Genetic Characterization of Four Groups of Chromosome-Borne Accessory Genetic Elements Carrying Drug Resistance Genes in Providencia

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Purpose: The aim of this study was to gain a deeper genomics and bioinformatics understanding of diversification of accessory genetic elements (AGEs) in Providencia.

Methods: Herein, the complete genome sequences of five Providencia isolates from China were determined, and seven AGEs were identified from the chromosomes. Detailed genetic dissection and sequence comparison were applied to these seven AGEs, together with additional 10 chromosomal ones from GenBank (nine of them came from Providencia).

Results: These 17 AGEs were divided into four groups: Tn6512 and its six derivatives, Tn6872 and its two derivatives, Tn6875 and its one derivative, and Tn7 and its four derivatives. These AGEs display high-level diversification in modular structures that had complex mosaic natures, and particularly different multidrug resistance (MDR) regions were presented in these AGEs. At least 52 drug resistance genes, involved in resistance to 15 different categories of antimicrobials and heavy metal, were found in 15 of these 17 AGEs.

Conclusion: Integration of these AGEs into the Providencia chromosomes would contribute to the accumulation and distribution of drug resistance genes and enhance the ability of Providencia isolates to survive under drug selection pressure.

Keywords: Providencia, integrative and conjugative elements, integrative and mobilizable elements, unit transposons, multidrug resistance

Introduction

Providencia species, mostly frequently identified as Providencia rettgeri and Providencia stuartii, are opportunistic pathogens causing urinary tract infections, diarrhea, and bacteremia in immunocompromised patients.1 Providencia is intrinsically resistant to penicillins and the first-generation cephalosporins due to inducible expression of AmpC β-lactamases,2 aminoglycosides by the reason of inducible expression of AAC(2’)-Ia,3 tetracyclines because of constitutive expression of a multidrug efflux pump AcrAB,4 and polymyxins resulting from the presence of a cell envelop that inhibits colistin to combine with the susceptible lipid target sites or the lipid A modification to reduce binding.5 In recent years, the wide use of aminoglycosides, β-lactams, and fluoroquinolones for antimicrobial therapy aggravates the acquisition and dissemination of diverse antimicrobial resistance genes in Providencia.6 Acquired antimicrobial
resistance genes in *Providencia* are commonly captured and horizontally transferred by accessory genetic elements (AGEs), such as integrative and conjugative elements (ICEs), integrative and mobilizable elements (IMEs), and unit transposons.

ICEs have the ability to transfer from one cell to another cell through conjugation and are autonomous in conjugation by mainly utilizing a type IV secretion system (T4SS) and a coupling protein.\(^7\) Up to now, three distinct families of ICEs, namely Tn6512 (R391),\(^8\) IECec2,\(^9\) and IECPlm\(^10\) have been reported in *Providencia*. Tn6512 is initially found in *Providencia rettgeri* in 1972,\(^8\) and since then a wealth of Tn6512-related ICEs have been identified in not only *Providencia*,\(^8,11\) but also *Enterobacteriaceae*,\(^12\) *Vibrionaceae*,\(^13\) *Shewanella*,\(^14\) *Actinobacillus*,\(^15\) *Alteromonas*,\(^16\) *Pseudoalteromonas*.\(^17\) Tn6512-related ICEs carry at least five hotspots (located within the intragenic or intergenic sites of their backbone regions) for integration of foreign resistance genes,\(^18\) which causes these ICEs being mosaic and diversified with respect to their modular structures.

IMEs do not encode the T4SS machinery and the coupling protein and thus is nonautonomous in conjugation.\(^19\) The conjugal transfer of an IME from a donor cell into a recipient cell relies on the T4SS gene sets of a helper conjugative element such as an IncA/C conjugative plasmid.\(^20\) So far, three distinct families of IMEs, namely Tn6523 (SGI1),\(^21\) Tn5888,\(^22\) and Tn6591\(^22\) have been reported in *Providencia*.

ICEs and IMEs can be transferred in an intercellular manner, while the Tn7-family unit transposons use a “cut-and-paste” transposition mechanism to transfer intracellularly, which is typically promoted by the core transposition determinants TnsA (endonuclease), TnsB (transposase), TnsC (transposition regulator), and TnsD plus TnsE (target-site selection proteins).\(^23\) Tn7-family transposons have widely been found as the vectors of diverse antimicrobial resistance genes in *Morganellaceae* including *Providencia rettgeri*,\(^22\) *Enterobacteriaceae*,\(^24\) *Pseudomonadaceae*,\(^25\) *Burkholderiaceae*,\(^26\) *Acinetobacter*,\(^27\) *Shewanella*,\(^24\) *Hahella*,\(^24\) *Pelobacter*,\(^24\) *Idiomarina*,\(^24\) *Acidithiobacillus*,\(^24\) *Neisseria* (eg *N. brasiliensis* with an accession number CP046027), *Nitrosomonas* (eg *N. stercoris* with an accession number AP019755), and *Bacillus*.\(^24\)

This study presented the complete sequences of seven chromosome-borne AGEs in five sequenced *Providencia* isolates from China. Detailed genetic dissection and comparison were applied to these seven AGEs, together with additional 10 chromosome-borne ones from GenBank (nine of them came from *Providencia*). These 17 AGEs could be classified into ICEs, IMEs, and Tn7 derivatives. Data presented here provided a deeper genomics and bioinformatics understanding of diversification of AGEs in *Providencia*.

