



Chromosomal Integration of Huge and Complex *bla*_{NDM}-Carrying Genetic Elements in Enterobacteriaceae

Xinhua Luo^{1†}, Zhe Yin^{1†}, Lijun Zeng^{1,2}, Lingfei Hu¹, Xiaoyuan Jiang¹, Ying Jing¹, Fangzhou Chen¹, Dongguo Wang³, Yajun Song¹, Huiying Yang^{1*} and Dongsheng Zhou^{1*}

OPEN ACCESS

Edited by:

Zhang Wan Jiang,
Chinese Academy of Agricultural
Sciences (CAAS), China

Reviewed by:

Ruichao Li,
Yangzhou University, China
Zhihai Liu,
Qingdao Agricultural University, China

*Correspondence:

Dongsheng Zhou
dongshengzhou1977@gmail.com
Huiying Yang
yhy324@aliyun.com

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Clinical Microbiology,
a section of the journal
Frontiers in Cellular and
Infection Microbiology

Received: 04 April 2021

Accepted: 17 May 2021

Published: 15 June 2021

Citation:

Luo X, Yin Z, Zeng L, Hu L, Jiang X,
Jing Y, Chen F, Wang D, Song Y,
Yang H and Zhou D (2021)
Chromosomal Integration of Huge and
Complex *bla*_{NDM}-Carrying Genetic
Elements in Enterobacteriaceae.
Front. Cell. Infect. Microbiol. 11:690799.
doi: 10.3389/fcimb.2021.690799

¹ State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China, ² The Fifth Medical Center, Chinese Peoples Liberation Army General Hospital, Beijing, China, ³ Department of Clinical Laboratory Medicine, Taizhou Municipal Hospital Affiliated With Taizhou University, Taizhou, China

In this study, a detailed genetic dissection of the huge and complex *bla*_{NDM}-carrying genetic elements and their related mobile genetic elements was performed in Enterobacteriaceae. An extensive comparison was applied to 12 chromosomal genetic elements, including six sequenced in this study and the other six from GenBank. These 12 genetic elements were divided into five groups: a novel IME Tn6588; two related IMEs Tn6523 (SGI1) and Tn6589; four related ICEs Tn6512 (R391), Tn6575 (ICEPvuChnBC22), Tn6576, and Tn6577; Tn7 and its derivatives Tn6726 and 40.7-kb Tn7-related element; and two related IMEs Tn6591 (GlsuI2) and Tn6590. At least 51 resistance genes, involved in resistance to 18 different categories of antibiotics and heavy metals, were found in these 12 genetic elements. Notably, Tn6576 carried another ICE Tn6582. In particular, the six *bla*_{NDM}-carrying genetic elements Tn6588, Tn6589, Tn6575, Tn6576, Tn6726, and 40.7-kb Tn7-related element contained large accessory multidrug resistance (MDR) regions, each of which had a very complex mosaic structure that comprised intact or residual mobile genetic elements including insertion sequences, unit or composite transposons, integrons, and putative resistance units. Core *bla*_{NDM} genetic environments manifested as four different Tn125 derivatives and, notably, two or more copies of relevant Tn125 derivatives were found in each of Tn6576, Tn6588, Tn6589, and 40.7-kb Tn7-related element. The huge and complex *bla*_{NDM}-carrying genetic elements were assembled from complex transposition and homolog recombination. Firstly identified were eight novel mobile elements, including three ICEs Tn6576, Tn6577, and Tn6582, two IMEs, Tn6588 and Tn6589, two composite transposons Tn6580a and Tn6580b, and one integron In1718.

Keywords: Enterobacteriaceae, chromosomal integration, *bla*_{NDM}, multidrug resistance, mobile genetic elements

INTRODUCTION

New Delhi metallo- β -lactamase (NDM) is able to hydrolyze nearly all β -lactams except aztreonam and thus mediates resistance to penicillins, cephalosporins, and carbapenems (Yong et al., 2009). It is hypothesized that the bla_{NDM} gene is originally integrated into the *Acinetobacter* chromosome from an unknown environmental species and then captured by two copies of ISAba125, giving rise to ISAba125-composite transposon Tn125 (Poirel et al., 2012). With the transposition of Tn125, bla_{NDM} is disseminated among *Acinetobacter*, Enterobacteriaceae and *Pseudomonas* species; Tn125 and its bla_{NDM}-carrying derivatives, with various truncations and deletions, can be found in the accessory resistance regions of bacterial plasmids or chromosomes (Wu et al., 2019). There are reports of chromosomal location of bla_{NDM} in Enterobacteriaceae species including *Escherichia coli* (Pfeifer et al., 2011; Poirel et al., 2011; Shen et al., 2017; Reynolds et al., 2019), *Providencia stuartii* (Poirel et al., 2011), *Proteus mirabilis* (Girlich et al., 2015), *Klebsiella pneumoniae* (Sakamoto et al., 2018), and *Proteus vulgaris* (Kong et al., 2020), but few of them are subjective to detailed genetic dissection of bla_{NDM}-carrying accessory resistance regions (Girlich et al., 2015; Sakamoto et al., 2018; Reynolds et al., 2019; Kong et al., 2020).

Integrative and conjugative elements (ICEs) and integrative and mobilizable elements (IMEs) (Bellanger et al., 2014; Delavat et al., 2017; Botelho and Schulenburg, 2021) are two different types of mobile genetic elements which are frequently integrated into bacterial chromosome, contributing to dissemination of resistance genes. ICEs have the ability to transfer between cells because of their self-encoded conjugation function. It is typically composed of *attL* (attachment site at the left end), *int* (integrase), *xis* (excisionase), *rlx* (relaxase), *oriT* (origin of conjugative replication), *cpl* (coupling protein), a P (TivB)- or F (TivF)-type T4SS machinery (mating pair formation), and *attR* (attachment site at the right end). IMEs are not self-transmissible, and they achieve the intercellular mobility with the help of other conjugative elements that encode proteins involved in complete conjugation function. IMEs typically have *attL*, *int*, *rlx*, *oriT*, and *attR*, but contained no conjugal transfer genes. Tn7 is a unit transposon with the ability to integrate into bacterial chromosomes and plasmids, and it encodes five core transposition determinants TnsA and TnsB (transposases), TnsC (regulator), and TnsD and TnsE (DNA-binding proteins), as well as three TnsB-binding sites and four TnsB-binding sites at its left and right ends, respectively (Peters, 2014).

In this work, whole-genome sequencing identified four bla_{NDM-1-3}-carrying genetic elements plus two additional genetic elements harboring other resistance genes in the chromosomes of four isolates of *Providencia rettgeri*, *Proteus mirabilis*, and *K. pneumoniae*. An extension sequence comparison was then applied to a collection of 12 chromosomal genetic elements (including the above six ones sequenced in this work) that could be grouped into ICEs, IMEs, and Tn7 unit transposon and its derivatives. Data presented here gave a detailed genetic dissection of the huge and complex

bla_{NDM}-carrying genetic elements and their related mobile genetic elements in multiple Enterobacteriaceae species.

MATERIALS AND METHODS

Bacterial Strains

The four chromosomal bla_{NDM}-carrying isolates (Table S1) were screened from more than two hundred bla_{NDM}-carrying Enterobacteriaceae isolates routinely collected from China hospitals and livestock farms. *Providencia rettgeri* 1701091 and *Proteus mirabilis* 1701092 (Table S1) were recovered in 2017 from the chicken intestinal contents in two different China livestock farms. *K. pneumoniae* QD23 and *Providencia rettgeri* 51003 were recovered from the urine specimens of two different patients with nosocomial infections in two Chinese public hospitals in 2015 and 2017, respectively. Bacterial species identification was performed using genome sequence-based average nucleotide identity analysis (<http://www.ezbiocloud.net/tools/ani>) (Richter and Rosselló-Móra, 2009).

Sequencing and Sequence Assembly

Bacterial genomic DNA was isolated using the UltraClean Microbial Kit (Qiagen, NW, Germany) and sequenced from a sheared DNA library with average size of 15 kb (ranged from 10 to 20 kb) on a PacBio RSII sequencer (Pacific Biosciences, CA, USA), as well as a paired-end library with an average insert size of 350 bp (ranged from 150 to 600 kb) on a HiSeq sequencer (Illumina, CA, USA). The paired-end short Illumina reads were used to correct the long PacBio reads utilizing *proovread* (Hackl et al., 2014), and then the corrected PacBio reads were assembled *de novo* utilizing *SMARTdenovo* (<https://github.com/ruanjue/smartdenovo>).

Sequence Annotation and Comparison

Open reading frames (ORFs) and pseudogenes were predicted using RAST 2.0 (<https://rast.nmpdr.org/>) (Brettin et al., 2015) combined with BLASTP/BLASTN searches (Boratyn et al., 2013) against the UniProtKB/Swiss-Prot database (https://web.expasy.org/docs/swiss-prot_guideline.html) (Boutet et al., 2016) and the RefSeq database (<https://www.ncbi.nlm.nih.gov/refseq/>) (O'Leary et al., 2016). Annotation of resistance genes, mobile elements, and other features were carried out using the online databases including CARD (<https://card.mcmaster.ca/browse>) (Jia et al., 2017), ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>) (Zankari et al., 2012), ISfinder (<https://www-is.biotoul.fr/>) (Sigquier et al., 2006), INTEGRALL (<http://integrall.bio.ua.pt/>) (Moura et al., 2009) and Tn Number Registry (<https://www.ucl.ac.uk/eastman/tn-number-registry>) (Roberts et al., 2008). Multiple and pairwise sequence comparisons were performed using MUSCLE 3.8.31 (Edgar, 2004) and BLASTN, respectively. Gene organization diagrams were drawn in Inkscape 1.0 (<https://inkscape.org/en/>). Heatmaps were plotted with MeV 4.9.0 (Saeed et al., 2003).

Conjugal Transfer

Conjugal transfer experiments were carried out with rifampin-resistant *Escherichia coli* EC600 or sodium azide-resistant *E. coli* J53 being used as a recipient, and the 1701092 or QD23 isolate as a donor. Three milliliters of overnight cultures of each of donor and recipient bacteria were mixed together, harvested and resuspended in 80 µl of Brain Heart Infusion (BHI) broth (BD Biosciences). The mixture was spotted on a 1 cm² hydrophilic nylon membrane filter with a 0.45 µm pore size (Millipore) that was placed on BHI agar (BD Biosciences) plate and then incubated for mating at 37°C for 12 to 18 h. Bacteria were washed from filter membrane and spotted on Muller–Hinton (MH) agar (BD Biosciences) plates, for selecting an *E. coli* transconjugant carrying bla_{NDM} or carrying tetA(C). Then 200 mg/L sodium azide (for J53) or 1,000 mg/L rifampin (for EC600), together with 4 mg/L imipenem (for bla_{NDM}) or 8 mg/L tetracycline [for tetA(C)] was used for transconjugant selection.

PCR Identification

All the wild-type and transconjugant strains was subjected to PCR amplification followed by amplicon sequencing, for determining the sequences of bacterial 16S rRNA genes (Frank et al., 2008), the presence of key markers such as bla_{NDM}, tetA (C), int, and parM, and also the location/boundary of mobile genetic elements such as Tn6588, Tn6589, Tn6576, Tn6577, and Tn6590 (data not shown).

Phenotypic Assays

Activity of Ambler class A/B/D carbapenemases in bacterial cell extracts was determined by a modified CarbaNP test (Feng et al., 2016). Bacterial antimicrobial susceptibility was tested by BioMérieux VITEK 2 and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

Nucleotide Sequence Accession Numbers

The complete chromosome sequences of the 1701091, 1701092, QD23, and 51003 isolates were submitted to GenBank under accession numbers CP042860, CP042857, CP042858, and CP042861 respectively.

RESULTS

Genome Sequencing for Dissection of Chromosomal bla_{NDM}-Carrying Genetic Elements

The complete genome sequences of four bla_{NDM}-carrying isolates *Providencia rettgeri* 1701091, *Proteus mirabilis* 1701092, *K. pneumoniae* QD23, and *Providencia rettgeri* 51003 were determined in this work through high-throughput genome sequencing. A total of six chromosome-borne accessory resistance regions were identified: bla_{NDM-1/-3}-carrying Tn6588, Tn6589, Tn6576, and 40.7-kb Tn7-related element from strains 1701091, 1701092, QD23, and 51003, respectively; tetA(C)- and bla_{CTX-M-14}-carrying Tn6577 were from strain 1701092; and strAB-carrying Tn6590 was from strain 51003.

