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ORIGINAL RESEARCH

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%.[2–6] *K. pneumoniae*, along with other highly important MDR pathogens, has been categorized as ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter aumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) organisms.[7,8] Many antibiotics, including aminoglycosides, fluoroquinolones, and carbapenems, are used for the treatment of infectious diseases caused by MDR pathogens.[9] 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.[10] 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.[11,12] Among all kinds of MGEs, plasmids play a key role in gene transfer processes.[13,14] *K. pneumoniae* acquired resistance through the successful acquisition of MDR plasmids, including those classified into IncFII, IncFIIY, IncHI2, and IncX1, incompatibility groups.[15–17] 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),[18] pCN1_1 (GenBank AC#CP015383), pRJ119-NDM1 (GenBank AC# KX636095),[19] 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.[20] The multilocus sequence typing (MLST) scheme for *K. pneumoniae* was followed as mentioned on the homepage (http://bigsdb.pasteur.fr/klebsiella)[3] by utilizing seven housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB*).[21] PCR screening of ESBLs,[22] carbapenem resistance,[23] quinolone resistance,[24] macrolide resistance[25] and common tetracycline resistance genes[26] 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 cm^2, 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.[27] As successful conjugation was not achieved, so electroporation experiments were carried out using *K. pneumonia* A1705, 911021, 1642 as the donor and *E.coli* DH5α as the recipient. The 2μL of plasmid DNA solution was added in 50 μL of *E.coli* DH5α 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.[28,29]

Antimicrobial Susceptibility Test

Antimicrobial susceptibility to ampicillin, compound sulfamethoxazole, ciprofloxacin, levofloxacin, 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.[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,[31] afterwards the contigs were congregated using Newbler 3.0.[32] 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[33] combined with BLASTP/BLASTN[34] results against UniProtKB/Swiss-Prot[35] and RefSeq databases.[36] Annotation of resistance genes, mobile elements, and other features were performed using online databases including ISfinder,[37] ResFinder,[38] and INTEGRALL.[39] Gene organization diagrams were drawn in Inkscape 0.48.1 (https://inkscape.org/en/).

GenBank Accession Numbers

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

Results

Characterization of *K. pneumoniae* Strains

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 $bla_{\text{KPC-2}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, *qnrS1, oqxAB, tetA*(A), *tetA*(D), $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1,}}$ and $bla_{\text{SHV-33}}$ genes in *K. pneumoniae* strain A1705 while the presence of the $bla_{\text{KPC-2}}$, *qnrS1, oqxAB, mph*(A), *tetA*(A), $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{SHV-11,}}$ and $bla_{\text{TEM-1}}$ genes in *K. pneumoniae* strain 911021 and the presence of $bla_{\text{KPC-2}}$, *qnrS1, mph*(A), *tetA*(A), $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{SHV-12,}}$ $bla_{\text{CTX-M-14}}$ genes in *K. pneumoniae* strain 1642. Later, three common drug resistance genes *qnrS1, tetA*(A), and $bla_{CTX\text{-}M\text{-}14}$ were co-transferred from *K. pneumoniae* strains A1705, 911021, 1642 into *E.coli* DH5α through electroporation, generating the transformants A1705-*qnrS*-DH5α, 911021-*tetA*-DH5α, and 1642-*tetA*-DH5α respectively. These results demonstrated the existence of these genes in all three plasmids of our study.

