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Genomic diversification of IncR plasmids from China

Ying Jing[a,b,1], Xiaoyuan Jiang[c,1], Zhe Yin[c], Lingfei Hu[c], Ying Zhang[c], Wenhui Yang[c], Huiying Yang[c], Bo Gao[c], Yuee Zhao[c], Dongsheng Zhou[c], Chengbin Wang[a,b,*], Yanping Luo[a,*]

[a] Medical Laboratory Center, General Hospital of People's Liberation Army, Beijing 100085, China
[b] School of Laboratory Medicine and Life Science, Wenzhou Medical University, Wenzhou 325035, China
[c] State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China

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ABSTRACT

Objectives: The aim of this study was to perform a detailed genomic characterisation of IncR plasmids from China.
Methods: Three IncR plasmids (p13190-tetA, p02085-tetA and p30860-tetA) from clinical isolates of*Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter cloacae*, respectively, were fully sequenced using high-throughput genome sequencing and were compared with five previously sequenced IncR plasmids (pHN84KPC, pSH-01, pK245, pKPC_P16 and pKPC-LK30) from China.
Results: The eight IncR plasmids from China possessed conserved IncR backbones composed of *repB*, *parAB*, *umuCD*, *retA* and *resD*. Resistance accessory modules integrated into the IncR backbones included multidrug resistance (MDR) regions in p30860-tetA, p02085-tetA, p13190-tetA and pK245, *bla*$_{KPC-2}$ regions in pHN84KPC, pKPC-LK30 and pKPC_P16, and the ΔTn*1721*–*sil* region in pSH-01. These resistance accessory modules were inserted at a site between *retA* and *vagD*, resulting in loss of the backbone genes *vagCD* in some of the plasmids. The resistance accessory modules differed dramatically from one another and carried distinct profiles of resistance markers. In particular, all of p13190-tetA, p02085-tetA, p30860-tetA, pHN84KPC, pSH-01 and pK245 carried tetracycline resistance *tet* gene modules, and the carbapenemase gene *bla*$_{KPC-2}$ was identified in pHN84KPC, pKPC-LK30 and pKPC_P16. In addition, one or more regions responsible for plasmid replication and/or maintenance were found in some of the resistance accessory modules, facilitating stable replication of corresponding IncR plasmids at steady-state copy numbers.
Conclusions: This detailed comparative genomics analysis of IncR plasmids from China provides a deeper insight into the diversification and evolution of IncR plasmids.

1. Introduction

The IncR plasmid replicon was originally discovered in a multidrug-resistant *Klebsiella pneumoniae* plasmid pK245 in 2006 [1] and often coexists with other replicons such as IncC, IncN, IncHI and IncFII in plasmid backbone regions, constituting multireplicon plasmids [1,2]. IncR plasmids are herein designated as those containing the sole IncR replicon and the partitioning genes *parAB* in their backbone regions. The IncR backbone is commonly composed of *repB* (replication initiation) and its iterons (RepB-binding sites), *parAB* (partition), *umuCD* (SOS mutagenesis), *retA* (group IIB intron-encoding reverse transcriptase), *vagCD* (toxin–antitoxin system) and *resD* (multimer resolvase). IncR plasmids lack conjugal transfer genes and thus are not self-transmissible [3]. The relatively small IncR backbones are able to integrate and accumulate various accessory modules, making IncR plasmids generally range in size from 40–160 kb [4].

This study presents complete nucleotide sequences of three IncR plasmids (p13190-tetA, p30860-tetA and p02085-tetA) from clinical *K. pneumoniae*, *Enterobacter cloacae* and *Citrobacter freundii* isolates, respectively. This genomic comparison of the above three

* Corresponding authors.
E-mail addresses: 527173138@qq.com (Y. Jing), 476115657@qq.com (X. Jiang), jerry9yin@163.com (Z. Yin), huling03@163.com (L. Hu), cherryzju@aliyun.com (Y. Zhang), fionyoung@163.com (W. Yang), 30235922@qq.com (H. Yang), gb5029@hotmail.com (B. Gao), zhaoyuee163@163.com (Y. Zhao), dongshengzhou1977@gmail.com (D. Zhou), wangcb301@126.com (C. Wang), ypluo301@yeah.net (Y. Luo).
[1] These two authors contributed equally to this work.

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plasmids together with all five previously sequenced IncR plasmids [pHN84KPC (accession no. KY296104; IncR reference), pSH-01 (accession no. KY486279), pK245 (accession no. DQ449578) [1], pKPC_P16 (accession no. KY689238) [5] and pKPC-LK30 (accession no. KC405622) [6] from China provides a deeper insight into the diversification and evolution of IncR plasmids.

