Novel IncR/IncP6 Hybrid Plasmid pCRE3-KPC Recovered from a Clinical KPC-2-Producing Citrobacter braakii Isolate

Dandan Dong,a Ziqiang Mi,b Dujun Li,c Mingming Gao,b Nan Jia,a Manli Li,b Yigang Tong,d Xianglilan Zhang,b Yuanqi Zhu*a

aDepartment of Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao, China
bState Key Laboratory of Pathogen & Biosecurity, Beijing Institute of Microbiology & Epidemiology, Beijing, China
cDepartment of Laboratory Medicine, Yeda Hospital of Yantai City, Yantai, China
dCollege of Information Science and Technology, Beijing University of Chemical Technology, Beijing, China

Dandan Dong and Ziqiang Mi contributed equally to this article. The order of the co-first authorship was determined by the order of contribution presentation.

ABSTRACT Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae have become widespread in hospitals and the environment. Here, we describe a blaKPC-2-carrying plasmid called pCRE3-KPC, which was recovered from a clinical multidrug-resistant Citrobacter braakii CRE3 strain in China. The complete nucleotide sequence of pCRE3-KPC was determined by combining MiSeq and MinION sequencing and then compared with those of three related plasmids. Plasmid conjugal transfer and electroporation tests, modified carbapenem inactivation method, and bacterial antimicrobial susceptibility test were carried out. We compared this plasmid with three related plasmids to verify that the backbone of pCRE3-KPC was composed of the backbones of the IncR plasmid and IncP6 plasmid. Further bioinformatics analysis showed that pCRE3-KPC carried two resistance-related regions (the blaKPC-2 gene cluster and the aacC2-tmrB-related region). The aacC2-tmrB-related region included two novel insertion sequences (ISChfr28 and ISChfr16).

IMPORTANCE Reports of human-pathogenic C. braakii strains, especially of strains showing resistance to carbapenems, are rare. To the best of our knowledge, our results represent the first detection of carbapenemase gene blaKPC-2 in C. braakii strains. In addition, we have studied detailed genetic characteristics of the novel IncR/IncP6 hybrid plasmid pCRE3-KPC, which was isolated from a clinical multidrug-resistant Citrobacter braakii CRE3 strain. Our results may provide further insight into the horizontal transfer of multidrug resistance genes in bacteria and into the genomic diversity and molecular evolution of plasmids.

KEYWORDS Citrobacter braakii, blaKPC-2, IncR, IncP6, plasmid, transposon

Klebsiella pneumoniae strains that produce K. pneumoniae carbapenemase (KPC) were initially identified in the United States in 2001 (1). Citrobacter braakii, as a member of the Citrobacter freundii complex, was identified in 1993 (2) and has rarely been reported as a human pathogen (3–6). The blaKPC-2 gene, as a subtype of KPC genes, has widely spread in Enterobacteriaceae, such as K. pneumoniae (1), Citrobacter freundii (7), C. portucalensis (8), and Escherichia coli (9) strains. However, the blaKPC-2 gene had not previously appeared in C. braakii strains. Moreover, it has been found to be carried on several plasmids to date, namely, IncR, IncP, IncFlI, IncLM, IncN, IncA, IncC, and IncX plasmids (10–12). As of 22 May 2019, 54 plasmids containing both the IncR replicon and the blaKPC-2 gene and 16 plasmids containing both the IncP6 replicon and the blaKPC-2 gene had been documented in the GenBank database, and there was no documented instance of an IncR/IncP6 hybrid plasmid (see Table S1 and S2 in the supplemental material).
The IncR replicon was first described in 2009 (13); since then, IncR plasmids have been increasingly reported in *Enterobacteriaceae* isolates (14). IncR replicons have also been found either as single replicons or as parts of multireplicon plasmids, which includes associations with IncA/C, IncF, IncFIIK, or nontypeable backbones (15). On the basis of prevalence statistics of plasmids containing both the IncR replicon and the *bla*<sub>KPC</sub> gene (Table S1), we found that these plasmids usually contain multiple replicons. The *bla*<sub>KPC</sub>-carrying plasmid unnamed3 (GenBank accession no. CP027150) contains one IncR replicon from the *K. pneumoniae* AR_0363 strain, which was that initially reported.