Antibiotic resistant *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), $bla_{CTX\text{-}M\text{-}14}$ 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 (Supplementary Figure 1 and Table 2). 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, 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)/Antimicrobial Susceptibility						
		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	≤2/R
	Piperacillin	≥128/R	≥128/R	≥128/R	≥128/R	≥128/R	≥128/R	≤4/S
	Piperacillin/tazobactam	≥128/R	≥128/R	≥128/R	≤4/S	≤4/S	≤4/S	≤4/S
Cephalosporins	Cefazolin	≥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	=4/S
	Cefuroxime axetil	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R	=4/S
	Cefotetan	≥64/R	≥64/R	≥64/R	≤4/S	≤4/S	≤4/S	≤4/S
	Ceftazidime	≥64/R	=32/R	≥64/R	≤1/S	≤1/S	=16/R	≤1/S
	Ceftriaxone	≥64/R	≥64/R	≥64/R	=32/R	≥64/R	≥64/R	≤1/S
	Cefepime	≥64/R	≥64/R	≥64/R	≤1/S	≤1/S	≤1/S	≤1/S
Monobactam	Aztreonam	≥64/R	≥64/R	≥64/R	=4/S	=4/S	≥64/R	≤1/S
Carbapenems	Imipenem	≥16/R	≥16/R	≥16/R	≤1/S	≤1/S	≤1/S	≤1/S
	Meropenem	≥16/R	≥16/R	≥16/R	≤0.25/S	≤0.25/S	≤0.25/S	≤0.25/S
Aminoglycosides	Amikacin	≤2/S	≥64/R	≥64/R	≤2/S	≤2/S	≤2/S	≤2/S
	Gentamicin	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R	≤1/S
Fluoroquinolones	Ciprofloxacin	≥4/R	≥4/R	≥4/R	=2/I	=0.5/S	=0.5/S	≤0.25/S
	Levofloxacin	≥8/R	≥8/R	≥8/R	=1/S	=1/S	=1/S	≤0.25/S
Furane	Nitrofurantoin	=128/R	≥512/R	≥512/R	≤16/S	≤16/S	≤16/S	≤16/S
Sulfanilamides	Compound sulfamethoxazole	≥320/R	≥320/R	≥320/R	≥320/R	≤20/R	≤20/R	≤20/R

Abbreviations: MIC, minimal inhibitory concentration; R, resistant; S, sensitive; I, intermediate resistant.

Table 2 Major Features of Plasmids Analyzed

Category	Plasmids							
	pA1705-qnrS	**p911021-tetA**	**p1642-tetA**	**pKPN-c22**	**pKPSH11**	**pKPN3-307_TypeC**	**pKPN3-307_TypeA**	**p6234-198.371kb**
Country	Shenyang Shengjing, China	Chongqing, China	Beijing, China	USA	Shafdan, Israel	Italy	Italy	Colombia, USA
Isolation source	urine sample	urine sample	alveolar lavage fluid	urine sample	Municipal water	Body fluid	Body fluid	Body fluid
Host bacterium	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
Collection date	April, 2013	August, 2014	August, 2014	2013	Oct, 2015	2014	2014	2012
Total length (bp)	271,255 bp	238,681 bp	237,591 bp	178,563 bp	186,474 bp	212,319 bp	227,989 bp	198,371 bp
Mean G+C content, %	51.89%	51.69%	51.77%	51.5%	51.5%	52.4%	52.2%	51.6%
Total number of ORF	335	301	300	303	342	303	356	349

Notes: pA1705-*qnrS*, p911021-*tetA* and p1642-*tetA* fully sequenced in this study. pKPN-c22, pKPSH11, pKPN3-307_TypeC, pKPN3-307_TypeA, p6234-198.371kb were derived from GenBank. Genetic comparisons of these plasmids interpreted in main text.
Abbreviation: ORF, open reading frame.

p1642-*tetA*, respectively)] of the 43-bp tandem repeat (gcaGatAAcCTgAcCgAcGAtGCAGGTGGGACCGTGGT CcCAG) 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 (orf*414*-orf*162* and orf*2340*-orf*543*) occurred in pKPSH11. Second the deletion of these two separate regions was found in pKPN3-307_TypeC, pKPN3-307_typeA, pA1705-*qnrS*, 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, pA1705-*qnrS*, 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′*

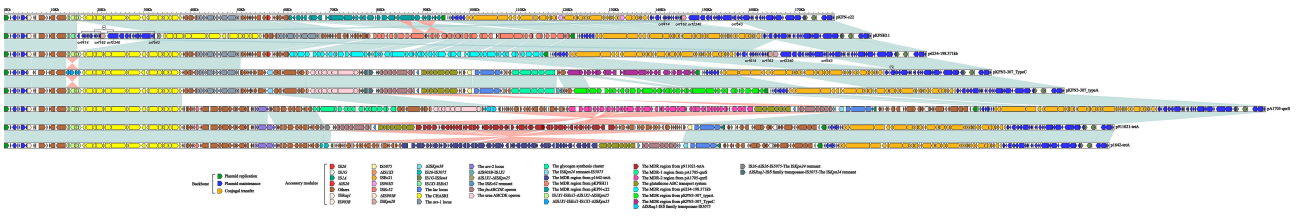

Figure 1 Linear comparison of the sequenced plasmid.
Notes: A linear comparison of the group of plasmids namely pKPN-c22, pKPSH11, p6234-198.371k, pKPN3-307_TypeC, pKPN3-307_typeA, pA1705-*qnrS*, p911021-*tetA* and p1642-*tetA* was performed containing the replication initiation genes *repA* and the *repB1*. Genes are indicated by arrows; genes, moving elements, and other regions are represented by different colors by function; shaded parts indicate regional nucleotide identity is greater than 95%.

