Structural Genomics of repA, repB1-Carrying IncFIB Family pA1705-qnrS, P911021-tetA, and P1642-tetA, Multidrug-Resistant Plasmids from Klebsiella pneumoniae

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Background: Multidrug-resistant plasmids carrying replication genes have been widely present in various strains of Klebsiella pneumoniae. RepA and repB1 were found in plasmids belong to the IncFIB, but their detailed structural and genomic characterization was not reported yet. This is the first study that delivers structural and functional insights of repA- and repB1-carrying IncFIB plasmids.

Methods: Klebsiella pneumoniae strains A1705, 911021, and 1642 were isolated from the human urine samples and bronchoalveolar fluids collected from different hospitals of China. Antibacterial susceptibility and plasmid transfer ability were tested to characterize the resistant phenotypes mediated by the pA1705-qnrS, p911021-tetA, and p1642-tetA. The complete nucleotide sequences of these plasmids were determined through high-throughput sequencing technology and comparative genomic analyses of plasmids belong to the same incompatibility group were executed to extract the genomic variations and features.

Results: The pA1705-qnrS, p911021-tetA, and p1642-tetA are defined as non-conjugative plasmids, having two replication genes, repA and repB1 associated with IncFIB family, and unknown incompatible group, respectively. Comparative genomic analysis revealed that relatively small backbones of IncFIB plasmids integrated massive accessory module at one “hotspot” that was located between orf312 and repB1. These IncFIB plasmids exhibited the distinct profiles of accessory modules including one or two multidrug-resistant regions, many complete and remnant mobile elements comprising integrons, transposons and insertion sequences. The clusters of resistant genes were recognized in this study against different classes of antibiotics including β-lactam, phenicol, aminoglycoside, tetracycline, quinolone, trimethoprim, sulfonamide, tunicamycin, and macrolide. It has been observed that all resistant genes were located in multidrug resistance regions.

Conclusion: It is concluded that multidrug-resistant repA and repB1-carrying IncFIB plasmids are a key source to mediate the resistance through mobile elements among Klebsiella pneumoniae. Current findings provide a deep understanding of horizontal gene transfer among plasmids of the IncFIB family via mobile elements that will be utilized in further in vitro studies.

Keywords: plasmids, repA, repB1, multidrug resistance, structural genomics, bioinformatics

Introduction

Klebsiella pneumoniae (K. pneumoniae) is a Gram-negative Enterobacteriaceae bacterium and the most concerning multidrug-resistant (MDR) pathogen in nosocomial infections.1 Due to MDR and limited treatment choices, K. pneumoniae has been
associated with a high mortality rate up to 40–50%. K. pneumoniae, along with other highly important MDR pathogens, has been categorized as ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) organisms. Many antibiotics, including aminoglycosides, fluoroquinolones, and carbapenems, are used for the treatment of infectious diseases caused by MDR pathogens. However, these antibiotics are tremendously threatened by MDR strains of K. pneumoniae because they harbor plasmids encoding resistance genes that lead to the increased disease burden. The widespread occurrence of these resistance genes highlights the significance of the underlying mechanism that needs to be elucidated.

A bacterial strain can transfer its antibiotic-resistant traits either horizontally or vertically to its filial generations or act as a donor for mobile genetic elements (MGEs) for instance plasmids and transposons. MGEs are the main effective vehicles for antibiotic resistance horizontal transfer from one bacterial strain to other strains, species, or genera. Among all kinds of MGEs, plasmids play a key role in gene transfer processes. K. pneumoniae acquired resistance through the successful acquisition of MDR plasmids, including those classified into IncFII, IncFIY, IncHI2, and IncX1, incompatibility groups. Plasmids harboring antimicrobial resistance markers in clinical strains are a severe threat to public health worldwide.

In the present study, three MDR plasmids pA1705-qnrS, p911021-tetA, and p1642-tetA isolated from K. pneumoniae strain A1705, 911021, and 1642, respectively, were sequenced. Each of pA1705-qnrS, p911021-tetA, and p1642-tetA harbored two different replication initiation genes repA (IncFIB-family) and repB1 of an unknown incompatibility group. Initially, repA and repB1 were found in pKPN-c22 (GenBank AC# CP009879.1) isolated from K. pneumoniae. Until now, total nine fully sequenced plasmids carrying repA and repB1 have been reported including pKPN-c22, pKPN3-307_typeA (GenBank AC# KY271404.1), pKPN3-307_TypeC (GenBank AC# KY271406.1), p6234-198.371kb (GenBank AC# CP010390.1) and pKPSH11 (GenBank AC# KT896504.1), pKPN3-307_typeD (GenBank AC# accession number: KY271407), pCN1_1 (GenBank AC#CP015383), pRJ119-NDM1 (GenBank AC# KX636095), and pKP301cro (GenBank AC# KY495890) isolated from K. pneumoniae (last accessed, 26 October 2017). However, among these plasmids, not a single one has been assigned to any incompatibility group. Although, the detailed structures of IncFIB plasmids carrying repA, repB1 have not been characterized and genomic comparison of this incompatibility group has not been performed.

The current study provides a deep understanding concerning structural genomics of pA1705-qnrS, p911021-tetA, and p1642-tetA and five other sequenced plasmids carrying repA, repB1 with the highest homology provide further insights into the incompatibility (FIB) group. The common features and differences of their backbone regions and accessory modules are extensively analyzed and elaborated. The detailed genomic characterization of MDR plasmids will contribute to improve the diagnostics and understand the epidemiological relevance of Klebsiella strains.

Materials and Methods

Bacterial Strains and Identification

K. pneumoniae A1705 and 911021 strains were isolated from the urine samples of patients attending teaching hospitals in Shenyang and Chongqing, respectively. K. pneumoniae 1642 was isolated from a bronchoalveolar lavage fluid of a patient in the 307th Hospital of the People’s Liberation Army in Beijing. Bacterial species were identified by the VITEK-2 automated system (BioMerieux Inc., Marcy-l’Etoile, France) and 16S rRNA gene sequencing. The multi locus sequence typing (MLST) scheme for K. pneumoniae was followed as mentioned on the homepage (http://bigd.bigdb.pasteur.fr/klebsiella) by utilizing seven housekeeping genes (gapA, infB, mdh, pgI, phoE, rpoB, and tonB). PCR screening of ESBLs, carbapenem resistance, quinolone resistance, macrolide resistance and common tetracycline resistance genes was carried out for each strain. All PCR amplicons were sequenced on an ABI 3730 platform (Applied Biosystems, CA, USA).