### Materials and Methods

*Providencia rettgeri* PROV275, PROV002, and PROV087 (Table S1) causing nosocomial infections were recovered from three different Chinese public hospitals in 2014, 2013, and 2016, respectively. *Providencia alcalifaciens* PROV023 and PROV013 (Table S1) were collected from two different China livestock farms in 2017. Whole-genome sequencing of these five *Providencia* isolates were conducted with a sheared DNA library with average size of 15 kb (ranged from 10 kb to 20 kb) on a PacBio RSII sequencer (Pacific Biosciences, CA, USA) and further genome sequencing of these five *Providencia* isolates were submitted to GenBank under accession numbers CP059298, CP059345, CP059348, CP059346, and CP059347, respectively. The GenBank accession numbers of all the six plasmids of these five isolates were listed in Table S1.
Results

Four Groups of 17 Chromosome-Borne AGEs

In this study, the complete genome sequences from Providencia rettgeri PROV275, PROV002, and PROV087 and Providencia alcalifaciens PROV023 and PROV013 were determined, and then a total of seven chromosome-borne AGEs were identified: i) Tn6860, Tn6861, and a 102.1-kb Tn7-related element T7RE<sub>PROV087</sub> from strains PROV275, PROV002, and PROV087, respectively; ii) Tn6862 and a 35.9-kb T7RE<sub>PROV023</sub> from strain PROV023; and iii) Tn6873 and Tn6876 from strain PROV013. Then, a detailed sequence comparison was applied to a collection of four groups of 17 chromosome-borne AGEs (16 of them from Providencia): i) seven related ICEs Tn6512, Tn6860, Tn6861, Tn6862, Tn6863<sup>18</sup>, Tn6864<sup>11</sup>, and Tn6865; ii) three related IMEs Tn6872, Tn6873, and Tn6874; iii) two related IMEs Tn6875 and Tn6876; and iv) Tn7 and its four derivatives T7RE<sub>PROV087</sub>, T7RE<sub>PROV023</sub>, a 58.0-kb T7RE<sub>MFI</sub>, and a 40.7-kb T7RE<sub>Pr-15.2-50</sub> (Table 1). At least 52 drug resistance genes, involved in resistance to 15 different categories of antimicrobials and heavy metal, were identified in 15 (expect for Tn6872 and Tn6875) of these 17 AGEs (Figure 1 and Table S4). All of these T7RE, T1696RE (see below), and T21RE (see below) elements could not be recognized as intact transposons due to truncation of relevant core transposition modules.

Six Tn6512-Related ICEs Tn6860, Tn6861, Tn6862, Tn6863, Tn6864, and Tn6865

All these seven ICEs including Tn6512 were integrated into the same 17-bp target site located within the 5' end of the chromosomal gene <i>pyrC</i> (peptide chain release factor 3).<sup>32</sup> These seven ICEs (Figure S1) had similar conserved backbones, containing <i>int</i> (integrate), <i>xis</i> (excisionase), <i>rlx</i> (relaxase), <i>oriT</i> (origin of conjugative replication), <i>cpl</i> (coupling protein), a TivF-type T4SS gene set, and <i>attL</i>/<i>R</i> (attachment sites at the left/right end). A toxin-antitoxin system was encoded by each of the five ICEs (hipB4 in Tn6512, and <i>abiE1–ii</i> in Tn6860, Tn6861, Tn6863, and Tn6864) except for Tn6862 and Tn6865. Type I restriction-modification system <i>hsdMSR</i> was carried by five of them except for Tn6512 and Tn6865.

The major differences were recognized at least 10 regions/sites (Figure 2 and Table S5) across the whole genomes of these seven ICEs. Firstly, a total of 23 events of acquisition of exogenous DNA region occurred at nine regions/sites within these seven ICEs. Secondly, a total of 12 events of backbone region deletion (10 of them were resulted from acquisition of exogenous DNA region) occurred at five regions/sites within these seven ICEs. Finally, totally 21 events of substitution of backbone region occurred at four regions/sites within six ICEs.

Each of these seven ICEs carried two to five accessory modules (Figure S1), including multidrug resistance (MDR) regions, composite transposons, IME, integron, insertion sequence (IS) elements, and so called “inserted regions”. Firstly, the 33.5-kb, 62.0-kb, 29.8-kb, 14.2-kb, 18.3-kb, and 33.0-kb MDR regions were integrated at the same site within Tn6860, Tn6861, Tn6862, Tn6863, Tn6864, and Tn6865, respectively. Secondly, an IS15DI (a minor variant of IS26)-composite transposon Tn6758 (containing <i>aphA1</i>), an IME Tn6912 (containing <i>bla</i><sub>HMS1</sub>-1), and an ISpu12-composite transposon Tn6896 were inserted into the different sites within Tn6512, Tn6860, and Tn6864, respectively. Tn6896 (Figure 3) was bracketed by 8-bp direct repeats (DRs; target site duplication signals for transposition), and carried <i>tetA</i> (C)-carrying ΔTn6309, and a concise class 1 integron In525 with the gene cassette array (GCA) <i>aadA2</i>–<i>ereA</i>–<i>dfrA32</i>. Thirdly, a class 9 integron In9-1 (containing <i>dfrA1</i>) was inserted within Tn6863. Finally, multiple IS elements and inserted regions were found at the different sites within these ICEs.