and *ΔtivF12-3′* 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 IS*903B*, IS*1X1*–IS*Ecl1*, ΔIS*1X1*–ΔIS*Kpn25*, CHASRI, IS*1G*–IS*Sen4*, 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 Tn*402*-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: *qacE1* [quaternary ammonium compound resistance]–*sul1* [sulfonamide resistance]–*orf5*–*orf6*), the Tn*402tni* module (*tniABQR*) and IRt (inverted repeat at the *tni* end), bounded by *5′*-bp DRs. In191 from the MDR region of pKPSH11/p6234-198.371kb/pKPN3-307_typeA was derived from the prototype Tn*402*-associated class 1 integron. In191 had IRi, 5′-CS, a single-gene (*dfrA14*) cassette (trimethoprim resistance), *mobC*–IRt–IS*6100*, IRt with the loss of 3′-conserved segment (*3′*-CS: Δ*qacEΔ1*–*sul1*–*orf5*–*orf6*) and *tni*, 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*, IS*6100* 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:*aacA4cr* [aminoglycoside resistance]–bla_{OXA-1} [β-lactam resistance]–*catB3* [phenicol resistance]–*arr3* [rifampin resistance]), the first copy of 3′-conserved segment (3′-CS1: Δ*qacEΔ1*[quaternary ammonium compound resistance]–*sul1*[sulfonamide resistance]), IS*CR1*, variable region 2 (VR2: *qnrA1*–ampR), the truncation of the second copy of 3′-conserved segment (Δ3′-CS2: Δ*qacEΔ1*–*sul1*–*orf5*–*orf6*), IRt (inverted repeat at the *tni* end), IS*6100* replacing the Tn*402tni* module and IRt. In pKPN3-307_typeA/pKPN3-307_TypeC, In37 had undergone the segmentation into a remnant (*aacA4cr*–bla_{OXA-1}–Δ*catB3*), which was inverted compared with that in pKPN-c22/pKPSH11/p6234-198.371kb (Figure 2).

Tn2 and the Truncated IS*26*-*tetA*(D)-*tetR*(D)-IS*26* Unit from MDR-1 Region of pA1705-*qnrS*

Tn*2*, a Tn*3*-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 Tn*2* segment (IRL–Δ*tnpA*), that was also found in p6234-198.371kb, but at the opposite orientation. An intact Tn*2* was identified in the MDR-1 region from pA1705-*qnrS*, while the Tn*2* had undergone disintegration to form two parts, namely Tn*2*-5′, Tn*2*-3′ in pKPSH11 and p6234-198.371kb. In pKPN3-307_typeA, ΔTn*2* (identical to Tn*2*-3′ from pKPSH11/p6234-198.371kb) and the Tn*2* remnant (Δ*tnpA*) were identified (Figure 2).

The IS*26*-*tetA*(D)-*tetR*(D)-IS*26* unit [also designated Tn*tet*(D)], derivative of the tetracycline resistance unit, was made up of two directly oriented IS*26* elements flanking a central region that contained *orf435, adh, tetA*(D) (tetracycline efflux protein), and *tetR*(D) (tetracycline repressor protein).[41] The truncated IS*26*-*tetA*(D)-*tetR*(D)-IS*26* unit found in MDR-1 region of pA1705-*qnrS* comprised *adh, tetA*(D), and *tetR*(D) with IS26 on both sides (Figure 2).