TABLE 1 | Major features of genetic elements characterized in this work.

Group	Genetic element	Accession number	Presence (+) or absence (-) of bla _{NDM}	Chromosomal nucleotide position	Length (bp)	Host bacterium	Reference
Novel IME	Tn6588	CP042860	+	4048158..4148181	100,024	<i>Providencia rettgeri</i> 1701091	This study
	Tn6523	AF261825	-	Not applicable	42,451	<i>Salmonella enterica</i> Typhimurium DT104	(Boyd et al., 2000)
Tn6572-related ICEs	Tn6589	CP042857	+	4033353..4127100	93,748	<i>Proteus mirabilis</i> 1701092	This study
	Tn6512	AY090559	-	Not applicable	88,549	<i>Providencia rettgeri</i> 107	(Bolter et al., 2002)
	Tn6575	MH160822	-	Not applicable	146,895	<i>Proteus vulgaris</i> BC22	(Kong et al., 2020)
	Tn6576	CP042858	+	4485620..5019721	534,102	<i>K. pneumoniae</i> D23	This study
	Tn6577	CP042857	-	3239622..3377168	137,547	<i>Proteus mirabilis</i> 1701092	This study
Tn7-related elements	Tn7	KX117211	-	Not applicable	14,067	<i>E. coli</i> 3..5-R3	(Peters and Craig, 2001)
	Tn6726	AP018750	+	5052592..5228430	175,839	<i>K. pneumoniae</i> KP64	(Sakamoto et al., 2018)
Tn6591-related IMEs	40.7-kb Tn7-related element	CP042861	+	31628.72403	40,776	<i>Providencia rettgeri</i> 51003	This study
	Tn6591	AE014073	-	2596547..2614010	15,464	<i>Shigella flexneri</i> 2457T	(Boyd et al., 2000)
	Tn6590	CP042861	-	2252695..2268315	15,621	<i>Providencia rettgeri</i> 51003	This study

For each group, all the fully sequenced and non-redundant bla_{NDM}-carrying genetic elements available in GenBank (last accessed 15 December 2019) are included.

A detailed sequence comparison was applied to a collection of 12 chromosomal genetic elements, which included the above mentioned six genetic elements sequenced in this study, together with six additional ones from GenBank (four reference/prototype ones Tn6523, Tn6512, Tn7, and Tn6591, and two bla_{NDM}-carrying ones Tn6575 and Tn6726). These 12 genetic elements could be further divided into five distinct groups: a novel IME Tn6588; two related IMEs Tn6523 and Tn6589; four related ICEs Tn6512, Tn6575, Tn6576 and Tn6577; Tn7 and its two derivatives Tn6726 and 40.7-kb Tn7-related element; and two related IMEs Tn6591 and Tn6590 (Table 1). Six (Tn6588, Tn6589, Tn6575, Tn6576, Tn6726, and 40.7-kb Tn7-related element) of them harbored bla_{NDM}. At least 51 resistance genes, involved in resistance to 18 different categories of antibiotics and heavy metals, were identified in these 12 elements (Figure 1 and Table S2).

A Novel IME Tn6588

Tn6588 (100.0 kb in length) was inserted into the chromosomal *orf1407* gene (cytochrome c551 peroxidase). Tn6588 had a 9.2-kb backbone (containing *int*) with insertion of two accessory modules: IS1A and a 90.1-kb multidrug resistance (MDR) region, and it had terminal 35-bp *attL/attR* pairs and were further bracketed by 5-bp direct repeats (DRs; target site duplication signals for transposition) (Figure 2A). The MDR region contained a total of 19 resistance genes including bla_{NDM-1} (Figure 1 and Table S2), which were located at eight different resistance loci: In1718, IS26-*mph*(E)-IS26 unit, In1247, a truncated ISCR2-*floR* unit, ISCR2-*sul2* unit, ISEc59-*aph*(4)-*Ia-aacC4*-IS26 unit, ΔTn4352 containing *aphA1*, and a 6.8-kb In27-carrying Tn6909-related region (Figure 2B).

In1718 was a concise class 1 integron with a gene cassette array (GCA) *aacA4cr-bla*_{OXA-1}-*catB3-arr-3*. In1247 was a complex class 1 integron containing VR1 (variable region 1=GCA: *aadA2-lnuF*), five copies of 6.4-kb repeat [VR2

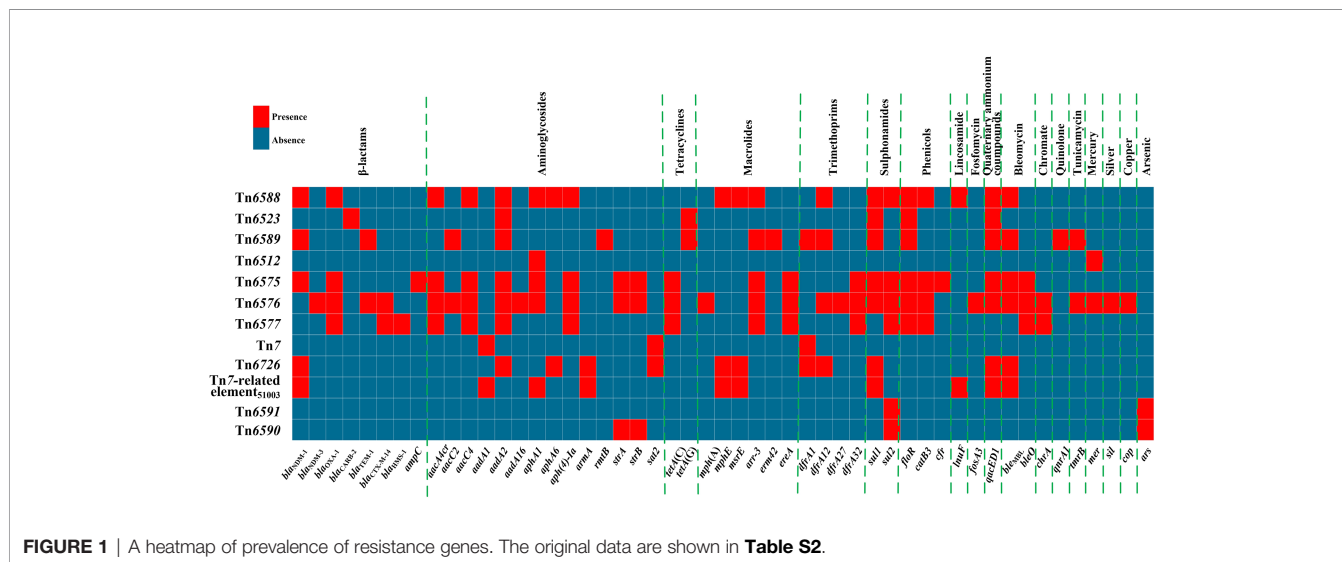
(ISCR1-*ble*_{MBL}-*bla*_{NDM-1}-*arr-3* unit) plus 3'-CS2 (a second 3'-conserve segment)], and VR3 (ISCR1-*aphA6* unit). In1247 plus a Tn21 core transposition module *tnpAR-res-tnpM* in this MDR region were genetically related to the Tn3-family unit transposon Tn6727 (Partridge et al., 2001), which was initially found in *Proteus vulgaris* and originally associated with Tn21. The 6.8-kb Tn6909-related region looked like a truncated version of Tn3-family unit transposon Tn6909 that was originally associated with Tn1696 and Tn21 (Partridge et al., 2001).

Two Related IMEs Tn6523 and Tn6589

Tn6523, a 42.4-kb IME initially found in *Salmonella enterica* serovar Typhimurium DT104 (Boyd et al., 2000), had a 27.2-kb backbone (containing *attL*, *int*, *xis*, *rlx*, *oriT*, and *attR*) with insertion of a single accessory module In127. Besides the GCA (*aadA2*), In127 captured additional two resistance loci: ISCR3-*tetA*(G)-*floR* unit and a bla_{CARB-2}-carrying In167. The backbone of Tn6589 was almost identical to Tn6523 but integrated with a 66.4-kb MDR region instead of In127 (Figure 3A). This MDR region contained a total of 16 resistance genes including bla_{NDM-1} (Figure 1 and Table S2), which were located at seven different resistance loci: In27, ISCR3-*tetA*(G)-*floR* unit, Tn2-*rmtB* region, ISCR2-*floR* unit, ISPa13-*erm42*-IS26 unit, *aacC2-tmrB* region, and In363 with a GCA *dfrA1-gcuC* (Figure 3B). In27 in this MDR region was a complex class 1 integron, which carried VR1 (GCA: *dfrA12-gcuF-aadA2*), and two copies of 11.8-kb repeat region [VR2 (ISCR1-*ble*_{MBL}-*bla*_{NDM-1}-*arr-3* unit) + 3'-CS2 + VR3 (ISCR1-*qnrA1* unit) + 3'-CS3] (Figure 3B).

Four Related ICEs Tn6512, Tn6575, Tn6576, and Tn6577

Tn6512, an 88.5-kb ICE initially found in *Providencia rettgeri* 107 (Boltner et al., 2002), was composed of an 82.4-kb backbone with insertion of three accessory modules: IS15DI-composite transposon Tn6578 (containing *aphA1*), ISPrre1 and ISPrre2 (Figure S1). Tn6512, Tn6575 (146.9 kb in length) (Kong et al., 2020), Tn6576 (534.1 kb in length), and Tn6577 (137.5 kb in



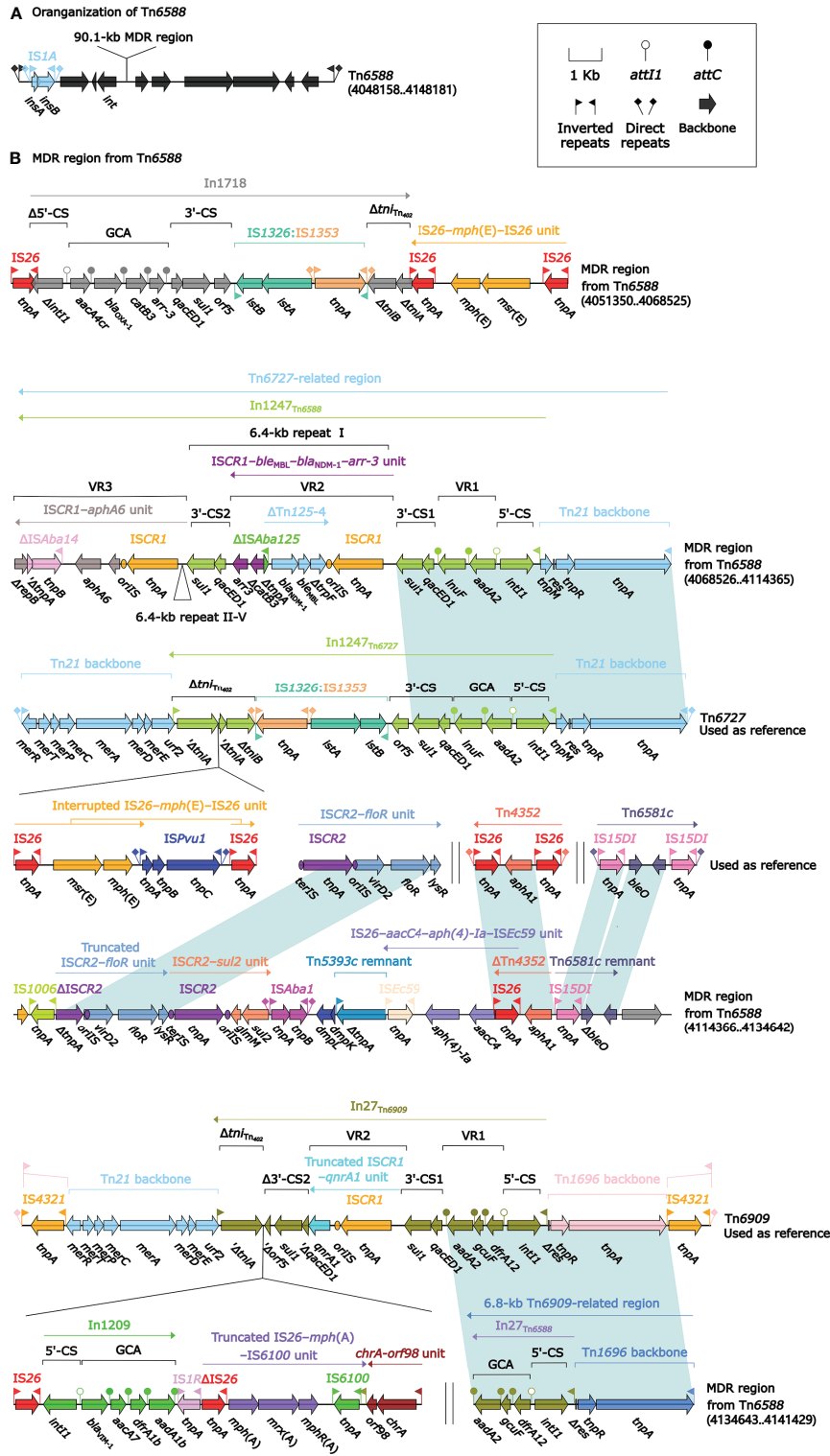


FIGURE 2 | Shown are the organization of Tn6588 (**A**), and MDR region from Tn6588 (**B**). Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within the chromosome of strain 1701091. The accession numbers of Tn6727, ISCR2-fluR unit, Tn4352 (Wrighton and Strike, 1987), Tn6581c, and Tn6909 used as reference are CP047346, CP042857, CP042858, CP042857, and CP032168, respectively.