IncP6 plasmids have a broad host range (16), and to date the *bla*<sub>KPC</sub>-carrying IncP6 plasmids have been found in *Pseudomonas aeruginosa* (16), *K. oxytoca* (GenBank accession no. KY913901), *Enterobacter cloacae* (GenBank accession no. CP018968), and *C. freundii* (17). Both *bla*<sub>KPC</sub>-carrying IncP6 plasmid pCOL-1 (GenBank accession no. KC609323) (18) and p10265-KPC (GenBank accession no. KU578314) (16) were recovered from *P. aeruginosa* strains.

In this work, we have reported the first isolation of a *bla*<sub>KPC</sub>-positive *C. braakii* strain. In addition, we determined the whole genomic sequence of a *bla*<sub>KPC</sub>-carrying plasmid that we have named pCRE3-KPC, which was isolated from a clinical multidrug-resistant *C. braakii* CRE3 strain. We compared this plasmid with the following three related plasmids: plasmid unnamed3 (GenBank accession no. CP027150), p10265-KPC (GenBank accession no. KU578314), and pCOL-1 (GenBank accession no. KC609323). Interestingly, we found that plasmid pCRE3-KPC contains both an IncR replicon and an IncP6 replicon belonging to a novel IncR/IncP6 hybrid plasmid. To the best of our knowledge, this is the first report of an IncR/IncP6 hybrid plasmid. Our results may offer insight into the horizontal transfer of resistance genes and provide an overview of plasmid diversity and evolution.

RESULTS AND DISCUSSION

Characterization of *C. braakii* CRE3. PCR screening revealed that the multiple antimicrobial resistance genes present in *C. braakii* CRE3 include *bla*<sub>KPC</sub>-2, *bla*<sub>TEM</sub>-1B, *bla*<sub>OXA</sub>-1<sub>Y</sub>, *bla*<sub>CMB</sub>-1<sub>B</sub>, *qnrB10*, and *aacC2*. Plasmid pCRE3-KPC failed to transfer to *E. coli* EC600 through conjugation experiments but was successfully transferred to *E. coli* DH5α by electroporation to generate the *bla*<sub>KPC</sub>-positive electroporant CRE3-KPC-DH5α. This result illustrates that pCRE3-KPC is a nonconjugative but mobilizable plasmid. The antimicrobial susceptibility tests showed that both the *C. braakii* CRE3 and *E. coli* electroporant CRE3-KPC-DH5α strains were highly resistant to ampicillin, piperacillin, cefuroxime, ceftiraxone, aztreonam, imipenem, meropenem, and gentamicin (Table 1). Moreover, carbapenemase was produced in both of the strains mentioned above, as revealed by the modified carbapenem inactivation method (mCIM) (19).

**Overview of plasmid pCRE3-KPC.** The circular DNA sequence of pCRE3-KPC is 62,673 bp in length, with mean G+C content of 56%. Furthermore, it contains 71 predicted open reading frames (ORFs) and two distinct replicons (IncR replicon repA and IncP6 replicon repB) (Table 2) (Fig. 1).

Linear comparisons of plasmid pCRE3-KPC with three related reference plasmids, namely, *bla*<sub>KPC</sub>-2-carrying IncR plasmid unnamed3 (GenBank accession no. CP027150), p10265-KPC (a *bla*<sub>KPC</sub>-2-carrying IncP6 plasmid first reported in China) (16), and pCOL-1 (a *bla*<sub>KPC</sub>-2-carrying IncP6 plasmid, initially identified in Colombia) (18), were conducted. The detailed comparisons revealed that the overall structure of plasmid pCRE3-KPC is highly mosaic and can be divided into the following three distinct modules (Fig. 1 and 2; see also Fig. S1 in the supplemental material): (i) a first module (~20.5 kb) that is high homologous (>98.6% identity) to plasmid unnamed3 from the *K. pneumoniae* AR_0363 strain reported in the United States and extends from the resolution site (res) of ΔTn1722 to gene *vagD* (virulence-associated gene); (ii) a second module (~27.8 kb) that shares >99.9% identity with plasmid p10265-KPC (16) from *P. aeruginosa* strain 10265 isolated in China and extends from the *bla*<sub>KPC</sub>-2 gene cluster to ΔTn5563; (iii) a third module comprising the other accessory modules (~13.8 kb) with two novel insertion
sequences (ISCfr28 and ISCfr16), the truncated aacC2-tmrB region, IS6221, and ΔISEc15. On the basis of the study of the hybrid plasmids p675920-1 (20, 21) and pK1034 (22), the majority of the backbone and accessory regions of unnamed3 and p10265-KPC were found to be present in pCRE3-KPC, so pCRE3-KPC may represent a combination resulting from plasmids like these. Compared to the backbone of unnamed3 and p10265-KPC, pCRE3-KPC lost part of its backbone genes (orf711 of unnamed3, Δorf1 and kfrA, and a fragment extending from mobE to orf5 of p10265-KPC) during the recombination process, suggesting that these genes may not be necessary in these plasmids. The gene functions of these plasmids are annotated in detail (see Data Set S1, S2, S3, and S4 in the supplemental material).

