Genomic characterization of novel IncFII-type multidrug resistant plasmids p0716-KPC and p12181-KPC from *Klebsiella pneumoniae*

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This study aimed to genetically characterize two fully-sequenced novel IncFII-type multidrug resistant (MDR) plasmids, p0716-KPC and p12181-KPC, recovered from two different clinical *Klebsiella pneumoniae* isolates. p0716-KPC and p12181-KPC had a very similar genomic content. The backbones of p0716-KPC/p12181-KPC contained two different replicons (belonging to a novel IncFII subtype and the Rep_3 family), the IncFII<sub>K</sub> and IncFII<sub>Y</sub> maintenance regions, and conjugal transfer gene sets from IncFII<sub>K</sub>-type plasmids and unknown origins. p0716-KPC and p12181-KPC carried similar three accessory resistance regions, namely ΔTn6209, a MDR region, and the *bla<sub>KPC-2</sub>* region. Resistance genes *bla<sub>KPC-2</sub>*, *mph(A)*, *strAB*, *aacC2*, *qacEΔ1*, *sul1*, *sul2*, and *dfrA25*, which are associated with transposons, integrons, and insertion sequence-based mobile units, were located in these accessory regions. p0716-KPC carried two additional resistance genes: *aphA1a* and *bla<sub>TEM-1</sub>*. Together, our analyses showed that p0716-KPC and p12181-KPC belong to a novel IncFII subtype and display a complex chimeric nature, and that the carbapenem resistance gene *bla<sub>KPC-2</sub>* coexists with a lot of additional resistance genes on these two plasmids.

Carbapenemases can be divided into three main categories: Ambler class A serine β-lactamases, class B metallo-β-lactamases, and the class D OXA group. *Klebsiella pneumoniae* carbapenemase (KPC) is a class A β-lactamase that was initially discovered in the USA in 1996. It has since disseminated worldwide among Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter* species, with *K. pneumoniae* being the most common species harboring *bla<sub>KPC</sub>* genes. KPC-producing bacteria are becoming endemic in certain hospitals, and are responsible for increasing numbers of outbreaks in healthcare facilities. KPC confers resistance or decreased susceptibility to almost all β-lactams, and KPC-producing isolates are often resistant to many other non-β-lactam drugs because of the co-occurrence of *bla<sub>KPC</sub>* with other classes of resistance gene. This multidrug resistance (MDR) leaves few available options for antimicrobial treatment, and thereby results in high mortality rates. The *bla<sub>KPC</sub>* genes have been found on IncFII-related plasmids such as pKPHS2 (GenBank accession number CP003224) and pKPC-LK30 (accession number KC405622) from *K. pneumoniae*. Conjugative IncFII<sub>K</sub> plasmid pKPHS2 has the core IncFII<sub>K</sub> backbone regions for plasmid replication (*repA*<sub>IncFII<sub>K</sub></sub>), maintenance (*parAB*, *stbAB*, *umuCD*, *psiAB*, *ardAB*, and *relBE*), and conjugal transfer (*tra* and *trb*), as well as additional replication genes *repA2*<sub>IncFII-Like</sub> and *repB*<sub>Rep_3-family/pKPHS2</sub>. pKPC-LK30 lacks plasmid conjugal transfer regions, which might result in it being nonconjugative. The pKPC-LK30 backbone is composed of a single replication gene, *repB*<sub>Rep_3-family/pKPHS2</sub>, a 36-kb IncFII<sub>K</sub>-type maintenance region homologous to a portion of the pKPHS2 maintenance regions, and a 16-kb IncFII<sub>Y</sub>-type plasmid maintenance region found in pKOX_NDM1, which is a *bla<sub>NDM-1</sub>*-carrying

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### Results

#### Clinical cases.

Patient 1 was a 59-year-old male admitted to Hospital 1 in October 2013, where he was diagnosed with a cardiac carcinoma. Nosocomial intra-abdominal infection occurred, and *K. pneumoniae* 0716 was isolated from ascitic fluid. The patient received intravenous administration of tigecycline, and the patient’s acute condition significantly improved.

Patient 2 was an 87-year-old man with chronic obstructive pulmonary disease, chronic bronchitis, pulmonary emphysema, coronary heart disease, and pancreatic carcinoma. Acute pancreatitis and peritonitis developed during treatment in Hospital 2 in September 2013. *K. pneumoniae* 12181 was isolated from a sputum sample, and the patient was treated with intravenous administration of meropenem plus ciprofloxacin. However, treatment was unsuccessful and the patient died.