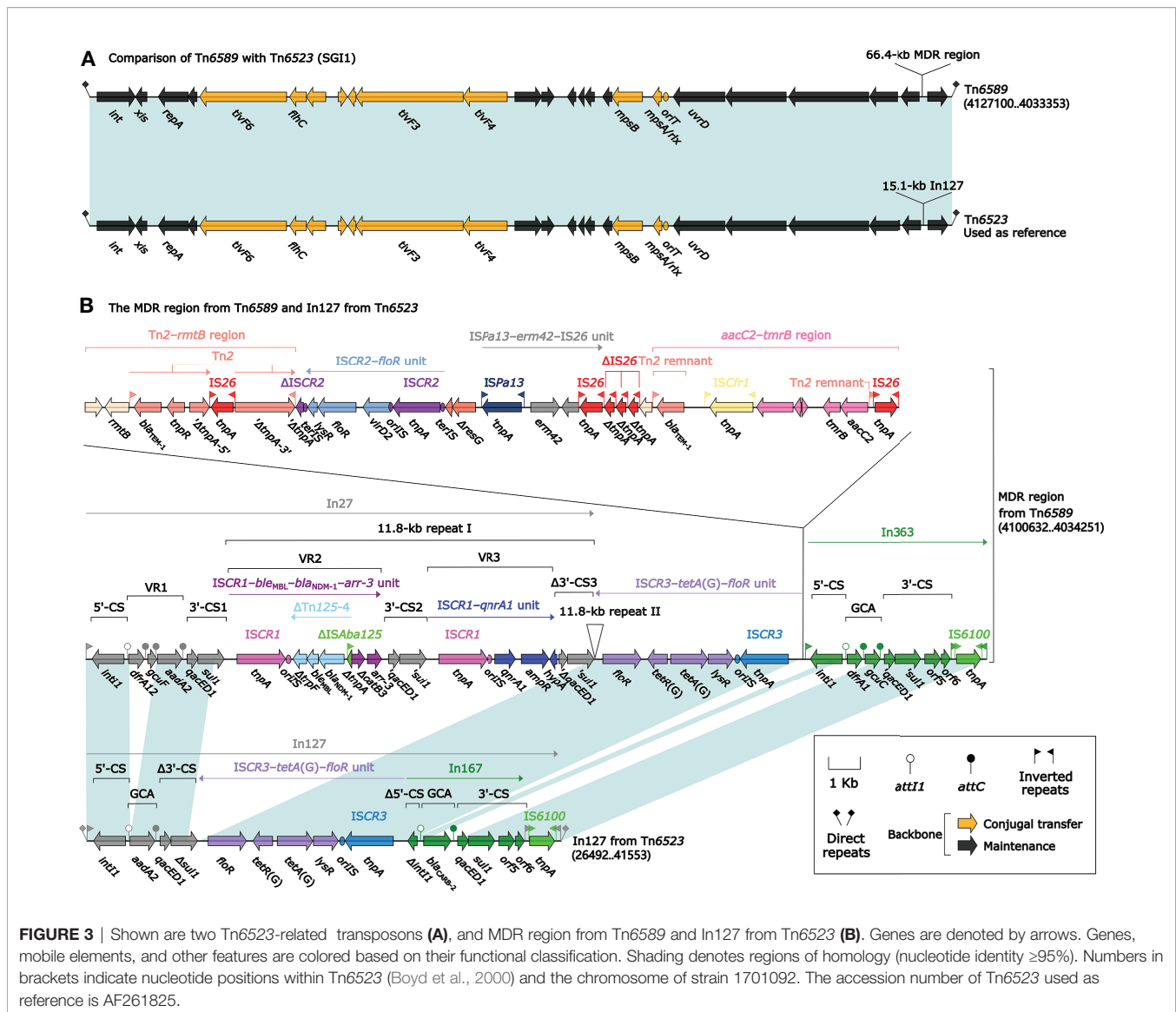


FIGURE 3 | Shown are two Tn6523-related transposons (A), and MDR region from Tn6589 and In127 from Tn6523 (B). Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within Tn6523 (Boyd et al., 2000) and the chromosome of strain 1701092. The accession number of Tn6523 used as reference is AF261825.

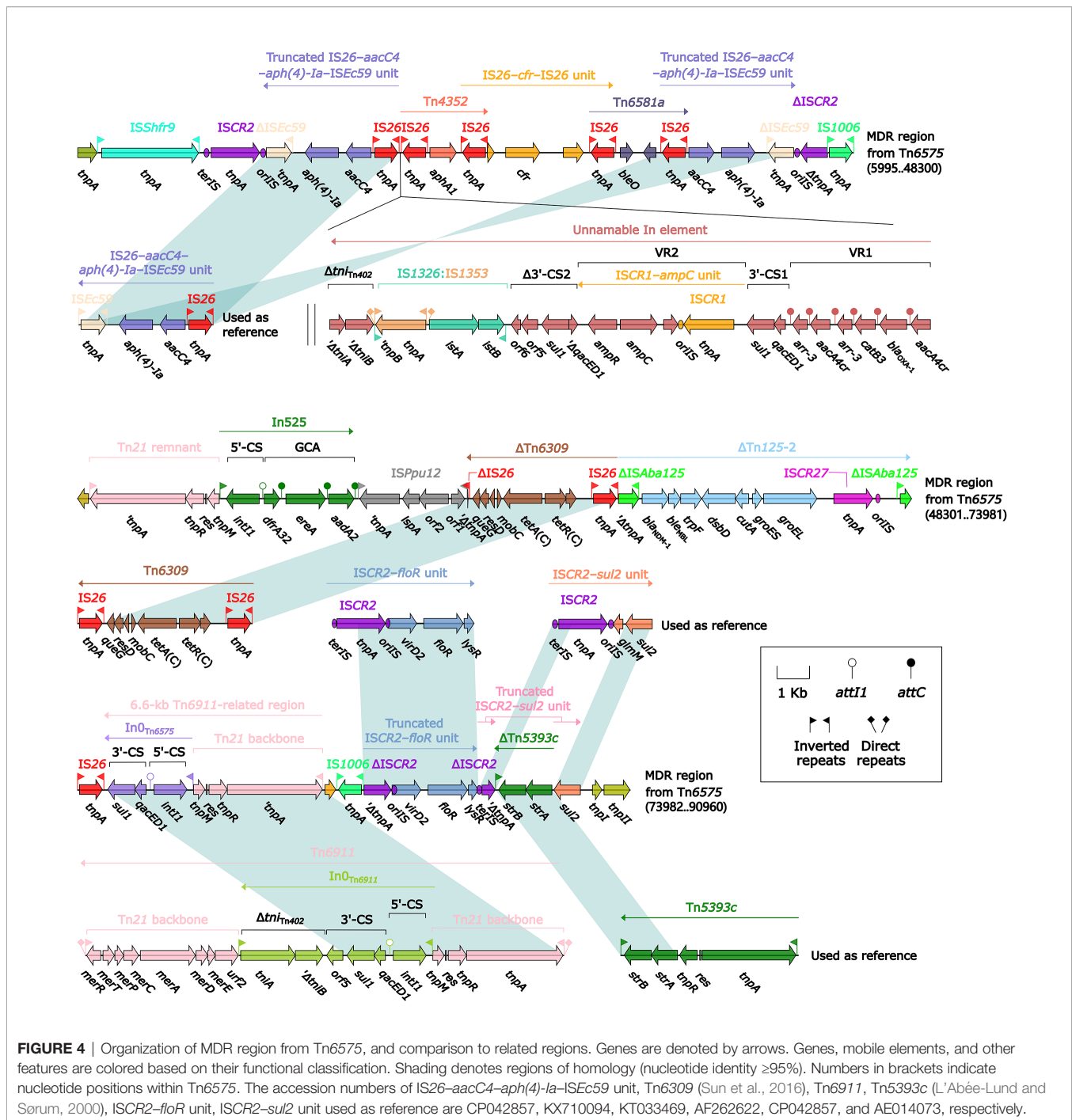
length) had similar backbones and, especially, shared the core backbone genes *attL*, *int*, *xis*, *rlx*, *ori*, *cpl*, a TivF-type T4SS gene set, and *attR* (Figure S1). All these four ICEs were integrated at the same site within the chromosomal gene *prfC* (peptide chain release factor 3).

Each of Tn6575, Tn6576, and Tn6577 had two accessory modules. Firstly, a 85.0-kb MDR region, a novel 406.4-kb ICE Tn6582, and a 11.1-kb *bla_{HMS-sul2}* region were inserted at the same site within *umuC* of Tn6575, Tn6576, and Tn6577, respectively, which led to different truncations of surrounding backbone regions of Tn6575 and Tn6577 but not Tn6576. Secondly, IS*Ppu12*, Tn6580*b* (55.2 kb in length), and Tn6580*a* (50.0 kb in length) were integrated at the same site downstream of *orf714* of Tn6575, Tn6576, and Tn6577, respectively (Figure S1).

The 85.0-kb MDR region of Tn6575 contained a total of 22 resistance genes including *bla_{NDM-1}* (Figure 1 and Table S2),

which were located at 12 different resistance loci: two copies of a truncated *ISEc59-aph(4)-Ia-aacC4-IS26* unit, an unnamable In element (harboring a long GCA *aacA4cr-bla_{OXA-1}-catB3-arr-3-aacA4cr-arr-3* but lacking the whole 5'-CS), *aphA1*-containing Tn4352, IS26-*cfr*-IS26 unit, IS26-composite transposon Tn6581*a* containing *bleO*, In525 (GCA: *dfrA32-ereA-aadA2*), *tetA(C)*-containing Δ Tn6309, *bla_{NDM-1}*-containing Δ Tn125-2, a 6.6-kb In0-carrying Tn6911-related region, a truncated ISCR2-*floR* unit, a truncated ISCR2-*sul2* unit, and *strAB*-containing Δ Tn5393*c* (Figure 4). The Tn6911-related region in this MDR region looked like a truncated version of Tn3-family unit transposon Tn6911 that was originally associated with Tn21 (Partridge et al., 2001).

Tn6582 had a complete set of core ICE backbone determinants and, moreover, a lot of accessory modules: two MDR (MDR-1 and MDR-2) regions, *sil-cop* region as found in IncHI2 plasmid R478 (Gilmour et al., 2004), *floR-strAB-sul2*



region (containing Δ Tn5393c and a truncated ISCR2-*floR* unit), and multiple intact or residue IS elements (Figure S2). The 38.9-kb MDR-1 region (Figure 5) comprised Δ Tn6029 (containing *sul2* and *strAB*), Tn6581b, an unnamable In37-like element (harboring the In37 GCA *aacA4cr*-*bla*_{OXA-1}-*catB3*-*arr-3* but lack of the whole 5'-CS), In27 (GCA: *dfrA12*-*gcuF*-*aadA2*), and two copies of 11.0-kb repeat (each copy harbored a truncated *aacC2*-*tmrB* region, Tn4352, IS26-*mph(A)*-IS6100 unit, and *chrA*-*orf98* unit). The 43.7-kb MDR-2 region

(Figure 6) comprised Δ Tn21 (containing *mer*), *aacC2*-*tmrB* region, and two copies of 21.4-kb repeat (each copy harbored IS26-*mph(A)*-IS6100 unit, *chrA*-*orf98* unit, In1021, and a truncated *aacC2*-*tmrB* region). In1021 was a complex class 1 integron containing VR1 (GCA: *aacA4cr*-*arr3*-*dfrA27*-*aadA16*) and VR2 (ISCR1-*ble*_{MBL}-*bla*_{NDM-3}-*arr-3* unit).