Tn*5403* from P6234-198.371kb and pKPN3-307_TypeC

Tn*5403*, a Tn*3* 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] Tn*5403* was intact in pKPN-c22, pKPSH11, and pKPN3-307_typeA, but has been found in fragmented forms; Tn*5403*-5′ and Tn*5403*-3′ in p6234-198.371kb and had undergone the loss of IRR_Tn*5403* 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
IS*903B*	IS*903B*	IS*903B*	ΔIS*903B*	IS*903B*	IS*903B*	IS*903B*	IS*903B*
IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*	IS*1X1*–ISEcl1	IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*	ΔIS*1X1*–ISEcl1–IS*1X1*-ΔIS*Kpn25*	IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*	IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*	IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*	IS*1X1*–ISEcl1–ΔIS*1X1*–ΔIS*Kpn25*
CHASRI	IS*Kpn28*	CHASRI	CHASRI	CHASRI	CHASRI	CHASRI	CHASRI
ars-1 seat	ΔIS*1X1*–ΔIS*Kpn25*	*ars*-1 seat	*ars*-1 seat	*ars*-1 seat	IS*Ec52*	IS*Ec52*	IS*Ec52*
IS*26*	CHASRI	IS*26*	ΔIS*Kpn38*	ΔIS*Kpn38*	*ars*-2 seat	*ars*-2 seat	*ars*-2 seat
Decentralized and functionally unkown areas	*ars*-1 seat	Decentralized and functionally unkown areas	ΔIS*1X3*	ΔIS*1X3*	MDR-1	IS*26*–ΔIS*26*–IS*5075*–IS*Kpn24* residual	IS*26*–IS*5075*
MDR region	ΔIS*26*–ΔIS*26*,	MDR region	Urea ABCDE operon	Urea ABCDE operon	ΔIS*1X3*	*fecABCDE*operon	MDR region
IS*Ec21*	Decentralized and functionally unkown areas	IS*Kpn28*–IS*Kpn28*	ΔIS*Raq1*–IS*5* family transposase–IS*5075*–IS*Kpn24* residual	ΔIS*Raq1*–IS*5* family transposase–IS*5075*–IS*Kpn24* residual	Urea ABCDE operon	IS*Ec62* residual	Glutathione ABC transfer system
IS*903D*	MDR region	IS*1G*–IS*Sen4*	*fecABCDE* operon	*fecABCDE*operon	ΔIS*Raq1*–IS*5 family transposase*–IS*5075*	Glutathione ABC transfer system	IS*Ec62* residual
IS*903D*	IS*1G*–IS*Sen4*	–	IS*Ec62* residual	IS*Ec62* residual	MDR-2	MDR region	*fecABCDE*operon
IS*Kpn28*	–	–	Glutathione ABC transfer system	Glutathione ABC transfer system	Glutathione ABC transfer system	IS*5075*	IS*Kpn24* residual–IS*5075*
IS*1G*–IS*Sen4*	–	–	IS*5075*	IS*5075*	IS*Ec62* residual	*Lac* seat	*Lac* seat
–	–	–	*Lac* seat	*Lac* seat	*fecABCDE* operon	IS*1A*	IS*1A*
–	–	–	IS*1G*	IS*1G*	IS*Kpn24* residual–IS*5075*	Decentralized and functionally unkown areas	Decentralized and functionally unkown areas
–	–	–	Glycogen synthesis cluster	Glycogen synthesis cluster	*Lac* seat	IS*Raq1*	IS*Raq1*

(*Continued*)

Table 3 (Continued).

pKPN-c22	pKPSH11	p6234-198.371kb	pKPN3-307_TypeC	pKPN3-307_typeA	pA1705-qnrS	p911021-tetA	p1642-tetA
–	–	–	Decentralized and functionally unkown areas	Δ*IS903B*–IS*1X1*	IS*1A*	IS*1G*–IS*Sen4*	IS*1G*–IS*Sen4*
–	–	–	MDR region	Decentralized and functionally unkown areas	Decentralized and functionally unkown areas	–	–
–	–	–	IS*1G*–IS*Sen4*	MDR region	IS*Raq1*	–	–
–	–	–		IS*1G*–IS*Sen4*	IS*1G*–IS*Sen4*	–	–

Tn*1721* Remnant and Unit IS*3000–qnrB1*–IS*26* 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 Tn*1721*. Gram-negative bacteria obtained mobilizable plasmids from different sources having complete or truncated variants of Tn*1721*.[43] Tn*1721* is a member of Tn*21* subgroup of Tn*3*-family unit bacterial transposons[44] 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. Tn*1721* consisted of a "basic transposon," Tn*1722*, which was cryptic, with the modular structure IRR–*tnpAR*–*mcp*–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 Δ*tnpA* (transposase) was discovered (Figure 2).

The IS*3000–qnrB1*–IS*26* unit, a *qnrB1* transmission vehicle, was first found in pPKPN1 from PittNDM01.[45] The *pspF–qnrB1–*Δ*orf909* was bracketed by two different ISs, namely IS*3000* and IS*26*. In each of pKPN-c22, pKPSH11, p6234-198.371kb, pKPN3-307_typeA, and pKPN3-307_TypeC, the IS*3000* within this unit was truncated at the 5′-terminal due to the transposition of Tn*5403*. The Δ*orf909* was truncated in pKPN-c22/pKPSH11/p6234-198.371kb, while in pKPSH11, IS*26* was truncated as well (Figure 2).