2. Materials and methods

2.1. Bacterial strains

Klebsiella pneumoniae 13190 was isolated in 2013 from a sputum specimen of a 72-year-old male with hepatitis and pulmonary infection in a teaching hospital in Hangzhou City, China. *Enterobacter cloacae* 30860 was recovered in 2013 from a blood specimen of a 52-year-old male with septic shock and bloodstream infection in a teaching hospital in Guangzhou City, China. *Citrobacter freundii* 02085 was isolated in 2015 from a bile specimen of a 55-year-old male with biliary tract infection in a public hospital in Beijing City, China [7]. All of the above infections were hospital-acquired.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using a VITEK®2 system with an AST GN09 card (bioMérieux, Marcy-l'Étoile, France) and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8].

2.3. Electroporation experiments

To prepare competent cells for electroporation, 200 mL of *Escherichia coli* EC600 in Super Optimal Broth (SOB) (40 g/L soy peptone, 10 g/L yeast extract, 0.6 g/L NaCl, 0.2 g/L KCl, 1.2 g/L $MgSO_4$ and 2 g/L $MgCl_2$) was cultured overnight to an optical density at 600 nm (OD_{600}) of 0.4–0.6 in the shaking incubator. The culture medium was washed three times with electroporation buffer (0.5 M mannitol and 10% glycerol) and was concentrated to a final volume of 2 mL. Then, 1 μg of DNA was mixed with 100 μL of competent cells for electroporation at 25 μF, 200 Ω and 2.5 kV. The resulting cells were suspended in 500 μL of SOB and an appropriate aliquot was spotted onto SOB agar plates containing 10 μg/mL tetracycline for selection of *tetA*-carrying electroporants.

2.4. Genomic sequencing and sequence assembly

Genomic DNA (gDNA) was isolated from each of strains 13190, 30860, and 02085 using a QIAGEN Blood & Cell Culture DNA Maxi Kit (Qiagen, Hilden, Germany). For strain 13190, gDNA was sheared to 15 kb (range 10–20 kb) by sonication using a Covaris E220 instrument (Covaris Inc., Woburn, MA, USA). Genome sequencing was performed with a sheared DNA library on a PacBio RSII sequencer (Pacific Biosciences, Menlo Park, CA, USA) as well as with a paired-end library for Illumina using a NEBNext® Ultra™ II DNA Library Prep Kit (Illumina Inc., San Diego, CA, USA) with an average insert size of 400 bp (range 150–600 kb) on a HiSeq sequencer (Illumina Inc.). The average sequence read length and average depth of sequencing coverage of the PacBio RSII sequencer were 9.12 kb and 110.63 kb, and those of the HiSeq sequencer were 155.63 bp and 171.78bp, respectively. Paired-end short Illumina reads were used to correct the long PacBio reads using proovread [9], and the corrected PacBio reads were then assembled de novo using SMARTdenovo (available from https://github.com/ruanjue/smartdenovo).

For strains 30860 and 02085, gDNA was sequenced from mate-pair libraries using a Nextera Mate Pair Sample Prep Kit (Illumina Inc.) with an average insert size of 5 kb (range 2–10 kb) on a MiSeq sequencer (Illumina Inc.), and DNA contigs were assembled based on their contig coverages using Newbler 2.6 [10]. Quality control, removing adapters and low-quality reads, was performed using Trimmomatic 0.36 [11]. The filtered clean reads were then assembled using Newbler 2.6 [10], followed by extraction of the consensus sequence with CLC Genomics Workbench 3.0 (QIAGEN Bioinformatics, Aarhus, Denmark). GapFiller v.1.11 [12] was used for gap closure. The sequence average read length was 154.55 bp and 155.29 bp and the average depth of sequencing coverage was 367.12 and 758.31 for strains 30860 and 02085, respectively.

2.5. Sequence annotation and comparison

Open-reading frames (ORFs) and pseudogenes were predicted using RAST 2.0 [13] combined with BLASTP/BLASTN [14] searches against the UniProtKB/Swiss-Prot database [15] and the RefSeq database [16]. Annotation of antimicrobial resistance genes, mobile genetic elements and other features was carried out using online databases including the Comprehensive Antibiotic Resistance Database (CARD) [17]. ResFinder [18], ISfinder [19], INTEGRALL [20] and the Tn number registry [21]. Multiple and pairwise sequence comparisons were performed using MUSCLE 3.8.31 [22] and BLASTN, respectively. Gene organisation diagrams were drawn in Inkscape 0.48.1 (https://inkscape.org/en/).

2.6. Nucleotide sequence accession numbers

The sequences of p13190-tetA, p02085-tetA and p30860-tetA were submitted to GenBank under accession nos. MG764549, MH477637 and MG764554, respectively.