Plasmid Transfer

Plasmid conjugal transfer was carried out by K. pneumoniae strains A1705, 911021, 1642 as donors and E.coli DH5α as the recipient. Overnight cultures of both strains (3mL), recipient and donor bacteria, were grown, mixed together and then harvested. The mixed culture was resuspended in 80ul of brain-heart infusion broth (BD Biosciences, CA, USA). The mixture was put onto a filter membrane of about 1 cm², subsequently placed onto a plate containing brain-heart infusion agar (BD Biosciences) and subsequently incubated at 37°C for mating for 12–18 h. The bacterial
culture washed from the filter membrane and spread onto Mueller-Hinton (MH) agar (BD Biosciences) plates which had 4 µg/mL ciprofloxacin and 4 µg/mL tetracycline for the screening of trans-conjugants.\textsuperscript{27} As successful conjugation was not achieved, so electroporation experiments were carried out using \textit{K. pneumonia} A1705, 911021, 1642 as the donor and \textit{E. coli} DH5\textalpha as the recipient. The 2µL of plasmid DNA solution was added in 50 µL of \textit{E. coli} DH5\textalpha and the cell suspension was transferred into ice-cooled electroporation cuvette followed by electroporation using a single pulse at the highest setting, corresponding to, 25 µF, 200 Ω, and 2.5 kV. After an electric shock, the cell suspension was diluted immediately by adding 1 mL volume of Super Optimal Broth (SOB) liquid medium and incubated for 1 hour at 37°C in a shaker at 220 rpm/min. An appropriate amount of suspension was applied to the resistant plate and incubated overnight at 37°C. Bacterial growth was re-enriched on the new resistant plate by picking up single isolated colonies. The transformation was confirmed by selection in ciprofloxacin 4ug/mL; (A1705) or tetracycline 4ug/mL (911021 and 1642) containing medium and plasmids.\textsuperscript{28,29}

**Antimicrobial Susceptibility Test**

Antimicrobial susceptibility to ampicillin, compound sulfamethoxazole, ciprofloxacin, levofoxacin, aztreonam, ceftazidime, piperacillin, cefazolin, cefuroxime, cefuroxime axetil, ceftriaxone, gentamicin, cefotetan, cefepime, imipenem, meropenem, nitrofurantoin, piperacillin/tazobactam, and amikacin was tested by broth dilution method and the minimum inhibitory concentration values were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines.\textsuperscript{27,30}

**Sequencing and Sequence Assembly**

Genomic DNA was extracted from each of the A1705, 911021, and 1642 isolates using a QIAGEN Blood & Cell Culture DNA Maxi Kit (Qiagen, Hilden, Germany). Genomic sequencing of A1705 and 911021 isolates was performed using a Single-MoleculeReal-Time technique on a PacBio RSII sequencer (Pacific Biosciences, CA, USA). Contigs were assembled using SMARTdenovo1.0 (https://github.com/ruanjue/smartdenovo).

Genomic DNA of the 1642 isolate was sequenced from a mate-pair library with an average insert size of 5000 bp, using a MiSeq sequencer (Illumina, CA, USA). Reads were trimmed to remove the poor quality sequences afterwards the contigs were congregated using Newbler 3.0.\textsuperscript{31} Gaps among contigs were bridged by utilizing a combination of PCR and Sanger sequencing using an ABI 3730 Sequencer (Applied Biosystems).

**Sequence Annotation and Comparison**

Open reading frames (ORFs) and pseudogenes were predicted using RAST server\textsuperscript{32} combined with BLASTP/BLASTN\textsuperscript{33} results against UniProtKB/Swiss-Prot\textsuperscript{34} and RefSeq databases.\textsuperscript{35} Annotation of resistance genes, mobile elements, and other features were performed using online databases including ISfinder,\textsuperscript{36} ResFinder,\textsuperscript{37} and INTEGRALL.\textsuperscript{38} Gene organization diagrams were drawn in Inkscape 0.48.1 (https://inkscape.org/en/).

**GenBank Accession Numbers**

The complete sequences of pA1705-\textit{qnrS}, p911021-\textit{tetA}, and p1642-\textit{tetA} were submitted to GenBank under the accession numbers MG764551, MG288679, and MF156696, respectively.

**Results**

**Characterization of \textit{K. pneumoniae} Strains**

\textit{K. pneumoniae} strains A1705 and 911021 were assigned to sequence type 449 and 11, respectively, while 1642 was given a new sequence type 2040, as determined by MLST. PCR screening confirmed the presence of \textit{bla}_{\textit{KPC}-2}, \textit{bla}_{\textit{TEM}-1}, \textit{bla}_{\textit{CTX}-M-14}, \textit{qnrS1}, \textit{oxa}4B, \textit{tetA}(A), \textit{tetA}(D), \textit{bla}_{\textit{CTX}-M-15}, \textit{bla}_{\textit{TEM}-1}, and \textit{bla}_{\textit{SHV}-33} genes in \textit{K. pneumoniae} strain A1705 while the presence of \textit{bla}_{\textit{KPC}-2}, \textit{qnrS1}, \textit{oxa}4B, \textit{mph}(A), \textit{tetA}(A), \textit{bla}_{\textit{CTX}-M-14}, \textit{bla}_{\textit{CTX}-M-65}, \textit{bla}_{\textit{SHV}-11}, and \textit{bla}_{\textit{TEM}-1} genes in \textit{K. pneumoniae} strain 911021 and the presence of \textit{bla}_{\textit{KPC}-2}, \textit{qnrS1}, \textit{mph}(A), \textit{tetA}(A), \textit{bla}_{\textit{TEM}-1}, \textit{bla}_{\textit{CTX}-M-65}, \textit{bla}_{\textit{SHV}-12}, \textit{bla}_{\textit{CTX}-M-14} genes in \textit{K. pneumoniae} strain 1642. Later, three common drug resistance genes \textit{qnrS1}, \textit{tetA}(A), and \textit{bla}_{\textit{CTX}-M-14} were co-transferred from \textit{K. pneumoniae} strains A1705, 911021, 1642 into \textit{E. coli} DH5\textalpha through electroporation, generating the transformants A1705-\textit{qnrS}-DH5\textalpha, 911021-\textit{tetA}-DH5\textalpha, and 1642-\textit{tetA}-DH5\textalpha respectively. These results demonstrated the existence of these genes in all three plasmids of our study.

Antibiotic resistant \textit{K. pneumoniae} strains (A1705, 911021, and 1642) and their respective transformants, were found to be resistant against ampicillin, ampicillin/ sulbactam, compound sulfamethoxazole, piperacillin, cefazolin, cefuroxime, cefuroxime axetil, ceftriaxone, and gentamicin. Additionally, these strains also exhibited resistance against cefotetan, cefepime, nitrofurantoin,
aztreonam, meropenem, imipenem, ceftazidime, piperacillin/tazobactam, levofloxacin, and ciprofloxacin. It also has been observed that 911021 and 1642 strains were resistant against amikacin, but strain A1705 was sensitive to amikacin (Table 1).

Overview of Plasmids pA1705-qnrS, P911021-tetA, and P1642-tetA

Clinical isolates of K. pneumoniae strains A1705, 911021, 1642 were resistant to β-lactams (including carbapenems), quinolones and carried qnrS1, tetA(A), blaCTX-M-14 plasmid-borne resistance genes. Considering this, we have chosen these isolates for further genome sequencing and detailed plasmid sequence analysis.

