

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 baumannii*, *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://bigsd.bpasteur.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 resistance²⁵ and common tetracycline resistance genes²⁶ 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* DH5 α 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.²⁷ As successful conjugation was not achieved, so electroporation experiments were carried out using *K. pneumoniae* 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 server³³ combined with BLASTP/BLASTN³⁴ results against UniProtKB/Swiss-Prot³⁵ 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*_{KPC-2}, *bla*_{NDM-1}, *bla*_{OXA-1}, *qnrS1*, *oqxAB*, *tetA(A)*, *tetA(D)*, *bla*_{CTX-M-14}, *bla*_{CTX-M-15}, *bla*_{TEM-1}, and *bla*_{SHV-33} genes in *K. pneumoniae* strain A1705 while the presence of the *bla*_{KPC-2}, *qnrS1*, *oqxAB*, *mph(A)*, *tetA(A)*, *bla*_{CTX-M-14}, *bla*_{CTX-M-65}, *bla*_{SHV-11}, and *bla*_{TEM-1} genes in *K. pneumoniae* strain 911021 and the presence of *bla*_{KPC-2}, *qnrS1*, *mph(A)*, *tetA(A)*, *bla*_{TEM-1}, *bla*_{CTX-M-65}, *bla*_{SHV-12}, *bla*_{CTX-M-14} genes in *K. pneumoniae* strain 1642. Later, three common drug resistance genes *qnrS1*, *tetA(A)*, and *bla*_{CTX-M-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-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 (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- <i>qnrS</i> -DH5 α	911021- <i>tetA</i> -DH5 α	1642- <i>tetA</i> -DH5 α	DH5 α
Penicillins	Ampicillin	$\geq 32/R$	$\geq 32/R$	$\geq 32/R$	$\geq 32/R$	$\geq 32/R$	$\geq 32/R$	$\leq 2/R$
	Piperacillin	$\geq 128/R$	$\geq 128/R$	$\geq 128/R$	$\geq 128/R$	$\geq 128/R$	$\geq 128/R$	$\leq 4/S$
	Piperacillin/tazobactam	$\geq 128/R$	$\geq 128/R$	$\geq 128/R$	$\leq 4/S$	$\leq 4/S$	$\leq 4/S$	$\leq 4/S$
Cephalosporins	Cefazolin	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\leq 4/S$
	Cefuroxime	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$=4/S$
	Cefuroxime axetil	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$=4/S$
	Cefotetan	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\leq 4/S$	$\leq 4/S$	$\leq 4/S$	$\leq 4/S$
	Ceftazidime	$\geq 64/R$	$=32/R$	$\geq 64/R$	$\leq 1/S$	$\leq 1/S$	$=16/R$	$\leq 1/S$
	Ceftriaxone	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$=32/R$	$\geq 64/R$	$\geq 64/R$	$\leq 1/S$
	Cefepime	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$\leq 1/S$	$\leq 1/S$	$\leq 1/S$	$\leq 1/S$
Monobactam Carbapenems	Aztreonam	$\geq 64/R$	$\geq 64/R$	$\geq 64/R$	$=4/S$	$=4/S$	$\geq 64/R$	$\leq 1/S$
	Imipenem	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\leq 1/S$	$\leq 1/S$	$\leq 1/S$	$\leq 1/S$
	Meropenem	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\leq 0.25/S$	$\leq 0.25/S$	$\leq 0.25/S$	$\leq 0.25/S$
Aminoglycosides	Amikacin	$\leq 2/S$	$\geq 64/R$	$\geq 64/R$	$\leq 2/S$	$\leq 2/S$	$\leq 2/S$	$\leq 2/S$
	Gentamicin	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\geq 16/R$	$\leq 1/S$
Fluoroquinolones	Ciprofloxacin	$\geq 4/R$	$\geq 4/R$	$\geq 4/R$	$=2/I$	$=0.5/S$	$=0.5/S$	$\leq 0.25/S$
	Levofloxacin	$\geq 8/R$	$\geq 8/R$	$\geq 8/R$	$=1/S$	$=1/S$	$=1/S$	$\leq 0.25/S$
Furane	Nitrofurantoin	$=128/R$	$\geq 512/R$	$\geq 512/R$	$\leq 16/S$	$\leq 16/S$	$\leq 16/S$	$\leq 16/S$
Sulfanilamides	Compound sulfamethoxazole	$\geq 320/R$	$\geq 320/R$	$\geq 320/R$	$\geq 320/R$	$\leq 20/R$	$\leq 20/R$	$\leq 20/R$

