Structure genomics of two chimera plasmids p675920-1 and p675920-2 coexisting in a multi-drug resistant *Klebsiella pneumoniae* isolate

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ABSTRACT

This study dealt with detailed genomic characterization of two multidrug resistant (MDR) plasmids p675920-1 and p675920-2 from a single clinical *Klebsiella pneumoniae* isolate 675920. p675920-1 was essentially a hybrid of the IncFII plasmid pHN7A8 and the IncR plasmid pKPC-LK30, and functioned as an IncFII plasmid with inactivation of the IncR replication gene. The backbone of p675920-2 was a hybrid of a novel replicon, three maintenance regions (22.0-, 2.7-, 2.6-kb in length, respectively) as found in pKPYL2, p10164-3 and pK1HV, respectively, and the entire 25.9-kb conjugal transfer region of pKPYL2. p675920-1 and p675920-2 carried a large number of resistance genes, which contributed to resistance to at least seven classes of antibiotics (β -lactams, quinolones, aminoglycosides, fosfomycins, sulphonamides, trimethoprim, and tetracyclines) and one kind of heavy metal (mercury). All of these resistance genes are associated with mobile elements such as insertion sequences, insertion sequence-based transposition units, and transposons, which constituted a total of three novel MDR regions, two in p675920-1 and another in p675920-2. Coexistence of two MDR plasmids p675920-1 and p675920-2 made *K. pneumoniae* 675920 tend to become extensively drug-resistant.

INTRODUCTION

Plasmids of the incompatibility group IncFII are usually low copy number plasmids (> 100 kb in size) with a narrow host range and circulated mainly among *Enterobacteriaceae* species [1]. Most of IncFII plasmids contain multiple replicons, which are composed of the master IncFII replicon together with one or more other replicons such as IncFIA (*repIA*) [2], IncFIB (*repIB*) [3], and that (*repB*) belonging to an uncharacterized incompatibility group [4]; by contrast, there are some IncFII plasmids, such as R100 (accession number AP000342)

[5] and R1 [6], which contain the sole IncFII replicon. IncFII plasmids with multiple replicons can overcome the incompatibility barrier with incoming plasmids [1]. The IncFII replicon is composed of a four-replication-gene array *repA2-6-1-4*: *repA1* is the core gene for replication initiation, and its expression is primarily dependent upon translation of leader peptide RepA6 and further regulated by the negative regulator RepA2 through inhibition of RepA6 translation [6]; the *repA4* region contains the *ter* (replication terminus) sites for replication termination, and it is also important for stable maintenance of plasmids [7]. IncFII plasmids have been found to carry various antibiotic genes

and play a key role in dissemination of antibiotic resistance across *Enterobacteriaceae* species [1].

pHN7A8 (accession number JN232517) is an IncFII single-replicon plasmid from *K. pneumoniae* and has the core IncFII backbone regions for plasmid replication initiation (*repA2*–*6*–*1*–*4*), maintenance (*tir*, *pemIK*, *stbAB*, *psiAB*, etc), and conjugal transfer (*tra*, *trb*, etc) [8]. All of resistance genes (*bla*_{CTX-M-65}, *bla*_{TEM-1}, *rmtB*, and *fosA3*) of this plasmid are harbored within a 14.5-kb MDR region, which is the sole accessory module of pHN7A8 and inserted at a site downstream of *pemIK*. These resistance genes are associated with mobile elements including one copy of Tn2, *IS1*, *ISEcp1*, *IS1294*, and *IS903D* and four copies of *IS26*, which denotes a complex history of transposition and homologous recombination.