Genome sequencing demonstrated that pA1705-qnrS, p911021-tetA, and p1642-tetA are circular DNA sequences of 271, 255bp, 238, 681bp, 237, 591bp and the average GC contents are 51.89%, 51.69%, 51.77%, annotated with 335, 301, 300 predicted ORFs, respectively (contents are 51.89%, 51.69%, 51.77%, annotated with 335, 301, 300 predicted ORFs, respectively (Supplementary Table S1)). The pA1705-qnrS, p911021-tetA, and p1642-tetA carry two replicons, an IncFIB-type of 1011-bp repA (replication initiator) gene and 1014-bp repB1 belong to unknown incompatibility group. These plasmids contain an IncFIB-type repA gene, so these are referred to as the IncFIB group.

In current research, a linear genomic comparison was performed with eight plasmids including (pKPN-c22 [first discovered plasmid, harboring repA and repB1] as the reference plasmid, pA1705-qnrS, p911021-tetA, p1642-tetA [three plasmids of this study], pKPN3-307_typeA, pKPN3-307_TypeC, pKPN3-307_TypeE, p6234-198.371kb, and pKPSH11) which showed the highest sequence homology to each of these three plasmids and shared replication genes (Supplementary Table S1). The molecular structure of each plasmid was divided into the conserved backbones, and a large number of separate accessory modules (Figure 1).

The conserved backbones comprising plasmid replication (repA and repB1), plasmid maintenance, and conjugal transfer regions. Plasmid maintenance region contained umuCD, parAB and the parB’s binding sites parC [copy number varied among plasmids (9, 9, 11, 4, 11, 9, 9, 4 for pKPN-c22, pKPSH11, p6234-198.371kb, pKPN3-307_TypeC, pKPN3-307_typeA, pA1705-qnrS, p911021-tetA, and

Table 1 Antimicrobial Drug Susceptibility Profiles

| Category      | Antibiotics     | MIC (mg/L) | A1705 | 911021 | 1642 | A1705-qnrS- DH5α | 911021-tetA- DH5α | 1642-tetA- DH5α | DH5α |
|---------------|-----------------|------------|-------|--------|------|-----------------|------------------|-----------------|------|
| Penicillins   | Ampicillin      | ≥32/R      | ≥32/R | ≥32/R  | ≥32/R| ≥32/R           | ≥32/R            | ≥32/R           | ≥2/R |
|               | Piperacillin    | ≥128/R     | ≥128/R| ≥128/R | ≥128/R| ≥128/R          | ≥128/R           | ≥128/R          | ≥4/S |
|               | Piperacillin/tazobactam | ≥128/R | ≥128/R | ≥128/R | ≥128/R | ≥128/R | ≥128/R | ≥128/R | ≥4/S |
| Cephalosporins| Cefazolin       | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Cefuroxime      | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Cefuroxime axetil| ≥64/R | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Cefotetan       | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Cefazidine      | ≥32/R      | ≥32/R | ≥32/R  | ≥32/R| ≥32/R           | ≥32/R            | ≥32/R           | ≥4/S |
|               | Ceftriaxone     | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Cefepime        | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
| Monobactam    | Aztreonam       | ≥64/R      | ≥64/R | ≥64/R  | ≥64/R| ≥64/R           | ≥64/R            | ≥64/R           | ≥4/S |
|               | Imipenem        | ≥16/R      | ≥16/R | ≥16/R  | ≥16/R| ≥16/R           | ≥16/R            | ≥16/R           | ≥4/S |
|               | Meropenem       | ≥16/R      | ≥16/R | ≥16/R  | ≥16/R| ≥16/R           | ≥16/R            | ≥16/R           | ≥4/S |
| Aminoglycosides| Amikacin        | ≤2/S       | ≤2/S  | ≤2/S   | ≤2/S | ≤2/S            | ≤2/S             | ≤2/S            | ≤2/S |
|               | Gentamicin      | ≥16/R      | ≥16/R | ≥16/R  | ≥16/R| ≥16/R           | ≥16/R            | ≥16/R           | ≥4/S |
| Fluoroquinolones| Ciprofloxacin  | ≥4/R       | ≥4/R  | ≥4/R   | ≥4/R | ≥4/R            | ≥4/R             | ≥4/R            | ≥4/S |
|               | Levofloxacin    | ≥2/R       | ≥2/R  | ≥2/R   | ≥2/R | ≥2/R            | ≥2/R             | ≥2/R            | ≥4/S |
| Furane        | Nitrofurantoin  | =128/R     | =128/R| =128/R | =128/R| =128/R          | =128/R           | =128/R          | =128/R |
| Sulfanilamides| Compound        | ≥320/R     | ≥320/R| ≥320/R | ≥320/R| ≥320/R          | ≥320/R           | ≥320/R          | =20/R |

Abbreviations: MIC, minimal inhibitory concentration; R, resistant; S, sensitive; I, intermediate resistant.
p1642-tetA, respectively) of the 43-bp tandem repeat (gcaGatAACGcATgACGATGCAGGGACCGTGGTCCcCAG) were found in these plasmids, a capital letter represents the base for all copies as the same and a lower letter differing bases between the copies. The conjugal transfer region composed of finO, a set of F-type conjugative DNA transfer genes including rlx, dtr, cpl, sfx, eex, tivF (tivF1 to tivF16, tivF18, and tivF19), traJQ, and trbEF (Supplementary Figure 1).

**Backbone Regions of Plasmids**

Pairwise comparison analyses showed that eight plasmids had >96% nucleotide identity across >77% of their backbone sequences (Supplementary Table S2). However, there were three major differences among the backbones of these eight plasmids: First, compared with pKPN-c22, the translocation of two separate regions (orf414-orf162 and orf2340-orf543) occurred in pKPSh11. Second, the deletion of these two separate regions was found in pKPN3-307_TypeC, pKPN3-307_typeA, p911021-tetA, and p1642-tetA. Third, nine modular differences were found in the conjugal transfer region. These differences are i): the gene finO was truncated in the plasmid pKPN3-307_TypeC, p911021-tetA, and p1642-tetA; ii): the gene tivF3 was interrupted into two parts, namely ΔtivF3−5' and ΔtivF3-3' in the plasmid pKPN-c22; iii): the gene tivF13 was truncated in the plasmid pKPN-c22; iv): the gene sfx was truncated in the plasmids pKPN3-307_TypeA, pA1705-qnrS, p911021-tetA, and p1642-tetA; v): the gene rlx was interrupted into three fragments in the plasmid pKPN3-307_TypeC; vi): the gene traQ was truncated in the plasmid pKPN3-307_TypeC; vii): the gene tivF12 was interrupted in two parts, ΔtivF12-5'.
and *AtivF12-3*’ in the plasmid pKPN3-307_TypeC; viii): the gene *tivF18* was truncated in the plasmids pKPN3-307_TypeC, pA1705-qnrS, p911021-tetA, and p1642-tetA; ix): the gene *tivF16* was truncated in plasmids pA1705-qnrS, p911021-tetA, and p1642-tetA (Figure 1).