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	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
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 (*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, 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 *tivF12-3'* 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, IS1X1-ISEcl1, ΔIS1X1-ΔISKpn25, CHASRI, IS1G-ISSen4, 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: *qacE1* [quaternary ammonium compound resistance]-*sulI* [sulfonamide resistance]-*orf5-orf6*), the Tn402*tni* 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 Tn402-associated class 1 integron. In191 had IRi, 5'-CS, a single-gene (*dfrA14*) cassette (trimethoprim resistance), *mobC*-IRt-*IS6100*, IRt with the loss of 3'-conserved segment (3'-CS: $\Delta qacE1-sulI-orf5-orf6$) 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*, *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: *aacA4cr* [aminoglycoside resistance]-*bla*_{OXA-1}

[β-lactam resistance]-*catB3* [phenicol resistance]-*arr3* [rifampin resistance]), the first copy of 3'-conserved segment (3'-CS1: $\Delta qacE1$ [quaternary ammonium compound resistance]-*sulI* [sulfonamide resistance]), *ISCR1*, variable region 2 (VR2: *qnrA1-ampR*), the truncation of the second copy of 3'-conserved segment ($\Delta 3'$ -CS2: $\Delta qacE1-sulI-orf5-orf6$), IRt (inverted repeat at the *tni* end), *IS6100* replacing the Tn402*tni* module and IRt. In pKPN3-307_typeA/pKPN3-307_TypeC, In37 had undergone the segmentation into a remnant (*aacA4cr-bla*_{OXA-1}- $\Delta catB3$), 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- $\Delta tnpA$), 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, Δ Tn2 (identical to Tn2-3' from pKPSH11/p6234-198.371kb) and the Tn2 remnant ($\Delta tnpA$) 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*, *tetA*(D) (tetracycline efflux protein), and *tetR*(D) (tetracycline repressor protein).⁴¹ The truncated IS26-*tetA*(D)-*tetR*(D)-IS26 unit found in MDR-1 region of pA1705-*qnrS* comprised *adh*, *tetA*(D), and *tetR*(D) 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	Δ IS903B	IS903B	IS903B	IS903B	IS903B
IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25	IS1X1-ISEc1	IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25	Δ IS1X1-ISEc1-IS1X1- Δ ISKpn25	IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25	IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25	IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25	IS1X1-ISEc1- Δ IS1X1- Δ ISKpn25
CHASRI	ISKpn28	CHASRI	CHASRI	CHASRI	CHASRI	CHASRI	CHASRI
ars-I seat	Δ IS1X1- Δ ISKpn25	ars-I seat	ars-I seat	ars-I seat	ISEc52	ISEc52	ISEc52
IS26	CHASRI	IS26	Δ ISKpn38	Δ ISKpn38	ars-2 seat	ars-2 seat	ars-2 seat
Decentralized and functionally unknwn areas	ars-I seat	Decentralized and functionally unknwn areas	Δ IS1X3	Δ IS1X3	MDR-1	IS26- Δ IS26- Δ IS5075-ISKpn24 residual	IS26- Δ IS5075
MDR region	Δ IS26- Δ IS26,	MDR region	Urea ABCDE operon	Urea ABCDE operon	Δ IS1X3	fecABCDEoperon	MDR region
ISEc21	Decentralized and functionally unknwn areas	ISKpn28-ISKpn28	Δ ISRaql-IS5 family transposase- Δ IS5075-ISKpn24 residual	Δ ISRaql-IS5 family transposase- Δ IS5075-ISKpn24 residual	Urea ABCDE operon	ISEc62 residual	Glutathione ABC transfer system
IS903D	MDR region	ISIG-ISSen4	fecABCDE operon	fecABCDEoperon	Δ ISRaql-IS5 family transposase- Δ IS5075	Glutathione ABC transfer system	ISEc62 residual
IS903D	ISIG-ISSen4	-	ISEc62 residual	ISEc62 residual	MDR-2	MDR region	fecABCDEoperon
ISKpn28	-	-	Glutathione ABC transfer system	Glutathione ABC transfer system	Glutathione ABC transfer system	IS5075	ISKpn24 residual- Δ IS5075
ISIG-ISSen4	-	-	IS5075	IS5075	ISEc62 residual	Lac seat	Lac seat
-	-	-	Lac seat	Lac seat	fecABCDE operon	ISIA	ISIA
-	-	-	ISIG	ISIG	ISKpn24 residual- Δ IS5075	Decentralized and functionally unknwn areas	Decentralized and functionally unknwn areas
-	-	-	Glycogen synthesis cluster	Glycogen synthesis cluster	Lac seat	ISRaql	ISRaql