3. Results

3.1. Major modular differences among IncR plasmids

Genome sequencing showed that p13190-tetA, p02085-tetA and p30860-tetA, respectively, had circularly closed DNA sequences of 77 583, 68 649 and 87 917 bp in length with average G + C contents of 53.86%, 52.88% and 51.49%, and contained 159, 135 and 151 predicted ORFs in total (Table 1; Supplementary Fig. S1).

A total of 109 fully sequenced IncR plasmids (Supplementary Table S1), including the 3 abovementioned plasmids, were collected from GenBank (last accessed 14 March 2019). The IncR core backbone regions of these 109 plasmids were 7.9–12.6 kb in length and harboured 8–15 ORFs. The 109 plasmids had *repB* genes with >95% nucleotide identity to pHN84KPC and displayed >96% nucleotide identity to pHN84KPC across >63% of their backbone sequences. These plasmids were disseminated among Enterobacteriaceae species mainly in the USA, Europe and China. All of these plasmids shared the backbone genes *repB*, *parAB* and *resD*, which encode essential functions of plasmid replication and maintenance.

Further comparative genomics analysis was applied to all eight IncR plasmids from China, which included p13190-tetA, p02085-tetA and p30860-tetA sequenced in this study as well as five previously sequenced plasmids (pHN84KPC, pSH-01, pK245, pKPC-LK30 and pKPC_P16). Each plasmid was composed of the backbone regions and one or two accessory modules that were defined as acquired DNA regions associated with or bordered by mobile genetic elements (Fig. 1; Table 2). Although pK245 was the first fully sequenced IncR plasmid, pHN84KPC was more appropriate as the reference because it had the most complete IncR backbone regions. These eight plasmids shared common IncR backbone genes *repB*, *parAB*, *umuCD*, *retA* and *resD*. At least three major modular differences were identified across their backbones

Table 1
Major features of the eight IncR plasmids analysed.

Category	Plasmid[a]							
	pHN84KPC	pSH-01	p13190-tetA	p02085-tetA	pK245	p30860-tetA	pKPC_P16	pKPC-LK30
Accession no.	KY296104	KY486279	MG764549	MH477637	DQ449578	MG764554	KY689238	KC405622
Host bacterium	*Enterobacter cloacae*	*Salmonella* sp.	*Klebsiella pneumoniae*	*Citrobacter freundii*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*K. pneumoniae*
Total length (bp)	39 367	43 986	77 583	68 649	99 909	87 917	86 958	87 766
Total no. of ORFs	76	70	159	135	199	151	164	165
Length of backbone (bp)	11 986	10 531	10 668	10 680	10 668	10 553	54 384	54 713
Mean G+C content (%)	55.65	51.77	53.86	52.88	51.79	51.49	55.97	55.97
Accessory modules	bla_{KPC-2} region[b]	ΔTn*1721–sil* region[b]	MDR region[b]	MDR region[b]	MDR region[b]	MDR region[b]	bla_{KPC-2} region[b] and IS*26*	bla_{KPC-2} region[b] and ΔIS*26*

ORF, open-reading frame; MDR, multidrug resistance.
[a] p13190-tetA, p02085-tetA and p30860-tetA were fully sequenced in this study, whereas pHN84KPC, pSH-01, pK245, pKPC_P16 and pKPC-LK30 were derived from GenBank. A genomic comparison of all eight plasmids is interpreted in the main text.
[b] Containing resistance gene.

(Fig. 1): (i) variations in copy number (14, 12, 14, 9, 9, 12, 17 and 17, respectively) of 36-bp tandem repeat within iterons; (ii) absence of *vagCD* in pSH-01, p13190-tetA, p02085-tetA, pK245 and p30860-tetA; and (iii) integration of partial $IncFII_K$ and $IncFII_Y$ backbone regions into pKPC_P16 and pKPC-LK30.

Each of the following plasmids had one resistance accessory module: a multidrug resistance (MDR) region in p13190-tetA, p02085-tetA, pK245 and p30860-tetA; a bla_{KPC-2} region in pHN84KPC, pKPC_P16 and pKPC-LK30; and a ΔTn*1721–sil* region in pSH-01; besides, pKPC_P16 and pKPC-LK30 contained individual insertion sequence (IS) elements IS*26* and ΔIS*26*, respectively (Table 1).