### Accessory Regions

Many different accessory modules were integrated at various sites within the backbones of these eight analyzed plasmids (Figure 1; Table 3). The IS903B, IS6100–IS6101, IS6100–IS6102, IS6100–IS6103, IS6100–IS6104, and MDR were found common in the all analyzed plasmids with slight differences. Notably, all these eight plasmids harbored resistance genes; all of which were located in the MDR regions (Table 4). MDR region from pKPN-c22, pKPSH11, p6234-198.371kb, pKPN3-307_TypeC, and pKPN3-307_TypeA, and the MDR-1 region from pA1705-qnrS has a complex mosaic structure and is sequentially organized (Figure 2 and Supplementary Table S3) while the MDR regions from p1642-tetA and p911021-tetA, and the MDR-2 from pA1705-qnrS shared similar structure with slight variations (Figure 3 and Supplementary Table S4).

In191, In37 from pKPSH11/p6234-198.371kb/pKPN3-307_typeA/pKPN3-307_TypeC and pKPN-C22

The prototype Tn402-associated class 1 integron was typically divided sequentially into IRi (inverted repeat at the integrase end), the 5’-conserved segment (5’-CS: *intI1*[integrase]–*attI1* [IntI1-recognizing recombination site]), the gene cassette array, the 3’-conserved segment (3’-CS: *qacE*Δ1 [quaternary ammonium compound resistance]–*sul1* [sulfonamide resistance]–*orf5*–*orf6*), the Tn402mini module (*miniABQR* and IRt (inverted repeat at the *mini* end), bounded by 5’-bp DRs. In191 from the MDR region of pKPSH11/p6234-198.371kb/pKPN3-307_typeA was derived from the prototype Tn402-associated class 1 integron. In191 had IRi, 5’-CS, a single-gene (*dfrA*14) cassette (trimethoprim resistance), *mobC*–IRt–IS6100, IRt with the loss of 3’-conserved segment (3’-CS: Δ*qacE*Δ1–*sul1*–*orf5*–*orf6*) and *mini*, bounded by 5’-bp DRs. In comparison with prototype Tn402-associated class 1 integron, the In191 in pKPN-c22 had undergone the loss of IRi, while the truncation at the 3’-end of *mobC*, IS6100 and the loss IRt occurred within In191 from pKPN3-307_TypeC (Figure 2).

A complex class 1 integron, In37 was surrounded by terminal 5’-bp DRs and included IRi, 5’-CS, variable region 1 (VR1:*aacA*4cr [aminoglycoside resistance]–*bla*OXA-1 [β-lactam resistance]–*catB3* [penicilol resistance]–*arr3* [rifampin resistance]), the first copy of 3’-conserved segment (3’-CS1: Δ*qacE*Δ1 [quaternary ammonium compound resistance]–*sul1* [sulfonamide resistance]), ISCR1, variable region 2 (VR2: *qnrA*1–ampR), the truncation of the second copy of 3’-conserved segment (Δ3’-CS2: Δ*qacE*Δ1–*sul1*–*orf5*–*orf6*), IRt (inverted repeat at the *mini* end), IS6100 replacing the Tn402mini module and IRt. In pKPN3-307_typeA/pKPN3-307_TypeC, In37 had undergone the segmentation into a remnant (*aacA*4cr–*bla*OXA-1–ΔcatB3), which was inverted compared with that in pKPN-c22/pKPSH11/p6234-198.371kb (Figure 2).

**Tn2 and the Truncated IS26-tetA(D)-tetR(D)-IS26 Unit from MDR-1 Region of pA1705-qnrS**

Tn2, a Tn3-family transposon was flanked by 5’-bp DRs and showed the following modular structure: IRL–*tnpA*–res(resolution site)–*tnpR*(resolvase)–*bla*TEM-1 (penicillin resistance)–IRR.40 pKPN-c22 carried a 2645-bp Tn2 segment (IRL–Δ*tnpA*), that was also found in p6234-198.371kb, but at the opposite orientation. An intact Tn2 was identified in the MDR-1 region from pA1705-qnrS, while the Tn2 had undergone disintegration to form two parts, namely Tn2-5’, Tn2-3’ in pKPSH11 and p6234-198.371kb. In pKPN3-307_typeA, ΔTn2 (identical to Tn2-3’ from pKPSH11/p6234-198.371kb) and the Tn2 remnant (Δ*tnpA*) were identified (Figure 2).

The IS26-tetA(D)-tetR(D)-IS26 unit [also designated Tn*tet(D)*], derivative of the tetracycline resistance unit, was made up of two directly oriented IS26 elements flanking a central region that contained *orf435, adh, tet*AT(D) (tetracycline efflux protein), and *tet*RD(D) (tetracycline repressor protein).41 The truncated IS26-tetA(D)-tetR(D)-IS26 unit found in MDR-1 region of pA1705-qnrS comprised *adh, tet*D(D), and *tet*RD with IS26 on both sides (Figure 2).

**Tn5403 from P6234-198.371kb and pKPN3-307_TypeC**

Tn5403, a Tn3 family unit transposon with typical 38-bp IRs at both ends, was first found in a *K. pneumoniae* strain and displayed the structure *tnpA–tnpR*.42 Tn5403 was intact in pKPN-c22, pKPSH11, and pKPN3-307_typeA, but has been found in fragmented forms; Tn5403-5’ and Tn5403-3’ in p6234-198.371kb and had undergone the loss of IRR_Tn5403 and a 5-bp truncation of *tnpR* at the 3’-end in pKPN3-307_TypeC (Figure 2).
| Table 3 Accessory Modules of Plasmids Analyzed |
|-----------------------------------------------|
| pKPN-c22 | pKPSH11 | p6234-198.371kb | pKPN3-307_TypeC | pKPN3-307_TypeA | pA1705-qnrS | p911021-tetA | p1642-tetA |
| IS903B   | IS903B  | IS903B          | IS903B          | IS903B          | IS903B      | IS903B      | IS903B      |
| IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 | IS1X1--ISEcl1--ΔIS1X1--ΔISKpn25 |
| CHASRI   | ISKpn28 | CHASRI          | CHASRI          | CHASRI          | CHASRI      | CHASRI      | CHASRI      |
| CHASRI   | ISKpn28 | CHASRI          | CHASRI          | CHASRI          | CHASRI      | CHASRI      | CHASRI      |
| IS26     | CHASRI  | IS26            | ΔISKpn38        | ΔISKpn38        | ars-2 seat  | ars-2 seat  | ars-2 seat  |
| Decentralized and functionally unknown areas | ΔIS26--ΔIS26, MDR region | MDR region | Decentralized and functionally unknown areas | ΔIS1X3 | ΔIS1X3 | MDR-1 | IS26--ΔIS26--IS5075--ISKpn24 residual |
| IS903D   | MDR region | IS1G--ISSen4 | fecABCDE operon | fecABCDE operon | ΔISRaq1--ISS family transposase--IS5075--ISKpn24 residual | Glutathione ABC transfer system | ISEc62 residual |
| IS903D   | IS1G--ISSen4 | – | ISRaq1--ISS family transposase--IS5075--ISKpn24 residual | Glutathione ABC transfer system | ISEc62 residual |
| ISKpn28  | – | – | IS5075 | IS5075 | ISEc62 residual | ISKpn24 residual--IS5075 |
| IS1G--ISSen4 | – | – | IS5075 | IS5075 | ISEc62 residual | Loc seat | IS1A |
| – | – | – | IS5075 | IS5075 | ISEc62 residual | IS1A |
| – | – | – | IS1G | IS1G | ISKpn24 residual--IS5075 | Decentralized and functionally unknown areas | Decentralized and functionally unknown areas |
| – | – | – | Glycogen synthesis cluster | Glycogen synthesis cluster | Loc seat | ISRaq1 | ISRaq1 |