(Continued)

Table 3 (Continued).

pKPN-c22	pKPSH11	p6234-198.371kb	pKPN3-307_TypeC	pKPN3-307_typeA	pAI705-qnrS	p911021-tetA	pI642-tetA
–	–	–	Decentralized and functionally unknown areas	Δ IS903B-IS1X1	IS1A	IS1G-ISSen4	IS1G-ISSen4
–	–	–	MDR region	Decentralized and functionally unknown areas	Decentralized and functionally unknown areas	–	–
–	–	–	IS1G-ISSen4	MDR region	ISRaql	–	–
–	–	–		IS1G-ISSen4	IS1G-ISSen4	–	–

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 Tn1721.⁴³ Tn1721 is a member of Tn21 subgroup of Tn3-family unit bacterial transposons⁴⁴ 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*–*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 IS3000–*qnrB1*–IS26 unit, a *qnrB1* transmission vehicle, was first found in pKPN1 from PittNDM01.⁴⁵ The *pspF*–*qnrB1*– Δ *orf909* 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 Δ *orf909* 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.⁴⁶ 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–*Sul2*–*strA*–*strB*–IS26 Unit from pAI705-*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 Tn5393.⁴⁷ It

Table 4 Drug Resistance Genes in Plasmids Analyzed

Plasmid	Resistance Gene	Resistance Phenotype	Nucleotide Position	Region Located
pKPN-c22	<i>catB3</i> <i>bla_{OXA-1}</i> <i>aacA4cr</i> <i>tetA(A)</i> <i>qnrB1</i> <i>dfrA14</i>	Phenicol resistance β-Lactam resistance Aminoglycoside resistance Tetracycline resistance Quinolone resistance Trimethoprim resistance	62,051.62494 62,632.63507 63,593.64192 66,136.67335 75,563.76207 79,066.79548	The MDR region
pKPSH11	<i>sul2</i> <i>strA</i> <i>strB</i> <i>bla_{TEM-1}</i> <i>bla_{CTX-M-15}</i> <i>tmrB</i> <i>aacC2</i> <i>catB3</i> <i>bla_{OXA-1}</i> <i>aacA4cr</i> <i>qnrB1</i> <i>dfrA14</i>	Sulfonamide resistance Aminoglycoside resistance Aminoglycoside resistance β-Lactam resistance β-Lactam resistance Tunicamycin resistance Aminoglycoside resistance Phenicol resistance β-Lactam resistance Aminoglycoside resistance Quinolone resistance Trimethoprim resistance	79,409.80224 80,285.81088 81,088.81942 82,645.83505 86,327.87202 96,444.96986 96,999.97859 98,093.98641 98,779.99654 99,740.100339 112,046.112690 115,412.115894	The MDR region
p6234-198.371kb	<i>Sul2</i> <i>strA</i> <i>strB</i> <i>bla_{CTX-M-15}</i> <i>tmrB</i> <i>aacC2</i> <i>catB3</i> <i>bla_{OXA-1}</i> <i>aacA4cr</i> <i>tetA(A)</i> <i>qnrB1</i> <i>dfrA14</i>	Sulfonamide resistance Aminoglycoside resistance Aminoglycoside resistance β-Lactam resistance Tunicamycin resistance Aminoglycoside resistance Phenicol resistance β-lactam resistance Aminoglycoside resistance Tetracycline resistance Quinolone resistance Trimethoprim resistance	66,393.67208 67,269.68072 68,072.68908 73,311.74186 88,190.88732 88,745.89605 90,362.90910 91,048.91923 92,009.92608 96,063.97262 106,318.106962 110,153.110635	The MDR region