The IS*Ppu12*-composite transposon Tn6580a contained a total of 17 resistance genes (Figure 1 and Table S2), which were located at nine different resistance loci: In525 (GCA:

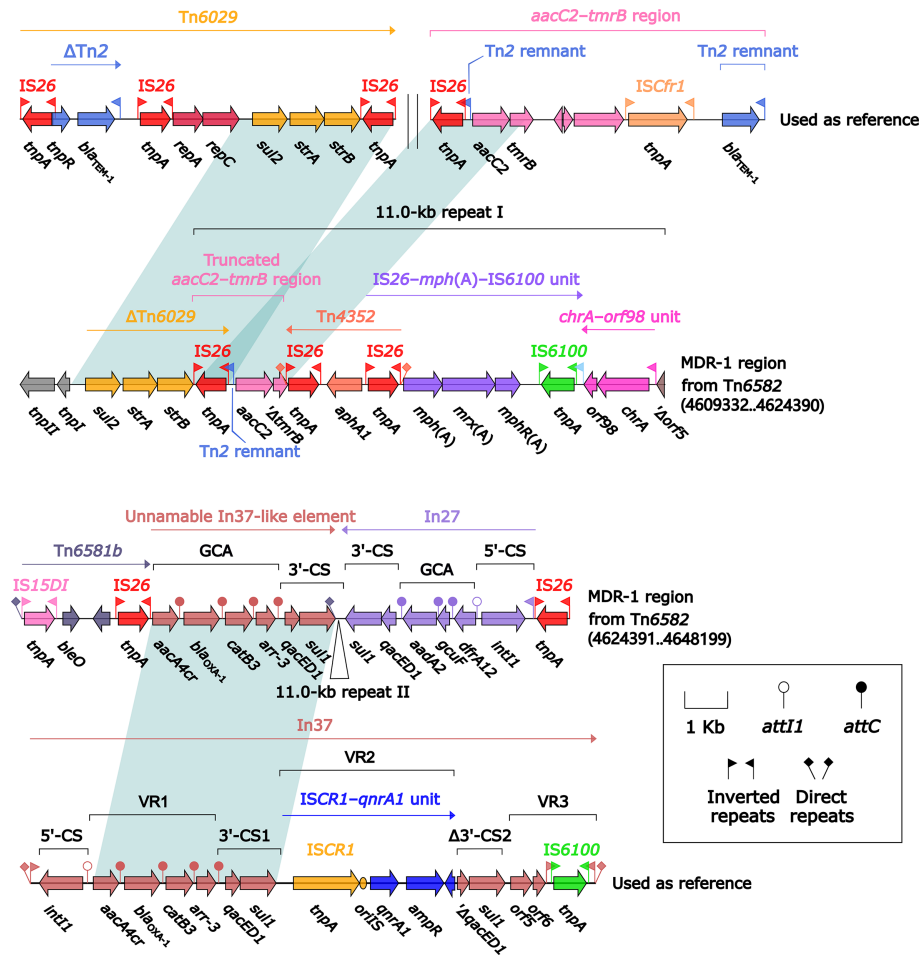


FIGURE 5 | Organization of MDR-1 region from Tn6582, and comparison to related regions. Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within the chromosome of strain QD23. The accession numbers of Tn6029 (Cain et al., 2010), *aacC2-tmrB* region (Partridge et al., 2012), and In37 (Wang et al., 2003) used as reference are GQ150541, JX101693, and AY259086, respectively.

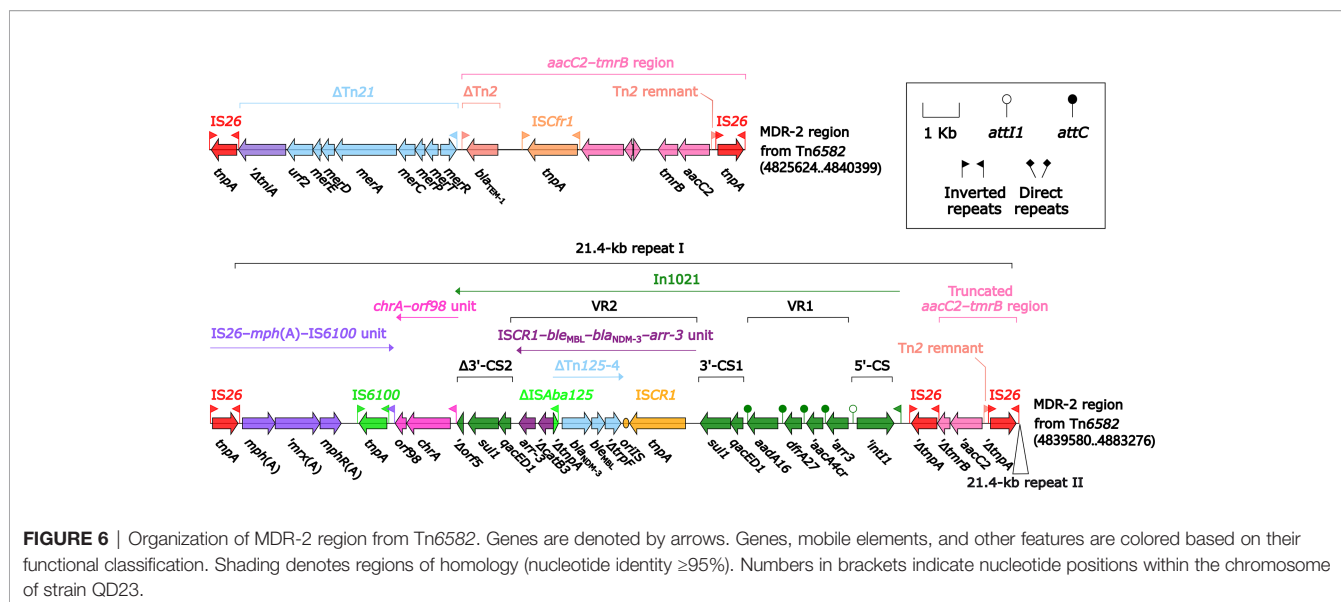
dfrA32-ereA-aadA2), a truncated *chrA-orf98* unit, Tn6581c, the unnamable In37-like element as described above, *ISec59-aph(4)-Ia-aacCA-IS26* unit, *ISCR2-sul2* unit, *ISCR2-flor* unit, *bla_{CTX-M-14}*-containing Δ Tn6503a, and *tetA(C)*-containing Δ Tn6309 (Figure 7). Three major modular differences were recognized in Tn6580b relative to Tn6580a: i) replacement of Tn6581c by Tn6581b; ii) inversion of the unnamable In37-like element; and iii) insertion of *IS26-fosA3-IS26* unit together with Tn4352 at a site between Δ Tn6503a and Δ Tn6309.

Three Related Unit Transposons Tn7, Tn6726, and the 40.7-kb Tn7-Related Element

All of Tn7 (14.1 kb in length), Tn6726 (175.8 kb in length) (Sakamoto et al., 2018), and 40.7-kb Tn7-related element were integrated at a site downstream of the chromosomal gene *glmS* (glutamine-fructose-6-phosphate aminotransferase). Tn6726

differed from Tn7 by acquisition of In2-3 (GCA: *dfrA1*) instead of In2-4 (GCA: *dfrA1-aadA1*) and, moreover, a 162.6-kb *ISKpn26*-composite transposon Tn6728 was inserted at a site within *intI2* of In2-3 (Figure 8). Tn6728 harbored a 40.9-kb MDR region as well as an array of IncHI3 core backbone genes (Figure S3). This 40.9-kb MDR region included a 9.4-kb Tn6909-related region together with Δ Tn1548 (these two shared In27), a truncated *ISAbal4-aphA6-ISAbal4* unit, and *bla_{NDM-1}*-carrying Δ Tn125-1 (Figure 9).

Compared with Tn7, 40.7-kb Tn7-related element underwent two insertion events: i) *tnsABCDE* was truncated by insertion of a 33.6-kb MDR region; and ii) *tnsB* was interrupted by insertion of *ISPrst3*; this element could not be discriminated as an intact Tn7-like transposon due to the presence of an incomplete *tnsABCDE* module (Figure 8). This 33.6-kb MDR region contained In2-16, Δ Tn1548, and *aphA1*-carrying Δ Tn4352. In2-16 carried VR1 (GCA: *lnu(F)-dfrA1-aadA1a*) and two



copies of 5.6-kb repeat [VR2 (ISCR1-*ble*_{MBL}-*bla*_{NDM-1} unit) plus $\Delta 3'$ -CS2] (Figure 10).

ISeC29-mph(E)-IS26/IS15DI unit and *ISCR1-arma* unit were presented in both Δ Tn1548 from Tn6728 and that from 40.7-kb Tn7-related element, whereas In27 was found in the former Δ Tn1548 rather than the later one; in addition, *ISeC29-mph(E)-IS26/IS15DI* unit was interrupted by insertion of two different IS elements *ISKpn21* and *ISAbA24*, respectively, in these two Δ Tn1548 (Figures 9 and 10).

Two Related IMEs Tn6591 and Tn6590

The 15.5-kb IME Tn6591 (*GI**sul2*) (Wei et al., 2003), initially found in *Shigella flexneri* 2457T, was integrated into the chromosomal gene *guaA* (glutamine-hydrolyzing GMP synthase) and had a 12.6-kb backbone (containing *attL*, *int*, *oriT* and *attR*) with insertion of a single accessory module *ISCR2-sul2* unit (Figure 11). Tn6590 (15.6 kb in length) was integrated at the same chromosomal site, and Tn6590 differed from Tn6591 by only truncation of *ISCR2-sul2* unit due to insertion of *strAB*-carrying Δ Tn5393c.

Plasmids of the Four Strains Sequenced in This Study

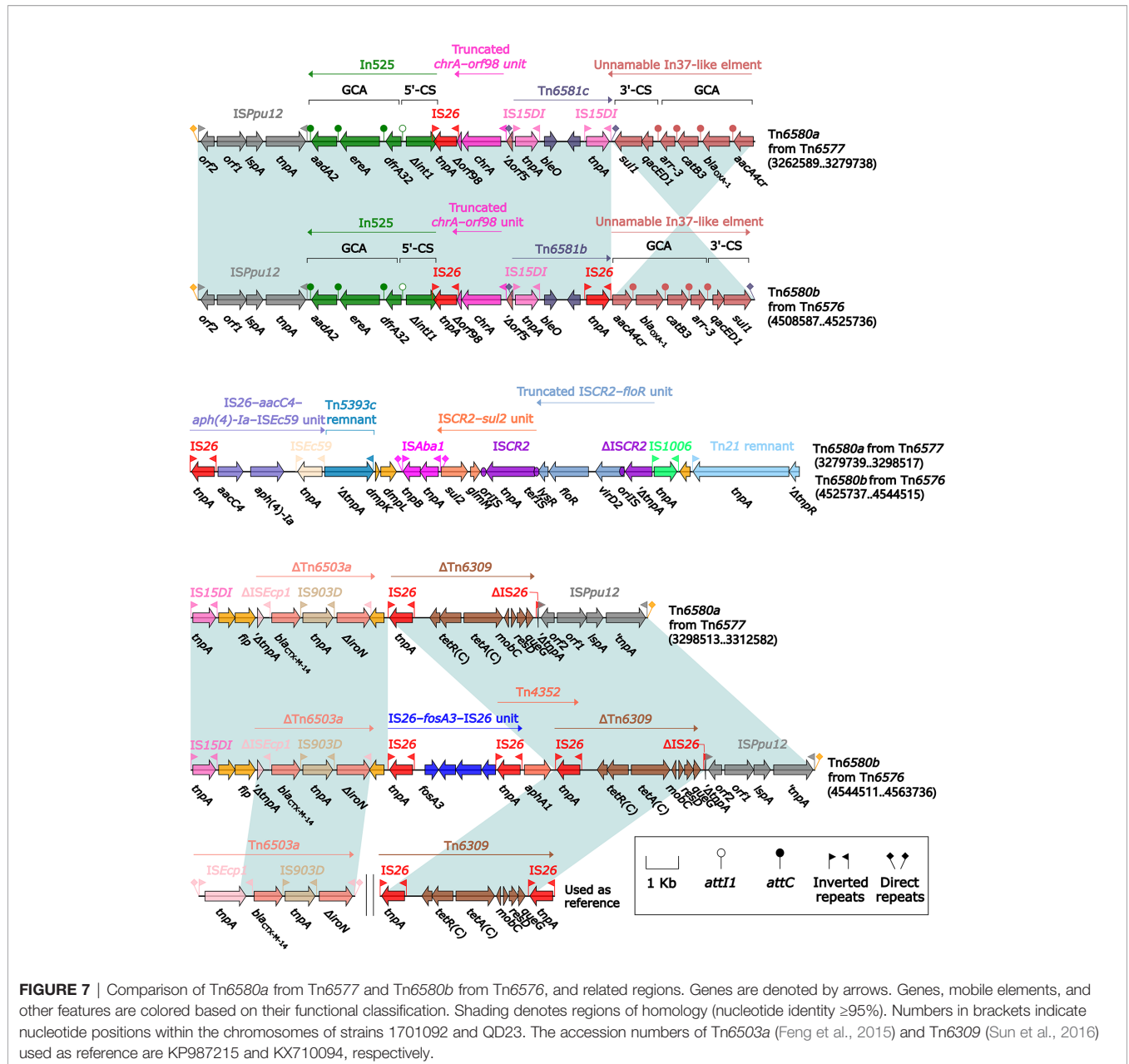
Proteus mirabilis 1701092 carried no plasmids, and all accessory resistance regions (Tn6577 and Tn6589) were located in the chromosome. Besides chromosome-borne accessory resistance regions, an IncFII plasmid p701091-FII (carrying no resistance genes), an IncI plasmid pQD23-CTXM [harboring *bla*_{CTX-M-104} and *erm(B)*], and an IncFII plasmid p51003-FII (containing *bla*_{TEM-1B} and *bla*_{CTX-M-3}) together with an Col3M plasmid p51003-qnrD (having *qnrD*) were identified in *Providencia* spp. 1701091, *K. pneumoniae* QD23, and *Providencia* spp. 51003, respectively. Coexistence of a large array of resistance genes in both chromosome and plasmids of a single bacterial isolate makes it tends to become extensively resistant.