Tn*6415* from pKPN3-307_typeA and pKPSH11

Tn*6415*, first appraised in plasmid unitig_2 (GenBank accession number CP021536) from *Escherichia coli* strain AR_0119, was an IS*26*-flanked composite transposon. It was bracketed by 8-bp DRs and arranged in the following order: IS*26, aacC2* (aminoglycoside resistance), *tmrB* (tunicamycin resistance), *orf222, orf891*, and IS*26*. Tn*6415* had undergone the deletion of DRs in pKPN3-307_typeA, the truncation upstream of right-hand IS*26* in p6234-198.371kb and further truncation at the 5′-end of the right-hand IS*26*, 3′-end of left-hand IS*26* in pKPSH11 (Figure 2).

IS*26*-*Cld*-IS*26* 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.[46] We found IS*26*-*cld*-IS*26* unit carrying *cld* (chlorite dismutase) and some genes of unknown function, encircled by two directly orientated IS*26* elements. In pKPN-c22, a 38-bp deletion was observed at the 5′-end of *orf1083*. In p6234-198.371kb, the left-hand IS*26* within this unit was truncated, while in pKPSH11, the left-hand IS*26* was further truncated, and the truncation of IS*26* in *orf384* at the right-hand was discovered (Figure 2).

Tn*5393c* and IS*26*–*Sul2*–*strA*–*strB*–IS*26* Unit from pA1705-*qnrS*, pKPSH11, P6234-198.371kb and pKPN3-307_typeA

Tn*5393c*, an active transposon, containing *strA–strB* was reported for the first time in the family of Tn*5393*.[47] It

Table 4 Drug Resistance Genes in Plasmids Analyzed

Plasmid	Resistance Gene	Resistance Phenotype	Nucleotide Position	Region Located
pKPN-c22	*catB3*	Phenicol resistance	62,051.62494	The MDR region
	bla_{OXA-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
	strA	Aminoglycoside resistance	80,285.81088	
	strB	Aminoglycoside resistance	81,088.81942	
	bla_{TEM-1}	β-Lactam resistance	82,645.83505	
	$bla_{CTX-M-15}$	β-Lactam resistance	86,327.87202	
	tmrB	Tunicamycin resistance	96,444.96986	
	aacC2	Aminoglycoside resistance	96,999.97859	
	catB3	Phenicol resistance	98,093.98641	
	bla_{OXA-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
	strA	Aminoglycoside resistance	67,269.68072	
	strB	Aminoglycoside resistance	68,072.68908	
	$bla_{CTX-M-15}$	β-Lactam resistance	73,311.74186	
	tmrB	Tunicamycin resistance	88,190.88732	
	aacC2	Aminoglycoside resistance	88,745.89605	
	catB3	Phenicol resistance	90,362.90910	
	bla_{OXA-1}	β-lactam resistance	91,048.91923	
	aacA4cr	Aminoglycoside resistance	92,009.92608	
	tetA(A)	Tetracycline resistance	96,063.97262	
	qnrB1	Quinolone resistance	106,318.106962	
	dfrA14	Trimethoprim resistance	110,153.110635	
pKPN3-307_typeA	*Sul2*	Sulfonamide resistance	129,146.129961	The MDR region
	strA	Aminoglycoside resistance	130,022.130825	
	strB	Aminoglycoside resistance	130,825.131661	
	bla_{TEM-1}	β-actam resistance	132,382.133242	
	$bla_{CTX-M-15}$	β-Lactam resistance	136,064.136939	
	tmrB	Tunicamycin resistance	141,538.142080	
	aacC2	Aminoglycoside resistance	142,093.142953	
	aacA4cr	Quinolone resistance	143,863.144462	
	bla_{OXA-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
	bla_{OXA-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*)

Table 4 (Continued).