pKPN3-307_typeA	<i>Sul2</i> <i>strA</i> <i>strB</i> <i>bla_{TEM-1}</i> <i>bla_{CTX-M-15}</i> <i>tmrB</i> <i>aacC2</i> <i>aacA4cr</i> <i>bla_{OXA-1}</i> <i>catB3</i> <i>qnrB1</i> <i>dfrA14</i>	Sulfonamide resistance Aminoglycoside resistance Aminoglycoside resistance β-actam resistance β-Lactam resistance Tunicamycin resistance Aminoglycoside resistance Quinolone resistance β-lactam resistance Phenicol resistance Quinolone resistance Trimethoprim resistance	129,146.129961 130,022.130825 130,825.131661 132,382.133242 136,064.136939 141,538.142080 142,093.142953 143,863.144462 144,548.145423 145,561.146019 153,364.154008 157,291.157773	The MDR region
pKPN3-307_TypeC	<i>aacA4cr</i> <i>bla_{OXA-1}</i> <i>catB3</i> <i>tetA(A)</i> <i>qnrB1</i> <i>dfrA14</i>	Quinolone resistance β-Lactam resistance Phenicol resistance Tetracycline resistance Quinolone resistance Trimethoprim resistance	124,667.125266 125,352.126227 126,365.126913 129,467.130666 138,855.139499 142,791.143264	The MDR region

(Continued)

Table 4 (Continued).

Plasmid	Resistance Gene	Resistance Phenotype	Nucleotide Position	Region Located
pA1705- <i>qnrS</i> (MDR-1)	<i>bla</i> _{TEM-1} <i>strB</i> <i>strA</i> <i>sul2</i> <i>tetA</i> (D)	β-Lactam resistance Aminoglycoside resistance Aminoglycoside resistance Sulfonamide resistance Tetracycline resistance	71,817.72677 72,942.73778 73,778.74581 74,642.75457 79,032.80216	
pA1705- <i>qnrS</i> (MDR-2)	<i>tetA</i> (A) <i>dfrA1</i> <i>qnrS1</i> <i>bla</i> _{LAP-2} <i>tmrB</i> <i>aacC2</i> <i>bla</i> _{CTX-M-14}	Tetracycline resistance Trimethoprim resistance Quinolone resistance β-Lactam resistance Tunicamycin resistance Aminoglycoside resistance β-Lactam resistance	124,510.125709 126,942.127415 139,821.140477 142,074.142931 147,263.147805 147,818.148678 158,542.159393	The MDR region
p911021- <i>tetA</i>	<i>bla</i> _{CTX-M-14} <i>aacC2</i> <i>tmrB</i> <i>bla</i> _{LAP-2} <i>qnrS1</i> <i>aphA1a</i> <i>mph(A)</i> <i>dfrA1</i> <i>tetA</i> (A)	β-Lactam resistance Aminoglycoside resistance Tunicamycin resistance β-Lactam resistance Quinolone resistance Aminoglycoside resistance Macrolide resistance Trimethoprim resistance Tetracycline resistance	90,093.90944 100,783.101643 101,656.101643 106,530.107387 108,984.109640 116,481.117296 118,273.119178 125,273.125746 126,979.128178	The MDR region
p1642- <i>tetA</i>	<i>tetA</i> (A) <i>dfrA1</i> <i>mph(A)</i> <i>bla</i> _{SHV-12} <i>qnrS1</i> <i>bla</i> _{LAP-2} <i>tmrB</i> <i>aacC2</i> <i>bla</i> _{CTX-M-14}	Tetracycline resistance Trimethoprim resistance Macrolide resistance β-Lactam resistance Quinolone resistance β-actam resistance Tunicamycin resistance Aminoglycoside resistance β-Lactam resistance	88,527.89726 90,959.91432 97,527.98432 100,242.101102 107,773.108429 110,026.110883 115,215.115757 115,770.116630 125,112.125963	The MDR region