Transferability and Antimicrobial Susceptibility

This work identified three ICEs Tn6577, Tn6582 and Tn6576 in total, all of which had essential conjugal transfer genes. Notably, Tn6582 was located within Tn6576. As for conjugation experiments, Tn6577 was transferred from the wild-type isolate (susceptible to rifampin) into *E. coli* EC600, generating the transconjugant Tn6577-TETA(C)-EC600; Tn6582 could be transferred from the wild-type isolate (non-susceptible to rifampin but susceptible to sodium azide) in *E. coli* J53 to obtain Tn6582-NDM-J53, but repeated conjugation attempts failed to transfer Tn6576 into *E. coli* J53. Tn6577-TETA(C)-EC600 was highly resistant to tetracycline and ceftriaxone owing to presence of *tetA(C)* and *bla*_{CTX-M-14}. Tn6582-NDM-J53 was highly resistant to ceftriaxone and imipenem resulted from production of NDM enzyme (data not shown). The Ambler class B carbapenemase activity was detected in Tn6582-NDM-J53 and its wild-type isolate.

DISCUSSION

Since the *bla*_{NDM} gene was initially identified in India in 2009 (Yong et al., 2009), it spread rapidly all over the world (Dortet et al., 2014). Although *bla*_{NDM} was initially discovered in a plasmid of *K. pneumoniae* (Yong et al., 2009), the chromosomal location of *bla*_{NDM} in Enterobacteriaceae species has been reported in recent years (Pfeifer et al., 2011; Poirel et al., 2011; Girlich et al., 2015; Shen et al., 2017; Sakamoto et al., 2018; Reynolds et al., 2019; Kong et al., 2020). There were few reports related to a detailed genetic dissection of different kinds of *bla*_{NDM}-carrying accessory resistance regions in the chromosomes (Girlich et al., 2015; Sakamoto et al., 2018; Reynolds et al., 2019; Kong et al., 2020), but none of them had

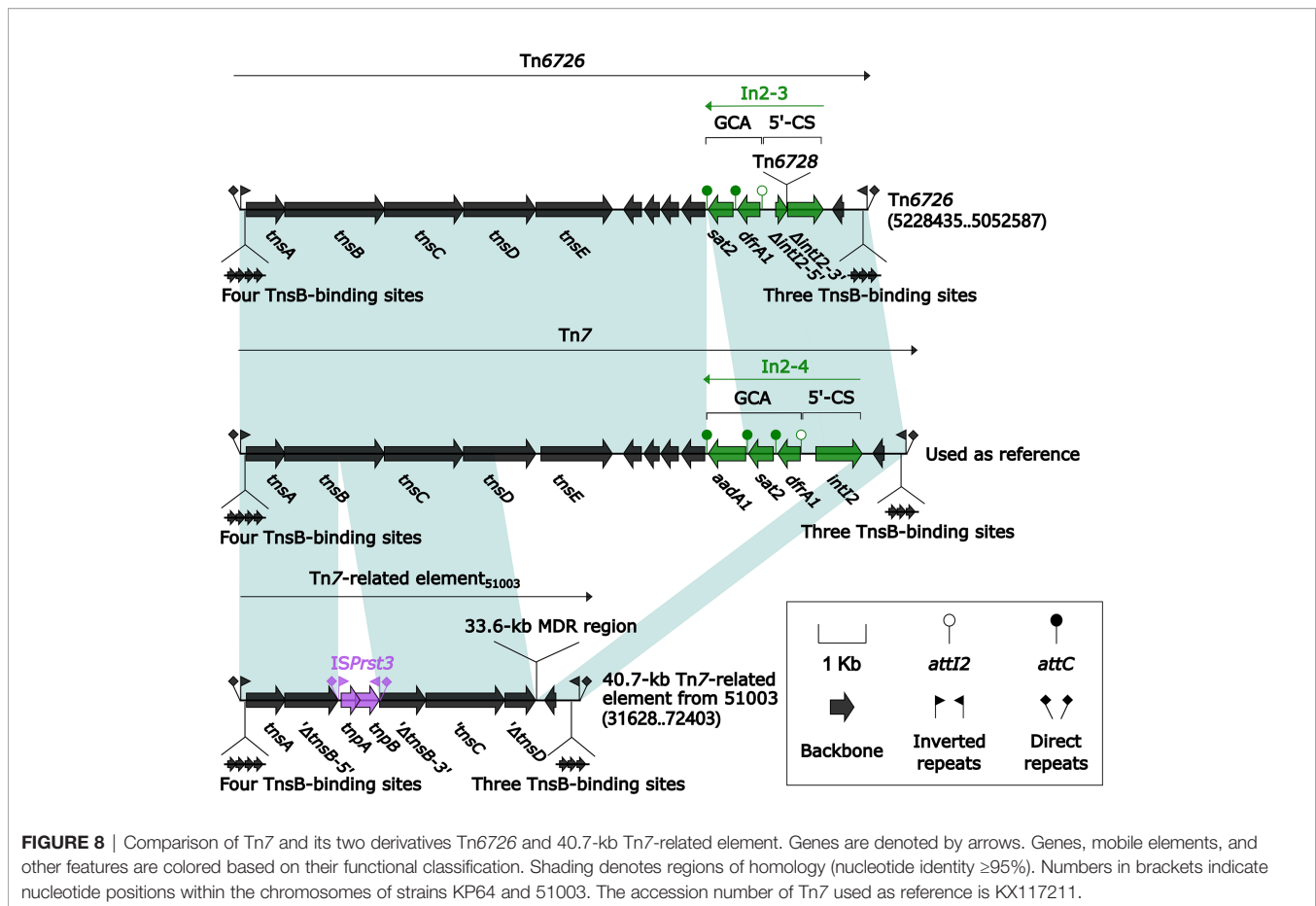


a systematic summary for these *bla*_{NDM}-carrying mobile genetic elements.

Data presented here involved a total of six chromosomal *bla*_{NDM}-carrying genetic elements Tn6575, Tn6726, Tn6588, Tn6589, Tn6576, and 40.7-kb Tn7-related element, and the last four were sequenced in this work. These six genetic elements belonged to three different categories: ICEs (Tn6575 and Tn6576), IMEs (Tn6588 and Tn6589), and two derivatives (Tn6726 and 40.7-kb Tn7-related element) of Tn7 unit transposon. Notably, Tn6576 carried another ICE Tn6582. These ICEs and IMEs would have the intercellular self-mobility as they carried essential conjugal transfer genes (Bellanger et al., 2014; Botelho and Schulenburg, 2021).

Tn6726 would have the intracellular mobility as it had a complete core transposition module *tnsABCDE*, while 40.7-kb Tn7-related element would lose its mobility due to lesion in *tnsABCDE*.

Tn6512-related ICEs were frequently reported in *Vibrio*, *Proteus*, and *Shewanella* (Burrus et al., 2006; Nonaka et al., 2012; Lei et al., 2016; Fang et al., 2018). Tn6575 and Tn6576 were the only two *bla*_{NDM}-carrying Tn6512-related ICEs (last accessed 15 December 2019). Tn6523-related IMEs were frequently reported in *Salmonella* and *Proteus mirabilis* (Hall, 2010; Siebor and Neuwirth, 2013; Sung et al., 2017). This study presented Tn6589, the first *bla*_{NDM}-carrying Tn6523-related IME. Tn7, and its derivatives had the ability to integrate into



bacterial plasmids and chromosomes (Peters, 2014). There were several reports of Tn7 derivatives located in bacterial chromosomes (Chen et al., 2018; Chen et al., 2019). To date, Tn6726 and 40.7-kb Tn7-related element were the only two bla_{NDM}-carrying Tn7 derivatives integrated into chromosomes. Different to 40.7-kb Tn7-related element, Tn6726 carried a series of backbone genes of IncHI3 plasmid, which means that bla_{NDM} together with its surrounding genetic environment in Tn6726 might be originated from a IncHI3 plasmid. In summary, Tn6512-related ICEs, Tn6523-related IMEs, and Tn7 derivatives recently began to be a reservoir of bla_{NDM} genes in Enterobacteriaceae.

Each of these six bla_{NDM}-carrying genetic elements had large accessory resistance regions: i) Tn6575, Tn6588, Tn6589, and 40.7-kb Tn7-related element; each had a single MDR region, 85.0 kb, 90.1 kb, 66.4 kb, and 33.8 kb in length, respectively; ii) Tn6726 contained a 162.6-kb ISKpn26-composite transposon Tn6728 integrated with a 40.9-kb MDR region; and iii) Tn6576 harbored a 406.4-kb ICE Tn6582 (containing two distinct MDR-1 and MDR-2 regions, 38.9 kb and 43.7 kb in length, respectively), and additionally a 55.2-kb ISPpu12-composite Tn6580b that as a whole could be considered as a MDR region. Each of these large MDR regions had a very complex mosaic structure, which was composed of intact or residue mobile genetic elements including

ISs, unit or composite transposons, integrons and putative resistance units, and likely assembled from complex transposition and homologous recombination.