**Genomic comparison of the backbone regions from pCRE3-KPC and related plasmids.** The backbone of each plasmid was further divided into the replication genes and the plasmid maintenance genes, without the conjugal-transfer genes, such that the hybrid pCRE3-KPC plasmid comprised the IncR and IncP6 backbones. The resultant backbone includes two replication genes (IncR replicon repA and IncP6 replicon repB) and two sets of partitioning system parAB genes (Fig. 1).

The IncR backbone from pCRE3-KPC was compared with plasmid unnamed3 (an IncR plasmid; GenBank accession no. CP027150), and their backbones were found to consist of the replication genes (IncR replicon and its iterons) as well as plasmid maintenance genes (parAB, umuC, and vagDC). However, two differences in their backbones were identified as follows: (i) the orf711 gene (hypothetical protein) is deleted in pCRE3-KPC but complete in plasmid unnamed3 and (ii) the orf258 gene (hypothetical protein) is interrupted into two parts by the insertion of the aac(6’)-Ib-cr-related region in plasmid unnamed3 (Fig. 1 and 2; see also Fig. S1).

Furthermore, p10265-KPC (16) and pCOL-1 (18) can be assigned to the IncP6 incompatibility group, according to replicon-based schemes. The IncP6 backbone of pCRE3-KPC was compared with those of both of the plasmids named above, and the

**TABLE 1** Antimicrobial susceptibility profiles

| Antibiotic            | MIC (mg/liter)/antimicrobial susceptibilitya |
|-----------------------|---------------------------------------------|
|                       | C. braakii CRE3 | Electroproant CRE3-KPC-DHSα | E. coli DHSα |
| Amoxicillin           | ≥32/R          | ≥32/R                       | ≤2/R        |
| Piperacillin          | ≥128/R         | ≥128/R                      | ≤4/S        |
| Cefuroxime            | ≥64/R          | ≥64/R                       | 4/S         |
| Ceftriaxone           | ≥64/R          | ≥64/R                       | ≤1/S        |
| Ceftazidime           | ≥64/R          | 4/S                         | ≤1/S        |
| Cefepime              | ≥64/R          | ≤1/S                        | ≤1/S        |
| Aztreonam             | ≥64/R          | ≥64/R                       | ≤1/S        |
| Imipenem              | ≥16/R          | ≥16/R                       | ≤1/S        |
| Meropenem             | ≥16/R          | ≥16/R                       | ≤0.25/S     |
| Amikacin              | 32/I           | ≤2/S                        | ≤2/S        |
| Gentamicin            | ≥16/R          | ≥16/R                       | ≤1/S        |
| Tobramycin            | ≥16/R          | 2/S                         | ≤2/S        |
| Ciprofloxacin         | ≥4/R           | ≤0.25/S                     | ≤0.25/S     |
| Levofloxacin          | 4/I            | ≤0.25/S                     | ≤0.25/S     |
| Nitrofurantoin        | 128/R          | ≤16/S                       | ≤16/S       |

The interpretation is derived from the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018) (S, sensitive; R, resistant; I, intermediately resistant).