3.2. The multidrug resistance (MDR) regions

The MDR regions (Fig. 2) of p30860-tetA, p02085-tetA, p13190-tetA and pK245 harboured different profiles of antimicrobial resistance genes and mobile genetic elements. The MDR region of p30860-tetA carried three resistance elements: (i) Tn*6309* that was an IS*26* composite transposon carrying *tetA*(C)–*tetR*(C) [23]; (ii) In*127* carrying a single gene cassette *aadA2*; and (iii) ΔTn*1696* that was a *mer*-carrying remnant of Tn*1696* [24]. In addition, this MDR region carried several type IV secretion system genes [25] and two metabolism-related gene loci, namely a *cps* region involved in capsular polysaccharides biosynthesis [26] as found in the chromosome of *C. freundii* CFNIH9 (GenBank accession no. CP026238) [27] and a *cyn*-related region (encoding cyanase and auxiliary proteins) with insertion of IS*5D* and a Tn*3*-family transposon remnant [28].

The MDR regions of p02085-tetA and p13190-tetA displayed similar modular structures (73% BLAST coverage and maximum 99% nucleotide identity) and shared two resistance elements: the IS*26*–*tetR*(D)–*tetA*(D)–IS*26* unit [29], and In*1021* that was a complex class 1 integron carrying variable region 1 (VR1) (a gene cassette *aacA4cr–arr3–dfrA27–aadA16*) and VR2 (harbouring *sdr* and *qnrB6*) [30]. In addition, these two MDR regions shared a 1.4-kb IncFI-family replicon and a 1.3-kb IncN1 plasmid maintenance region (*stbAB*). Truncated versions of the IS*26*–bla_{SHV-2}–IS*26* unit, the *aacC2–tmrB* region and Tn*6029*, which carried bla_{SHV-2}, *aacC2*, *tmrB* and bla_{TEM-1}, and *strB* and *sul2*, respectively, were found in the MDR region of p13190-tetA rather than p02085-tetA. p02085-tetA carried an intact Tn*6322*, a *mer3*-carrying Tn*3*-family transposon

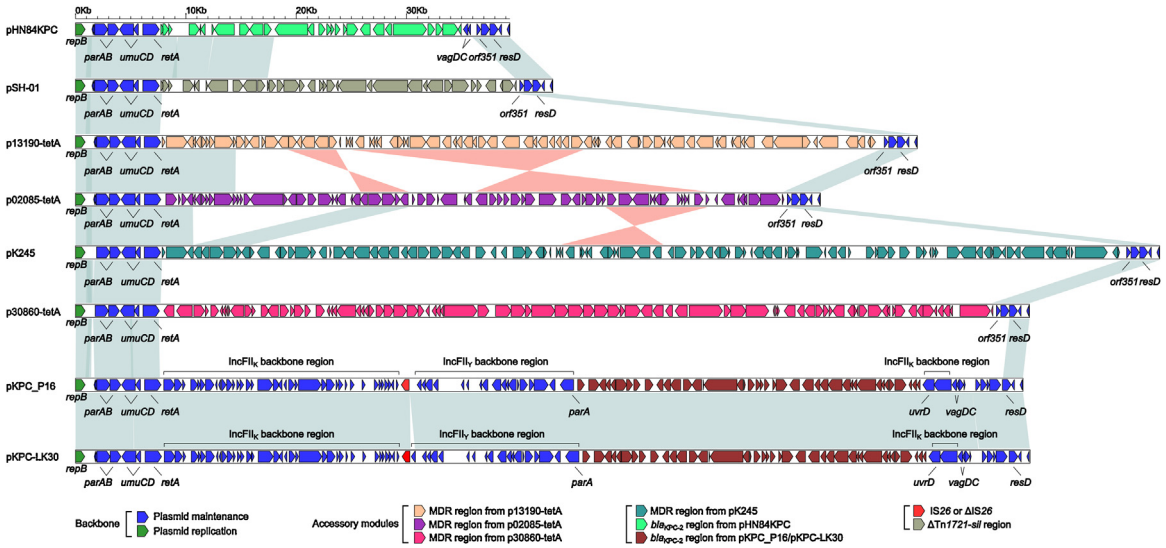

Fig. 1. Linear comparison of plasmid genome sequences. A linear comparison was carried out for the complete DNA sequences of pHN84KPC (accession no. KY296104), pSH-01 (accession no. KY486279), p13190-tetA (this study), p02085-tetA (this study), pK245 (accession no. DQ449578), p30860-tetA (this study), pKPC_P16 (accession no. KY689238) and pKPC-LK30 (accession no. KC405622). Genes are denoted by arrows. Genes, mobile genetic elements and other features are coloured based on function classification. Shading denotes regions of homology (>95% nucleotide identity).

Table 2
Drug resistance genes in sequenced IncR plasmids.