These six MDR regions (Figure 4) shared only a 0.5-kb Tn3-family Tn remnant together with an ISS<i>hrf</i>9 element, and they carried dramatically different profiles of resistance loci. The 33.5-kb MDR region carried three resistance loci: a truncated ISCR2–<i>flor</i>U region, a 12.6-kb region (containing <i>bla<sub>CTX-M-3</sub></i>-carrying ΔTn6502a) from IncM plasmid R69, and IS<i>Ec29</i>–<i>mpf</i>(E)–IS15DI unit. The 62.0-kb MDR region was composed of five resistance loci: a 23.4-kb Tn1969-related element T1696RE<sub>PROV002</sub> (see below), <i>tetA</i>(C)-carrying Tn6309, a concise class 1 integron In1793 (GCA: <i>gacG2</i>–<i>aadA6</i>–<i>gacG2</i>), a truncated IS26–mpf(A)–IS6100 unit, and <i>mer</i> region. The 29.8-kb, 14.2-kb, and 18.3-kb MDR regions shared a truncated ISCR2–<i>sul2</i> unit (containing <i>strAB</i>-carrying ΔTn5393c), but each of them integrated one or two additional resistance loci: i) an interrupted ISCR2–<i>flor</i>U region and an IS1006-composite transposon Tn6976 (which was bracketed by 5-bp DRs and contained two resistance loci: <i>aphA1</i>-carrying ΔTn4352 and IS26–<i>aacC4</i>–<i>aph</i>
(4)-Ia–ISEc59 unit) in 29.8-kb MDR region; ii) ISCR2–floR unit in 14.2-kb MDR region; and iii) ISCR2–erm42 unit and a truncated ISCR2–tet(X6) unit in 18.3-kb MDR region. The 33.0-kb MDR region had the sole resistance locus In1571, whose 5'-conserved segment (5'-CS: intI–attI1) was interrupted by IS26. In1571 was a complex class 1 integron, which contained two resistance loci: GCA (also called VR1: variable region 1) fosC2–aacA4'-8–ereA1e–blaOXA-21–dfrA1r, and VR2 composed of ISCR1–qnrVCI unit and a truncated ISCR1–blaPER-4 unit.

| Group | Accessory Genetic Element | Accession Number | Chromosomal Nucleotide Position | Length (bp) | Host Bacterium | Reference |
|-------|---------------------------|------------------|---------------------------------|-------------|----------------|-----------|
| Tn6512-related ICEs | Tn6512 | AY090559 | Not applicable | 88,549 | Providencia rettgeri | 107 |
| | Tn6860 | CP059298 | 575696.691964 | 116,269 | Providencia rettgeri | PROV275 |
| | Tn6861 | CP059345 | 727272.869956 | 142,685 | Providencia rettgeri | PROV002 |
| | Tn6862 | CP059348 | 704853.810761 | 105,909 | Providencia alcalifaciens | PROV023 |
| | Tn6863 | GQ463139 | Not applicable | 96,586 | Providencia alcalifaciens | Bari |
| | Tn6864 | MT219827 | Not applicable | 107,207 | Providencia rettgeri | RF14-2 |
| | Tn6865 | CP031123 | 751328.820050 | 68,723 | Providencia huaxiensis | WCHP000369 |
| Tn6872-related IMEs | Tn6872 | LR134189 | 025117.3045606 | 20,490 | Providencia rustigianii | NCTC6933 |
| | Tn6873 | CP059346 | 2973270.3043055 | 69,786 | Providencia alcalifaciens | PROV013 |
| | Tn6874 | AP022371 | 3358722.3420122 | 61,401 | Providencia rettgeri | BML2496 |
| Tn6875-related IMEs | Tn6875 | CP031508 | 1032159.1038038 | 5880 | Providencia stuartii | FDAARGOS_87 |
| | Tn6876 | CP059346 | 2583916.2605820 | 21,905 | Providencia alcalifaciens | PROV013 |
| Tn7-related elements | Tn7 | KX117211 | Not applicable | 14,067 | Escherichia coli | 3.5-R3 |
| | 102.1-kb T7REPROV087 | CP059347 | 33162.135304 | 102,143 | Providencia rettgeri | PROV087 |
| | 35.9-kb T7REPROV023 | CP059348 | 47906.83831 | 35,926 | Providencia alcalifaciens | PROV023 |
| | 58.0-kb T7REMF1 | CP048621 | 1077700.1135740 | 58,041 | Providencia stuartii | MFI |
| | 40.7-kb T7REPr-15-2-50 | CP039844 | 31494.72272 | 40,779 | Providencia rettgeri | Pr-15-2-50 |
Three Related IMEs Tn6872, Tn6873, and Tn6874

All these three IMEs were integrated 102-bp upstream the chromosomal gene *mutS* (DNA mismatch repair protein). The prototype IME Tn6872 was initially found in *Providencia rustigianii* NCTC6933 (accession number LR134189). These three IMEs (Figure 5) shared the core IME backbone markers *int*, *oriT* and *attL/R*. Tn6872 harbored its three unique backbone regions *orf201*–to–*orf207*, *orf627*–to–*orf1068*, and *orf1338*–to–*uvrD*; correspondingly, Tn6873/Tn6874 carried their unique backbone regions *hsdMSR* (type I restriction-modification system)–*mrr*–*orf411*, *orf597*–to–*orf1107*, and *orf1311*–to–*hnhc*, instead of the above three Tn6872-unique regions, respectively. No accessory modules were found in Tn6872, but a 47.0-kb MDR region and a 38.5-kb MDR region were integrated at the same site within Tn6873 and Tn6874, respectively.

The 47.0-kb MDR region (Figure 6) contained six resistance loci: a concise class 1 integron In27 (GCA: *dfrA12*–*gcuF*–*aadA2*), ΔTn4352 containing *aphA1*, IS26–*aph(4)-Ia*–*aacC4*–IS*Ec59* unit, IS*CR2*–*sul2* unit, a truncated IS*CR2*–*floR* unit, and a Tn21-related transposon Tn6974 (see below). The 38.5-kb MDR region carried an IME Tn6977, and a Tn1696-related transposon Tn6910 (see below).