The IncR replicon was initially discovered in 2009, and often coexists as an auxiliary replicon with other replicons such as IncA/C, IncF, and IncH [9], constituting multi-replicon plasmids. The IncR replicon alone can promote plasmid replication, and IncR plasmids are herein designated as those containing the sole IncR replicon. The core backbone regions of IncR plasmids are composed of genes or gene loci responsible for plasmid replication initiation (*repB*) and maintenance (*parAB*, *umuCD*, *vagCD*, and *resD*) but lack conjugal transfer regions, and thus IncR plasmids are not self-transmissible [10]. An array of IncR plasmids, such as pEFER (IncR reference plasmid, accession number CU928144), pKPS30 (accession number KF793937) [9], pKPS77 (accession number KF954150) [9], pKP1780 (accession number JX424614) [11] and pKPC-LK30 (accession number KC405622) [12], have been fully sequenced. IncR plasmids have been increasingly reported in *Enterobacteriaceae* species, and carry various kinds of antibiotic resistance genes.

pKPC-LK30 (accession number KC405622) [12] from *K. pneumoniae* is an untypical IncR plasmid because its backbone contains IncFII-type maintenance regions (carrying *parAB*, *relEB*, *stbAB*, *psiAB*, *umuCD*, and *ardAB*) beside the core IncR backbone regions. pKPC-LK30 has a total of two accessory modules, one is a truncated *IS26* element and another is called the *bla*_{KPC-2} region (see below) carrying two resistance genes *bla*_{KPC-2} and *bla*_{SHV-11}.

This study dealt with detailed genomic characterization of two novel MDR plasmids p675920-1 and p675920-2 coexisting in a clinical *K. pneumoniae* isolate. These two plasmids displayed complex chimera structures, and harbored not only the backbone regions but the accessory modules composed of a large number of resistance markers and mobile elements.

RESULTS AND DISCUSSION

Coexistence of p675920-1 and p675920-2 in *K. pneumoniae*

The multilocus sequence typing (MLST) showed that the 675920 isolate belonged to the *K. pneumoniae*

sequence type 11 (ST11), with an allelic profile 3–3–1–1–1–1–4 corresponding to the seven housekeeping genes *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*. PCR screening indicated the presence of *bla*_{KPC-2} but none of the other carbapenemase genes were found in the 675920 isolate.

High-throughput sequencing with genomic DNA of the 675920 isolate generated the circularly closed sequences of two plasmids p675920-1 and p675920-2, which were 164.0 kb and 79.4 kb in length, with average G+C contents of 53.6% and 54.1%, and contained 216 and 83 predicted open reading frames (ORFs) in total, respectively (Supplementary Table 1). The modular structure of each plasmid was divided into the backbone regions and the separate accessory modules, which were defined as acquired DNA regions associated with and bordered by mobile elements and inserted at different sites of the backbone (Figure 1, Supplementary Figure 1 and Supplementary Table 1). A total of ten non-redundant genes or gene loci (*bla*_{KPC-2}, *bla*_{CTX-M-65}, *bla*_{TEM-1}, *bla*_{LAP-2}, *qnrS1*, *rmtB*, *tetA(A)*, *sul2*, *fosA3*, and the *mer* locus), which were involved in resistance to antimicrobials and heavy metal, were found in the accessory modules of these two plasmids (Supplementary Table 1 and Supplementary Table 2).

p675920-2, but not p675920-1, could be transferred into *Escherichia coli* EC600 through conjugation, generating the transconjugant 675920-tetA-EC600. p675920-1 could be transferred into *E. coli* TOP10 through electroporation, yielding the electroporant 675920-KPC-TOP10. Class A carbapenemase activities were detected in 675920 and 675920-KPC-TOP10 rather than 675920-tetA-EC600, which were due to production of KPC-2 in the corresponding strains.

The 675920 isolate was resistant to ampicillin, ceftazidime, meropenem, aztreonam, amikacin, tetracycline, ciprofloxacin, azithromycin, trimethoprim, sulfamethoxazole, and nitrofurantoin, but remained susceptible to tigecycline and colistin (Supplementary Table 3). 675920-KPC-TOP10 was resistant to the first six drugs but susceptible to all the other tested. 675920-tetA-EC600 was resistant to tetracycline, trimethoprim and sulfamethoxazole, and intermediately resistant to ampicillin and ciprofloxacin, but remained susceptible to all the other drugs tested.