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 NK245.⁴⁸ It was comprised of remnant (*strA-strB*-IRR_Tn5393c) of the Tn5393 family unit 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).

In363, Tn1721 Remnant and IS26-*bla*_{SHV-12}-IS26 Unit from P1642-*tetA*/P911021-*tetA* and pA1705-*qnrS*

In363, a *dfrA1* gene and a gene array of undefined function was classified in class 1 integrons.⁴⁹ We observed the In363 from the MDR region of p1642-*tetA*/p911021-*tetA* and found that it was similar to the prototype Tn402-associated class 1 integron. In363 had Δ5'-CS (Δ*intI1-attI1*), GCA (*dfrA1* [trimethoprim resistance]-*gcuC* [unknown function]), 3'-CS and IRt. Compared with the prototype Tn402-associated class 1 integron, In363 has gone through the truncation of *intI1*, the loss of IRi and *tnti*. The In363 in the MDR-2 region of pA1705-*qnrS* have been found to be inserted with IS*Kpn26* (Figure 3).

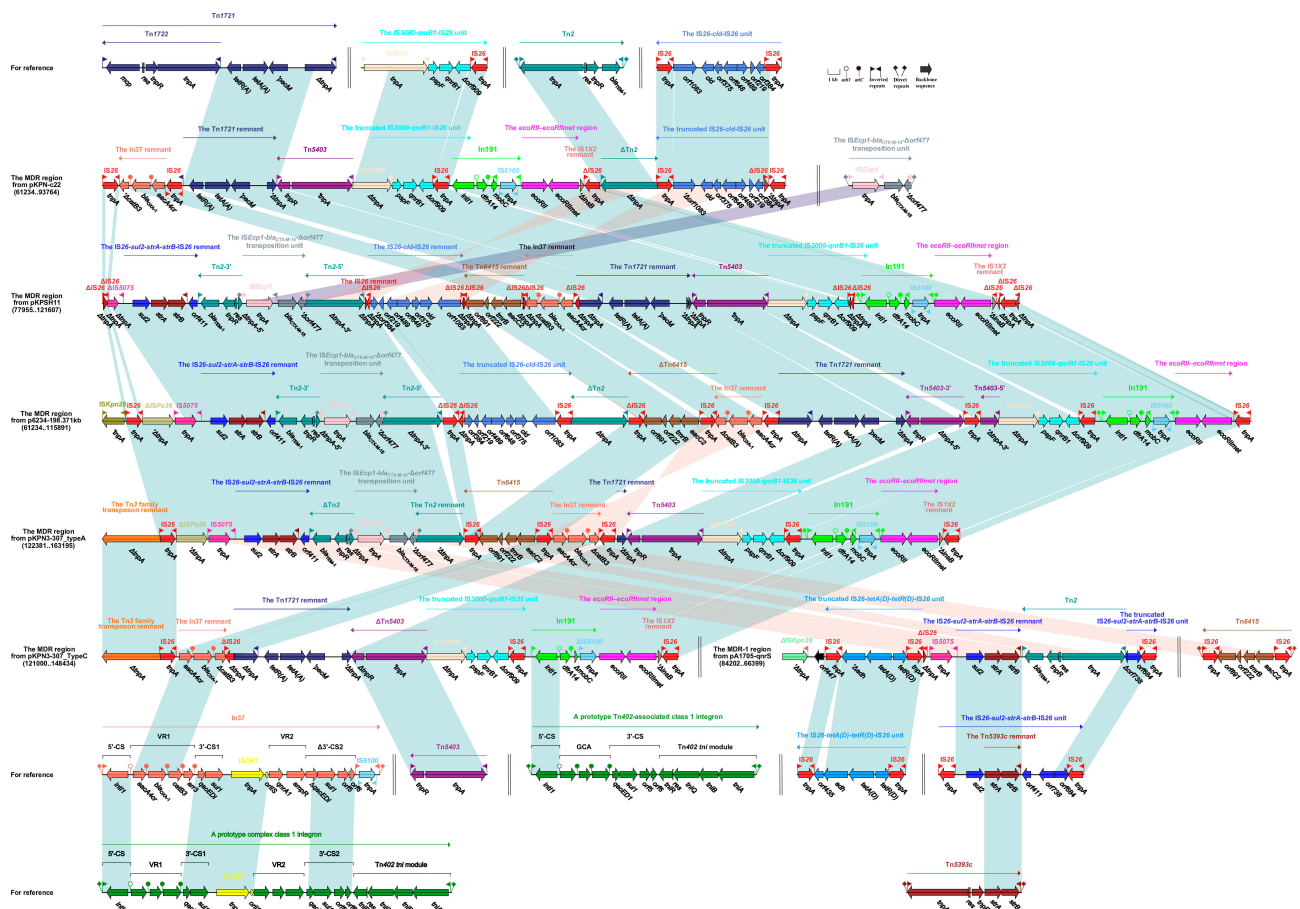


Figure 2 Accessory modules of the MDR region from pKPN-c22, pKPSH11, p6234-198.371kb, pKPN3-307_TypeC, and pKPN13-307_typeA and the MDR-1 region from pA1705-qnrS.