Strains 0716 and 12181 were resistant to multiple antibiotics, including ampicillin, β-lactamase inhibitors (amoxicillin/clavulanic acid and piperacillin/tazobactam), cephalosporins (cefazolin and ceftriaxone), carbapenems (imipenem and meropenem), aztreonam, macrodantin, fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (amikacin and tobramycin), and trimethoprim/sulfamethoxazole, but remained susceptible to tetracycline (data not shown).

#### Overview of p0716-KPC and p12181-KPC.

PCR screening and sequencing indicated the presence of *bla*<sub>KPC-2</sub>, but none of the other carbapenemase genes tested for, in strains 0716 and 12181. The *bla*<sub>KPC</sub> markers could be transferred from strains 0716 and 12181 into TOP10 through electroporation, generating the *E. coli* transformants and transconjugants demonstrating class A carbapenemase activity.

As expected, the resulting *E. coli* transformants and transconjugants demonstrated *KPC* activity, and were resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, ceftriaxone, imipenem, meropenem, and aztreonam, but remained susceptible to macrolactam antibiotics (data not shown).

### Backbone regions of p0716-KPC and p12181-KPC.

Two representative *bla*<sub>KPC-2</sub>-carrying plasmids pKPHS2 and pKPC-LK30 were included in the genomic comparison with p0716-KPC and p12181-KPC (Fig. 1). p0716-KPC and p12181-KPC were most closely related to pKPHS2 (67% coverage and 99% nucleotide identity; last accessed on August 15, 2015). pKPC-LK30 was selected because it contains a large plasmid maintenance region that is not found in pKPHS2 but was identified in p0716-KPC and p12181-KPC (see below).

The backbone regions of p0716-KPC and p12181-KPC were almost identical with respect to genetic content, p0716-KPC and p12181-KPC both contained two novel replicons, including a *rep*<sub>B</sub> gene and a *rep*<sub>AIcFII-family</sub> gene, both of which were very different from their counterparts in pKPHS2 and pKPC-LK30. The *rep*<sub>AIcFII-family</sub> gene was most closely related to the IncFII plasmid pEA49-KPC (accession number KU318419) with a nucleotide identity of 94% (last accessed on December 25, 2016). p0716-KPC and p12181-KPC also contained the complete 16-kb IncFII<sup>+</sup>-type pKOX<sub>NDM1</sub> maintenance region, and almost the entire non-redundant IncFII<sup>-</sup>-type plasmid from *Klebsiella oxytoca* isolated in China. Although highly unusual, these backbone components can function together to promote the replication and stability of pKPC-LK30 in *K. pneumoniae*.

This work presents the complete sequences of two novel MDR plasmids, p0716-KPC and p12181-KPC, from *K. pneumoniae* strains isolated from China (Table 1). The two closely related plasmids belong to a novel IncFII subtype, and displayed a complex chimeric nature with respect to both the plasmid backbone (closely related to pKPHS2 and pKPC-LK30) and the accessory resistance regions. Co-occurrence of *bla*<sub>KPC-2</sub> (carbapenemase resistance) with *mph*(A) (macrolide resistance), *strAB* and *aacC2* (aminoglycoside resistance), *qacEΔ1* (quaternary ammonium compound resistance), *sul1* and *sul2* (sulphonamide resistance), and *dfrA25* (trimethoprim resistance) was observed in both plasmids.

| Category | Plasmid | p0716-KPC | p12181-KPC |
|----------|---------|-----------|------------|
| Accessory resistance regions | ΔTn6029 | MDR region | ΔTn6029 | MDR region |
| Resistance genes | *strAB*, and sul2 | *aphA1*, Δ*tnrB*, *aacC2*, *mph*(A), *sul1*, *qacEΔ1*, and *dfrA25* | *mph*(A), *sul1*, *qacEΔ1*, and *dfrA25* | Δ*tnrB*, *aacC2*, and *bla*<sub>NDM1</sub> |
| Host bacterium | *K. pneumoniae* 0716 | *K. pneumoniae* 12181 |
| Bacterial isolation | Recovered from ascitic fluid from Patient 1 in Hospital 1 | Recovered from sputum from Patient 2 in Hospital 2 |