Modular comparison of p675920-1 with pCT-KPC, pHN7A8, and pKPC-LK30

p675920-1 and the IncFII plasmid pCT-KPC (accession number KT185451) from *K. pneumoniae* constituted a novel group of plasmids with highly similar backbones (86% BLAST query coverage and 99% maximum nucleotide identity; last accessed on October 24, 2016). The backbones of p675920-1 and pCT-KPC had the identical replication initiation genes (see below), and displayed only two major modular differences: i) a

15.2-kb backbone region (from *orf375* to *parA*) was not found in pCT-KPC, and ii) the conjugal transfer gene Δ traI-5' was truncated in pCT-KPC (Supplementary Figure 2). The accessory modules of p675920-1 were composed of the MDR region, the *bla*_{KPC-2} region, the Tn6346-associated region, and IS26, which were highly similar to pCT-KPC with the exception that the 13.6-

kb MDR region of p675920-1 split into two separate regions (a 1.9-kb Tn2-related region and a 14.3-kb MDR region) inserted downstream of *pemK* and Δ retA-5', respectively, in the backbone of pCT-KPC.

p675920-1 and pCT-KPC were the hybrids of a 75.9-kb region derived from pHN7A8 [8], an 80.3-kb region derived from pKPC-LK30 [12], and

Figure 1: Schematic maps of p675920-1, pCT-KPC, and p675920-2. Genes are denoted by arrows, and the backbone and accessory module regions are highlighted in black and grey, respectively. The innermost circle presents GC-skew [(G-C)/(G+C)], with a window size of 500 bp and a step size of 20 bp. The next-to-innermost circle presents GC content.

a 7.0-kb Δ GI*Isul2*– Δ Tn6346 region not found in both pHN7A8 and pKPC-LK30 (Supplementary Figure 2). First, the pHN7A8-derived region of p675920-1 differed modularly from the entire pHN7A8 sequence by truncation of IS1294 (Supplementary Figure 2). Second, the pKPC-LK30-derived region of p675920-1 was composed of the whole pKPC-LK30 backbone, an intact IS26 element (but the counterpart in pKPC-LK30 was truncated), and the *bla*_{KPC-2} region and the Tn6346-associated region (these two accessory regions were originated but slightly differed from the *bla*_{KPC-2} region of pKPC-LK30; see below). In addition, the *repB* gene of p675920-1 was pseudogenized and would not participate in plasmid replication initiation. The pKPC-LK30-derived region was inserted within the *traI* gene of the pHN7A8-derived region, which might result in p675920-1 being nonconjugative (Supplementary Figure 2). Third, p675920-1 still contained a 4.9-kb conjugal transfer region as observed in the IncFII_K plasmid pKPHS2 (accession number CP003224) [13], which was located within the pKPC-LK30-derived region of p675920-1 but not found in either pKPC-LK30 or pHN7A8 (Supplementary Figure 2).

The hybrid structure of p675920-1 was validated by a set of PCR amplifications that targeted several key joining fragments (Supplementary Figure 3), using genomic DNA of the 675920 isolate as template.

The MDR regions of pHN7A8, pCT-KPC, and p675920-1

Two major steps of insertion occurred to eventually assemble the MDR region of pHN7A8 (Supplementary Figures 4 and 2): insertion of the Tn2–*rmtB* element at a site within *insB* of IS1*R* (making interruption and further 214-bp truncation of *insB*), and that of a 9.4-kb region (organized in order of IS26, Δ Tn6377, the IS26–*fosA3*–IS26 unit [14], and IS1294) at a site between *tnpA* and *tnpR* of Tn2 (making truncation of both *tnpA* and *tnpR*, and loss of *res*). The *rmtB* (aminoglycoside resistance)-carrying Tn2–*rmtB* element [15] and its variants [16] were widely found in resistance plasmids from *Enterobacteriaceae* species. Tn6377 was initially found in pKP96 from *K. pneumoniae* [17], and generated from insertion of the *ISEcp1*–*bla*_{CTX-M-65}–IS903*D* unit [18] at a site within the methyl-accepting chemotaxis gene *mcp* of the Tn3-family unit transposon Tn1722 [19]. In pHN7A8, Tn6377 was truncated at both ends due to connection of two flanking IS26 elements, generating Δ Tn6377 with a Δ ISE*cp1*–*bla*_{CTX-M-65}–IS903*D*–*iroN*– Δ *mcp* structure.