**TABLE 2** Major features of plasmids in this work

| Plasmid | Accession no. or source | Species | Inc group | Country of origin | Total length (bp) | Total no. of ORFs | Mean G+C content (%) | Accessory module(s) (resistance genes harbored) |
|---------|-------------------------|---------|-----------|-------------------|-------------------|------------------|----------------------|-----------------------------------------------|
| unnamed3 | CP027150              | K. pneumoniae | IncR     | United States    | 65,684            | 72               | 55                   | MDR region, Tn4401a, aac(6’)-Ib-cr-related region, blaKPC-2 gene cluster, aacC2-tmrB-related region |
| pCRE3-KPC | This study           | C. braakii | IncR-P6 | China             | 62,673            | 71               | 56                   | blaKPC-2 gene cluster                                |
| p10265-KPC | KUS78314            | P. aeruginosa | IncP6   | China             | 38,939            | 46               | 58                   | blaKPC-2 gene cluster                                |
| pCOL-1     | KC609323             | P. aeruginosa | IncP6   | Colombia          | 31,529            | 34               | 60                   | Tn4401b                                       |
backbones were found to comprise the replication genes (IncP6 replicon and its iterons) and plasmid maintenance genes (kfrA, parABC, the mob gene cluster, the msrB-msrA-yfcG-corA-orf8 gene cluster, and paeR7IR). Three differences were notable among them (Fig. 1 and 2; see also Fig. S1): (i) pCRE3-KPC has lost genes (Δorf1 and kfrA) and a fragment extending from mobE (auxiliary protein) to orf5 (hypothetical protein); (ii) the numbers of copies of the 17-bp tandem repeat (GCGCCTGCCTTTGAGTA) within the iterons were 11 in pCRE3-KPC, 6 in p10265-KPC, and 12 in pCOL-1; and (iii) the Δorf8-corA-yfcG-msrA-msrB gene cluster was found to be inverted in pCOL-1.

**Genomic comparison of the blaKPC-2 gene region from pCRE3-KPC with those from related plasmids.** The blaKPC-2 gene is associated with the core blaKPC platform (Tn3-ISKpn27-blaKPC-ΔISKpn6) in most Chinese Enterobacteriaceae strains (23–25). This core platform is integrated into a DItec33-associated blaKPC-2 cluster, which was initially discovered in the p10265-KPC plasmid from a *P. aeruginosa* strain (16). In the blaKPC-2 gene cluster of p10265-KPC, the primary genetic structure, Tn3-ISKpn27-blaKPC-2-ΔISKpn6-korC-orf6-kIC-ΔrepB, may have undergone two evolutionary events (16): (i) insertion of a ΔblaTEM-1 gene between ISKpn27 and the Tn3 IRR (right inverted repeat) and (ii) disruption of the tnpA gene (transposase) from Tn3, resulting in its becoming two separate parts, an event caused by insertion of a composite transposon, ISApu1-
orf7-ISApu2. The blaKPC-2-carrying pCRE3-KPC plasmid was detected in an inpatient at a tertiary care hospital in China, and the BLASTN analysis of it showed that the surrounding genetic environment of the blaKPC-2 gene in pCRE3-KPC is highly similar to that in p10265-KPC. The ΔISApu1-orf7-ISApu2 composite transposon is also present in pCOL-1, but it has not been inserted into Tn3 and occurs downstream of ΔTn5403. Furthermore, the blaKPC-2 gene cluster is located downstream of ΔTn1722. Tn1722, a Tn3-family transposon, consists of an IRL (left inverted repeat), tnpA, tnpR (resolvase), res, mcp (methyl-accepting chemotaxis protein), and an IRR (26). ΔTn1722 contains an IRR, tnpA, tnpR, and res in pCRE3-KPC, which is also present in plasmid unnamed3 (GenBank accession no. CP027150) (Fig. 3).

However, the Tn3-family Tn4401 transposon has contributed to the rapid dissemination of the blaKPC-2 gene in Europe and the Americas. A number of previously reported isoforms of Tn4401, which differ by a 100- to 200-bp sequence upstream of blaKPC-2, are currently known (27–29). For example, Tn4401b, which is a Tn4401 isofrom, contains IRL, tnpA, tnpR, ISKpn7, blaKPC-2, ISKpn6, and IRR. Plasmid pCOL-1 (18) and plasmid unnamed3 (GenBank accession no. CP027150) originated from Colombia and the United States, respectively. The blaKPC-2 genes carried by plasmid pCOL-1 and plasmid unnamed3 are embedded in Tn4401b and Tn4401a, respectively. Compared with the complete Tn4401b, Tn4401a in plasmid unnamed3 (GenBank accession no. CP027150) has lost a 135-bp sequence upstream of blaKPC-2 (Fig. 3).