Plasmid	Resistance marker	Resistance phenotype	Nucleotide position	Region located
pHN84KPC	*tetA*(A)	Tetracycline resistance	15788 . . . 16987	bla_{KPC-2} region
	bla_{KPC-2}	Carbapenem resistance	25820 . . . 26701	
pSH-01	*tetA*(A)	Tetracycline resistance	15199 . . . 16398	Δ*Tn1721–sil* region
	qnrS1	Quinolone resistance	21053 . . . 21709	
	silE	Silver resistance	35863 . . . 36294	
p13190-tetA	*mer* locus	Mercuric resistance	10930 . . . 14539	MDR region
	bla_{SHV-2}	β-Lactam resistance	15761 . . . 16625	
	tetA(D)	Tetracycline resistance	21719 . . . 22903	
	sul1	Sulfonamide resistance	35159 . . . 35998	
	qacED1	QAC resistance	35992 . . . 36339	
	qnrB6	Quinolone resistance	36534 . . . 37214	
	sul1	Sulfonamide resistance	40625 . . . 41464	
	qacED1	QAC resistance	41458 . . . 41805	
	aadA16	Aminoglycoside resistance	41922 . . . 42767	
	dfrA27	Trimethoprim resistance	42948 . . . 43421	
	arr3	Rifampicin resistance	43554 . . . 44006	
	aacA4cr	Fluoroquinolone and aminoglycoside resistance	44103 . . . 44702	
	aacC2	Fluoroquinolone and aminoglycoside resistance	48615 . . . 49475	
	tmrB	Tunicamycin resistance	48060 . . . 48602	
	bla_{TEM-1}	β-Lactam resistance	51436 . . . 52296	
	Δ*tetA*(A)	Tetracycline resistance	52458 . . . 53345	
	floR	Bicyclomycin resistance	53945 . . . 55159	
	Δ*strA*	Aminoglycoside resistance	60353 . . . 60638	
	sul2	Sulfonamide resistance	60699 . . . 61514	
p02085-tetA	*mer* locus	Mercuric resistance	10894 . . . 14870	MDR region
	floR	Bicyclomycin resistance	22013 . . . 23227	
	tetA(D)	Tetracycline resistance	26559 . . . 27743	
	aacA4cr	Fluoroquinolone and aminoglycoside resistance	37541 . . . 38140	
	arr3	Rifampicin resistance	38237 . . . 38689	
	dfrA27	Trimethoprim resistance	38822 . . . 39295	
	aadA16	Aminoglycoside resistance	39476 . . . 40321	
	qacED1	QAC resistance	40438 . . . 40785	
	sul1	Sulfonamide resistance	40779 . . . 41618	
	qnrB6	Quinolone resistance	45035 . . . 45715	
	qacED1	QAC resistance	45910 . . . 46257	
	sul1	Sulfonamide resistance	46251 . . . 47090	
pK245	*tetA*(D)	Tetracycline resistance	12188 . . . 13372	MDR region
	qnrS1	Quinolone resistance	22081 . . . 22737	
	bla_{LAP-2}	β-Lactam resistance	24334 . . . 25191	
	tmrB	Tunicamycin resistance	29523 . . . 30065	
	aacC2	Fluoroquinolone and aminoglycoside resistance	30078 . . . 30938	
	bla_{SHV-2}	β-Lactam resistance	36291 . . . 37151	
	dfrA14	Trimethoprim resistance	53708 . . . 54190	
	catA2	Phenicol resistance	57095 . . . 57736	
	strB	Aminoglycoside resistance	61482 . . . 62318	
	strA	Aminoglycoside resistance	62318 . . . 63121	
	sul2	Sulfonamide resistance	63182 . . . 63997	
p30860-tetA	*tetA*(C)	Tetracycline resistance	14054 . . . 15244	MDR region
	aadA2	Aminoglycoside resistance	18541 . . . 19320	
	qacED1	QAC resistance	19484 . . . 19831	
	sul1	Sulfonamide resistance	19825 . . . 20664	
	mer locus	Mercuric resistance	23079 . . . 27055	
pKPC_P16	bla_{SHV-12}	β-Lactam resistance	55881 . . . 56741	bla_{KPC-2} region
	bla_{KPC-2}	Carbapenem resistance	64729 . . . 65610	
	mer locus	Mercuric resistance	70220 . . . 74182	
pKPC-LK30	bla_{SHV-12}	β-Lactam resistance	56419 . . . 57279	bla_{KPC-2} region
	bla_{KPC-2}	Carbapenem resistance	65267 . . . 66148	
	mer locus	Mercuric resistance	70758 . . . 74720	

QAC, quaternary ammonium compound; MDR, multidrug resistance.

[31], whilst p13190-tetA harboured a truncated Tn*6322* with a partial region of *mer3*.