Plasmid	Resistance Gene	Resistance Phenotype	Nucleotide Position	Region Located
pA1705-*qnrS*(MDR-1)	*bla*$_{TEM-1}$	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	Quninolone resistance	139,821.140477	
	bla$_{LAP-2}$	β-Lactam resistance	142,074.142931	
	tmrB	Tunicamycin resistance	147,263.147805	
	aacC2	Aminoglycoside resistance	147,818.148678	
	bla$_{CTX-M-14}$	β-Lactam resistance	158,542.159393	
p911021-*tetA*	*bla*$_{CTX-M-14}$	β-Lactam resistance	90,093.90944	The MDR region
	aacC2	Aminoglycoside resistance	100,783.101643	
	tmrB	Tunicamycin resistance	101,656.101643	
	bla$_{LAP-2}$	β-Lactam resistance	106,530.107387	
	qnrS1	Quninolone resistance	108,984.109640	
	aphA1a	Aminoglycoside resistance	116,481.117296	
	mph(A)	Macrolide resistance	118,273.119178	
	dfrA1	Trimethoprim resistance	125,273.125746	
	tetA(A)	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$_{SHV-12}$	β-Lactam resistance	100,242.101102	
	qnrS1	Quninolone resistance	107,773.108429	
	bla$_{LAP-2}$	β-actam resistance	110,026.110883	
	tmrB	Tunicamycin resistance	115,215.115757	
	aacC2	Aminoglycoside resistance	115,770.116630	
	bla$_{CTX-M-14}$	β-Lactam resistance	125,112.125963	

showed the modular structure as follows; *tnpA-res-tnpR-strA-strB* with direct and inverted repeats at both ends. The IS*26*–*sul2*–*strA*–*strB*–IS*26* unit was an IS*26*-flanked transposition unit and first discovered in pK245 from *K. pneumoniae* strain NK245.[48] It was comprised of remnant (*strA*–*strB*–IRR_Tn*5393c*) of the Tn*5393* family unit transposon Tn*5393c*, the upstream *sul2*, and the downstream *orf411*–*orf738*–*orf684*–IS*26*. 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 Tn*2*. Only a 3607-bp remnant (*sul2*–*strA*–*strB*–IRR_Tn*5393c*–*orf411*) was observed in pKPSH11/p6234-198.371kb/pKPN3-307_typeA (Figure 2).

In*363*, Tn*1721* Remnant and IS*26*-*bla*$_{SHV-12}$-IS*26* Unit from P1642-*tetA*/P911021-*tetA* and pA1705-*qnrS*

In*363*, a *dfrA1* gene and a gene array of undefined function was classified in class 1 integrons.[49] We observed the In*363* from the MDR region of p1642-*tetA*/p911021-*tetA* and found that it was similar to the prototype Tn*402*-associated class 1 integron. In*363* had Δ5′-CS (Δ*intI1*–*attI1*), GCA (*dfrA1* [trimethoprim resistance]–*gcuC* [unknown function]), *3′*-CS and IRt. Compared with the prototype Tn*402*-associated class 1 integron, In*363* has gone through the truncation of *intI1*, the loss of IRi and *tni*. The In*363* in the MDR-2 region of pA1705-*qnrS* have been found to be inserted with IS*Kpn26* (Figure 3).

