026158 (F127), CP026162-CP026163 (F13), CP026164-CP026166 (F81), CP026159 (F89-1), and CP026160-CP026161 (F93-1).

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thus suggesting the phylogenetically high similarity of these strains. Furthermore, there were seven core SNPs between F1 and F127 and 4 to 14 core SNPs among F5, F77, F132, and F138, while there were 50 to 55 core SNPs differentiating the strains of F1 and F127 from the strains of F5, F77, F132, and F138. According to a recently published study, the distance of strains of F1 and F127 from the strains of F5, F77, F132, and F138, while there were 50 to 55 core SNPs differentiating the strains. Furthermore, there were seven core SNPs between F1 and F127 and 4 to 14 core SNPs among F5, F77, F132, and F138. According to a recently published study, the distance of strains of F1 and F127 from the strains of F5, F77, F132, and F138, while there were 50 to 55 core SNPs differentiating the strains. Furthermore, there were seven core SNPs between F1 and F127 and 4 to 14 core SNPs among F5, F77, F132, and F138. 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Figure 1: (A) The phylogeny analysis of *K. pneumoniae* strains. SNPs across the 27 examined genomes were called. The core SNPs were used to construct phylogenetic trees using the ML method based approach FastTreeMP with 1000 bootstraps. *K. variicola* X39 and DSM 15968 was included as the out-group sequences. The branch bearing double hatch marks indicates that it has been truncated and is not proportional to the rest. (B) Venn diagram of the tally of homologous proteins shared by or unique among *K. pneumoniae* strains of the XDR, MDR, and S groups. The core genome for XDR, MDR, or S group was determined as homologous proteins presenting in all of the isolates inside the group. The “supercore” genome was determined as the core genes that were present in each of the groups. The dispensable genome of each group were created by subtracting the “supercore” from each group’s core genome. (C) Distribution of COG functional categories among DI, DII, and DIII. All proteins in DI, DII, and DIII were aligned against the COG database and comparisons among DI, DII, and DIII were conducted. COG: Clusters of Orthologous Groups; *K. pneumoniae*: Klebsiella pneumoniae; MDR: Multidrug-resistant; ML: Maximum-likelihood; SNPs: Single-nucleotide polymorphisms; XDR: Extensively drug resistant.
group, compared with that of only 0.33 and 0 in the MDR group and susceptible group, respectively. The number of prophages identified in the strains of XDR (13.33) group was much larger than that of MDR (4.00) and S (4.25) groups (P = 0.0238 and 0.0048, respectively), whereas there was no significant difference between MDR and S groups (P = 0.8857). Similarly, the average numbers of genomic islands in XDR group (21.17) were larger than that in MDR group (15.67) and S group (5.75) significantly (P = 0.0238 and 0.0048, respectively), yet not in the S group compared with that of the three MDR genomes (P = 0.0857). The distributions of prophages and genomic islands of the XDR group strains were similar, but there were significant differences between groups. The detailed information of prophages and genomic islands was listed in Supplementary Table 4, http://links.lww.com/CM9/A323.

Different predicted functional genes between XDR strains and MDR and S strains

There were 4264 core genes in the MDR strains and 4155 genes in the S strains, markedly lower than the number (5036) of core genes in the XDR group. Pan-genome analysis based on the three groups showed that there were a total of 5158 genes comprising their pan-genome. In the pan-genome, 4009 genes were identified to be shared by all three groups; these were defined as the supercore genome. The non-supercore genes within the XDR, MDR, and S groups were assigned to dispensable genomes and defined as, respectively, DI (1027), DII (255), and DIII (146) [Figure 1B].