The MDR regions from pCT-KPC and p675920-1 were genetically closely related to that of pHN7A8 (Figure 2). Compared to the MDR region of pHN7A8, there were three major modular differences in the

counterparts of pCT-KPC: i) the MDR region of pHN7A8 split into two separate regions, namely the Tn2-related region and the MDR region, in pCT-KPC; ii) IS1294 was segmented into two parts Δ IS1294-5' and Δ IS1294-3' in pCT-KPC, which was followed by inversion of IS26–*fosA3*–IS26– Δ IS1294-5', and iii) an IS26 element was inserted at a site between Δ Tn6377 and Δ IS1294-5' in pCT-KPC. The above segmentation and inversion occurred also in the MDR region of p675920-1, but there was further deletion of IRL_{IS26} (inverted repeat left)– Δ IS1294-5', generating a truncated IS26–*fosA3*–IS26 unit.

The *bla*_{KPC-2} regions of pKPC-LK30, pCT-KPC and p675920-1

The modular structures of the *bla*_{KPC-2} region of pKPC-LK30 could be divided sequentially as a Tn3-family transposon remnant, a truncated IS26–*bla*_{SHV-12}–IS26 unit, Δ Tn6296-1, and a Tn21-related region (Figure 3). The Tn3-family transposon remnant contained a typical 38-bp IRL element, a truncated core transposition module *tnpAR*, *orf612*–*orf396*–*orf408* and *orf543* encoding hypothetical proteins, *htrC* (heat shock protein), and IS*Kpn26* inserted at a site between *orf408* and *orf543*. Tn6296 was originally identified in the MDR plasmid pKP048 from *K. pneumoniae* [20], and generated from insertion of the core *bla*_{KPC-2} genetic platform (Tn6376–*bla*_{KPC-2}– Δ IS*Kpn6*–*korC*–*orf6*–*k1cA*– Δ *repB*) into Tn1722, resulting in truncation of *mcp*. The IS26–*bla*_{SHV-12}–IS26 transposition unit had a structure IS26–*bla*_{SHV-12}–the *ygb* operon–IS26, and its mobilization was catalyzed by the two flanking copies of IS26 [21]. In pKPC-LK30, the 3'-terminal Δ *mcp*–Tn6376 region of Tn6296 was lost to form Δ Tn6296-1, and the connection of Δ Tn6296-1 with the IS26–*bla*_{SHV-12}–IS26 unit resulted in truncation of the 5'-end IS26. The Tn21-related region in pKPC-LK30 was organized sequentially as a 7.3-kb *mer* (mercuric resistance)-carrying Δ Tn21 region with insertion of IS5075 into the inverted repeat right (IRR), a 288-bp IS903*B* remnant, and IS*Kpn14* [22].

The *bla*_{KPC-2} regions of p675920-1, pCT-KPC and pKPC-LK30 were genetically closely related to each other (Figure 3). The loss of the Tn3-family transposon remnant accounted for the sole major modular change in the *bla*_{KPC-2} region of pCT-KPC relative to pKPC-LK30. Compared to pKPC-LK30, there were three major modular changes in the *bla*_{KPC-2} region of p675920-1: i) the truncated IS26–*bla*_{SHV-12}–IS26 unit was entirely deleted, ii) the 5'-terminal region of Δ Tn6296-1 was replaced by an IS26 element, which generated a further truncated structure designated Δ Tn6296-2; and iii) the Tn3-family transposon remnant was moved into another accessory module named the Tn6346-associated region (see below).