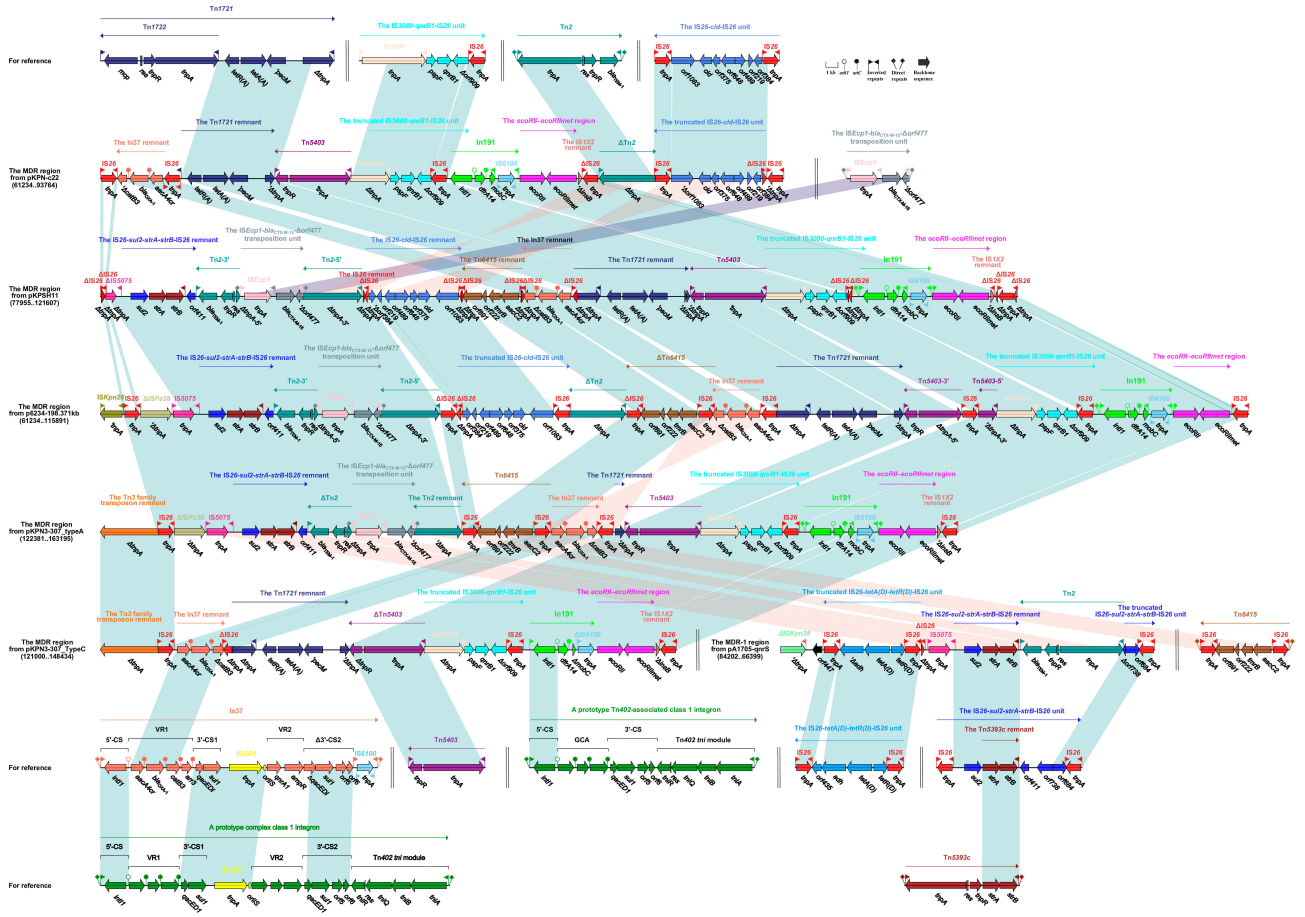

Figure 2 Accessory modules of the MDR region from pKPN-c22, pKPSH11, p6234-198.371kb, pKPN3-307_TypeC, and pKPN13-307_typeA and the MDR-1 region from pA1705-*qnrS*.

Notes: Genes are indicated by arrows; genes, moving elements, and other regions are represented by different colors by function; shaded parts indicate regional nucleotide identity greater than 95%.

In plasmids pA1705-*qnrS*, p1642-*tetA*, and p911021-*tetA*, Tn*1721* have been dislocated into two fragments, namely the Tn*1721* remnant (IRL_Tn*1721*–Δ*mcp*) and ΔTn*1721*[*tetR*(A)–*tetA*(A)–*pecM*–Δ*tnpA*–IRR-2_Tn*1721*]. The 5-bp DRs are locating upstream of the Tn*1721* remnant and downstream of ΔTn*1721* indicate that the disruption occurred after transposition. Remarkably, the lengths of the Tn*1721* remnants varied among plasmids (1455-, 1601-, and 1707-bp for p1642-*tetA*, MDR-2 pA1705-*qnrS*, and p911021-*tetA* respectively) (Figure 3).

IS*26*-bla_{SHV-12}-IS*26* locus was found in different genetic environments, including class 1 integrons and transposons.[50] These elements could play an important role in the spread of bla_{SHV-12} and are suggestive of multiple recombination events. IS*26*-bla_{SHV-12}-IS*26* carried bla_{SHV-12}–*deoR*-*yjbJ*–*yjbK*–*yjbL*–*yjbM* genes, encircled by IS*26* at both ends. Two fragments (IS*26*–bla_{SHV-12}–*deoR* and *yjbJ*–*yjbK*–*yjbL*–*yjbM*–IS*26*) were identified in the opposite direction in p1642-*tetA*. Nonetheless, a single portion (Δ*deoR*–*yjbJ*–*yjbK*–*yjbL*–Δ*yjbM*) of the IS*26*-bla_{SHV-12}-IS*26* unit was found in pA1705*qnrS*/p911021-*tetA* (Figure 3).