**Table 1.** Major features of p0716-KPC and p12181-KPC and their antibiotic resistance genes and host bacteria.
IncFII \textit{K}-type maintenance region found in both pKPHS2 and pKPC-LK30 (Fig. 1). Compared with pKPHS2 and pKPC-LK30, p0716-KPC and p12181-KPC contained two unique backbone regions: a 5.6-kb plasmid maintenance region connected to the \textit{repB}_{\textit{Rep}_3\text{-family/p0716-KPC}} gene, and a novel 3.2-kb conjugal transfer region contained the \textit{pld} gene (conjugal transfer endonuclease). This 3.2-kb region was most closely related (93% query coverage and 98% maximum nucleotide identity; last accessed on December 25, 2016) to the MOB F family plasmid pEA49-KPC (Fig. 1).

p0716-KPC and p12181-KPC belonged to a novel IncFII subtype since it contained a novel \textit{repA} gene belonging to the IncFII family, together with IncFII \textit{K}/\textit{II Y}-type maintenance regions. Based on the three key regulatory DNA transfer genes \textit{traM}, \textit{traJ}, and \textit{finO} and the ATPase gene \textit{traC}, all of which encoded key proteins of a type IV secretion system, p0716-KPC and p12181-KPC were assigned into the subgroup A of the IncF/MOB \textit{F12} group7.

Accessory regions of p0716-KPC and p12181-KPC. p0716-KPC contained three accessory modules in total, namely \textit{Δ}Tn6029, the MDR region, and the \textit{bla}_{KPC-2} region, which were inserted at different sites in the backbone (Fig. 2). \textit{Δ}Tn6029 is a partial fragment of the IS26-based composite transposon Tn6029\textsuperscript{8}, and comprises a \textit{strAB} module flanked by two inverted IS26 elements. As initially characterized in the IncHI plasmid pSRC27-H, Tn6029 and Tn4352 are two overlapping transposons likely generated from complex recombination events between IS26, Tn2, and IS\textit{I\textsubscript{26}}\textsuperscript{9}.

**Figure 1.** Linear comparison of sequenced plasmids. Genes are denoted by arrows and are colored based on gene function classification. Shaded regions denote regions of homology (>95% nucleotide similarity). The sequences of p0716-KPC and p12181-KPC were determined in this study, while those of pKPC-LK30 and pKPHS2 are derived from GenBank.

**Figure 2.** Organization and alignment of resistance regions. The genetic organization of the resistance regions from p0716-KPC, p12181-KPC, pKPC-LK30, and pKPHS2 is shown, and relevant mobile elements are included for reference. Genes are denoted by arrows and are colored based on gene function classification. Shaded regions denote regions of homology (>95% nucleotide similarity).
The MDR region was further divided into an aacC2/\Delta tetr-B-related region, In207, and a Tn21-related region. The aacC2/\Delta tetr-B-related region harbored the aphA1a (aminoglycoside resistance)-carrying Tn4352, which lacked target site duplication signals of transposition. Tn4352 was further connected to a region composed of \Delta tetr-B, aacC2, and a 135-bp Tn2 remnant containing its inverted repeat right (IRR), resulting in truncation of tetrB (tunicamycin resistance). The association of aacC2-tetrB with Tn2 and IS26, as observed in pCTX-M3 and pU302L, constitutes one example of the multifarious aacC2-harboring environments. In207 was identified in p0716-KPC and p12181-KPC, and is an In4-like integron containing a single dfrA25 cassette. Notably, In207 is connected to the macrolide resistance unit IS26-mph(A)-mxr-mphR(A)-IS6100, a structure frequently associated with class 1 integrons, at its 3’ region. This likely occurred through Is6100-mediated recombinolation, and resulted in the deletion of the inverted repeat terminal (IRr). In207 from p0716-KPC and p12181-KPC appeared to be more complete than the prototype In207 (GenBank accession number AB280920), which is only an integron cassette array fragment. The Tn21-related region was organized sequentially as follows: IS26, Tn21, a 288-bp IS903B remnant, and ISKpn14. Tn21, a Tn3-family unit transposon, contained the core transposition module tnpA (transposase)-tnpR (resolvase), tnpM, inserted integron In2,urf2, and the mercuric resistance (mer) operon. The transposase was flanked by 38-bp IRL (inverted repeat left) and IRR sequences, and the In2 insertion was shown to disrupt a presumed ancestral urf2M gene, resulting in urf2 and tnpM. The ΔTn21 element from p0716-KPC was composed of ΔttnA202, the mer operon, and the IS075-disrupted IRrTn. The IS111-family element IS075 targets the terminal inverted repeats of the Tn21-subgroup transposons of the Tn3 family.