(Continued)
Tn1721 Remnant and Unit IS3000–qnrB1–IS26 from pKPN-C22, pKPSH11, P6234-198.371kb, and pKPN3-307_TypeC, pKPN3-307_typeA

The tetracycline resistance gene of hybridization class A [tet(A)] is associated with non-conjugative transposon Tn1721. Gram-negative bacteria obtained mobilizable plasmids from different sources having complete or truncated variants of Tn1721. Tn1721 is a member of Tn21 subgroup of Tn3-family unit bacterial transposons having three 38 bp inverted repeats, with the following modular structure: IRR–mcp(methyl-accepting chemotaxis protein)–res (resolution site)–tnpR(resolvase)–tnpA(transposase)–IRL–1–tetR(A)–tetA(A)–pecM(PecM-like protein)–tnpA–IRL-2. Tn1721 consisted of a “basic transposon,” Tn1722, which was cryptic, with the modular structure IRR–tnpAR–tnpC–IRL. The tetR(A)–tetA(A)–pecM module remained same in each of pKPN-c22, pKPSH11, P6234-198.371kb, and pKPN3-307_TypeC, while in pKPN3-307_typeA, only a small segment of ΔtnpA (transposase) was discovered (Figure 2).

The IS3000–qnrB1–IS26 unit, a qnrB1 transmission vehicle, was first found in pKPN1 from PittNDM01. The pspF–qnrB1–Δorf909 was bracketed by two different ISs, namely IS3000 and IS26. In each of pKPN-c22, pKPSH11, P6234-198.371kb, pKPN3-307_typeA, and pKPN3-307_TypeC, the IS3000 within this unit was truncated at the 5′-terminal due to the transposition of Tn5403. The Δorf909 was truncated in pKPN-c22/pKPSH11/P6234-198.371kb, while in pKPSH11, IS26 was truncated as well (Figure 2).

Tn6415 from pKPN3-307_typeA and pKPSH11

Tn6415, first appraised in plasmid unitig_2 (GenBank accession number CP021536) from Escherichia coli strain AR_0119, was an IS26-flanked composite transposon. It was bracketed by 8-bp DRs and arranged in the following order: IS26, aacC2 (aminoglycoside resistance), tmrB (tunicamycin resistance), orf222, orf891, and IS26. Tn6415 had undergone the deletion of DRs in pKPN3-307_typeA, the truncation upstream of right-hand IS26 in p6234-198.371kb and further truncation at the 5′-end of the right-hand IS26, 3′-end of left-hand IS26 in pKPSH11 (Figure 2).

IS26-cld-IS26 Unit in pKPN-C22, P6234-198.371kb, and pKPSH11

In previous studies, genomic analyses suggested that chlorite dismutase originates in the perchlorate respiratory islands, from which it is transferred into transposons in the chlorate respirers. The cld gene behaves like a rogue and is predicted as a result of horizontal gene transfer. We found IS26-cld-IS26 unit carrying cld (chlorite dismutase) and some genes of unknown function, encircled by two directly orientated IS26 elements. In pKPN-c22, a 38-bp deletion was observed at the 5′-end of orf1083. In p6234-198.371kb, the left-hand IS26 within this unit was truncated, while in pKPSH11, the left-hand IS26 was further truncated, and the truncation of IS26 in orf384 at the right-hand was discovered (Figure 2).

Tn5393c and IS26–Su2–strA–strB–IS26 Unit from pA1705-qnrS, pKPSH11, P6234-198.371kb and pKPN3-307_typeA

Tn5393c, an active transposon, containing strA–strB was reported for the first time in the family of Tn5393. It