Two Related IMEs Tn6875 and Tn6876

Tn6875 and Tn6876 were integrated 494-bp downstream the chromosomal gene *smpB* (SsrA-binding protein). The prototype IME Tn6875 was initially found in *Providencia stuartii* FDAARGOS_87 (accession number CP031508). Tn6875 and Tn6876 (Figure 7) shared the core IME backbone markers *int* and *attL/R*, but they displayed two major modular variations: i) Tn6875 contained its unique backbone region *rimI*–to–*orf567*, while Tn6876 harbored its unique backbone region containing *orf288*; and ii) Tn6875 carried no accessory modules, but Tn6876 acquired a 20.0-kb MDR region composed of *tetA(B)*-containing Tn10 and 10.4-kb T1696REPROV013 (see below).

Four Tn7 Derivatives T7REPROV087, T7REPROV023, T7RE_MF1, and T7RE_Pr-15-2-50

Tn7 and its three derivatives T7REPROV087, T7REPROV023, and T7RE_Pr-15-2-50 were inserted 25-bp downstream of the chromosomal gene glmS (glutamine-fructose-6-phosphate aminotransferase), while the remaining one T7RE_MF1 was inserted within the chromosomal gene *orf1389* (carbohydrate porin). T7REPROV087, T7REPROV023, T7RE_MF1, and...
Figure 2 Massive gene acquisition and loss across whole-genomes of seven related ICEs Tn6512, Tn6860, Tn6861, Tn6862, Tn6863, Tn6864, and Tn6865. 

Notes: Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading in light blue or light orange denotes regions of homology (nucleotide identity ≥90%), light pink (nucleotide identity <90%). The seven Tn6512-related ICEs are listed at vertical coordinates, while the 10 genomic regions/sites (numbers in circles) with major modular differences (for details in Table S5) are shown at horizontal coordinates. Please also refer to the detailed genetic organization of the accessory modules inserted within regions 1, 5, 6, 7, 9, and 10, plus site 4 (Figure S1), site 3 (Figure 4), and region 8 (Figures 3 and S1).
Figure 3 Organization of Tn6896 from Tn6864, and comparison to related regions.

**Notes**: Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading in light blue denotes regions of homology (nucleotide identity ≥95%). Numbers in brackets indicate nucleotide positions within Tn6864. Accession number of Tn6309<sup>48</sup> used as reference is KX710094.

Figure 4 Comparison of MDR regions from Tn6860, Tn6861, Tn6862, Tn6863, Tn6864, and Tn6865.

**Notes**: Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading in light blue or light orange denotes regions of homology (nucleotide identity ≥90%), light pink (nucleotide identity < 90%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains PROV275 and PROV023, Tn6863, Tn6864; and the chromosomes of strains PROV002 and WCHPr000369, respectively. Accession numbers of ISCR1–<i>bla<sub>PER-4</sub></i> unit,<sup>49</sup> Tn6502a<sup>50</sup>, Tn501<sup>51</sup>, ISCR2–<i>tet(X6)</i> unit,<sup>52</sup> and Tn5993c<sup>2</sup> used as reference are KU133341, KF914891, Z00027, CP047340, and AF262622, respectively.
T7RE<sub>Pr-15-2-50</sub> (Figure 8) differed from Tn<sub>7</sub> by acquisition of 95.9-kb, 29.7-kb, 51.8-kb, and 33.6-kb MDR regions, respectively, instead of In<sub>2</sub>-4 (GCA: <i>dfrA1</i>–<i>sat2</i>–<i>aadA1</i>). The above acquisition events resulted in the same truncation of <i>tnsABCDE</i> in these four Tn<sub>7</sub> derivatives. Moreover, <i>tnsB</i> of T7RE<sub>Pr-15-2-50</sub> was interrupted by insertion of IS<sub>Prst3</sub>.

These four MDR regions (Figure 9) shared only a 1.7-kb Tn<sub>1722</sub> remnant together with an IS<sub>26</sub> element, and they displayed considerable modular diversification: i) a concise class 2 integron In<sub>2</sub>-16 with GCA <i>lnu(F)</i><sub>1b</sub>–<i>dfrA1</i>–<i>aadA1</i> in the MDR regions from T7RE<sub>PROV087</sub>, T7RE<sub>PROV023</sub>, and T7RE<sub>MF1</sub>, while a complex In<sub>2</sub>-16 carrying VR1/GCA as
above and additionally two copies of VR2 (ISCR1–bla<sub>MBL</sub>–bla<sub>NDM-1</sub> unit) in the MDR region from T7RE<sub>Pr-15-2-50</sub>; ii) a truncated ISCR2–floR unit, ISCR2–sul2 unit, IS26–aacC4–aph(4)-Ia–ISEc59 unit, and a truncated chrA–orf98 unit shared by the MDR regions from T7RE<sub>PROV087</sub> and T7RE<sub>MF1</sub>; iii) a 71.2-kb Tn21-related element T21RE<sub>PROV087</sub> (see below) and a 21.2-kb T21RE<sub>MF1</sub> (see below) in the MDR regions from T7RE<sub>PROV087</sub> and T7RE<sub>MF1</sub>, respectively; iv) IS26–mph(E)–IS26 unit acquired by the MDR regions from T7RE<sub>MF1</sub> and T7RE<sub>PROV023</sub>; v) intact or truncated Tn4352 (containing aphA1) in the MDR regions from T7RE<sub>MF1</sub> and T7RE<sub>Pr-15-2-50</sub>; vi) a complex class 1 integron In37 with four resistance loci: VR1/GCA (aacA4cr–bla<sub>OXA-1</sub>–catB3–arr-3), a disrupted Tn2 (containing bla<sub>TEM-1</sub>), ΔTn6502a (containing bl<sub>EC29</sub>–ampC–mx–mecC), and VR2 (ISCR1–rmtB unit) in the MDR region from T7RE<sub>PROV023</sub>; and vii) ΔTn1548 carrying ISCR1–armA unit and an interrupted ISEc29–mph(E)–IS15DI unit in the MDR region from T7RE<sub>Pr-15-2-50</sub>.