Similar to p13190-tetA, the MDR region of pK245 contained the IS*26*–*tetR*(D)–*tetA*(D)–IS*26* unit, a truncated *aacC2*–*tmrB* region, a truncated IS*26*–bla_{SHV-2}–IS*26* unit, a 1.3-kb IncN1-type *stbAB* region and a 1.4-kb IncFI-family replicon; in addition, it carried Tn*6557*, a truncated IS*26*–bla_{LAP-2}–*qnrS*–IS*26* unit, ΔTn*6029* carrying *strAB*, and a 5.7-kb $repA_{IncpA1763-KPC}$-carrying backbone region [32] with insertion of ΔIS*Ec52*. Tn*6557* was a novel IS*26* composite transposon bracketed by 3-bp direct repeats (DRs) and carried In*191*, *catA2* and *ecoRIIM*–*ecoRIIR* (type II restriction–modification system).

3.3. The bla_{KPC-2} regions and the Δ*Tn*1721–sil *region*

The bla_{KPC-2} regions (Fig. 3) were found in pHN84KPC, pKPC-LK30 and pKPC_P16 and carried two Tn*6296* [33] derivatives, namely $\Delta Tn6296_{pHN84KPC}$ and $\Delta Tn6296_{pKPC-LK30/pKPC\_P16}$, serving as core genetic environments of bla_{KPC-2}. Truncations within 3′-terminal regions of Tn*6296* generated these two highly similar ΔTn*6296* elements. Each of them was connected with an additional resistance region: ΔTn*1721* carrying *tetA*(A)–*tetR*(A) [34] in pHN84KPC, and a truncated IS*26*–bla_{SHV-12}–IS*26* unit harbouring bla_{SHV-12} [35] in pKPC-LK30 and pKPC_P16. The ΔTn*1721*–*sil* region (Fig. 3) of pSH-01 harboured ΔTn*1721* (as found in pHN84KPC),

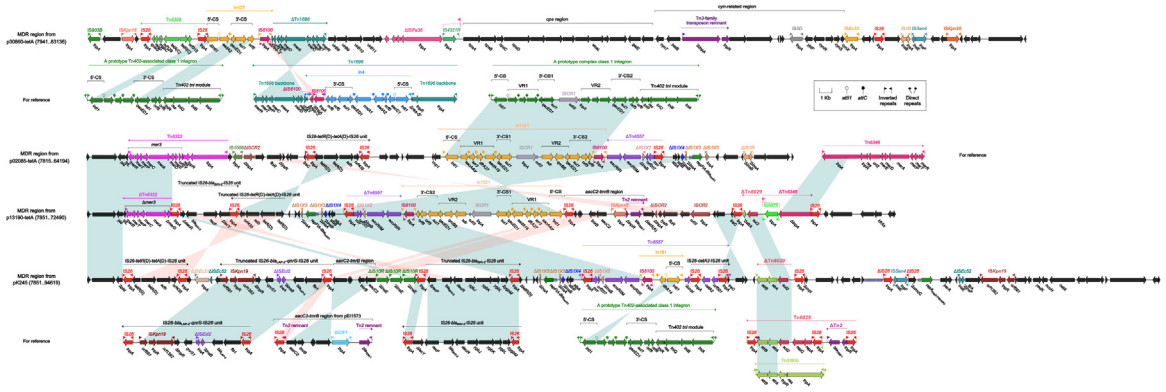

Fig. 2. The multidrug resistance (MDR) regions and comparison with related regions. Genes are denoted by arrows. Genes, mobile genetic elements and other features are coloured based on function classification. Shading denotes regions of homology (>95% nucleotide identity). Numbers in brackets indicate nucleotide positions within the corresponding plasmids. The accession numbers of Tn*1696*, IS*26*–bla_{LAP-2}–*qnrS*–IS*26* unit, *aacC2*–*tmrB* region, IS*26*–bla_{SHV-2}–IS*26* unit and Tn*6029* for reference are U12338, HF545433, AF262622, AJ245670 and HQ840942, respectively.