The *Tn6346*-associated regions of p675920-1 and pCT-KPC

The *Tn6346*-associated region of p675920-1 was organized in order of the *Tn3*-family transposon remnant

(see above), *IS26*, *Glul2* remnant, $\Delta Tn6346$, and *IS26* (Figure 3). *Tn6346* was a *Tn3*-family transposon with a structure *IRL*–*tnpAR*–*res*–*mer*–*IRR* [23]. The *mer*-carrying 3'-region of *Tn6346* was replaced by an *IS26* element, which left a 1.4-kb remnant composed of the

Figure 2: The MDR regions from pHN7A8, pCT-KPC and p675920-1, and comparison with related regions. Genes are denoted by arrows. Genes, mobile elements and other features are colored based on function classification. Shading denotes regions of homology (> 95% nucleotide identity). Numbers in brackets indicate the nucleotide positions within the corresponding plasmids.

IS5075-disrupted IRL (IRL_{Tn} 6346:IS5075), *tnpA*, and Δ *tnpR* in p675920-1. *GI*sul2 is a large integrative and mobile element carrying *int* (integrase), the resolvase gene *resG*, several conjugation transfer genes, the sulphonamide resistance gene *sul2* and the ISCR2 element, as found in various bacterial species [24]. The *GI*sul2 remnant was located between the Tn3-family transposon remnant and Δ Tn6346 in p675920-1 and composed of only *resG* and an unknown function gene *orf225*. In the Tn6346-associated region of pCT-KPC, an IS26 element (instead of the fragment composed of the Tn3-family transposon remnant, IS26 and the *GI*sul2 remnant as observed in p675920-1) was connected with Δ Tn6346, leading to truncation of IRL_{Tn} 6346:IS5075 (Figure 3). The Tn6346-associated regions from both p675920-1 and pCT-KPC carried none of resistance genes.

Modular comparison of p675920-2 with pKPYL2

The whole sequence of p675920-2 was mostly similar to pKPYL2 (accession number CP013340) from *Raoultella ornithinolytica*, with 68% query coverage

and 98% nucleotide identity (last accessed October 24, 2016). pKPYL2 and p675920-2 contained two different replication initiation *repA* genes with extremely low identity, both of which could not be assigned into any of known incompatibility groups. The entire conjugal transfer region of p675920-2 was essentially identical to pKPYL2. The plasmid maintenance regions of p675920-2 could be divided into three separate parts (48.8-, 2.7-, 2.6-kb in length, respectively), which were also observed in pKPYL2, p10164-3 (accession number KX710094) [25] and pK1HV (accession number HF545434) [26], respectively (Figure 1). pKPYL2 contained a total of 12 accessory modules, associated with Tn5403, a Tn3-family transposon remnant and various IS elements, but carried none of resistance genes. p675920-2 harbored a single accessory module, namely the 24.7-kb MDR region, which was inserted at a site between p10164-3 and pK1HV derived maintenance regions.

The MDR region of p675920-2

The MDR region of p675920-2 could be divided into the Tn1721- Δ GI*sul2* region and the truncated

Figure 3: The *blaKPC-2* regions from pKPC-LK30, pCT-KPC and p675920-1, the Tn6346-associated regions from p675920-1 and pCT-KPC, and comparison with related regions. Genes are denoted by arrows. Genes, mobile elements and other features are colored based on function classification. Shading denotes regions of homology (> 95% nucleotide identity). Numbers in brackets indicate the nucleotide positions within the corresponding plasmids.

IS26-*bla*_{LAP-2}-*qnrS1*-IS26 unit (Figure 4). The Tn1721- Δ GI*su*l2 region was generated from insertion of the IS26- Δ Tn6346- Δ GI*su*l2-IS26 unit (flanked by two oppositely oriented IS26 elements) into Tn1721 [19] containing the class A tetracycline resistance module *tetA*(A)-*tetR*(A), which led to truncation of *mcp* as well as loss of *res*-*tnpR*A. Δ GI*su*l2 was a 3'-terminal region of GI*su*l2 with a *resG*-*orf225*-ISCR2-*glmM*-*su*l2 structure.