Functional classification analysis of DI, DII, DIII, and the supercore genome was carried out by comparison against the COG database [Figure 1C, Supplementary Table 6, http://links.lww.com/CM9/A325]. We classified the COG functional categories into four classes, namely, metabolism, information storage and processing, cellular processes and signaling, and “poorly characterized.” An apparently high proportion of genes in DI (32.47%) were assigned to COG categories involved in information storage and processing, in comparison with that in supercore genome (18.61%), DII (17.60%), and DIII (16.33%) (P = 0.0329, 0.0206 and 0.0124, respectively) [Supplementary Table 6, http://links.lww.com/CM9/A325]. In particular, the proportion of COG categories [X] (mobility: prophages, transposons; 13.89%) within DI is apparently higher than that in the supercore genome, DII, or DIII (0.0002, 0.0054, and 0.0054, respectively), suggesting the presence of a large amount of MGEs and implying that much of the genetic and phenotypic diversity amongst the clinical isolates may be a result of horizontal gene transfer. As expected, a relatively low proportion of genes dispersed in the DI were predicted to be involved in metabolic profiles (32.64%) compared with that in supercore genome (48.90%) (P = 0.0295), reflecting a relatively low proportion of essential genes supporting basic bacterial activities and, therefore, a relative abundance of unessential genes such as MGEs. For genes involved in cellular processes and signaling and in the “poorly characterized” class, there was no significant discrepancy between supercore genome, DI, DII, and DIII.

Linking unique large genomic regions in XDR genomes to MGEs

We conducted a further inspection of a large number of genes unique to the XDR ST11 CP-Kp strains with the idea that these unique genomic regions may be related to MGEs that may have contributed to the wide dissemination of these strains. Gene names and genomic positions for the 818 genes unique to the core genome of XDR ST11 CP-Kp strains were extracted and manually inspected: this identified 11 large (>9 Kb) genomic regions that ranged in size from 9.21 to 143.20 Kb. Three regions were in the IncFII plasmids, and eight were in the chromosomes. These 11 unique large genomic regions in the XDR group strains basically match to five MGEs carrying ARGs and eight MGEs carrying adaptation associated genes.

Of the five MGEs carrying ARGs, three were located on IncFII plasmids and two on chromosomes. For plasmids, XDR group-specific MGEs included a 16.05 Kb IncFII plasmid partial backbone and two prophages (ProphagePlas1_1 and ProphagePlas1_2) [Figure 2A, Supplementary Table 7, http://links.lww.com/CM9/A326, and Supplementary Table 8, http://links.lww.com/CM9/A327]. The IncFII plasmid itself is a large MGE, and many IncFII plasmid carry blaKPC-2 as well as other ARGs.[41] Homologous sequences of this region in GenBank are from K. pneumoniae, Proteus mirabilis, Escherichia coli, and Escherichia albertii which are all opportuntic pathogens of the Enterobacteriaceae. BlaKPC-2 is carried by ProphagePlas1_2 and is located within a transposon that is a variant of the ΔTn1721-blaKPC-2.[19] We named it as ΔΔTn1721-blaKPC-2 [Figure 2B]. The unique 25.45 Kb prophage region (ProphagePlas1_1) carries a chloramphenicol acetyltransferase catI, which can inactivate chloramphenicol.[42] Homologous sequences of this region in GenBank are from opportunistic pathogens of the Enterobacteriaceae such as K. pneumoniae, E. coli, Citrobacter freundii, or Enterobacter cloacae. The composite transposon of catI composed of catII and a transferase of IS26, tnpAIS26), the ProphagePlas1_1, and the IncFII plasmid might collectively contribute the horizontal genetic exchange of catI. Besides, the two XDR group-specific chromosome-bearing MGEs carrying ARGs included a class 1 integron In127 (carrying sul1 and aadA) that was carried by ProphageChr1_6, and another class 1 integron In610 (carrying cmlA, ANT(2")-Ia, APH(3")-I, APH(6)-I, and a gene for a puromycin acetyltransferase) that was carried by ICE_F1 [Figure 3, Supplementary Table 7, http://links.lww.com/CM9/A326, and Supplementary Table 8, http://links.lww.com/CM9/A327].