### Table 3 (Continued)

| pKPN-c22 | pKPSH11 | p6234-198.371kb | pKPN3-307_TypeC | pKPN3-307_typeA | pA1705-qnrS | p911021-tetA | p1642-tetA |
|----------|---------|----------------|----------------|----------------|-------------|-------------|------------|
| –        | –       | –              | Decentralized and functionally unknown areas | ΔIS9038–IS1X1 | IS1A | ISG-ISSen4 | ISG-ISSen4 |
| –        | –       | –              | MDR region | Decentralized and functionally unknown areas | Decentralized and functionally unknown areas | – | – |
| –        | –       | –              | ISG-ISSen4 | MDR region | ISRoq1 | – | – |
| –        | –       | –              | ISG-ISSen4 | ISG-ISSen4 | – | – | – |
| Plasmid            | Resistance Gene | Resistance Phenotype      | Nucleotide Position | Region Located          |
|-------------------|-----------------|---------------------------|---------------------|-------------------------|
| pKPN-c22          | catB3           | Phenicol resistance       | 62,051.62494        | The MDR region          |
|                   | blOXA-1         | β-Lactam resistance       | 62,632.63507        |                         |
|                   | aacA4cr         | Aminoglycoside resistance | 63,593.64192        |                         |
|                   | tetA(A)         | Tetracycline resistance   | 66,136.67335        |                         |
|                   | qnrB1           | Quinolone resistance      | 75,563.76207        |                         |
|                   | dfrA14          | Trimethoprim resistance   | 79,066.79548        |                         |
| pKPSH11           | sul2            | Sulfonamide resistance    | 79,409.80224        | The MDR region          |
|                   | staA            | Aminoglycoside resistance | 80,285.81088        |                         |
|                   | strB            | Aminoglycoside resistance | 81,088.81942        |                         |
|                   | blOXA-1         | β-Lactam resistance       | 82,645.83505        |                         |
|                   | blCTX-M-1       | β-Lactam resistance       | 86,327.82702        |                         |
|                   | tmrB            | Tunicamycin resistance    | 96,444.96986        |                         |
|                   | aacC2           | Aminoglycoside resistance | 96,999.97859        |                         |
|                   | catB3           | Phenicol resistance       | 98,093.98641        |                         |
|                   | blOXA-1         | β-Lactam resistance       | 98,779.99654        |                         |
|                   | aacA4cr         | Aminoglycoside resistance | 99,740.100339       |                         |
|                   | qnrB1           | Quinolone resistance      | 112,046.112690      |                         |
|                   | dfrA14          | Trimethoprim resistance   | 115,412.115894      |                         |
| p6234-198.371kb   | Sul2            | Sulfonamide resistance    | 66,393.67208        | The MDR region          |
|                   | staA            | Aminoglycoside resistance | 67,269.68072        |                         |
|                   | strB            | Aminoglycoside resistance | 68,072.68908        |                         |
|                   | blOXA-1         | β-Lactam resistance       | 73,311.74186        |                         |
|                   | blCTX-M-1       | β-Lactam resistance       | 88,190.88732        |                         |
|                   | tmrB            | Tunicamycin resistance    | 88,745.89605        |                         |
|                   | aacC2           | Aminoglycoside resistance | 90,362.90910        |                         |
|                   | catB3           | Phenicol resistance       | 91,048.91923        |                         |
|                   | blOXA-1         | β-Lactam resistance       | 92,009.92608        |                         |
|                   | aacA4cr         | Aminoglycoside resistance | 92,063.97262        |                         |
|                   | tetA(A)         | Tetracycline resistance   | 106,318.106962      |                         |
|                   | qnrB1           | Quinolone resistance      | 110,153.110635      |                         |
|                   | dfrA14          | Trimethoprim resistance   | 115,412.115894      |                         |
| pKPN3-307_typeA   | Sul2            | Sulfonamide resistance    | 129,146.129961      | The MDR region          |
|                   | staA            | Aminoglycoside resistance | 130,022.130825      |                         |
|                   | strB            | Aminoglycoside resistance | 130,825.131661      |                         |
|                   | blOXA-1         | β-Lactam resistance       | 132,382.133242      |                         |
|                   | blCTX-M-1       | β-Lactam resistance       | 136,064.136939      |                         |
|                   | tmrB            | Tunicamycin resistance    | 141,538.142080      |                         |
|                   | aacC2           | Aminoglycoside resistance | 142,093.142953      |                         |
|                   | aacA4cr         | Quinolone resistance      | 143,863.144462      |                         |
|                   | blOXA-1         | β-Lactam resistance       | 144,548.145423      |                         |
|                   | catB3           | Phenicol resistance       | 145,561.146019      |                         |
|                   | qnrB1           | Quinolone resistance      | 153,364.154008      |                         |
|                   | dfrA14          | Trimethoprim resistance   | 157,291.157773      |                         |
| pKPN3-307_TypeC   | aacA4cr         | Quinolone resistance      | 124,667.125266      | The MDR region          |
|                   | blOXA-1         | β-Lactam resistance       | 125,352.126227      |                         |
|                   | catB3           | Phenicol resistance       | 126,365.126913      |                         |
|                   | tetA(A)         | Tetracycline resistance   | 129,467.130666      |                         |
|                   | qnrB1           | Quinolone resistance      | 138,855.139499      |                         |
|                   | dfrA14          | Trimethoprim resistance   | 142,791.143264      |                         |

(Continued)
showed the modular structure as follows; *tnpA-res-tnpR-strA-strB* with direct and inverted repeats at both ends. The IS26–sul2–strA–strB–IS26 unit was an IS26-flanked transposition unit and first discovered in pK245 from *K. pneumoniae* strain NK245. It was comprised of remnant (*strA–strB–IRR_Tn5393c*) of the Tn5393 family transposon Tn5393c, the upstream sul2, and the downstream orf411–orf738–orf684–IS26. In pA1705-qnrS, this unit had undergone segmentation into two parts of different lengths (1478 and 3152 bp) resulting from the insertion of an intact Tn2. Only a 3607-bp remnant (sul2–strA–strB–IRR_Tn5393c–orf411) was observed in pKPSH11/ p6234-198.371kb/pKPN3-307_typeA (Figure 2).

**Table 4 (Continued).**

| Plasmid          | Resistance Gene | Resistance Phenotype | Nucleotide Position | Region Located |
|------------------|-----------------|----------------------|---------------------|----------------|
| pA1705-qnrS(MDR-1) | **bla**<sub>TEM-1</sub> | β-Lactam resistance | 71,817.72677        |                |
|                  | strB            | Aminoglycoside resistance | 72,942.73778        |                |
|                  | strA            | Aminoglycoside resistance | 73,778.74581        |                |
|                  | sul2            | Sulfonamide resistance | 74,642.75457        |                |
|                  | tetA(D)         | Tetracycline resistance | 79,032.80216        |                |
| pA1705-qnrS(MDR-2) | tetA(A)         | Tetracycline resistance | 124,510.125709      | The MDR region |
|                  | dfra1           | Trimethoprim resistance | 126,942.127415      |                |
|                  | qnrS1           | Quinolone resistance | 139,821.140477      |                |
|                  | sul2            | Tetracycline resistance | 142,074.142931      |                |
|                  | tetA(A)         | Aminoglycoside resistance | 147,263.147805      |                |
|                  | strB            | β-Lactam resistance | 147,818.148678      |                |
|                  | tetA(D)         | Aminoglycoside resistance | 158,542.159393      |                |
| p911021-tetA     | **bla**<sub>LAP-2</sub> | β-Lactam resistance | 90,093.90944        | The MDR region |
|                  | aocC2           | Tetracycline resistance | 100,783.101643      |                |
|                  | tmrB            | Aminoglycoside resistance | 101,656.101643      |                |
|                  | sul2            | Macrolide resistance | 106,530.103738      |                |
|                  | qnrS1           | Tetracycline resistance | 108,984.109640      |                |
|                  | tetA(A)         | Aminoglycoside resistance | 116,481.112796      |                |
|                  | dfra1           | Quinolone resistance | 118,273.119178      |                |
|                  | tetA(A)         | Trimethoprim resistance | 125,273.125746      |                |
|                  | strB            | Tetracycline resistance | 126,979.128178      |                |
| p1642-tetA       | tetA(A)         | Tetracycline resistance | 88,527.89726        | The MDR region |
|                  | dfra1           | Trimethoprim resistance | 90,959.91432        |                |
|                  | mph(A)          | Macrolide resistance | 97,527.98432        |                |
|                  | **bla**<sub>SHV-12</sub> | β-Lactam resistance | 100,242.101102      |                |
|                  | qnrS1           | Quinolone resistance | 107,733.108429      |                |
|                  | sul2            | Aminoglycoside resistance | 110,026.110883      |                |
|                  | tetA(A)         | Tetracycline resistance | 115,215.115757      |                |
|                  | dfra1           | Macrolide resistance | 115,770.116630      |                |
|                  | tetA(A)         | β-Lactam resistance | 125,112.125963      |                |
In plasmids pA1705-qnrS, p1642-tetA, and p911021-tetA, Tn\textsubscript{1721} have been dislocated into two fragments, namely the Tn\textsubscript{1721} remnant (IRL\textsubscript{1721}–\textDelta mcp) and \textDelta Tn\textsubscript{1721}\textsubscript{(tetR(A)–tetA(A)–pecM–\textDelta tnpA–IRR-2_Tn\textsubscript{1721}}). The 5-bp DRs are locating upstream of the Tn\textsubscript{1721} remnant and downstream of \textDelta Tn\textsubscript{1721} indicate that the disruption occurred after transposition. Remarkably, the lengths of the Tn\textsubscript{1721} remnants varied among plasmids (1455-, 1601-, and 1707-bp for p1642-tetA, MDR-2 pA1705-qnrS, and p911021-tetA respectively) (Figure 3).