**Three Tn1696 Derivatives Tn6910, T1696RE<sub>PROV002</sub>, and T1696RE<sub>PROV013</sub>**

Herein, a detailed sequence comparison (Figure 10) was applied to the three Tn1696 derivatives Tn6910, T1696RE<sub>PROV002</sub>, and T1696RE<sub>PROV013</sub> (identified as the inner components of Tn6874, Tn6861, and Tn6876, respectively; see above), together with Tn1696, Tn6338, and Tn6909 derived from GenBank. Tn1696 was one of the Tn3-family prototype unit transposons, and it was originally found in plasmid R1003 from *Pseudomonas aeruginosa* and contained a core backbone structure: IRL (inverted repeat left)–tnpA–tnpR (resolvase)–res (resolution site)–mer–IRR (inverted repeat right). These five Tn1696 derivatives exhibited three major modular differences across the core backbones: i) an intact version of *tnpAR–res* in Tn6338, Tn6909, and Tn6910, while a truncated version in T1696RE<sub>PROV002</sub> and T1696RE<sub>PROV013</sub>; ii) a T1696 mer locus in Tn6338 and Tn6910, and a Tn21 mer locus instead in Tn6909; none of mer locus in T1696RE<sub>PROV002</sub> and T1696RE<sub>PROV013</sub>; and iii) intact IRL/R in Tn6910, and IRL/R interrupted by insertion of IS4321 in Tn6909; only IRL in T1696RE<sub>PROV013</sub>, and IRL interrupted by insertion of IS5075 in Tn6338; only IRR in Tn6338; none of IRL/R in T1696RE<sub>PROV002</sub>.

Each of these five Tn1696 derivatives acquired one or more integrons into res site, instead of In4 (GCA: *aacC1–gcuE–aadA2–cmlA1*) in Tn1696: a complex class 1 integron In469 in Tn6338, a different complex In469 in T1696RE<sub>PROV002</sub>, a concise class 1 integron In27 (GCA: *dfrA12–gcuF–aadA2*) in T1696RE<sub>PROV013</sub>, a distinct complex In27 in Tn6909, and the four tandem concise class 1 integrons including In1849 (GCA: *dfrA1–aacA4′–bla<sub>OXA-1</sub>*) , two copies of In994 (GCA: *bla<sub>MMA-1</sub>–*), and In151 (GCA: *catB8–lmu(F)*) in Tn6910. These two complex In469 harbored the same VR1/GCA (arr-3–dfrA27–aadA16), but differed from each other by capturing different additional VR modules: VR2 (ISCR1–qnrB4–bla<sub>NDM-1</sub> unit) in In469 from Tn6338, and VR2 (ISCR1–bla<sub>PER-1</sub> unit) together with VR3 (a truncated ISCR1–aphA6 unit, ΔISSod18,
and ISEc29) in In469 from T1696REPROV087. The complex In27 from Tn6909 carried GCA (VR1) as above and still captured four additional resistance modules: a truncated ISCR1–qnrA1 unit (VR2), chrA–orf98 unit, a truncated IS26–mph (A)–IS6100 unit, and a concise class 1 integron In1209 (GCA: aadA1b–dfrA1b–aacA7–blaVIM-1).
Three Tn21 Derivatives Tn6974, T21REPROV087, and T21REMF1

Herein, a detailed sequence comparison (Figure 11) was applied to the three Tn21 derivatives Tn6974, T21REPROV087, and T21REMF1 (identified as the inner components of Tn6873, T7REPROV087, and T7REMF1, respectively; see above), together with Tn21, Tn502a, Tn548, and Tn125 used as reference are X61367, AF262622, CP042858, HM749967, KF914891, CP042858, AF550415, and JN872328, respectively.

Figure 9 Comparison of MDR regions from four Tn7 derivatives.

Notes: Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading in light blue or light orange denotes regions of homology (nucleotide identity ≥ 95%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains PROV087, MF1, PROV023, and Pr-15-2-50, respectively. Accession numbers of Tn1722, Tn5393c, chrA-orf98 unit, Tn2, Tn502a, Tn548, Tn1548, and Tn125 used as reference are X61367, AF262622, CP042858, HM749967, KF914891, CP042858, AF550415, and JN872328, respectively.
differences: i) a Tn21 mer locus in Tn6975, and a Tn1696 mer locus instead in Tn6974; none of mer locus in T21RE_MF1 and T21RE_PROV087; and ii) IRL/R in Tn6974 and Tn6975, while only IRL in T21RE_PROV087 and T21RE_MF1.

Each of these four Tn21 derivatives acquired an integron instead of In2 (GCA: aadA1α) in Tn21: a concise class 1 integron In1808 (GCA: aacA4cr–blaOXA1–ΔcatB3–arr–3–dfrA27–aadA16) in Tn6974, a concise class 1 integron In27 in Tn6975, a distinct complex In27 in T21RE_MF1, and a complex class 1 integron In263 in T21RE_PROV087. The concise In27 contained three resistance loci: dfrA12–gcuF–aadA2 (GCA), chrA–orf98 unit, and IS26–mph(A)–IS6100 unit. The
Figure 11 Comparison of Tn21 and its three derivatives.