Genomic comparison of the aacC2-tmrB-related region from pCRE3-KPC with those from related plasmids. The aacC2-tmrB-related region from pCRE3-KPC is composed of ΔTn5563, two novel insertion sequences (ISCfr28 and ISCfr16), the truncated aacC2-tmrB region, ISEc21, and ΔISEc15. The Tn5563 element is organized sequentially with an IRL, tnpR, orf2 (hypothetical protein), pilT (PII domain-containing protein), tnpA, merP (mercuric transport protein periplasmic component), merR (mercuric transport protein), merR (mercuric resistance operon regulatory protein), and an IRR. In p10265-KPC (16), Tn5563, which is located upstream of two consecutive backbone genes (Δorf1 and kfrA), differs from the prototype Tn5563 from pRA2 (30) with a 286-bp insertion occurring between merP (mercuric transport protein periplasmic component) and merR (mercuric transport protein). However, ΔTn5563 has undergone the deletion of a fragment extending from merR to the IRR in pCRE3-KPC (Fig. 2 and 3).

In addition, two novel insertion sequences (ISCfr28 and ISCfr16) are inserted downstream of ΔTn5563. ISCfr28, containing two transposase genes, tnpA and tnpB, and a Tn3 family element, is bordered by 13-bp IRs (IRL, GTCAAGCCAAAGAAG; IRR, CTCTGAGG CTCAGC) (Fig. 4). The 1,025-bp ISCfr16 insertion sequence, a Tn3 family element, is made up of a transposase gene (tnpA) and 13-bp IRs (IRL, TAACTGAGGCGG; IRR, CGCTCGC AGCTAA). The aacC2 (aminoglycoside resistance)-tmrB (tunicamycin resistance) region...
is derived from transposon Tn2, and Tn2 has undergone the following molecular evolutionary changes (31, 32): (i) the trpR-res-trpA segment of Tn2 has been replaced by the aacC2-tmrB-orf192-orf228-orf1182-IS26 module and (ii) the IS26 insertion sequence has been inserted at the right-hand end of Tn2. The complete aacC2-tmrB region was discovered in pEl1573 from *E. cloacae* (33), and its truncated forms have been integrated into transposon Tn6411 from the chromosome of *P. aeruginosa* 12939.
(34). Because ISEc21 had inserted upstream of ISEc15, this may have led to the truncation of ISEc15 (Fig. 4).

MATERIALS AND METHODS

Bacterial isolates and identification. The clinical C. braakii CRE3 strain was isolated from a drainage sample from a patient at a tertiary care hospital in China on 5 May 2018. Bacterial identification was carried out using a Vitek compact-2 automated system (bioMérieux, France) and was confirmed by 16S rRNA sequencing (35). The genes encoding extended-spectrum β-lactamase (36), carbapenemase (37), fluoroquinolone (38), and aminoglycoside (39) were detected by PCR. All the PCR amplicons were sequenced on an ABI 3730 platform (Applied Biosystems, USA).

Plasmid conjugal transfer. The pCRE3-KPC plasmid was recovered from a clinical multidrug-resistant C. braakii CRE3 isolate. Conjugation experiments were carried out with cells of rifampin-resistant Escherichia coli strain EC600 as the recipient cells, and the transformation experiments were conducted using cells of E. coli DH5α-Electro-Cells (TaKaRa, China) as the recipient cells for the plasmid electroporation. Plasmid pCRE3-KPC was extracted from the cells using a Qiagen Plasmid Midi kit (Qiagen, Germany). The plasmid conjugal transfer and electroporation tests were performed as described previously (40, 41).

Antimicrobial susceptibility and carbapenemase activity detection. Antimicrobial susceptibility testing was conducted using a Vitek compact-2 automated system (bioMérieux, France). The results were interpreted according to the CLSI (Clinical and Laboratory Standards Institute) 2018 performance standards (42). Carbapenemase activities were detected using mCIM (19).