IS\textsubscript{26}-bla\textsubscript{SHV-12}-IS\textsubscript{26} locus was found in different genetic environments, including class 1 integrons and transposons. These elements could play an important role in the spread of bla\textsubscript{SHV-12} and are suggestive of multiple recombination events. IS\textsubscript{26}-bla\textsubscript{SHV-12}-IS\textsubscript{26} carried bla\textsubscript{SHV-12}–deoR–yjbL–yjbK–yjbL–yjbM genes, encircled by IS\textsubscript{26} at both ends. Two fragments (IS\textsubscript{26}–bla\textsubscript{SHV-12}–deoR and yjbJ–yjbK–yjbL–yjbM–IS\textsubscript{26}) were identified in the opposite direction in p1642-tetA. Nonetheless, a single portion (\textDeltadeoR–yjbJ–yjbK–yjbL–\textDeltayjbM) of the IS\textsubscript{26}-bla\textsubscript{SHV-12}-IS\textsubscript{26} unit was found in pA1705qnrS/p911021-tetA (Figure 3).

IS\textsubscript{26}-mph(A)–mrx–mphR(A)–IS\textsubscript{6100}, IS\textsubscript{26}-bla\textsubscript{LAP-2}–qnrS–IS\textsubscript{26} and aacC2–tmrB Transposition Units from P1642-tetA/P911021-tetA and pA1705-qnrS

The IS\textsubscript{6100} transposable unit is based on IS\textsubscript{26}, IS\textsubscript{6100} and includes mph(A)–mrx–mphR(A) operon. The insertion sequences IS\textsubscript{26} and IS\textsubscript{6100} belong to the IS\textsubscript{6} family, contain almost identical IRs of 14 bp in length. The complete IS\textsubscript{26}–mph(A)–mrx–mphR(A)–IS\textsubscript{6100} transposable unit appears in plasmid p1642-tetA/p911021-tetA, but only a small residue was found in plasmid pA1705-qnrS (Figure 3).

The IS\textsubscript{26}-bla\textsubscript{LAP-2}–qnrS–IS\textsubscript{26} transposition unit was first discovered in plasmid pE66An in E. coli E66An (AC # HF545433). The transposable unit structure is...
IS26, ftsI, bla\_LAP\_2, orf657, ΔSEcI2, qnrS1, ΔnpR, ISKpn19, and IS26. The unit lost its IS26 at its right-hand end in plasmids p1642-tetA, pA1705-qnrS, and p911021-tetA (Figure 3).

Furthermore, The complete aacC2–tmrB transposition unit was found in at least 13 plasmids, including plasmid pEl1573 in Enterobacter cloacae El1573. The structure of the aacC2-tmrB unit is IS26, Tn2 residual, aacC2, tmrB, orf192, orf228, orf1158, ISCfrI, and Tn2 residues. There is only a small portion (aacC2–tmrB–orf192–orf228–Δorf1158) of this unit that was found in each p1642-tetA, pA1705-qnrS, and p911021-tetA (Figure 3).

**Discussion**

Antibiotics such as aminoglycosides, β-lactams, quinolones and macrolides, consumption have been spread globally, but the emergence of MDR K. pneumoniae often lead to the failure of clinical antibiotics. It is important to distinguish and elucidate the drug resistance genes in plasmid and genetic environment, thereby illustrating the drug resistance mechanism mediated by MDR plasmids. There are many mechanisms involved in K. pneumoniae resistance to multiple antibiotics, among those, horizontal transfer of resistance genes is the most significant.

All of the eight analyzed plasmids from environmental and clinical isolates belonging to K. pneumoniae were obtained from different countries including China, USA, Italy, and Israel. The transferability of plasmids through conjugation was still unsuccessful due to the lack of some conjugal transfer genes even after the repetitive attempts of plasmid transformation. However, the mobility of plasmids was verified by the transformation experiments. The findings showed that pA1705-qnrS, p911021-tetA, and p1642-tetA could be transferred from K. pneumoniae strains A1705, 911021, 1642 into A1705-qnrS-DH5α, 911021-tetA-DH5α, and 1642-tetA-DH5α through electroporation. Although conjugation is necessary for plasmid maintenance, it has been found that compensatory adaptation plays a possible role in plasmid stability by eliminating the plasmid carriage cost together with positive selection for antibiotic resistance. Compensatory adaptation is sufficient to maintain the plasmid stability and possibly explain the reasons behind the existence of non-conjugative plasmids. Bacteria become resistant by picking up such MDR plasmids carrying resistance genes.

The comparison of pKPN-c22 (reference plasmid) backbone structure and other plasmids demonstrate genetic conservation in terms of gene contents and organization even omitting the deletion and inversion of some
segments. Interestingly, backbones of plasmids could integrate a large number of accessory modules, mostly integrated at one “hotspot”, located between orf312 and repB1. It is interesting that the insertion of MDR regions occurs at the same site in the backbone of all plasmids, but occasionally with the insertion of two MDR regions. It is worth determining whether there are specific mechanisms associated with this incompatibility group plasmid that promote their involvement in the complex processes of acquisition of foreign genetic material.

The distribution of antibiotic resistance genes, particularly multidrug resistance genes, via transposable elements is an important concerning issue globally. In the current study, we found that pKPN-c22, pA1705-qnrS, p911021-tetA, p1642-tetA, pKPN3-307_typeA, pKPN3-307_TypeC, p6234-198.371kb, and pKPSH11 resistant plasmids encompass genes that are involved in resistance against seven different classes of antibiotics including aminoglycosides, quinolones, sulfonamides, tetracycline, trimethoprim, tunicamycin, phenicol, and macrolides. Notably, the presence of redundant resistance genes formed highly resistant strains A1705, 911021, and 1642 correspond to the classes of antibiotics, including aminoglycosides (aacC2), quinolones (qnrB1), sulfonamides (sul2), and macrolides (mph (A)). This resistance limits the selection of antibiotics for the therapies of infections caused by these bacterial strains.