Notes: Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading in light blue denotes regions of homology (nucleotide identity ≥95%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains PROV013, MF1, and PROV087, respectively. Accession numbers of Tn21\(^35\) and Tn6975\(^37\) used as reference are AF071413 and CP041301, respectively.
complex In263 had a VR1/GCA (aacA4cr–arr-3) and additionally nine copies of VR2 (truncated ISCR1–bla_DHA-1 unit).

Transferability and Antimicrobial Susceptibility
Tn6862, which was selected to represent Tn6512-related ICEs, could be transferred from the wild-type PROV023 isolate into E. coli EC600 through conjugation, generating the transconjugant EC600/Tn6862. Both PROV023 and EC600/Tn6862 were highly resistant to amikacin with a minimum inhibitory concentration (MIC) value ≥64 μg/mL (Table S3) owing to production of aminoglycoside 3′-phosphotransferase.

Plasmids in the Five Isolates Sequenced in This Study
Providencia rettgeri PROV087 and Providencia alcalifaciens PROV023 carried no plasmids. Identified were an IncC plasmid pPROV275-1NDM (carrying blaNDM-1, bladMY-6, aacA4, rmtC, sul1, and qacED1), an IncPGZ276-NDM plasmid pPROV275-2NDM (having blaNDM-1, blaper-4, blaOXA-10, aadA1, aadB, aphA6, catA1, catB8, sul1, and qacED1) together with a Co3M plasmid pPROV275-qnrD (containing qnrD1) in Providencia rettgeri PROV275, an IncW plasmid pPROV002-IMP (carrying blIMP-4, blaNDM-1, aacA4, catB3, sul1, and qacED1) in Providencia rettgeri PROV002, and an IncPGZ276-NDM plasmid pPROV013-PER [containing blaper-4, blaoXa-1, aacA4, mph(E), msr(E), arr-3, catB3, iimu(G), sul1, and qacED1] together with an IncFII plasmid pPROV013-NR (harboring no resistance genes) in Providencia alcalifaciens PROV013.

Discussion
This work presents the complete sequences of seven chromosomal AGEs in Providencia, and they can be further divided into four groups: three Tn6512-related ICEs Tn6860, Tn6861, and Tn6862; one Tn6872-related IME Tn6873; one Tn6875-related IME Tn6876; and two Tn7-related elements T7REPROV087 and T7REPROV023. Newly identified are five (except for T7REPROV087 and T7REPROV023) of the above seven AGEs, together with one unit transposon Tn6974, one composite transposon Tn6976, and two integrons In1793 and In1808, all of which are located within these five AGEs. There are additional 10 newly designated (firstly designated in this study, but with previously determined sequences) AGEs: i) one IME Tn6912 located within Tn6860 sequenced in this study; ii) one ICEs Tn6865, four IMEs Tn6872, Tn6874, Tn6875, and Tn6977, two unit transposons Tn6910 and Tn6975, one composite transposon Tn6896, and one IS element ISPpre11 which come from GenBank and are included in the sequence comparison herein. This is also the first report of identifying Tn6872 and Tn6875 in Providencia. The two previously designated ICEs ICEPreChnRF14-2 were renamed herein as standard Tn designations Tn6863 and Tn6864, respectively.

The transposition of Tn7-related unit transposons, generating bracketed 5-bp DRs, is generally site-specific at 25-bp downstream of glmS, and these target sites with a consensus CCgcGtAAccTGcaAAacGcTcAGcTGATGta together with Tn7-related elements are presented in a wide range of bacteria as mentioned above. Three of the four Tn7-related elements (all bracketed by 5-bp DRs) characterized herein, as expected, are located downstream of glmS, but T7RE_MF1 is presented within orf1389. The presence of bracketed 5-bp DRs denotes that T7RE_MF1 is most likely transposed directly into orf1389 that does not contain the above consensus target site; similarly, additional target sites, not resembling that downstream of glmS, have been proposed previously for integration of Tn7-related unit transposons. Tn6512-related ICEs have 17-bp highly conserved attP (attachment site on the ICE) sequences with a consensus ATcATcTCtGACCCtGA. Integration of these ICEs needs the 17-bp relatively non-conserved attB (attachment site on the chromosome) target sites within the 5′ end of prfC, and can occur with up to 23.5% sequence mismatch between attB and attP. These attB sites together with Tn6512-related ICEs are widely distributed in Gram-negative bacteria as mentioned above. Taken together, Tn7-related unit transposons and Tn6512-related ICEs have a wide host range.

Conjugal transfer experiments confirm that Tn6512-related ICEs with the complete gene sets for conjugal transfer have the ability to transfer from the wild-type isolates of Proteus, Vibrio, Shewanella, and Providencia (this study) to the recipient bacteria E. coli, and that a circular extrachromosomal Tn6512 has the ability to transfer into the recipient bacteria Salmonella, Enterobacter, and Serratia, indicating that this group of ICEs have a very broad host range.