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, Tn1721 have been dislocated into two fragments, namely the Tn1721 remnant (IRL_Tn1721-Δmcp) and ΔTn1721[tetR(A)-tetA(A)-pecM-ΔtnpA-IRR-2_Tn1721]. The 5-bp DRs are locating upstream of the Tn1721 remnant and downstream of ΔTn1721 indicate that the disruption occurred after transposition. Remarkably, the lengths of the Tn1721 remnants varied among plasmids (1455-, 1601-, and 1707-bp for p1642-tetA, MDR-2 pA1705-qnrS, and p911021-tetA respectively) (Figure 3).

IS26-bla_{SHV-12}-IS26 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. IS26-bla_{SHV-12}-IS26 carried bla_{SHV-12}-deoR-yjbJ-yjbK-yjbL-yjbM genes, encircled by IS26 at both ends. Two fragments (IS26-bla_{SHV-12}-deoR and yjbJ-yjbK-yjbL-yjbM-IS26) were identified in the

opposite direction in p1642-tetA. Nonetheless, a single portion (ΔdeoR-yjbJ-yjbK-yjbL-ΔyjbM) of the IS26-bla_{SHV-12}-IS26 unit was found in pA1705qnrS/p911021-tetA (Figure 3).

IS26-mph(A)-mrx-mphR(A)-IS6100, IS26-bla_{LAP-2}-qnrS-IS26 and aacC2-tmrB Transposon Units from P1642-tetA/P911021-tetA and pA1705-qnrS

The IS6100 transposable unit is based on IS26, IS6100 and includes mph(A)-mrx-mphR(A) operon.⁵¹ The insertion sequences IS26 and IS6100 belong to the IS6 family, contain almost identical IRs of 14 bp in length. The complete IS26-mph(A)-mrx-mphR(A)-IS6100 transposable unit appears in plasmid p1642-tetA/p911021-tetA, but only a small residue was found in plasmid pA1705-qnrS (Figure 3).