Sequencing and sequence assembly. The bacterial genomic DNA extracted from the CRE3 isolate using a Wizard Genomic DNA purification kit (Promega, USA) was sequenced using the MiSeq (Illumina, USA) and the MinION (Oxford Nanopore Technologies) platforms. The DNA library was constructed in accordance with a NEB Next Ultra II DNA Library Prep kit for Illumina, and the Illumina sequencing read length used was 300. The library preparations for the MinION platform were performed by the use of a rapid barcoding sequencing kit (SQK-RBK004) according to the protocol of the manufacturer (Oxford Nanopore Technologies), and the results were then loaded into the flow cell (FLO-MIN106D, Oxford Nanopore Technologies) for sequencing. Short Illumina reads were trimmed to remove poor-quality reads using Trimmomatic, and the contigs were assembled using Newbler3.0 (43). The long reads from MinION were combined with the short Illumina reads, which were subjected to hybrid assembly using SPAdesv3.11.1 (44). The hybrid assembly produced several scaffolds, and further bioinformatics analysis verified that the scaffold of the pCRE3-KPC plasmid was successfully cyclized by our in-house script. The correctness was then demonstrated by mapping the Illumina reads to the cyclized scaffold using CLC Genomics Workbench 9.0 (CLC Bio, Denmark), with an average level of read mapping coverage of 817×. The final consensus sequence obtained from CLC Genomics Workbench 9.0 was considered to represent the complete sequence of plasmid pCRE3-KPC.

Sequence annotation and genome comparisons. Annotation of open reading frames (ORFs) and pseudogenes was performed using RAST2.0 (the complete sequence of plasmid pCRE3-KPC). Annotation of open reading frames (ORFs) and pseudogenes was performed using RAST2.0 (the complete sequence of plasmid pCRE3-KPC). Annotation of open reading frames (ORFs) and pseudogenes was performed using RAST2.0 (the complete sequence of plasmid pCRE3-KPC). Annotation of open reading frames (ORFs) and pseudogenes was performed using RAST2.0.
REFERENCES

1. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC. 2001. Novel carbapenem-hydrolyzing ß-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 45:1151–1161. https://doi.org/10.1128/AAC.45.4.1151-1161.2001.

2. Brenner DJ, Grimont PA, Steigerwalt AG, Fanning GR, Ageron E, Riddle CF. 1993. Classification of citrobacteria by DNA hybridization: designation of Citrobacter farmeri sp. nov., Citrobacter youngae sp. nov., Citrobacter braakii sp. nov., Citrobacter wernkmanii sp. nov., Citrobacter sedlakii sp. nov., and three unnamed Citrobacter genomospecies. Int J Syst Bacteriol 43:645–650. https://doi.org/10.1099/00207713-43-4-645.

3. Samonis G, Karageorgopoulou DE, Koteridis DP, Matthaiou DK, Sidiro-poulou V, Maraki S, Falagas ME. 2009. Citrobacter infections in a general hospital: characteristics and outcomes. Eur J Clin Microbiol Infect Dis 28:61–68. https://doi.org/10.1007/s10096-008-0598-z.

4. Lai CC, Tan CK, Lin SH, Liu WL, Liao CH, Huang YT, Hsueh PR. 2010. Genetic organization of transposase regions surrounding a blaKPC-2 and blaNDM-1: characterization by whole genome sequencing. Sci Rep 6:30670. https://doi.org/10.1038/srep03067.

5. Carlini A, Mattei R, Mazzotta L, Lucartoti I, Pioi R, Bartellioli A, Antonelli A. 2005. Citrobacter braakii, an unusual organism as cause of acute peritonitis in PD patients. Perit Dial Int 25:405–406. https://doi.org/10.1111/j.1077-9868.2005.005471.x.

6. Gupta R, Rauf SJ, Singh S, Smith J, Agraharkar ML. 2003. Sepsis in a renal transplant patient due to Citrobacter braakii. South Med J 96:796–798. https://doi.org/10.1097/01.SMJ.00000656.25066.E2.

7. Wu W, Espedido B, Feng Y, Zong Z. 2016. Citrobacter freundii carrying blaKPC-2 and blaNDM-1: characterization by whole genome sequencing. J Genic doi.org/10.1038/srep03067.

8. Huang J, Ding H, Shi Y, Zhao Y, Hu X, Ren J, Huang G, Wu R, Zhao Z. 21 August 2018, posting date. Further spread of a blaKPC-harboring un-typeable plasmid in Enterobacteriaceae in China. Front Microbiol https://doi.org/10.3389/fmicb.2018.01938.

9. Chen YT, Lin JC, Fung CP, Lu PL, Chuang YC, Wu TL, Siu LK. 2014. Complete nucleotide sequence of two multidrug-resistant IncR plasmids from Klebsiella pneumoniae. Antimicrob Agents Chemother 57:269–276. https://doi.org/10.1128/AAC.00175-10.