As the acquisition of multiple-resistance genes is associated with a variety of mobile elements, such as insertion sequences, integrons (In363, In37, In191) and transposons (Tn4352, Tn1721, Tn6415, Tn2) which mediate the gene transfer events. The presence of mobile elements containing antibiotic resistance genes is a concern since it can promote the dissemination of resistance. This study also demonstrated the various truncated versions of mobile elements that carried the multiple resistance genes. These findings could explain the evolution of these eight plasmids through complex transposition and homologous recombination events.

Conclusion
Comparative genomic analysis of non-conjugative, repA, repB1-carrying plasmids pA1705-qnrS, p911021-tetA, p1642-tetA were carried out to determine the structural insights of these plasmids. The backbone regions of IncFIB plasmids are small as compared to the accessory regions, and the accessory regions are composed of a large number of mobile elements. Multidrug resistance mediated through these MGE’s, which contained the resistant genes, among K. pneumoniae strains. Stable inheritance and replication of these plasmids are promoted by the coordination of replicons with maintenance gene sets and the conjugation regions. This study provides a detailed genetic characterization of IncFIB plasmids, an important route for horizontal transfer of the resistance genes through mobile elements among IncFIB-family plasmids. Additionally, current findings provided a primary cause of Enterobacteriaceae epidemiology, especially MDR K. pneumoniae. The prevalence of resistant IncFIB plasmids carrying repA and repB1 at various geographic areas is still required to determined from clinical settings cultures especially those from immuno-compromised patients. Moreover, the identification and evaluation of specific factors and underlying mechanisms associated in the spread of these resistant plasmids also needs to be elucidated. There is still extensive experimental, clinical, and Bioinformatics techniques are required to reduce the dissemination of virulence and antimicrobial resistant plasmids in hospital settings. Furthermore, epidemiological studies and regular inspection of repA and repB1-carrying IncFIB plasmids will be of great importance.

Ethics Statement
Ethics approval and informed consent were not required. All the bacterial isolates involved in this study were part of the routine hospital laboratory procedure.

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Disclosure
The authors state that they have no conflicts of interest.

References
1. Yan J, Pu S, Jia X, et al. Multidrug resistance mechanisms of carbapenem resistant Klebsiella pneumoniae strains isolated in Chongqing, China. Ann Lab Med. 2017;37(5):398–407. doi:10.3343/alm.2017.37.5.398
2. Gomez-Simmonds A, Uhlemann AC. Clinical implications of genomic adaptation and evolution of carbapenem-resistant Klebsiella pneumoniae. J Infect Dis. 2017;215(suppl_1):S18–S27. doi:10.1093/infdis/jiw378
39. Moura A, Soares M, Pereira C, Leitão N, Henriques I, Correia A. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics. 2009;25(8):1096–1098. doi:10.1093/bioinformatics/btp105

40. Partridge SR, Zong Z, Iredell JR. Recombination in IS26 and Tn2 in the evolution of multiresistance regions carrying blaCTX-M-15 on conjugative IncF plasmids from Escherichia coli. Antimicrob Agents Chemother. 2011;55(11):4971–4978. doi:10.1128/AAC.00225-11

41. Anantham S, Harmer CJ, Hall RM. p39R861-4, a type 2 A/C2 plasmid carrying a segment from the A/C1 plasmid RA1. Microb Drug Res. 2015;21(6):571–576. doi:10.1089/mdr.2015.0133

42. Rinkel M, Hubert J-C, Roux B, Lett M-C. Transposon Tn 5403, a mobilization-helper element: complete nucleotide sequence and distribution in aquatic strains. FEMS Microbiol Ecol. 1994;15(1–2):89–95. doi:10.1111/j.1574-6941.1994.tb00233.x

43. Pasquali F, Kehrenberg C, Manfreda G, Schwarz S. Physical linkage of Tn 3 and part of Tn 1721 in a tetracycline and ampicillin resistance plasmid from Salmonella Typhimurium. J Antimicrob Chemother. 2005;55(4):562–565. doi:10.1093/jac/dkh553

44. Allmeier H, Crenar B, Greck M, Schmidt R. Complete nucleotide sequence of Tn1721: gene organization and a novel gene product with features of a chemotaxis protein. Gene. 1992;111(1):11–20. doi:10.1016/0378-1119(92)90097-T

45. Doi Y, Hazen TH, Boitano M, et al. Whole-genome assembly of Klebsiella pneumoniae coproducing NDM-1 and OXA-232 carbapenemases using single-molecule, real-time sequencing. Antimicrob Agents Chemother. 2014;58(10):5947–5953. doi:10.1128/AAC.0180-14

46. DuBois JL, Ojha S. Production of dioxygen in the dark: dismutases of oxyanions. In: Sustaining Life on Planet Earth: Metalloenzymes Mastering Dioxygen and Other Chewy Gases. Springer; 2015:45–87. doi:10.1007/978-3-319-12415-5_3

47. Trine M, Sørum H. Functional Tn5393-like transposon in the R plasmid pRAS2 from the fish pathogen Aeromonas salmonicida subspecies salmonicida isolated in Norway. Appl Environ Microbiol. 2000;66(12):5533–5535. doi:10.1128/AEM.66.12.5533-5535.2000

48. Chen Y-T, Shu H-Y, Li L-H, et al. Complete nucleotide sequence of pK245, a 98-kilobase plasmid conferring quinolone resistance and extended-spectrum-β-lactamase activity in a clinical Klebsiella pneumoniae isolate. Antimicrob Agents Chemother. 2006;50(11):3861–3866. doi:10.1128/AAC.00456-06

49. Zautner AE, Bunk B, Pfeifer Y, et al. Monitoring microevolution of OXA-48-producing Klebsiella pneumoniae ST147 in a hospital setting by SMRT sequencing. J Antimicrob Chemother. 2017;72(10):2737–2744. doi:10.1093/jac/dlx216

50. Chen CM, Yu WL, Huang M, et al. Characterization of IS26-composite transposons and multidrug resistance in conjugative plasmids from Enterobacter cloacae. Microbiol Immunol. 2015;59(9):516–525. doi:10.1111/1348-0421.12289

51. Noguchi N, Takada K, Katayama J, Emura A, Sasatsu M. Regulation of transcription of the mph (A) gene for Macrolide 2′-Phosphotransferase I in Escherichia coli: characterization of the regulatory gene mphR (A). J Bacteriol. 2000;182(18):5052–5058. doi:10.1128/JB.182.18.5052-5058.2000

52. Partridge SR, Ginn AN, Paulsen IT, Iredell JR. pEl1573 carrying blaIMP-4, from Sydney, Australia, is closely related to other IncL/M plasmids. Antimicrob Agents Chemother. 2012;56(11):6029–6032. doi:10.1128/AAC.01189-12

53. San Millan A, Peña-Miller R, Toll-Riera M, et al. Positive selection and compensatory adaptation interact to stabilize non-transmissible plasmids. Nat Commun. 2014;5:5208. doi:10.1038/ncomms6208