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

The IS*6100* transposable unit is based on IS*26*, IS*6100* and includes *mph*(*A*)–*mrx*–*mphR*(*A*) operon.[51] The insertion sequences IS*26* and IS*6100* belong to the IS*6* family, contain almost identical IRs of 14 bp in length. The complete IS*26*–*mph*(*A*)–*mrx*–*mphR*(*A*)–IS*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*26*-bla_{LAP-2}–*qnrS*-IS*26* transposition unit was first discovered in plasmid pE66An in *E. coli* E66An (AC # HF545433). The transposable unit structure is

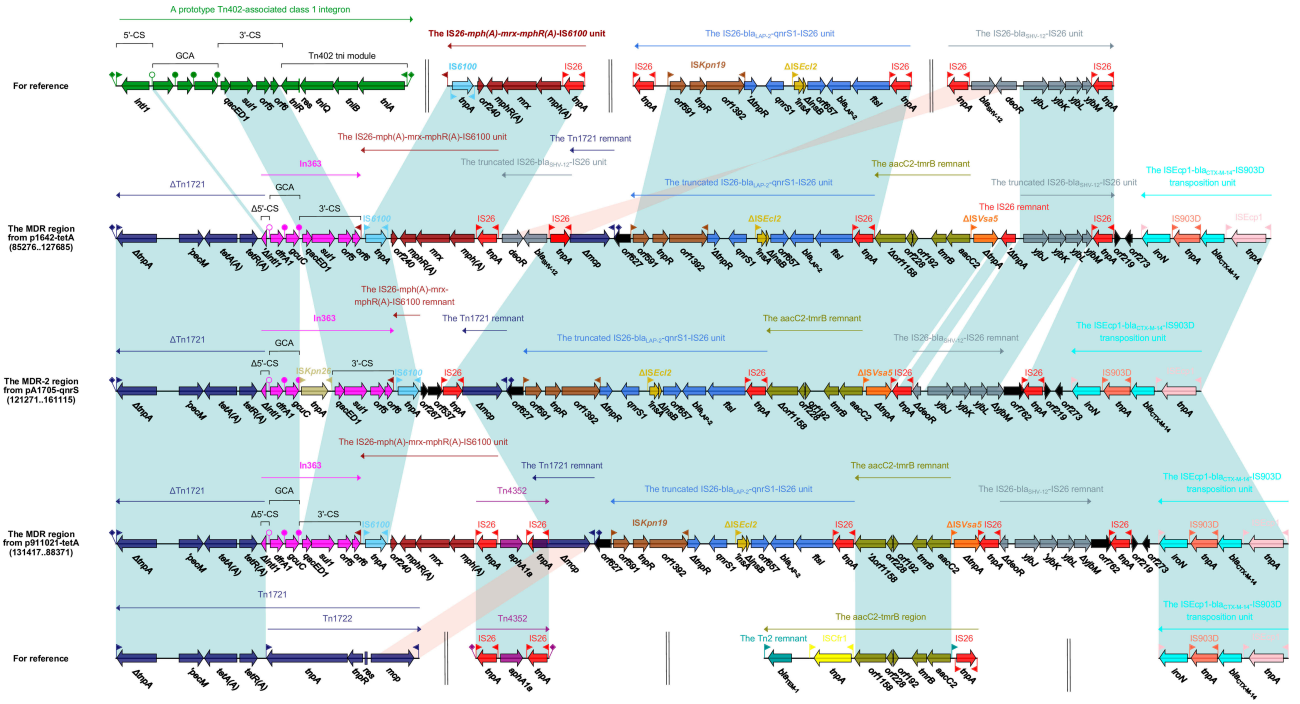

Figure 3 Accessory modules of the MDR region from p1642-*tetA* and p911021-*tetA*, and the MDR-2 region from pA1705-*qnrS*.
Notes: Genes are indicated by arrows; genes, moving elements, and other regions are represented by different colors by function; shaded parts indicate regional nucleotide identity is greater than 95%.

IS*26*, *ftsI*, bla_{LAP-2}, *orf657*, ΔIS*Ecl2*, *qnrS1*, *ΔtnpR*, IS*Kpn19*, and IS*26*. The unit lost its IS*26* 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.[52] The structure of the *aacC2-tmrB* unit is IS*26*, Tn*2* residual, *aacC2, tmrB*, orf192, *orf228, orf1158*, IS*Cfr1*, and Tn*2* 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.[53] 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 (In*363*, In*37*, In*191*) and transposons (Tn*4352*, Tn*1721*, Tn*6415*, Tn*2*) 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.

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