The bla\(\text{KPC-2}\) region consisted of a Tn2-related region, \(\Delta\text{Tn6296-2}\), and a Tn3-family transposon remnant. Tn6296 (designated in this work) was originally identified in the MDR plasmid pKP048 from K. pneumoniae, and is generated from the insertion of a core bla\(\text{KPC-2}\)-genetic platform (ΔTn21:ISKpn27-bla\(\text{KPC-2}\)-ΔISKpn6-\text{Kpn6-corf6-klcA}ΔrepT) into Tn212. Various Tn6296 derivatives, with deletions, insertions, and rearrangements at different sites, such as ΔTn6296-1 in pKPHS2/pKPC-LK30, ΔTn6296-2 in p0716-KPC, and ΔTn6296-3 in p12181-KPC, have been identified in KPC-encoding plasmids from K. pneumoniae strains isolated in China. A four-gene cluster coding for proteins of unknown function was located at the 5’ end of the Tn2-related region, followed by a partial bla\(\text{TEM-1}\) (β-lactamase)-carrying Tn213 region, plus IS26. The cryptic Tn3-family transposon remnant contained a typical 38-bp IRL element, a core transposition module (tnpA-tnpR), and inserted IS elements ISKpn26 and IS26.

p12181-KPC also contained ΔTn6029, the MDR region, and the bla\(\text{KPC-2}\)-region, which resembled their counterparts in p0716-KPC. Tn4352 in the MDR region and the Tn2-related region in the bla\(\text{KPC-2}\)-locus were not found in p12181-KPC. Therefore, p0716-KPC contained two additional resistance genes, aphA1a and bla\(\text{TEM-1}\), compared with p12181-KPC. Notably, aphA1a and bla\(\text{TEM-1}\) are redundant determinants accounting for resistance to aminoglycosides and β-lactams, respectively, in p0716-KPC. In addition, extensive rearrangement of large fragments was observed not only within, but between the MDR region and the bla\(\text{KPC-2}\)-region of p12181-KPC relative to p0716-KPC. These rearrangements were likely promoted by IS26-based replicative transposition as multiple copies of IS26 were identified in these two accessory regions. p12181-KPC still maintained three small accessory regions: two IS903D copies and group IIB retro-transposable intron Sm.a.I1, none of which were found in p0716-KPC. One copy of IS903D was inserted into a region between trbF and trbB, while traV (an essential gene encoding a core protein of type IV secretion system) was disrupted by a second copy, rendering p12181-KPC non-conjugative.

pKPC-LK30 contained a single accessory resistance region (the bla\(\text{KPC-2}\)-region) containing two antibiotic resistance genes: bla\(\text{PC-3}\) and bla\(\text{SHV-11}\). The \(\text{KPHS2}\) carried two accessory resistance regions, namely the bla\(\text{PC-3}\)-region and the Tn2-related region, each of which contained a single antibiotic resistance gene (bla\(\text{PC-3}\) and bla\(\text{SHV-11}\), respectively). All these accessory resistance regions were genetically related to their counterparts from p0716-KPC and p12181-KPC. Compared with pKPC-LK30 and pKPHS2, p0716-KPC and p12181-KPC appear to have acquired many more accessory regions containing several additional resistance genes.

**Discussion**

The \(\text{bla}\text{KPC}\) genes are largely associated with \(\text{Tn4401}\) and \(\text{Tn6296}\), which constitute the core \(\text{bla}\text{KPC}\)-genetic environments. \(\text{bla}\text{KPC}\)-carrying \(\text{Tn4401}\) and its close derivatives are frequently found on plasmids from bacteria isolated in European and American countries. \(\text{Tn4401}\) is rarely found in China, with \(\text{Tn6296}\) and its derivatives more frequently identified as the \(\text{bla}\text{KPC}\)-platforms, such as those located in \(\text{pKP048}\), \(\text{pKPHS2}\), \(\text{pKPC-LK30}\), and \(\text{p0716-KPC}\) and \(\text{p12181-KPC}\) (this study).

\(\text{bla}\text{KPC}\) genes are also commonly identified in plasmids belonging to various incompatibility groups, including IncF, IncI, IncA/C, IncN, IncX, IncR, IncP, IncU, IncW, IncL/M, and ColE1, ranging in size from 10–300 kb. The IncF replicons can be classified into the groups FIA, FIB, FIC, and FII. The IncFII plasmids are commonly low copy number plasmids and carry the primary FII replicon, often in association with additional replicons such as FIA and FIB. Moreover, the FII replicons can be further divided into various subtypes, including FIA, FIB, and FII, generating many compatible variants that can be used to overcome the incompatibility barrier with incoming plasmids.