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This work presents the complete sequences of seven chromosomal AGEs in Providencia, and they can be further divided into four groups: three Tn6512-related ICEs Tn6860, Tn6861, and Tn6862; one Tn6872-related IME Tn6873; one Tn6875-related IME Tn6876; and two Tn7-related elements T7REPROV087 and T7REPROV023. Newly identified are five (except for T7REPROV087 and T7REPROV023) of the above seven AGEs, together with one unit transposon Tn6974, one composite transposon Tn6976, and two integrons In1793 and In1808, all of which are located within these five AGEs. There are additional 10 newly designated (firstly designated in this study, but with previously determined sequences) AGEs: i) one IME Tn6912 located within Tn6860 sequenced in this study; ii) one ICEs Tn6865, four IMEs Tn6872, Tn6874, Tn6875, and Tn6977, two unit transposons Tn6910 and Tn6975, one composite transposon Tn6896, and one IS element ISPpre11 which come from GenBank and are included in the sequence comparison herein. This is also the first report of identifying Tn6872 and Tn6875 in Providencia. The two previously designated ICEs ICEPreChnRF14-2 were renamed herein as standard Tn designations Tn6863 and Tn6864, respectively.

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Conjugal transfer experiments confirm that Tn6512-related ICEs with the complete gene sets for conjugal transfer have the ability to transfer from the wild-type isolates of Proteus, Vibrio, Shewanella, and Providencia (this study) to the recipient bacteria E. coli, and that a circular extrachromosomal Tn6512 has the ability to transfer into the recipient bacteria Salmonella, Enterobacter, and Serratia, indicating that this group of ICEs have a very broad host range.
Integrons of Tn6872-related IMEs needs 24-bp highly conserved attB sequences (with a consensus AACCCTAcAATTAACCTACCTTA) located 102-bp upstream of mutS. These attB sequences together with Tn6872-related elements are mainly found in Morganellaceae including *Providencia* (this study), *Proteus* (eg *Proteus mirabilis* with an accession number CP053894), and Morganella (eg *M. morganii* with an accession number CP064830). Integration of Tn6875-related IMEs recognizes 11-bp highly conserved attB sequences (with a consensus CcTatTTTATC) located 494-bp downstream of smpB. These attB sites together with Tn6875-related elements are mainly distributed in Morganellaceae including *Providencia* (this study), *Proteus* (eg *Proteus mirabilis* with an accession number CP042857), and Morganella (eg *M. morganii* with an accession number CP068145), as well as Enterobacteriaceae including *Salmonella* (eg *S. enterica* with an accession number CP053319) and *Escherichia* (eg *E. coli* with an accession number CP056565). It seems that these two groups of IMEs spread mainly in Morganellaceae and/or Enterobacteriaceae.

Five (except for Tn6862 and Tn6865) of the seven Tn6512-related ICEs encode the toxin-antitoxin systems: HipBA in Tn6512, and AbiEi-ii in Tn6860, Tn6861, Tn6863, and Tn6864. These toxin-antitoxin systems have been shown to stabilize Tn6512-related ICEs by preventing their loss when they are extrachromosomal.\(^43,44\) The loss of the AbiEi-ii toxin-antitoxin system from Tn6512 and Tn6862 is due to the substitution of ICE backbone region 7 as shown in Figure 2, while that from Tn6865 is caused by the insertion of the MDR region within the ICE backbone gene umuC (site 3 in Figure 2). Five (except for Tn6512 and Tn6865) of the seven Tn6512-related ICEs and two (except for Tn6872) of the three Tn6872-related IMEs carry a type I restriction-modification system HsdMSR. This system can degrade the unmethylated incoming DNA to stabilize AGEs against challenges by competitor elements.\(^45\) Tn6512 lose the HsdMSR system because of the substitution of backbones region 5 (Figure 2). The loss of this system from Tn6865 is on account of the insertion of the MDR region. Tn6862 do not harbor this system due the substitution of orf201–to–orf207 region (Figure 5). It is speculated herein that the loss of toxin-antitoxin or restriction-modification systems might lead to the instability of relevant ICEs and IMEs after they are excised from the chromosomes.\(^46\)

Tn7-related unit transposons accomplish transposition using the core transposition module tnsABCDE.\(^23\) All the four Tn7-related elements analyzed herein have undergone the truncation of tnsABCDE, which results from the integration of MDR regions. The lesion of tnsABCDE in these four Tn7-related elements would lose their ability of intracellular transfer.

Each of 13 of the totally characterized 17 AGEs carries a MDR region, and notably the resulting 13 MDR regions carry at least 49 drug resistance genes, which can be grouped into 15 different categories of antimicrobials and heavy metal, including β-lactam, aminoglycoside, macrolide, tetracycline, trimethoprim, phenicol, sulphonamide, lincosamide, rifampicin, quinolone, fosfomycin, bleomycin, chromate, quaternary ammonium compound, and mercury. Among these 49 drug resistance genes, predominantly found are those resistant to aminoglycosides (n=14) and β-lactams (n=9), which might be due to the wide clinical use of aminoglycosides and β-lactams against *Providencia*-induced infections. Intact or truncated versions of Tn21- and Tn1696-related transposons, Tn4352, Tn5393c, In27, ISCR2–floR unit, ISCR2–sul2 unit, ISEc29–mph(E)–IS15DI unit, and IS26–aacC4–aph(4)-Ia–ISEc59 unit are frequently found within these 13 MDR regions, indicating the assembly of these MDR regions from various collections of AGEs and associated resistance genes via complex transposition and homologous recombination. In addition, there are so called “concise” accessory modules (Tn6912, Tn6578, Tn6896, In2-4, In9-1, and mer region) that are presented in six AGEs, including four harboring MDR regions and two not. These “concise” accessory modules harbor at least 10 drug resistance genes, which mediate the resistance to six different categories of antimicrobials and heavy metal, including aminoglycoside, β-lactam, tetracycline, trimethoprim, macrolide, and mercury.