We also detected a variety of adaptation associated genes that were carried by 8 of the eleven XDR group-specific MGEs from chromosomes, including six prophages, one genomic island, and one ICE. The functions of these adaptation associated genes could be classified into five classes: cell wall/membrane/envelope, DNA operation, defense mechanisms, cell motility, and respiratory chain [Table 2]. Of particular note, we found that cell wall/membrane/envelope associated genes—responsible for the biosynthesis of lipopolysaccharide and biofilms—were present in ProphageChr1_2 of the XDR strains. Moreover, we found on ICE_F1 both LytM and the regulator LytR,
Figure 2: (A) Alignment of the plasmid sequences of the *K. pneumoniae* strains. BLASTN-based whole genome comparison was performed and visualized using BRIG to exhibit the architecture and gene repertoire of a total of twelve *K. pneumoniae* plasmids, using the plasmid sequence of pF1_1 as a reference. Part of the IncFII plasmid backbone region, ProphagePlas1_1, ProphagePlas1_2, and the significant genes are indicated by rectangles. (B) Transposon carrying *bla*<sup>KPC</sup>-2 located in our isolates (ΔΔ*Tn1721*-*bla*KPC-2) was compared with that in pKPHS2 (Δ*Tn1721*-*bla*KPC-2) and that in the unnamed plasmid of SWU01 (ΔΔ*Tn1721*-*bla*KPC-2). Regions of synteny between adjacent schematics are indicated by the shaded areas; the matching percentage nucleotide sequence identity for each such region is indicated. These schematics are drawn to scale. *K. pneumoniae*: Klebsiella pneumoniae.
which are known to participate in the synthesis of bacterial cell wall and the formation of biofilms and which likely enhance virulence, and the ability of anti-innate immune killing\(^{43-45}\); these may also participate in maintaining the integrity of bacterial plasma membrane and promoting β-lactam resistance.\(^{46}\)

Furthermore, to confirm preliminarily that these MGEs were XDR CP-Kp group specific, we detected some genes of these XDR-specific MGEs by PCR using a total of 98 K. pneumoniae strains we collected subsequently as templates [Supplementary Table 9, http://links.lww.com/CM9/A328]. The percentage of positive genes detected in XDR strains was larger than that in MDR \((P = 0.0045)\) and S \((P < 0.0001)\) strains significantly, whereas there was no significant difference between MDR and S strains \((P = 0.7081)\) [Supplementary Table 10, http://links.lww.com/CM9/A329].

**Discussion**

ST11 is a high-risk clone often presented as the XDR phenotype, which is associated with KPC-2 dissemination, and is commonly found in Asian countries especially in
China, and in Latin American countries. The ST11 genomes were found to be highly heterogeneous based on the patterns of SNPs. In the present study, we found and WGS confirmed a dissemination of ST11 XDR CP-Kp strains in a Fujian tertiary teaching hospital within eight months in 2014, and it has a warning role on the infection prevention and control in this hospital. Two extremely close clones were identified in these ST11 blaKPC-2-bearing K. pneumoniae strains, and they were considered to spread in the ICU of the hospital with open genomes and with evolution of chromosomes or plasmids mutations and/or genetic recombination from a common ancestor or clone.

Then, we found that the unique genes in the XDR group strains are related to MGEs either functionally or structurally. Horizontal gene transfer and subsequent recombination was considered another considerable factor for the widespread dissemination of ST11 XDR CP-Kp strains apart from clonal spread. We will discuss this MGEs from two aspects: MGEs carrying ARGs and MGEs carrying adaptation associated genes.