The IS26-*bla*_{LAP-2}-*qnrS1*-IS26 unit, as observed in pE66An from *E. coli* [26], had two flanking copies of IS26 in direct orientation. This unit contained the core *qnrS1* (quinolone resistance) region displaying an Δ ISE*cl*2-*qnrS1*- Δ *tnpR*-ISK*pn*19 structure, which was widely found in *qnrS1*-carrying plasmids from *Enterobacteriaceae* species [25]. The upstream ISE*cl*2 element could play an important role in original acquisition of *qnrS1* [27], while ISK*pn*19 might contribute to mobilization of the core *qnrS1* region. The *bla*_{LAP-2} gene was located upstream of the core *qnrS1* region and conferred resistance to narrow-spectrum β -lactams. In p675920-1, IS26- Δ *ftsI*-5' and IS26 were absent at 5' - and 3'-ends of the IS26-*bla*_{LAP-2}-*qnrS1*-IS26 unit, respectively.

MATERIALS AND METHODS

Bacterial strain and identification

The use of human specimens and all related experimental protocols were approved by the Committee

on Human Research of the First People's Hospital of Lianyungang, and carried out in accordance with the approved guidelines. The research involving biohazards and all related procedures were approved by the Biosafety Committee of the Beijing Institute of Microbiology and Epidemiology. *K. pneumoniae* isolate 675920 was isolated in 2015 from a sputum specimen from an elderly male with pulmonary infection in a public hospital in Lianyungang City, China. Bacterial species identification was performed by 16S rDNA gene sequencing [28] and by PCR detection of *K. pneumoniae*-specific *khe* gene [29]. The MLST scheme for *K. pneumoniae* was derived from the PasteurMLST database (<http://bigsdb.pasteur.fr/klebsiella/>). The major plasmid-borne carbapenemase and ESBL genes were screened for by PCR [30]. All PCR amplicons were sequenced on ABI 3730 Sequencer (LifeTechnologies, CA, USA) using the PCR primers.

Sequencing and annotation

Genomic DNA was isolated from the 675920 isolate using a Qiagen large construct kit, and sequenced from a mate-pair library with average insert size of 5,000 bp, using a MiSeq sequencer (Illumina, CA, USA). Sequence assembly and annotation were performed as described previously [31]. Briefly, the contigs were assembled using Newbler 2.6 [32]. Open reading frames and pseudogenes were predicted using RAST 2.0 [33] combined with BLASTP/BLASTN searches [34] against the UniProtKB/Swiss-Prot database [35] and the RefSeq

Figure 4: The MDR region from p675920-2, and comparison with related regions. Genes are denoted by arrows. Genes, mobile elements and other features are colored based on function classification. Shading denotes regions of homology (>95% nucleotide identity). Numbers in brackets indicate the nucleotide positions within p675920-2.

database [36]. Annotation of resistance genes, mobile elements, and other features was carried out using the online databases including CARD [37], ResFinder [38], BacMet [39], ISfinder [40], INTEGRALL [41], and the Tn Number Registry [42]. Multiple and pairwise sequence comparisons were performed using MUSCLE 3.8.31 [43] and BLASTN, respectively. Gene organization diagrams were drawn in Inkscape 0.48.1.

Plasmid transfer

Plasmids were transferred in attempt from the 675920 isolate into *E. coli* TOP10 and EC600 (highly resistant to rifampicin) through electroporation and conjugal transfer, respectively [31]. For selection of electroporant or transconjugant containing the *tetA*(A) or *bla*_{KPC} marker, 10 µg/ml tetracycline, 2 µg/ml imipenem, and 1000 µg/ml rifampicin were used in accordance with specific circumstances.