The IS26-bla_{LAP-2}-qnrS-IS26 transposon unit was first discovered in plasmid pE66An in E. coli E66An (AC # HF545433). The transposable unit structure is

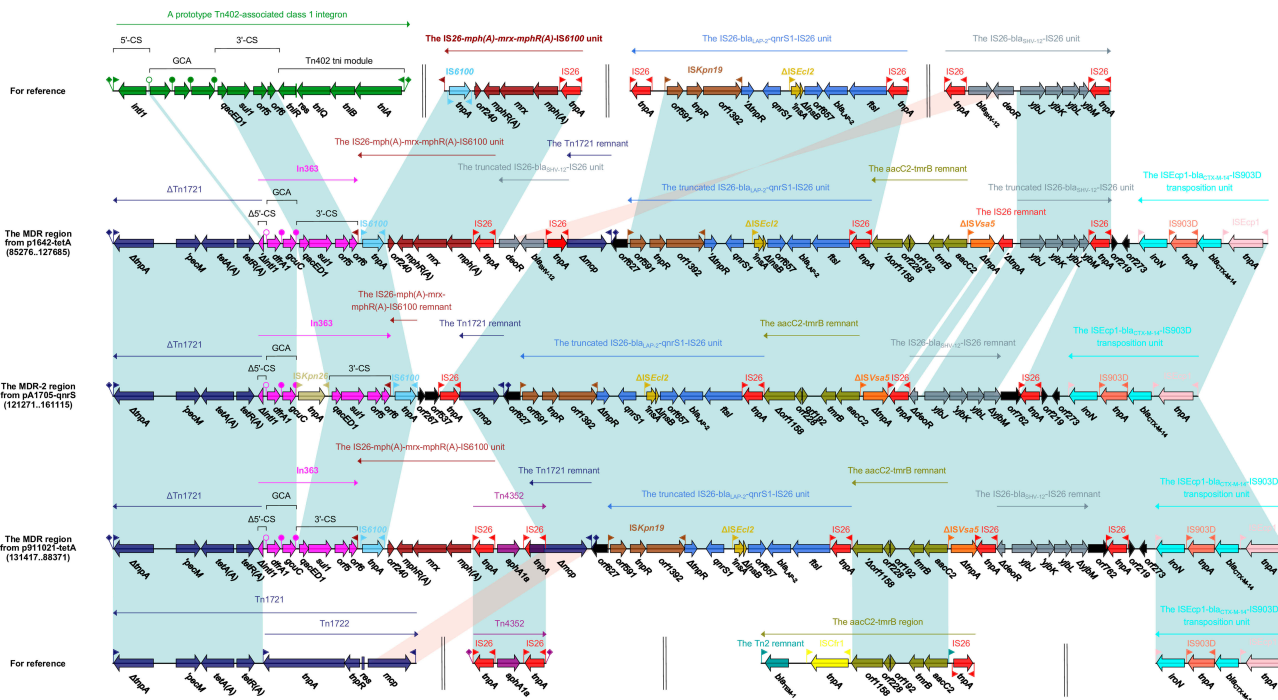


Figure 3 Accessory modules of the MDR region from p1642-*tetA* and p911021-*tetA*, and the MDR-2 region from pA1705-*qnrS*.

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%.

IS26, *ftsI*, *bla_{LAP-2}*, *orf657*, ΔISEcl2, *qnrS1*, *AtnpR*, 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 pE11573 in *Enterobacter cloacae* E11573.⁵² The structure of the *aacC2-tmrB* unit is IS26, Tn2 residual, *aacC2*, *tmrB*, *orf192*, *orf228*, *orf1158*, *ISCfr1*, 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.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 (*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 be 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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