Four different Tn125 derivatives, namely Δ Tn125-1, Δ Tn125-2, Δ Tn125-3, and Δ Tn125-4 (Figure 12), were identified from the relevant MDR regions of these six bla_{NDM}-carrying genetic elements. Δ Tn125-1 from Tn6726 and Δ Tn125-2 from Tn6575 highly resembled the prototype Tn125: Δ Tn125-1 resulted from the insertion of ISEc33 into the left copy of ISAbal25, while terminal truncation of both copies of ISAbal25 generated Δ Tn125-2. It was thought that Δ Tn125-1 and Δ Tn125-2 were generated from transposition of Tn125 into Tn6726 and Tn6575, followed by further modular modifications such as insertion and truncation. Δ Tn125-3 from 40.7-kb Tn7-related element and Δ Tn125-4 from Tn6588, Tn6589 and Tn6576 had very short bla_{NDM-1/-3}-carrying structures. Δ Tn125-3 or Δ Tn125-4 was captured by ISCR1, generating ISCR1-ble_{MBL}-bla_{NDM-1} or ISCR1-ble_{MBL}-bla_{NDM-1/-3}-arr-3 unit, respectively. Furthermore, the former unit was integrated into In2-16 (Figure 10) while the later one into In1247_{Tn6588} (Figure 2), In27_{Tn6589} (Figure 3) and In1021_{Tn6576} (Figure 6), manifesting as the VR2 regions of these integrons. Notably, two or more copies of bla_{NDM-1/-3} genes were found in each of Tn6576, Tn6588, Tn6589, and 40.7-kb Tn7-related element, which resulted from the presence of multiple ≥ 5.6 -kb repeats (each harboring a

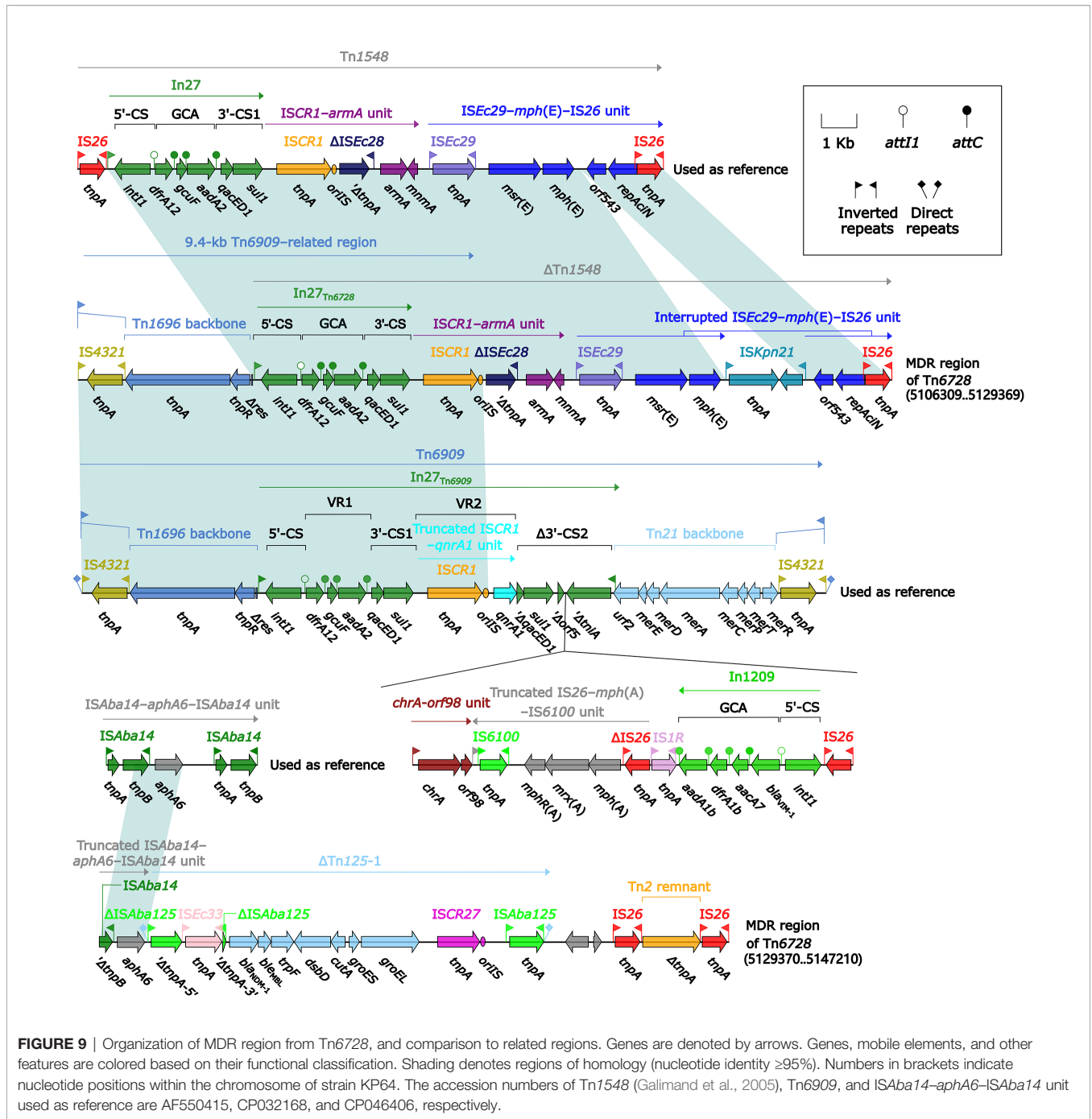


FIGURE 9 | Organization of MDR region from Tn6728, and comparison to related regions. Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within the chromosome of strain KP64. The accession numbers of Tn1548 (Galimand et al., 2005), Tn6909, and ISAb_a14-aphA6-ISAb_a14 unit used as reference are AF550415, CP032168, and CP046406, respectively.

Tn125 derivative and the other components) in these four genetic elements.

Multiple copies of *bla*_{NDM} located in a single plasmid or chromosome were reported in previous studies (Jovčić et al., 2013; Shen et al., 2017; Feng et al., 2018), and all these *bla*_{NDM} genes were around ISCR1. Similarly, Tn6576, Tn6588, Tn6589, and 40.7-kb Tn7-related element in this study also contained ISCR1-around *bla*_{NDM} genes. It was confirmed that ISCR1 captured adjacent genes (frequently including antibiotic resistance genes) at the end of its initiation of replication (*oriS*) through rolling-circle

transposition (Toleman et al., 2006). Our sequencing data suggested that ISCR1 might experience multiple rounds of capturing *bla*_{NDM} and further integrating *bla*_{NDM} into the integrons, resulting in the presence of multiple copies of ISCR1-associated *bla*_{NDM} genes in a single genetic element.

There were eight novel (firstly identified in this study) mobile genetic elements, including three ICEs Tn6576, Tn6577, and Tn6582, two IMEs Tn6588 and Tn6589, two composite transposons Tn6580a and Tn6580b, and one integron In1718. Additional 12 genetic elements (IME: Tn6590; composite

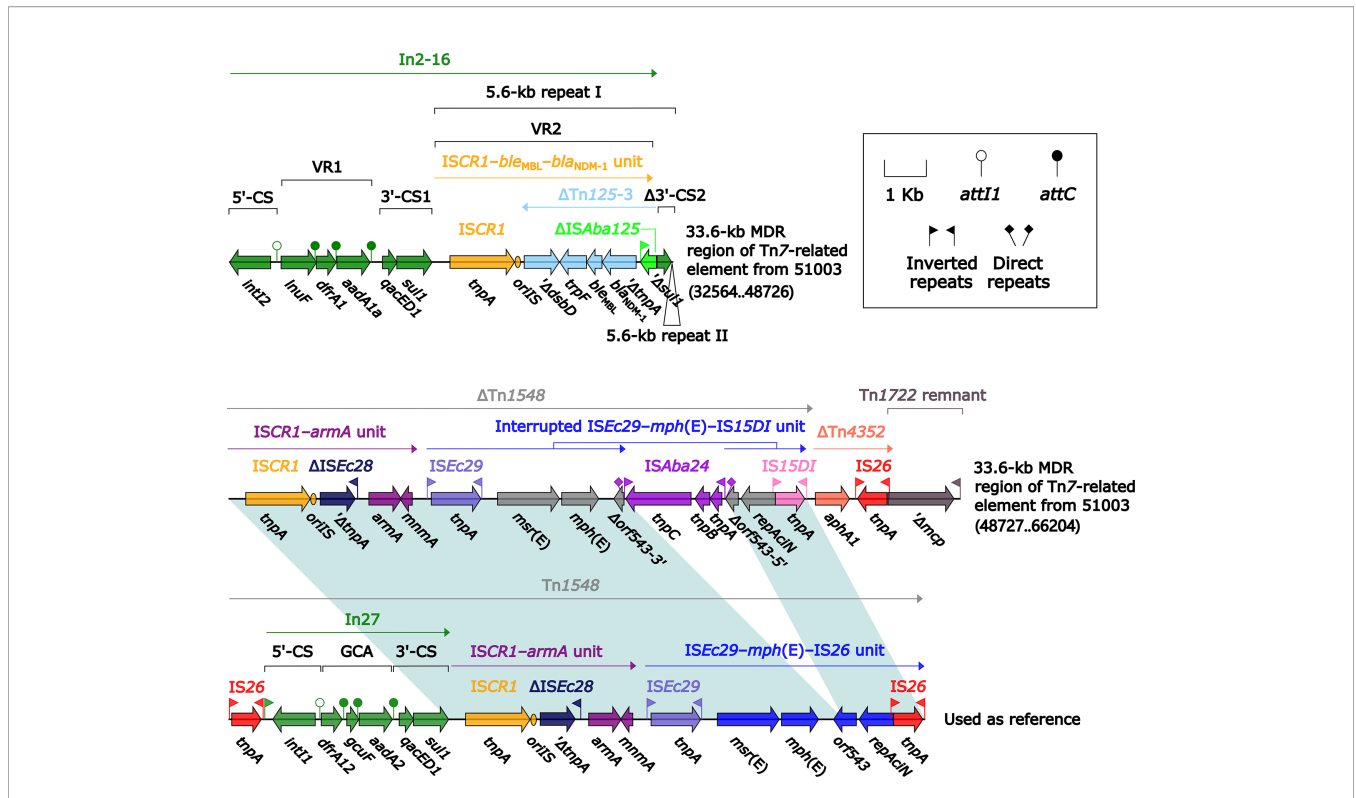


FIGURE 10 | Organization of MDR region from 40.7-kb Tn7-related element, and comparison to related regions. Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within the chromosome of strain 51003. The accession number of Tn1548 (Galimand et al., 2005) used as reference is AF550415.