### Table 2: Adaptation associated genes on MGEs unique to the chromosomes of XDR *Klebsiella pneumoniae* strains.

| Number | Gene ID   | Annotation                                      | Class                                | Reference |
|--------|-----------|-------------------------------------------------|--------------------------------------|-----------|
| 1      | F1GM001432 | DNA damage inducible protein DinI                | DNA operation                        | [69]      |
| 2      | F1GM001446 | Putative membrane protein, related to biofilm formation | Cell wall/membrane/envelope          | [70]      |
| 3      | F1GM001571 | DNA adenine methylase Dam                        | DNA operation                        | [71]      |
| 4      | F1GM001579 | XRE family transcriptional regulator with helix-turn-helix structure | Defense mechanisms                  | [63]      |
| 5      | F1GM001598 | Glycopeptide alpha-N-acetylgalactosaminidase (GalNAc) | Cell wall/membrane/envelope          | [72]      |
| 6      | F1GM001619 | O-antigen ligase-like membrane protein           | Cell wall/membrane/envelope          | [73]      |
| 7      | F1GM001622 | Type 1 fimbrial protein                          | Cell motility                        | [67]      |
| 8      | F1GM001968 | dTDP-4-dehydrorhamnose reductase rfbD            | Cell wall/membrane/envelope          | [74]      |
| 9      | F1GM001969 | dTDP-4-dehydrorhamnose 3, 5-epimerase rfbC       | Cell wall/membrane/envelope          | [74]      |
| 10     | F1GM001977 | O-antigen export-TMD component                   | Cell wall/membrane/envelope          | [75]      |
| 11     | F1GM001978 | O-antigen export-NBD component                   | Cell wall/membrane/envelope          | [75]      |
| 12     | F1GM002223 | Pathogenic specific protein                      | Cell wall/membrane/envelope          | [70]      |
| 13     | F1GM002228 | Uropathogenic specific protein                   | Cell wall/membrane/envelope          | [70]      |
| 14     | F1GM003286 | DNA adenine methylase Dam                        | DNA operation                        | [71]      |
| 15     | F1GM003481 | 5-Methylcytosine-specific restriction endonuclease McrA | DNA operation                      | [76]      |
| 16     | F1GM003483 | SOS-response transcriptional repressor LexA      | DNA operation                        | [60]      |
| 17     | F1GM003484 | DNA damage inducible protein DinI                | DNA operation                        | [69]      |
| 18     | F1GM003786 | DNA adenine methylase Dam                        | DNA operation                        | [74]      |
| 19     | F1GM004955 | Arabinose operon transcriptional regulator AraC  | Defense mechanisms                   | [66]      |
| 20     | F1GM004973 | Arabinose efflux permease                        | Defense mechanisms                   | [62]      |
| 21     | F1GM005017 | DNA cytosine methyltransferase Dcm               | DNA operation                        | [67]      |
| 22     | F1GM005040 | Soluble lytic murein transglycosylase LytM       | Cell wall/membrane/envelope          | [43,46]   |
| 23     | F1GM005041 | LytR family DNA-binding response regulator       | Cell wall/membrane/envelope          | [44,45]   |
| 24     | F1GM005054 | DNA repair protein RadC                          | DNA operation                        | [61]      |
| 25     | F1GM005082 | Excision nuclease subunit A uvrA                 | DNA operation                        | [64]      |
| 26     | F1GM005085 | NADH dehydrogenase NDH                           | Respiratory chain                    | [68]      |
multiple replicons, and exist widely in different species of the Enterobacteriaceae[53,54] especially amongst opportunistic pathogens. We found that \(\text{bla}_{\text{KPC-2}}\) genes in the ST11 CP-Kp strains are located on transposons nested into prophages and plasmids.

Notably, a \(\Delta\text{TN1721-bla}_{\text{KPC-2}}\) transposon carried by ProphagePlas1_2 in all of the six ST11 CP-Kp strains sequenced has never been reported before: it might have been transposed and then truncated by an IS903D and an ISKpn8 that is flanked on the left and right sides of a region encompassing \(\text{IS5075}\) (harboring the anti-mercury operon) \(\Delta\text{TN2-I326-\Delta\text{TN3}}\) located on pKPHS2 (\(\Delta\text{TN1721-bla}_{\text{KPC-2}}\)).[59] A study found that the deletion of \(\text{tnpR\text{TN3}}\) increased the transposition frequency by 16-fold.[55]

Likewise, we speculate that the bacteria proactively lost the aforementioned IS5075-\(\Delta\text{TN2-IS26-\Delta\text{TN3}}\) genetic region in order to increase the transposition frequency, thereby promoting horizontal gene transfer. Additionally, the bacteria might passively lose the genetic region \(\text{IS5075}\)-\(\Delta\text{TN2-\Delta\text{IS26-\Delta\text{TN3}}}\) harboring the anti-mercury operon due to the lack of mercury pressure in the environment. Furthermore, considering that the \(\text{bla}_{\text{KPC-2}}\) bearing transposon found in the most similar plasmid from SWU01[56] resembles \(\Delta\text{TN1721-bla}_{\text{KPC-2}}\) in our sequences, we suspect that \(\Delta\text{TN1721-bla}_{\text{KPC-2}}\) in our sequences was transposed, with or without ProphagePlas1_2 or IncFIII plasmid, to the unnamed plasmid of SWU01. Besides \(\text{bla}_{\text{KPC-2}}\), there are also other ARGs carried by MGEs. The selective advantage of XDR phenotypes due to these ARGs may have played an important role in promoting ST11 CP-Kp strains to flourish in the face of heavy antibiotic pressure in the healthcare environment and may have favored their dissemination.