10. Shi L, Feng J, Zhan Z, Zhao Y, Zhou H, Mao G, Gao Y, Zhang Y, Yin Z, Gao B, Tong Y, Luo Y, Zhang D, Zhou D. 2018. Comparative analysis of bla KPC-2 and mltB-carrying IncFlI-family pKPC-LK30/pHN7A8 hybrid plasmids from Klebsiella pneumoniae CG258 strains disseminated among multiple Chinese hospitals. Infect Drug Resist 11:1783–1793. https://doi.org/10.2147/IDR.S171953.

11. Liu J, Xie J, Yang L, Chen D, Peters BM, Xu Z, Shirtliff ME. 2018. Identification of the KPC plasmid pCT-KPC34: new insights on the evolution pathway of epidemic plasmids harboring fosA3-blaKPC-2 genes. Int J Antimicrob Agents 52:510–511. https://doi.org/10.1016/j.ijantimicag.2018.04.013.

12. Liang D-R, Li J-J, Sheng Z-K, Yu H-Y, Deng M, Bi S, Hu F-S, Chen W, Xue J, Zhou Z-B, Dui Y, Shi J-F, Li L-L. 2015. Complete sequence of a novel IncN F33:A-:B-:plasmid, pKP1034, harboring fosA3, blaKPC-2, blaCTX-M-65, blaSHV-12, and mtlB from an epidemic Klebsiella pneumoniae sequence type 11 strain in China. Antimicrob Agents Chemother 60:1343–1348. https://doi.org/10.1128/AAC.01488-15.

13. Shen P, Wei Z, Jiang Y, Du X, Ji S, Yu Y, Li L. 2009. Novel genetic environment of the carbapenem-hydrolyzing ß-lactamase KPC-2 among Enterobacteriaceae-comparative genomics. J Clin Microbiol 57:2636–2644. https://doi.org/10.1128/JCM.01934-08.

14. Li G, Zhang Y, Bi D, Shen P, Al F, Liu H, Tian Y, Ma Y, Wang B, Rajakumar K, Ou X, Ji F, Liu Y, Ji X, Shi L. 2015. First report of a clinical, multidrug-resistant Enterobacteriaceae isolate coharboring fosfomycin resistance gene fosA3 and carbapenemase gene blaKPC-2 on the same transposon, Tn1721. Antimicrob Agents Chemother 59:3338–3433. https://doi.org/10.1128/AAC.00620-09.

15. Chen L, Hu H, Chavda KD, Zhao S, Liu R, Liang H, Zhang W, Wang X, Jacobs MR, Bonomo RA, Kreiswirth BN. 2014. Complete sequence of a KPC-producing IncN multidrug-resistant plasmid from an epidemic Escherichia coli sequence type 131 strain in China. Antimicrob Agents Chemother 58:2422–2425. https://doi.org/10.1128/AAC.02587-13.

16. Allmeier H, Crensner B, Greck M, Schmitt R. 1992. Complete nucleotide sequence of Tn1721: gene organization and a novel gene product with putative mercuric ion transport proteins located on plasmid pRA2 of Pseudomonas alcaligenes. FEMS Microbiol Lett 165:253–260. https://doi.org/10.1111/j.1574-6968.1998.tb13154.x.

17. Yeo CC, Tham JM, Kwong SM, Yiin S, Poh CL. 1998. Tn5563, a transposon involved in blaKPC gene mobilization. Antimicrob Agents Chemother 57:269–276. https://doi.org/10.1128/AAC.00175-10.

18. Partridge SR. 2011. Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev 35:820–855. https://doi.org/10.1111/j.1574-6976.2010.00277.x.

19. Liang Q, Yin Z, Zhao Y, Liang F, Jeng Z, Zhou Z, Wang H, Song Y, Tong
Y. Wu W, Chen W, Wang J, Jiang L, Zhou D. 2017. Sequencing and comparative genomics analysis of the IncH1 plasmids pTS282-mplA and p122298-catA and the IncH5 plasmid pVNYK001-ddfA. Int J Antimicrob Agents 49:709–718. https://doi.org/10.1016/j.ijantimicag.2017.01.021.

33. Partridge SR, Ginn AN, Paulsen IT, Reddell JR. 2012. pE1573 carrying blaIMP-4, from Sydney, Australia, is closely related to other IncL/M plasmids. Antimicrob Agents Chemother 56:6029–6032. https://doi.org/10.1128/AAC.01189-12.