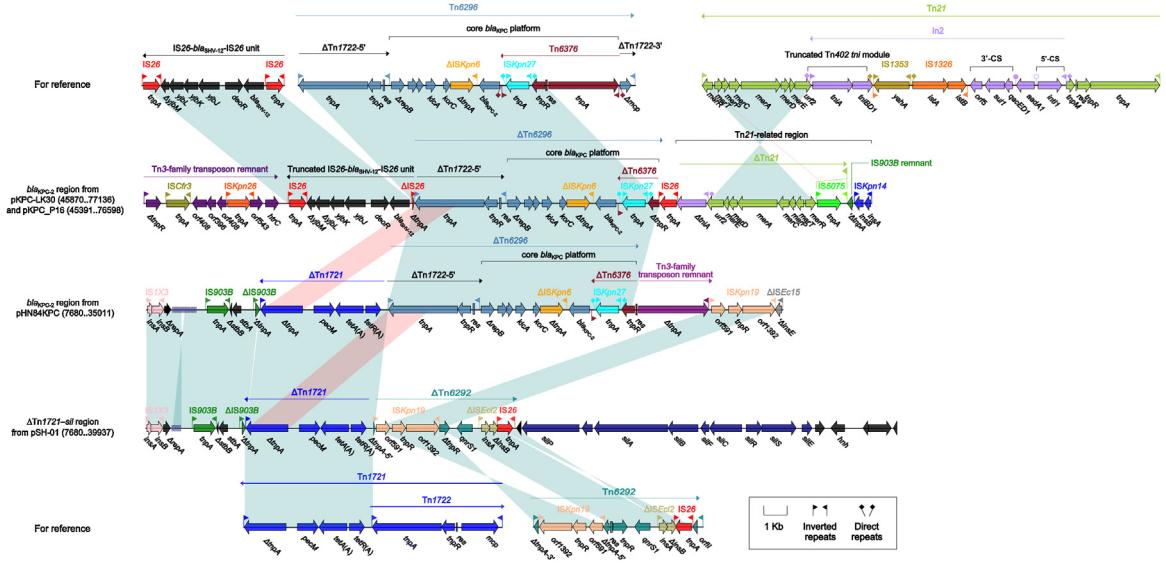

Fig. 3. The bla_{KPC-2} regions and the ΔTn*1721*–*sil* region and comparison with related regions. Genes are denoted by arrows. Genes, mobile genetic elements and other features are coloured based on function classification. Shading denotes regions of homology (>95% nucleotide identity). Numbers in brackets indicate nucleotide positions within the corresponding plasmids. The accession numbers of Tn*1721*, Tn*6292*, IS*26*–bla_{SHV-12}–IS*26* unit, Tn*6296* and Tn*21* for reference are X61367, KX711879, CP003684, FJ628167 and AF071413, respectively.

which was further connected with ΔTn*6292* containing *qnrS1* [30] and a silver resistance (*sil*) region [as observed in pAPEC-O2-R (GenBank accession no. AY214164)] [36].

3.4. Transferability and antimicrobial susceptibility

p13190-tetA, p02085-tetA and p30860-tetA could be transferred from strains 13190, 02085 and 30860 into *E. coli* EC600 through electroporation, generating the electroporants 13190-tetA-EC600, 02085-tetA-EC600 and 30860-tetA-EC600, respectively. All of the above strains carried *tet* genes (Table 2) and, as expected, were resistant to tetracycline and minocycline (Table 3).

4. Discussion

Comparison of eight sequenced IncR plasmids from China showed that the IncR backbones of these plasmids shared core genes *repB*, *parAB*, *umuCD*, *retA* and *resD*; moreover, loss of *vagCD* in some of these plasmids occurred due to integration of accessory modules. When the comparison analysis was applied to a collection of 109 sequenced IncR plasmids available from public databases, the core backbone genes included only *repB*, *parAB* and *resD*, which might represent the minimum core backbone of IncR plasmids. Notably, pKPC-LK30 and pKPC_P16 integrated three different IncFII backbone regions (Fig. 4), including a 21.7-kb $IncFII_K$ backbone region (containing *stbAB*, *ardAB*, *parB* and *psiAB*) and another 2.8-kb $IncFII_K$ backbone region (containing *urvD*) as observed in pKPHS2 (GenBank accession no. CP003224) [37], and a 15.3-kb $IncFII_Y$ backbone region (carrying *psiAB*, *ardA*, *umuCD* and *parAB*) as observed in pKOX_NDM1 (GenBank accession no. JQ314407) [38].

The accessory modules of these eight IncR plasmids contained IS elements, transposons and integrons, and among them were three types of resistance accessory modules (Fig. 4), including the MDR regions in p13190-tetA, p02085-tetA, pK245 and p30860-tetA, the bla_{KPC-2} regions in pHN84KPC, pKPC-LK30 and pKPC_P16, and the ΔTn*1721*–*sil* region in pSH-01. A 71.7-kb large chimera region (composed of the bla_{KPC-2} region, the three different IncFII backbone

Table 3
Antimicrobial susceptibility profiles.