Studies found that prophages, genomic islands, and ICEs of CP-Kp strains always contain some proteins which have putative functions associated with bacterial virulence[57-59] that are speculated to make a contribution to the strong fitness of these strains. XDR group-specific MGEs (on chromosome) including six prophages, one genomic island, and one ICE, carry adaptation associated genes. These adaptation associated genes participate, for example, in SOS responses and help repair damaged DNA,[60,61] potentially protecting ST11 CP-Kp strains from environmental attacks and enhancing of the resistance of these strains to antibiotics,[46,62,63] UV radiation,[64] and other chemical stresses.[65] These adaptation associated genes also likely increase the strain’s abilities for host cell invasion and virulence,[66,67] as they have functional annotations relating to the progress of bacterial recognition, adhesion, and pathogenesis.

Due to the lack of available effective antibiotics, infections caused by CP-Kp strains have significantly higher morbidity and mortality than non-CP-Kp strains.[12-14] The higher risk of CP-Kp infection is associated with a number of patient factors, including being admitted to the ICU, being older, or presenting with pulmonary infection.[14-18] It bears emphasis that the XDR group strains isolated and analyzed in our study were from the ICU and that four of the patients from which these strains were isolated eventually died. Thus, the adaptation associated genes which we identified in these strains are attractive potential drug targets in the future.[68] Moreover, it will be interesting to track possible horizontal gene transfer of MGEs from these strains, as such transfer could promote the strong adaptive traits in other bacteria and thereby favoring their survival and spread.

There were some limitations in our research. This retrospective study could not provide immediate assistance to effectively control CP-Kp, and real-time WGS are urgently required to improve the surveillance and management of nosocomial infection. The sample size was limited, and although PCR confirmation was performed with some genes unique to XDR group in another 98 strains, further verifications based on larger-scale bioinformatics analysis and even biological experiments are waiting to be performed to confirm our findings. However, to some extent, our results could illustrate the cause of the success of XDR ST11 CP-Kp strains, and could provide an idea of identifying important genetic factors of this tricky infection caused by XDR ST11 CP-Kp strains clinically.

In summary, to the best of our knowledge, this is a novel study to investigate the widespread mechanism of the dominant ST11 XDR CP-Kp strains based on WGS and comparative genomics in China. Our study demonstrated a dissemination of ST11 XDR CP-Kp strains in a Chinese hospital, indicating that prevention and control strategies for CP-Kp nosocomial infection needed to be investigated. Using SMRT sequencing, which enables analysis of long and repetitive sequence, we identified MGEs carrying ARGs and adaptation associated genes potentially contribute to the strong fitness of the ST11 XDR CP-Kp strains, and helped probe the genetic basis of their widespread dissemination. Meanwhile, a new \(\text{bla}_{\text{KPC-2}}\)-bearing \(\Delta\text{TN1721-bla}_{\text{KPC-2}}\) transposon identified in all of the ST11 XDR CP-Kp we sequenced was reported. Both clonal spread and horizontal gene transfer were related to the ST11 XDR CP-Kp strains dissemination. Our study assists to define potential new targets to develop more effective strategies for the control and prevention of CP-Kp.

Data availability statement
The datasets generated for this study can be found in the GenBank (https://www.ncbi.nlm.nih.gov/genome/genomes/815).

Acknowledgements
The authors thank Dr. Xing Shi (Department of Respiratory and Critical Care Medicine, Peking University People’s Hospital, Beijing, China) for the technical help in figure-drawing assistance. The authors thank the Company of Novogene, Beijing, China for the technical help in WGS data analysis.

Funding
This work was supported by the Chinese Ministry of Science and Technology (No. 2016YFC0903800), the
National Natural Science Foundation of China (No. 81870010), and the Natural Science Foundation of Beijing Municipality (No. 7192217).

Conflicts of interest
None.

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How to cite this article: Li DX, Zhai Y, Zhang Z, Guo YT, Wang ZW, He ZL, Hu SN, Chen YS, Kang Y, Gao ZC. Genetic factors related to the widespread dissemination of ST11 extensively drug-resistant carbapenemase-producing Klebsiella pneumoniae strains within hospital. Chin Med J 2020;133:2573–2585. doi: 10.1097/CM9.00000000000011101.