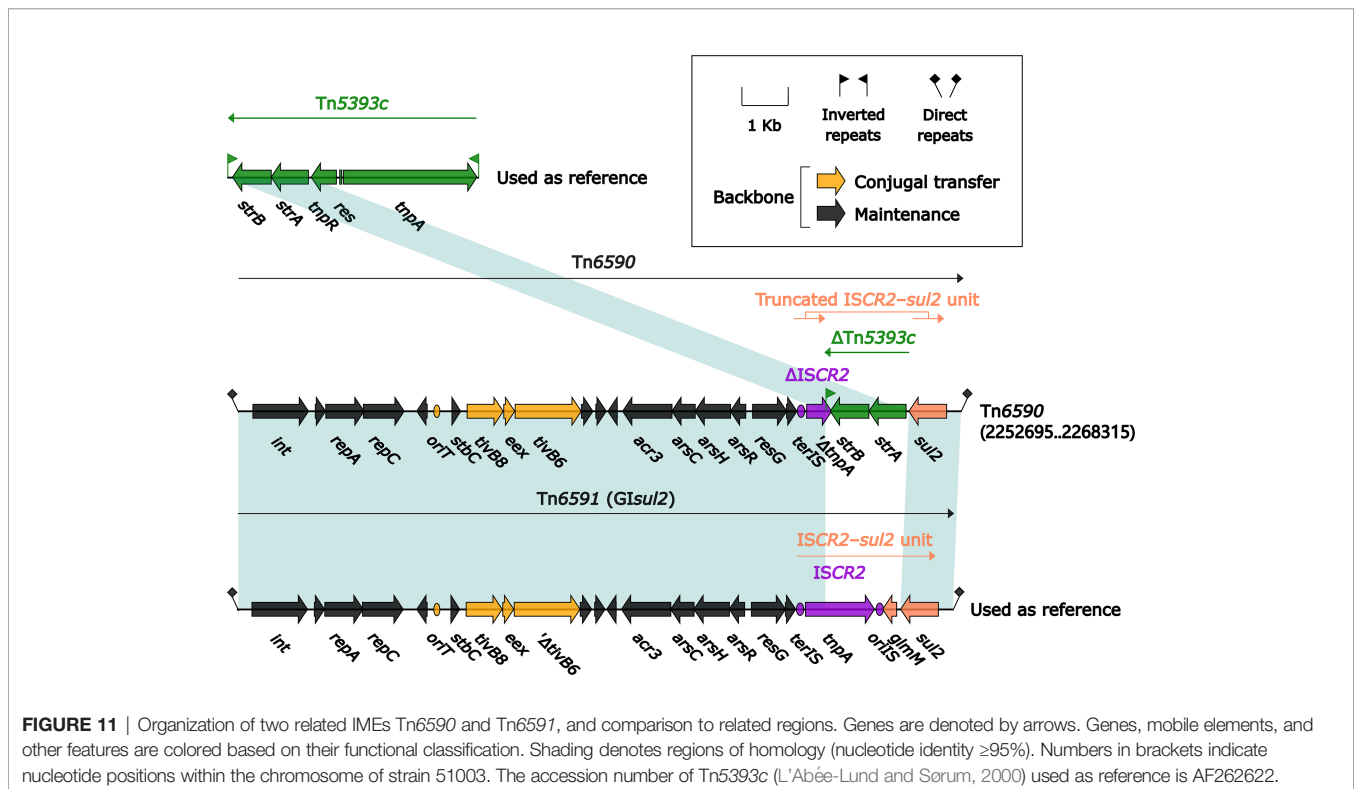
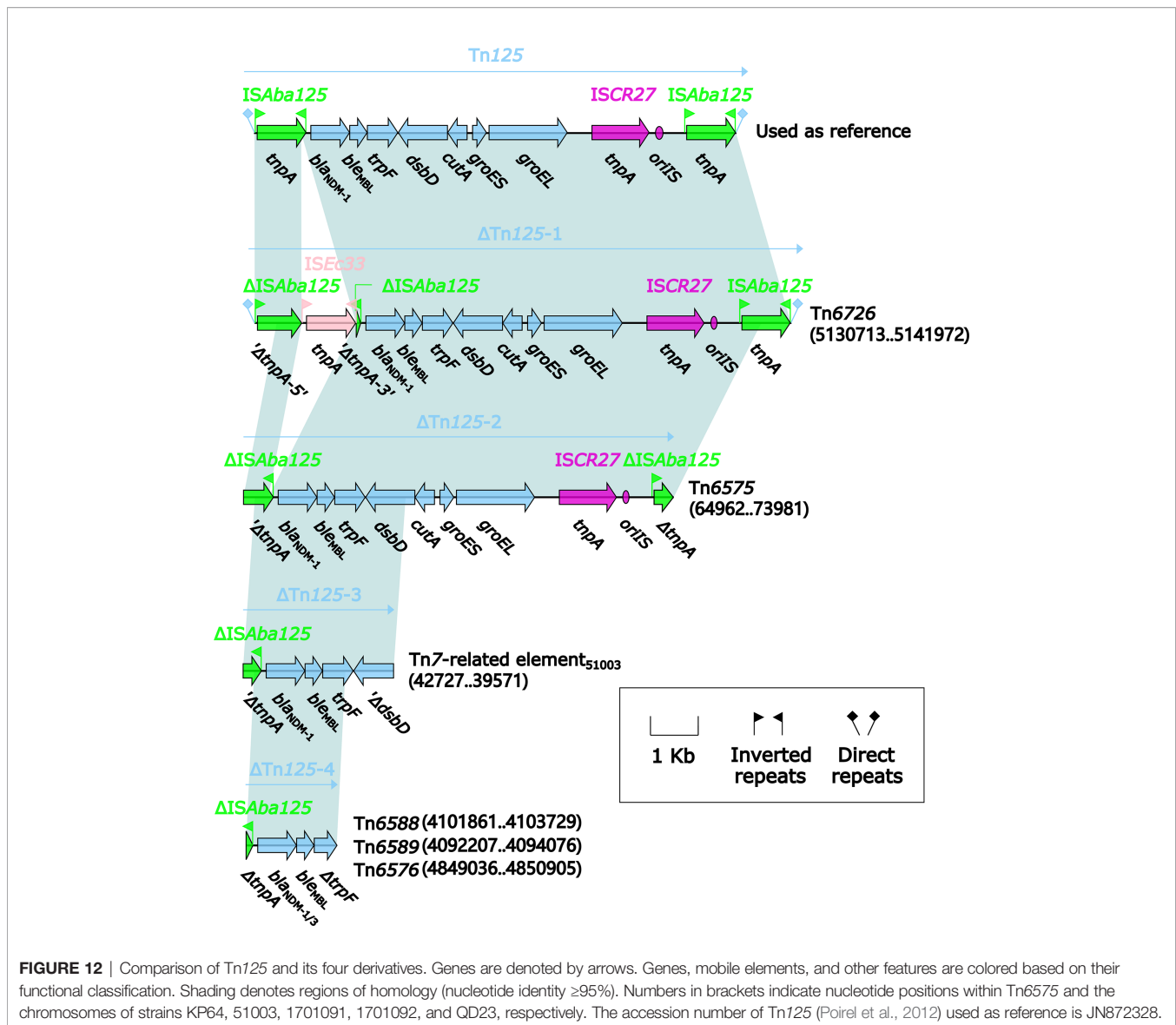


FIGURE 11 | Organization of two related IMEs Tn6590 and Tn6591, and comparison to related regions. Genes are denoted by arrows. Genes, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity $\geq 95\%$). Numbers in brackets indicate nucleotide positions within the chromosome of strain 51003. The accession number of Tn5393c (L'Abée-Lund and Sorum, 2000) used as reference is AF262622.



transposons: Tn6578, Tn6581a, Tn6581b, Tn6581c, and Tn6728; unit transposons: Tn6726, Tn6727, Tn6909, and Tn6911; IS: *ISPvu1*; and 40.7-kb Tn7-related element) were newly designated (firstly designated in this study, but with previously determined sequences). The four previously designated ICEs/IMEs SGI1, R391, ICEPvuChnBC22, and *GIsul2* were renamed as standard Tn designations Tn6523, Tn6512, Tn6575, and Tn6591, respectively. All the putative resistance units presented in this work were annotated and collected in a custom and yet unpublished database.

CONCLUSION

This study dealt with an extensive sequence comparison of 12 chromosomal genetic elements, including six bla_{NDM}-carrying ones. All these bla_{NDM}-carrying genetic elements had huge and complex MDR regions. The core bla_{NDM} genetic environments

manifested as four different Tn125 derivatives. Notably, two or more copies of bla_{NDM} were found in each of the four genetic elements. Eight novel mobile elements were firstly identified, including three ICEs Tn6576, Tn6577, and Tn6582, two IMEs Tn6588 and Tn6589, two composite transposons Tn6580a and Tn6580b, and one integron In1718. This study would provide a deeper genetic insight into the chromosomal integration of bla_{NDM}-carrying genetic elements in Enterobacteriaceae.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the complete chromosomal nucleotide sequences of 1701091, 1701092, QD23 and 51003, which were submitted to GenBank under accession numbers CP042860, CP042857, CP042858 and CP042861, respectively.

ETHICS STATEMENT

This study uses the bacterial isolates obtained from the Chinese livestock farm and public hospitals as listed in **Table S1**. The local legislation did not require the study to be reviewed or approved by an ethics committee, because the bacterial isolates involved in this study was part of the routine laboratory procedures. The research involving biohazards and all related procedures were approved by the Biosafety Committee of the Beijing Institute of Microbiology and Epidemiology.

AUTHOR CONTRIBUTIONS

DZ and HY conceived the study and designed experimental procedures. XL, YJ, and FC performed the experiments. XL, XJ, and LZ analyzed the data. LH, DW and YS contributed to reagents and materials. XL and ZY wrote the original draft. DZ

REFERENCES

- Bellanger, X., Payot, S., Leblond-Bourget, N., and Guedon, G. (2014). Conjugative and Mobilizable Genomic Islands in Bacteria: Evolution and Diversity. *FEMS Microbiol. Rev.* 38 (4), 720–760. doi: 10.1111/1574-6976.12058
- Boltner, D., MacMahon, C., Pembroke, J. T., Strike, P., and Osborn, A. M. (2002). R391: A Conjugative Integrating Mosaic Comprised of Phage, Plasmid, and Transposon Elements. *J. Bacteriol.* 184 (18), 5158–5169. doi: 10.1128/jb.184.18.5158-5169.2002
- Boratyn, G. M., Camacho, C., Cooper, P. S., Coulouris, G., Fong, A., Ma, N., et al. (2013). BLAST: A More Efficient Report With Usability Improvements. *Nucleic Acids Res.* 41 (Web Server issue), W29–W33. doi: 10.1093/nar/gkt282
- Botelho, J., and Schulenburg, H. (2021). The Role of Integrative and Conjugative Elements in Antibiotic Resistance Evolution. *Trends Microbiol.* 29 (1), 8–18. doi: 10.1016/j.tim.2020.05.011
- Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., Bansal, P., Bridge, A. J., et al. (2016). UniProtKB/Swiss-Prot, the Manually Annotated Section of the UniProt KnowledgeBase: How to Use the Entry View. *Methods Mol. Biol.* 1374, 23–54. doi: 10.1007/978-1-4939-3167-5_2
- Boyd, D. A., Peters, G. A., Ng, L., and Mulvey, M. R. (2000). Partial Characterization of a Genomic Island Associated With the Multidrug Resistance Region of *Salmonella Enterica* Typhimurium Dt104. *FEMS Microbiol. Lett.* 189 (2), 285–291. doi: 10.1111/j.1574-6968.2000.tb09245.x
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., et al. (2015). Rasttk: A Modular and Extensible Implementation of the RAST Algorithm for Building Custom Annotation Pipelines and Annotating Batches of Genomes. *Sci. Rep.* 5, 8365. doi: 10.1038/srep08365
- Burrus, V., Marrero, J., and Waldor, M. K. (2006). The Current ICE Age: Biology and Evolution of SXT-Related Integrating Conjugative Elements. *Plasmid* 55 (3), 173–183. doi: 10.1016/j.plasmid.2006.01.001
- Cain, A., Liu, X., Djordjevic, S., and Hall, R. (2010). Transposons Related to Tn1696 in IncHI2 Plasmids in Multiply Antibiotic Resistant *Salmonella Enterica* Serovar Typhimurium From Australian Animals. *Microbial Drug Resistance (Larchmont N. Y.)* 16 (3), 197–202. doi: 10.1089/mdr.2010.0042
- Chen, Y. P., Lei, C. W., Kong, L. H., Zeng, J. X., Zhang, X. Z., Liu, B. H., et al. (2018). Tn6450, A Novel Multidrug Resistance Transposon Characterized in a *Proteus Mirabilis* Isolate From Chicken in China. *Antimicrob. Agents Chemother.* 62 (4), e02192–17. doi: 10.1128/aac.02192-17
- Chen, Y., Lei, C., Zuo, L., Kong, L., Kang, Z., Zeng, J., et al. (2019). A Novel Cfr-Carrying Tn7 Transposon Derivative Characterized in *Morganella Morganii* of Swine Origin in China. *J. Antimicrob. Chemother.* 74 (3), 603–606. doi: 10.1093/jac/dky494
- CLSI (2020). *Performance Standards for Antimicrobial Susceptibility Testing* (Wayne, PA: Clinical and Laboratory Standards Institute).

and HY reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Science and Technology Major Project (2018ZX10733402) of China, and the Foundation of the Public Welfare Program of Natural Science Foundation (LGF19H200006) of Zhejiang.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2021.690799/full#supplementary-material>