Antimicrobial agent	MIC (mg/L)/antimicrobial susceptibility						
	13190	13190-tetA-EC600	02085	02085-tetA-EC600	30860	30860-tetA-EC600	EC600
Ceftazidime	≥64/R	2/S	≥64/R	≤1/S	≥64/R	≤1/S	4/S
Cefepime	≥64/R	≤1/S	≥64/R	≤1/S	≥64/R	≤1/S	≤1/S
Imipenem	4/R	≤1/S	≥16/R	≤1/S	≥16/R	≤1/S	≤1/S
Meropenem	≥16/R	≤0.25/S	8/R	≤0.25/S	≥16/R	≤0.25/S	≤0.25/S
Aztreonam	≥64/R	≤1/S	≥64/R	≤1/S	≥64/R	≤1/S	≤1/S
Amikacin	≤2/S	≤2/S	≤2/S	≤2/S	32/I	≤2/S	≤2/S
Tobramycin	≥16/R	8/I	8/I	4/S	≥16/R	≤1/S	≤1/S
Ciprofloxacin	≥4/R	2/I	≥4/R	1/S	≥4/R	0.5/S	≤0.25/S
Levofloxacin	≥8/R	1/S	≥8/R	1/S	≥8/R	0.5/S	0.25/S
Tetracycline	4/R	4/R	4/R	4/R	4/R	4/R	<1/S
Minocycline	4/R	4/R	4/R	4/R	4/R	4/R	<1/S
SXT	≥320/R	≥320/R	≥320/R	≥320/R	160/R	160/R	≤20/S

MIC, minimum inhibitory concentration; S, susceptible; R, resistant; I, intermediate-resistant; SXT, trimethoprim/sulfamethoxazole.

regions and an IS*26* or ΔIS*26* element) was inserted at a site between *retA* and *vagD* in pKPC-LK30 and pKPC_P16, whilst the bla_{KPC-2} region, the MDR region and the ΔTn*1721–sil* region alone was integrated at the same site in other corresponding plasmids (Fig. 4). The integration events in p13190-tetA, p02085-tetA, pK245, p30860-tetA and pSH-01 likely resulted in a 1.2-kb deletion containing *vagCD* (Fig. 4). These resistance accessory modules, displaying separate evolutionary histories, differed dramatically from one another and carried distinct profiles of resistance markers (Table 2). In particular, all of p13190-tetA, p02085-tetA and p30860-tetA (sequenced in this study) as well as pHN84KPC, pSH-01 and pK245 previously sequenced carried tetracycline resistance *tet* gene modules, which could be further divided into classes A, C and D. The carbapenemase gene bla_{KPC-2} was identified in pHN84KPC, pKPC-LK30 and pKPC_P16. Diversified resistance accessory modules carried by different IncR plasmids promoted accumulation and spread of antimicrobial resistance genes among bacterial species.

At least three copies of IS*26* plus IS*6100* were found in each of the MDR regions of p30860-tetA, p02085-tetA, p13190-tetA and pK245. The two IS*6*-family elements IS*26* and IS*6100* possessed almost identical 14-bp inverted repeat (IR) sequences and thereby they would be together involved in complex homologous recombination events [39], promoting assembly of the MDR regions in p30860-tetA, p02085-tetA, p13190-tetA and pK245. Notably, each of these three MDR regions acquired one or more regions responsible for plasmid replication and/or maintenance, which would facilitate stable replication of these IncR plasmids at steady-state copy numbers.

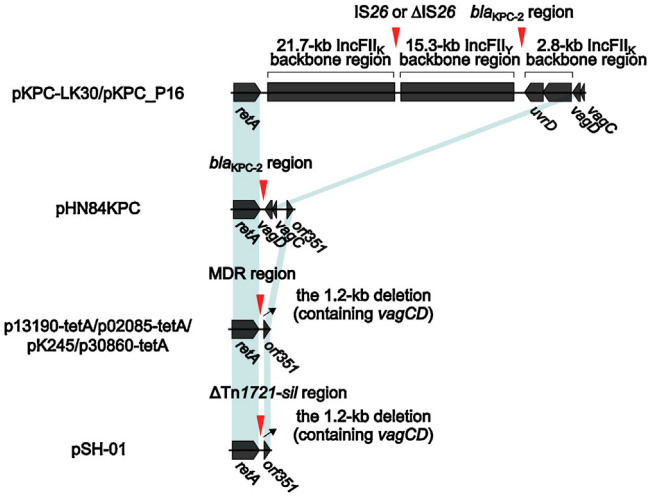

Fig. 4. Integration of foreign regions into IncR backbones. Genes are denoted by arrows. Genes, mobile genetic elements and other features are coloured based on function classification. Shading denotes regions of homology (>95% nucleotide identity).

5. Conclusion

In this study, three plasmids (p13190-tetA, p02085-tetA and p30860-tetA) were fully sequenced and were compared with five available sequenced IncR plasmids (pHN84KPC, pSH-01, pK245, pKPC_P16 and pKPC-LK30) from China. These plasmids shared relatively small and conserved IncR backbones that were able to integrate various resistance accessory modules carrying different profiles of antimicrobial resistance markers, thereby promoting accumulation and spread of antimicrobial resistance among bacterial species. The data presented here encourage us to accurately dissect backbone and accessory modules of IncR plasmids and to gain a deeper understanding of the evolution and diversification of IncR plasmids.

Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.06.007.

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