The IncFII plasmid family can replicate in many different enterobacterial species, and is clearly playing an important role in the dissemination of antimicrobial resistance genes, including \(\text{bla}\text{KPC}\) amongst Enterobacteriaceae. The \(\text{bla}\text{KPC-1}\)-carrying \(\text{IncFII}\text{x}\text{K}\) plasmid \(\text{pKPQI}\) and its close derivatives have spread in European and American countries, \(\text{bla}\text{KPC-2}\)-carrying \(\text{IncFII}\text{x}\text{K}\) plasmids from China, such as \(\text{pKP048}\) and \(\text{pKPHS2}\), have very similar core backbone regions but limited overall sequence similarity to \(\text{pKPQI}\). It seems that the \(\text{pKPQI}\)-like and \(\text{pKP048}\)-like plasmids followed distinct evolutionary routes after separating from their common ancestor.

The recovery of the closely related plasmids p0716-KPC and p12181-KPC from two independent cases of nosocomial infection from two different hospitals indicates the potential of trans-regional spread and circulation of
these plasmids in hospital settings. p0716-KPC and p12181-KPC belong to a novel IncFII subtype, and display a complex chimeric nature, as observed within the backbone as well as the accessory resistance regions. The replication and stable inheritance of these two plasmids is likely promoted by the coordinated action of the IncFII and Rep3-family replicons and the IncFIIK and IncFII maintenance gene sets, respectively.

Production of KPC-2 makes strains containing p0716-KPC or p12181-KPC resistant to almost all β-lactams, including carbapenems. The situation is exacerbated by the presence of five additional classes of antibiotic resistance genes [mpf(A), strAB and aacC2, qacEΔ1, sul1 and sul2, and dfrA25] on these two plasmids. The accumulation of various antibiotic resistance genes on a plasmid have resulted from complex horizontal genetic transfer events under selection pressure of multiple antibiotics, and a bacterium will become resistant to multiple antibiotics at once by picking up such a MDR plasmid.

p0716-KPC is conjugative and contains the complete IncFIIK conjugal transfer gene content, while p12181-KPC has become non-conjugative likely because of the presence of multiple genetic lesions in the conjugal transfer regions. Non-transmissible plasmids rely largely on vertical transmission to be maintained in populations. Although classical models of plasmid evolution predict that conjugation is necessary for plasmid maintenance, it has been found that compensatory adaptation to ameliorate the cost of plasmid carriage coupled to rare (positive) selection for plasmid-encoded antibiotic resistance is sufficient to stabilize non-transmissible plasmids, explaining why non-conjugative plasmids are common.

Materials and Methods

Bacterial strains and identification. The use of human specimens and all related experimental protocols was approved by the Committee on Human Research of all the institutions (Beijing Institute of Microbiology and Epidemiology, the 307th Hospital of the People’s Liberation Army, and Navy General Hospital), and was carried out in accordance with the approved guidelines. Informed consent was obtained from patients where indicated. Our research was carried out in accordance with the Declaration of Helsinki.

Imipenem-non-susceptible K. pneumoniae strains 0716 and 12181 were isolated from two inpatients with hospital-acquired infections from two different public hospitals, and there was no epidemiological link between the two patients. Bacterial species identification was performed by 16S rRNA gene sequencing. The major hospital-acquired infections from two different public hospitals, and there was no epidemiological link between populations. Although classical models of plasmid evolution predict that conjugation is necessary for plasmid maintenance, it has been found that compensatory adaptation to ameliorate the cost of plasmid carriage coupled to rare (positive) selection for plasmid-encoded antibiotic resistance is sufficient to stabilize non-transmissible plasmids, explaining why non-conjugative plasmids are common.

Plasmid conjugal transfer. Plasmid conjugal transfer experiments were carried out using rifampin-resistant Escherichia coli strain EC600 as the recipient, and K. pneumoniae strains 0716 and 12181 as donors. Aliquots (3 ml) of overnight culture of each donor and recipient strain were mixed, harvested, and resuspended in 20 μl of Brain Heart Infusion (BHI) broth (BD Biosciences). The mixtures were spotted on 1 cm² hydrophilic nylon membrane filters with a 0.45-μm pore size (Millipore), which were then placed on BHI agar (BD Biosciences) plates and incubated at 37 °C for 12–18 h. Bacteria were washed from the filter membranes and spotted on Muller-Hinton (MH) agar (BD Biosciences) plates containing 1 mg/ml rifampin and 2 μg/ml imipenem for selection of blaKPC-positive E. coli transconjugants.