Phenotypic assays

Activity of Ambler class A/B/D carbapenemases in bacterial cell extracts was determined by a modified CarbaNP test [31]. Bacterial antimicrobial susceptibility was tested by the broth dilution method, and interpreted as per CLSI guidelines [44].

Nucleotide sequence accession numbers

The p675920-1 and p675920-2 sequences were submitted to GenBank under accession numbers MF133495 and MF133496, respectively.

CONCLUSIONS

The two coexisting MDR plasmids p675920-1 and p675920-2 display complex chimera structures. p675920-1 is essentially a hybrid of the IncFII single-replicon plasmid pHN7A8 and the IncR plasmid pKPC-LK30, and almost all of the backbone and accessory regions of pKPC-LK30 and pHN7A8 are presented in p675920-1. p675920-1 still contains two additional regions, i.e., a 4.9-kb conjugal transfer region and a 7.0-kb Δ GI*sul2*– Δ Tn6346 region, which are not presented in both pKPC-LK30 and pHN7A8. p675920-1 is not self-transmissible because the conjugal transfer region originated from pHN7A8 is interrupted by insertion of the pKPC-LK30-derived region. p675920-1 functions as an IncFII plasmid because the IncR replicon is inactivated.

p675920-1 contains a total of four accessory modules inserted at different sites across the backbone, and among them are two large resistance regions, which harbor a lot of resistance genes or gene loci (such as *bla*_{KPC-2}, *rmtB*, *fosA3*, and *mer*) and associated mobile elements (such as insertion sequences, insertion sequence-based transposition units, and transposons). A total of six copies of IS26 are

presented in the two large resistance regions of p675920-1. All of the regions flanked by two IS26 elements lack paired short direct repeats (DRs; target site duplication signals for transposition) at both ends and therefore cannot be annotated as typical IS26-composite transposons. The common component IS26 would act as an adaptor to mediate massive recombination and transposition events [45–47] and thereby contribute to the assembly of these two large resistance regions in p675920-1.

Whilst the preparation of this manuscript, plasmid pKp_Goe_414-4 (accession number CP018341) from *K. pneumoniae*, which shows 98% query coverage and 99% nucleotide identity to p675920-2, was released in GenBank. p675920-2 and pKp_Goe_414-4 display very low levels of homology to available sequences in public databases and, thus, represent a novel group of plasmids. The backbone of p675920-2 displays a mosaic structure composed of a novel replicon, three maintenance regions as found in pKPYL2, p10164-3 and pK1HV, respectively, and the entire conjugal transfer region of pKPYL2. This novel replicon is able to coordinate with the maintenance and conjugal transfer regions originated from various plasmids, and maintain stable replication of pKPYL2 at a steady-state copy number. In addition, p675920-2 has evolved to integrate a sole accessory module that carries multiple resistance determinants such as *qnrS1*, *tetA*(A), *sul2*, and *bla*_{LAP-2}. p675920-2 is self-transmissible because it contains the intact conjugal transfer region originated from pKPYL2.

Coexistence of p675920-1 and p675920-2 makes *K. pneumoniae* 675920 tend to become extensively drug-resistant, thus resulting in limited choice of antibiotics for treatment and increased risks of death. Surveillance and epidemiological studies are needed to evaluate prevalence of p675920-1- and p675920-2-like plasmids among *K. pneumoniae* isolates.

Abbreviations

MDR: multidrug resistance; ESBL: extended-spectrum β -lactamase; ORFs: open reading frames; IS: insertion sequence; IRL: inverted repeat left; IRR: inverted repeat right; DRs: direct repeats.

Author contributions

DS.Z., HJ.Z. and JL.W. conceived the study and designed experimental procedures. J.F., Z.Y., Z.Z. and HF.M. performed the experiments. J.F., DS.Z., and Y.Z. analyzed the data. XY.J., LJ.Z., WH.Y and HY.Y. contributed to reagents and materials. DS.Z., J.F., HJ.Z., and JL.W. wrote this manuscript.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

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