34. Zhan Z, Hu L, Jiang X, Zeng L, Feng J, Wu W, Chen W, Yang H, Yang W, Gao B, Yin Z, Zhou D. 2018. Plasmid and chromosomal integration of four novel blaIMP-carrying transposons from Pseudomonas aeruginosa, Klebsiella pneumoniae and an Enterobacter sp. J Antimicrobial Chemother. 73:3005–3015. https://doi.org/10.1093/jac/dky288.

35. Dubois D, Leyssene D, Chacornac JP, Kostrzewa M, Schmit PO, Talon R, Bonnet R, Delmas J. 2010. Identification of a variety of Staphylococcus species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 48:941–945. https://doi.org/10.1128/JCM.00413-09.

36. Galas M, Decousser J-W, Breton N, Godard T, Allouch PY, Pina P. 2008. Nationwide study of the prevalence, characteristics, and molecular epidemiology of extended-spectrum-β-lactamase-producing Enterobacteriaceae in France. Antimicrobial Agents Chemother 52:786–789. https://doi.org/10.1128/AAC.00906-07.

37. Poirel L, Walsh TR, Cuvelier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70:119–123. https://doi.org/10.1016/j.diagmicrobio.2010.12.002.

38. Shao H, Zhou H, Zhang T, Zhao X, Jiang Z, Wang Q. 18 December 2018, posting date. Preparation of molecularly imprinted hybrid monoliths for the selective detection of fluoroquinolones in infant formula powders. J Chromatogr A https://doi.org/10.1016/j.jchroma.2018.12.038.

39. Zurfluh K, Tasara T, Stephan R. 2016. Full-genome sequence of Escherichia coli K-15KW01, a uropathogenic E. coli B2 sequence type 127 isolate harboring a chromosomally carried blaCTX-M-15 gene. Genome Announc 4:e00927-16. https://doi.org/10.1128/genomeA.00927-16.

40. Srijan A, Margulieux KR, Ruekt S, Snxrud E, Maybank R, Serichantelgers O, Kormann R, Sukhatch P, Sriyabhat J, Hinkle M, Crawford JM, McNally A, Swierczewski BE. 2018. Genomic characterization of nonclonal mcr-1-positive multidrug-resistant Klebsiella pneumoniae from clinical samples in Thailand. Microb Drug Resist 24:403–410. https://doi.org/10.1089/mdr.2017.0400.

41. Cuzon G, Naas T, Villegas M-V, Correa A, Quinn JP, Nordmann P. 2011. Wide dissemination of Pseudomonas aeruginosa producing β-lactamase blakpc2 gene in Colombia. Antimicrob Agents Chemother 55:5350–5353. https://doi.org/10.1128/AAC.00297-11.

42. CLSI. 2018. Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement M100-S28. CLSI, Wayne, PA.

43. Nederbragt AJ. 2014. On the middle ground between open source and commercial software - the case of the Newbler program. Genome Biol 15:113. https://doi.org/10.1186/gb1473.

44. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

45. Brettin T, Davis JJ, Dizis T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomson JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.

46. O’Leary NA, Wright MW, Brisier JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Artashyun A, Badretdin A, Bao Y, Blinkova O, Brover V, Chevernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McIlravey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, et al. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:8. https://doi.org/10.1093/nar/gkv1189.

47. Boutet E, Lieberherr D, Tognoli M, Schneider M, Bansal P, Bridge AJ, Poux S, Bougueleret L, Xenarios I. 2016. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: how to use the entry view. Methods Mol Biol 1374:23–54. https://doi.org/10.1007/978-1-4939-3167-5_2.

48. Kleinheinz KA, Joensen KG, Larsen MW. 2014. Applying the ResFinder and VirulenceFinder Web-services for easy identification of acquired antibiotic resistance and E. coli virulence genes in bacteriophage and prophage nucleotide sequences. Bacteriophage 4:e27943. https://doi.org/10.4161/bact.27943.

49. Moura A, Soares M, Pereira C, Leitao N, Henriques I, Correia A. 2009. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics 25:1096–1098. https://doi.org/10.1093/bioinformatics/btp105.

50. Siguer P, Perezcho J, Lestrade L, Mahillon J, Chandler M. 1 January 2006, posting date. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 25:1096–1098. https://doi.org/10.1093/nar/gkj014.

51. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.

52. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340.