Plasmid electroporation. To prepare competent E. coli TOP10 cells for plasmid electroporation, 200 ml of overnight culture in Super Optimal Broth (SOB) at an optical density (OD₆₀₀) of 0.4–0.6 were washed three times with electroporation buffer (0.5 M mannitol and 10% glycerol), and then concentrated into a final volume of 2 ml. A 1-μg aliquot of plasmid DNA, isolated from strain 0716 or 12181 using a Qiagen Plasmid Midi Kit, was centrifuged at 10000 x g for 10 min at 4 °C. Aliquots (50 μl) of the supernatant (the enzymatic bacterial suspension) were individually mixed with 50 μl of substrates I–V, followed by incubation at 37 °C for a maximum of 2 h. The substrates consisted of: (I) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8); (II) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem; (III) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem, 0.8 mg/μl tazobactam; (IV) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem, 3 mM EDTA (pH 7.8); (V) 0.054% phenol red plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl mg imipenem, 0.8 mg/μl tazobactam, 3 mM EDTA (pH 7.8).

Detection of carbapenemase activity. Activity of class A/B/D carbapenemases in bacterial cell extracts was determined via a modified CarbaNP test. Briefly, overnight bacterial culture in MH broth was diluted 1:100 into 3 ml of fresh MH broth, and then incubated at 37 °C with shaking at 200 rpm to an OD₆₀₀ of 1.0–1.4. If required, ampicillin was used at 200 μg/ml. Bacterial cells were harvested from 2 ml of the above culture and washed twice with 20 mM Tris-HCl (pH 7.8). Cell pellets were resuspended in 200 μl of 20 mM Tris-HCl (pH 7.8), lysed by sonication, and then pelleted by centrifugation at 10000 × g for 5 min at 4 °C. Aliquots (50 μl) of the supernatant (the enzymatic bacterial suspension) were individually mixed with 50 μl of substrates I–V, followed by incubation at 37 °C for a maximum of 2 h. The substrates consisted of: (I) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8); (II) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem; (III) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem, 0.8 mg/μl tazobactam; (IV) 0.054% phenol red, 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl imipenem, 3 mM EDTA (pH 7.8); (V) 0.054% phenol red plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/μl mg imipenem, 0.8 mg/μl tazobactam, 3 mM EDTA (pH 7.8).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was conducted using the VITEK 2 system (bioMérieux) according to the manufacturer’s instructions, and interpreted as per the Clinical and Laboratory Standards Institute guidelines.

Plasmid sequencing and annotation. Plasmid DNA was isolated from E. coli transformants using a Qiagen Large Construct Kit, and then sequenced from a paired-end library with an average insert size of 500 bp, and a mate-pair library with average insert size of 5,000 bp, using an Illumina MiSeq sequencer. The circled DNA contigs were assembled using Newbler 2.6. Open reading frames and pseudogenes were predicted using RAST 2.0 combined with BLASTP/BLASTN searches against the UniProtKB/Swiss-Prot and RefSeq databases. Annotation of resistance genes, mobile elements, and other features was carried out using CARD, ResFinder,
ISfinder\textsuperscript{35}, INTEGRALL\textsuperscript{36}, and the Tn Number Registry\textsuperscript{37}. Multiple and pairwise sequence comparisons were performed using MUSCLE 3.8.31\textsuperscript{10} and BLASTN, respectively. Gene organization diagrams were drawn in Inkscape 0.48.1.

**Nucleotide sequence accession numbers.** The complete nucleotide sequences of p0716-KPC and p12181-KPC were submitted to GenBank under accession numbers KY270849 and KY270850, respectively.

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Author Contributions
D.S.Z., Y.L., and J.W. conceived the study and designed experimental procedures. J.F., Z.Y., Q.Z., Y.Z., D.Z., and X.J. performed the experiments. J.F., D.S.Z., Y.Z., and D.Z. analyzed the data. Q.Z., W.W., W.C., H.W., Y.S., and Y.T. contributed reagents and materials. D.S.Z., Y.L., J.W., and J.F. wrote this manuscript.

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