- Delavat, F., Miyazaki, R., Carraro, N., Pradervand, N., and van der Meer, J. R. (2017). The Hidden Life of Integrative and Conjugative Elements. *FEMS Microbiol. Rev.* 41 (4), 512–537. doi: 10.1093/femsre/fux008
- Dortet, L., Poirel, L., and Nordmann, P. (2014). Worldwide Dissemination of the NDM-Type Carbapenemases in Gram-Negative Bacteria. *BioMed. Res. Int.*, 249856. doi: 10.1155/2014/249856
- Edgar, R. C. (2004). MUSCLE: Multiple Sequence Alignment With High Accuracy and High Throughput. *Nucleic Acids Res.* 32 (5), 1792–1797. doi: 10.1093/nar/gkh340
- Fang, Y., Wang, Y., Li, Z., Liu, Z., Li, X., Diao, B., et al. (2018). Distribution and Genetic Characteristics of SXT/R391 Integrative Conjugative Elements in *Shewanella* Spp. From China. *Front. Microbiol.* 9, 920. doi: 10.3389/fmicb.2018.00920
- Feng, Y., Liu, L., McNally, A., and Zong, Z. (2018). Coexistence of Two bla_{NDM-5} Genes on an IncF Plasmid as Revealed by Nanopore Sequencing. *Antimicrob. Agents Chemother.* 62 (5), e00110–18. doi: 10.1128/AAC.00110-18
- Feng, J., Qiu, Y., Yin, Z., Chen, W., Yang, H., Yang, W., et al. (2015). Coexistence of a Novel KPC-2-Encoding MDR Plasmid and an NDM-1-Encoding pNDM-HN380-Like Plasmid in a Clinical Isolate of *Citrobacter Freundii*. *J. Antimicrob. Chemother.* 70 (11), 2987–2991. doi: 10.1093/jac/dkv232
- Feng, W., Zhou, D., Wang, Q., Luo, W., Zhang, D., Sun, Q., et al. (2016). Dissemination of IMP-4-Encoding pIMP-HZ1-Related Plasmids Among *Klebsiella Pneumoniae* and *Pseudomonas Aeruginosa* in a Chinese Teaching Hospital. *Sci. Rep.* 6, 33419. doi: 10.1038/srep33419
- Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., and Olsen, G. J. (2008). Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. *Appl. Environ. Microbiol.* 74 (8), 2461–2470. doi: 10.1128/aem.02272-07
- Galimand, M., Sabtcheva, S., Courvalin, P., and Lambert, T. (2005). Worldwide Disseminated Arma Aminoglycoside Resistance Methylase Gene Is Borne by Composite Transposon Tn1548. *Antimicrob. Agents Chemother.* 49 (7), 2949–2953. doi: 10.1128/aac.49.7.2949-2953.2005
- Gilmour, M. W., Thomson, N. R., Sanders, M., Parkhill, J., and Taylor, D. E. (2004). The Complete Nucleotide Sequence of the Resistance Plasmid R478: Defining the Backbone Components of Incompatibility Group H Conjugative Plasmids Through Comparative Genomics. *Plasmid* 52 (3), 182–202. doi: 10.1016/j.plasmid.2004.06.006
- Girlich, D., Dortet, L., Poirel, L., and Nordmann, P. (2015). Integration of the bla_{NDM-1} Carbapenemase Gene Into Proteus Genomic Island 1 (PGI1-PmPEL) in a *Proteus Mirabilis* Clinical Isolate. *J. Antimicrob. Chemother.* 70 (1), 98–102. doi: 10.1093/jac/dku371
- Hackl, T., Hedrich, R., Schultz, J., and Forster, F. (2014). Proovread: Large-Scale High-Accuracy PacBio Correction Through Iterative Short Read Consensus. *Bioinformatics* 30 (21), 3004–3011. doi: 10.1093/bioinformatics/btu392

- Hall, R. M. (2010). Salmonella Genomic Islands and Antibiotic Resistance in Salmonella Enterica. *Future Microbiol.* 5 (10), 1525–1538. doi: 10.2217/fmb.10.122
- Jia, B., Raphenya, A. R., Alcock, B., Waglechner, N., Guo, P., Tsang, K. K., et al. (2017). CARD 2017: Expansion and Model-Centric Curation of the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res.* 45 (D1), D566–D573. doi: 10.1093/nar/gkw1004
- Jovčić, B., Lepsanović, Z., Begović, J., Rakonjac, B., Perovanović, J., Topisirović, L., et al. (2013). The Clinical Isolate *Pseudomonas Aeruginosa* MMA83 Carries Two Copies of the bla_{NDM-1} Gene in a Novel Genetic Context. *Antimicrob. Agents Chemother.* 57 (7), 3405–3407. doi: 10.1128/aac.02312-12
- Kong, L. H., Xiang, R., Wang, Y. L., Wu, S. K., Lei, C. W., Kang, Z. Z., et al. (2020). Integration of the bla_{NDM-1} Carbapenemase Gene Into a Novel SXT/R391 Integrative and Conjugative Element in *Proteus Vulgaris*. *J. Antimicrob. Chemother.* 75 (6), 1439–1442. doi: 10.1093/jac/dkaa068
- L'Abée-Lund, T. M., and Sorum, H. (2000). Functional Tn5393-like Transposon in the R Plasmid pRAS2 From the Fish Pathogen *Aeromonas Salmonicida* Subspecies *Salmonicida* Isolated in Norway. *Appl. Environ. Microbiol.* 66 (12), 5533–5535. doi: 10.1128/aem.66.12.5533-5535.2000
- Lei, C. W., Zhang, A. Y., Wang, H. N., Liu, B. H., Yang, L. Q., and Yang, Y. Q. (2016). Characterization of SXT/R391 Integrative and Conjugative Elements in *Proteus Mirabilis* Isolates From Food-Producing Animals in China. *Antimicrob. Agents Chemother.* 60 (3), 1935–1938. doi: 10.1128/aac.02852-15
- Moura, A., Soares, M., Pereira, C., Leitão, N., Henriques, I., and Correia, A. (2009). INTEGRALL: A Database and Search Engine for Integrons, Integrases and Gene Cassettes. *Bioinformatics* 25 (8), 1096–1098. doi: 10.1093/bioinformatics/btp105
- Nonaka, L., Maruyama, F., Miyamoto, M., Miyakoshi, M., and Kurokawa, K. (2012). And Masuda, MNovel Conjugative Transferable Multiple Drug Resistance Plasmid pAQU1 From Photobacterium Damselae Subsp. Damselae Isolated From Marine Aquaculture Environment. *Microbes Environ.* 27 (3), 263–272. doi: 10.1264/jsm.2.me11338
- O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., et al. (2016). Reference Sequence (RefSeq) Database at NCBI: Current Status, Taxonomic Expansion, and Functional Annotation. *Nucleic Acids Res.* 44 (D1), D733–D745. doi: 10.1093/nar/gkv1189
- Partridge, S. R., Brown, H. J., Stokes, H. W., and Hall, R. M. (2001). Transposons Tn1696 and Tn21 and Their Integrons In4 and In2 Have Independent Origins. *Antimicrob. Agents Chemother.* 45 (4), 1263–1270. doi: 10.1128/aac.45.4.1263-1270.2001
- Partridge, S. R., Ginn, A. N., Paulsen, I. T., and Iredell, J. R. (2012). Pel1573 Carrying bla_{IMP-4}, From Sydney, Australia, Is Closely Related to Other IncL/M Plasmids. *Antimicrob. Agents Chemother.* 56 (11), 6029–6032. doi: 10.1128/aac.01189-12
- Peters, J. E. (2014). Tn7. *Microbiol. Spectr.* 2 (5). doi: 10.1128/microbiolspec.MDNA3-0010-2014
- Peters, J. E., and Craig, N. L. (2001). Tn7: Smarter Than We Thought. *Nat. Rev. Mol. Cell Biol.* 2 (11), 806–814. doi: 10.1038/35099006
- Pfeifer, Y., Witte, W., Holfelder, M., Busch, J., Nordmann, P., and Poirel, L. (2011). NDM-1-Producing Escherichia Coli in Germany. *Antimicrob. Agents Chemother.* 55 (3), 1318–1319. doi: 10.1128/AAC.01585-10
- Poirel, L., Bonnin, R. A., Boulanger, A., Schrenzel, J., Kaase, M., and Nordmann, P. (2012). Tn125-Related Acquisition of bla_{NDM}-Like Genes in *Acinetobacter Baumannii*. *Antimicrob. Agents Chemother.* 56 (2), 1087–1089. doi: 10.1128/aac.05620-11
- Poirel, L., Dortet, L., Bernabeu, S., and Nordmann, P. (2011). Genetic Features of bla_{NDM-1}-Positive Enterobacteriaceae. *Antimicrob. Agents Chemother.* 55 (11), 5403–5407. doi: 10.1128/AAC.00585-11
- Reynolds, M. E., Phan, H. T. T., George, S., Hubbard, A. T. M., Stoesser, N., Maciuga, I. E., et al. (2019). Occurrence and Characterization of Escherichia Coli ST410 Co-Harboring bla_{NDM-5}, bla_{CMY-42} and bla_{TEM-190} in a Dog From the UK. *J. Antimicrob. Chemother.* 74 (5), 1207–1211. doi: 10.1093/jac/dkz017
- Richter, M., and Rosselló-Móra, R. (2009). Shifting the Genomic Gold Standard for the Prokaryotic Species Definition. *Proc. Natl. Acad. Sci. U. S. A.* 106 (45), 19126–19131. doi: 10.1073/pnas.0906412106
- Roberts, A. P., Chandler, M., Courvalin, P., Guedon, G., Mullany, P., Pembroke, T., et al. (2008). Revised Nomenclature for Transposable Genetic Elements. *Plasmid* 60 (3), 167–173. doi: 10.1016/j.plasmid.2008.08.001
- Saeed, A. I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., et al. (2003). TM4: A Free, Open-Source System for Microarray Data Management and Analysis. *Biotechniques* 34 (2), 374–378. doi: 10.2144/03342mt01
- Sakamoto, N., Akeda, Y., Sugawara, Y., Takeuchi, D., Motooka, D., Yamamoto, N., et al. (2018). Genomic Characterization of Carbapenemase-Producing *Klebsiella Pneumoniae* With Chromosomally Carried Bla_{ndm-1}. *Antimicrob. Agents Chemother.* 62 (12), e01520–18. doi: 10.1128/aac.01520-18
- Shen, P., Yi, M., Fu, Y., Ruan, Z., Du, X., Yu, Y., et al. (2017). Detection of an Escherichia Coli Sequence Type 167 Strain With Two Tandem Copies of bla_{NDM-1} in the Chromosome. *J. Clin. Microbiol.* 55 (1), 199–205. doi: 10.1128/jcm.01581-16
- Siebor, E., and Neuwirth, C. (2013). Emergence of Salmonella Genomic Island 1 (SGI1) Among Proteus Mirabilis Clinical Isolates in Dijon, France. *J. Antimicrob. Chemother.* 68 (8), 1750–1756. doi: 10.1093/jac/dkt100
- Siguier, P., Perochon, J., Lestrade, L., Mahillon, J., and Chandler, M. (2006). ISfinder: The Reference Centre for Bacterial Insertion Sequences. *Nucleic Acids Res.* 34 (Database issue), D32–D36. doi: 10.1093/nar/gkj014
- Sung, J. Y., Kim, S., Kwon, G., and Koo, S. H. (2017). Molecular Characterization of Salmonella Genomic Island 1 in Proteus Mirabilis Isolates From Chungcheong Province, Korea. *J. Microbiol. Biotechnol.* 27 (11), 2052–2059. doi: 10.4014/jmb.1708.08040
- Sun, F., Zhou, D., Sun, Q., Luo, W., Tong, Y., Zhang, D., et al. (2016). Genetic Characterization of Two Fully Sequenced Multi-Drug Resistant Plasmids pP10164-2 and pP10164-3 From Leclercia Adecarboxylata. *Sci. Rep.* 6, 33982. doi: 10.1038/srep33982
- Toleman, M. A., Bennett, P. M., and Walsh, T. R. (2006). ISCR Elements: Novel Gene-Capturing Systems of the 21st Century? *Microbiol. Mol. Biol. Rev.* 70 (2), 296–316. doi: 10.1128/MMBR.00048-05
- Wang, M., Tran, J. H., Jacoby, G. A., Zhang, Y., Wang, F., and Hooper, D. C. (2003). Plasmid-Mediated Quinolone Resistance in Clinical Isolates of Escherichia Coli From Shanghai, China. *Antimicrob. Agents Chemother.* 47 (7), 2242–2248. doi: 10.1128/aac.47.7.2242-2248.2003
- Wei, J., Goldberg, M. B., Burland, V., Venkatesan, M. M., Deng, W., Fournier, G., et al. (2003). Complete Genome Sequence and Comparative Genomics of Shigella Flexneri Serotype 2a Strain 2457T. *Infection Immun.* 71 (5), 2775–2786. doi: 10.1128/iai.71.5.2775-2786.2003
- Wrighton, C. J., and Strike, P. (1987). A Pathway for the Evolution of the Plasmid NTP16 Involving the Novel Kanamycin Resistance Transposon Tn4352. *Plasmid* 17 (1), 37–45. doi: 10.1016/0147-619x(87)90006-0
- Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., and Zong, Z. (2019). NDM Metallo-β-Lactamases and Their Bacterial Producers in Health Care Settings. *Clin. Microbiol. Rev.* 32 (2), e00115–18. doi: 10.1128/cmr.00115-18
- Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., et al. (2009). Characterization of a New Metallo-Beta-Lactamase Gene, bla_{NDM-1b}, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella Pneumoniae Sequence Type 14 From India. *Antimicrob. Agents Chemother.* 53 (12), 5046–5054. doi: 10.1128/AAC.00774-09
- Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., et al. (2012). Identification of Acquired Antimicrobial Resistance Genes. *J. Antimicrob. Chemother.* 67 (11), 2640–2644. doi: 10.1093/jac/dks261

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Luo, Yin, Zeng, Hu, Jiang, Jing, Chen, Wang, Song, Yang and Zhou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.