On the one hand, these four groups of AGEs have a wide range of hosts including *Providencia*, and they are stable in the host bacteria and further able to transfer across different bacterial species. On the other hand, these four groups of AGEs display high-level diversification in modular structures, which have complex mosaic natures and carry a large number of drug resistance genes, and particularly different MDR regions are presented in these AGEs. Integration of these AGEs into the *Providencia* chromosomes contributes to the accumulation and distribution of drug resistance genes and enhances the ability of *Providencia* isolates to survive under drug selection pressure.
Data Sharing Statement
The complete chromosome sequences of the PROV275, PROV002, PROV023, PROV013, and PROV087 isolates were submitted to GenBank under accession numbers CP059298, CP059345, CP059348, CP059346, and CP059347, respectively. The GenBank accession numbers of all the six plasmids of these five isolates were listed in Table S1.

Ethics Statement
This study used the bacterial isolates obtained from the Chinese public hospitals as listed in Table S1. The bacterial isolation was part of the routine hospital laboratory procedures and the bacterial isolates involved in this study were not isolated from patients directly. The genetic analysis of accessory genetic elements in this study had no experimentation involving humans or animals. According to relevant legislation/policy of China (http://www.nhc.gov.cn/fzs/s3576/201808/14ee8ab2388440c4a44ecee0f24e064c.shtml), this study was not subject to ethical review. The research involving biohazards and all related procedures were approved by the Biosafety Committee of the Beijing Institute of Microbiology and Epidemiology.

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Disclosure
The authors report no conflicts of interest in this work.

References
1. O’Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 2000;13(4):534–546. doi:10.1128/CMR.13.4.534
2. Stock I, Wiedemann B. Natural antibiotic susceptibility of Providencia stuartii, P. rettgeri, P. alcalifaciens and P. rustigianii strains. J Med Microbiol. 1998;47(7):629–642. doi:10.1099/00222615-47-7-629
3. Szwiatlo E, Kocka FE. Inducible expression of an aminoglycoside-acetylating enzyme in Providencia stuartii. J Antimicrob Chemother. 1987;19(1):27–30. doi:10.1093/jac/19.1.27
4. Yaghoubi S, Zekiy AO, Krutova M, et al. Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review. Eur J Clin Microbiol Infect Dis. 2021;1–20.
5. Samonis G, Korbila IP, Manaki S, et al. Trends of isolation of intrinsically resistant to colistin Enterobacteriaceae and association with colistin use in a tertiary hospital. Eur J Clin Microbiol Infect Dis. 2014;33(9):1505–1510. doi:10.1007/s10096-014-2097-8
6. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2):10. doi:10.1128/microbiolspec.VMBF-0016-2015
7. Johnson CM, Grossman AD. Integrative and conjugative elements (ICEs): what they do and how they work. Annu Rev Genet. 2015;49(1):577–601. doi:10.1146/annurev-genet-112414-055018
8. Coetzee JN, Datta N, Hedges RW. R factors from Proteus rettgeri. J Gen Microbiol. 1972;72(3):543–552. doi:10.1099/00221287-72-3-543
9. Olaitan AO, Diene SM, Assous MV, Relain J-M. Genomic plasticity of multidrug-resistant NDM-1 positive clinical isolate of Providencia rettgeri. Genome Biol Evol. 2016;8(3):723–728. doi:10.1093/gbe/evv195
10. Flannery EL, Mody L, Mobley HLT. Identification of a modular pathogenicity island that is widespread among urease-producing uropathogens and shares features with a diverse group of mobile elements. Infect Immun. 2009;77(11):4887–4894. doi:10.1128/IAI.00705-09
11. Li R, Lu X, Peng K, et al. Deciphering the structural diversity and classification of the mobile tigecycline resistance gene tet(X)-bearing plasmidome among bacteria. mSystems. 2020;5(2):e00134–e00120. doi:10.1128/mSystems.00134-20
12. Ryan MP, Armshaw P, O’Halloran J, Pembroke JT. Analysis and comparative genomics of R997, the first SXT/R391 integrative and conjugative element (ICE) of the Indian Sub-Continent. Sci Rep. 2017;7(1):8562. doi:10.1038/s41598-017-08735-y
13. Taviani E, Ceccarelli D, Lazzaro N, et al. Environmental Vibrio spp., isolated in Mozambique, contain a polymorphic group of integrative conjugative elements and class 1 integrons. FEMS Microbiol Ecol. 2008;64(1):45–54. doi:10.1111/j.1574-6941.2008.00455.x
14. Pembroke JT, Piterina AV. A novel ICE in the genome of Shewanella putrefaciens W3-18-1: comparison with the SXT/R391 ICE-like elements. FEMS Microbiol Lett. 2006;256(1):80–88. doi:10.1111/j.1574-6968.2006.00452.x
15. Xu J, Jia H, Cui G, et al. ICEApChn1, a novel SXT/R391 integrative conjugative element (ICE), carrying multiple antibiotic resistance genes in Actinobacillus pleuropneumoniae. Vet Microbiol. 2018;220:18–23. doi:10.1016/j.vetmic.2018.05.002
16. López-Pérez M, Gonzalez A, Rodriguez-Valera F. Genomic diversity of “deep ecotype” Alteromonas macidclisi isolates: evidence for Pan-Mediterranean clonal frames. Genome Biol Evol. 2013;5(6):1220–1232. doi:10.1093/gbe/evt089
17. Wang H, Sun B, Xie G, Wan X, Huang J, Song X. Spotlight on a novel bactericidal mechanism and a novel SXT/R391-like integrative and conjugative element, carrying multiple antibiotic resistance genes, in *Pseudoalteromonas flavipulchra* strain CDM8. *Microbiol Res.* 2021;242:126598. doi:10.1016/j.micres.2021.126598

18. Wozniak RAF, Fouts DE, Spagnolletti M, et al. Comparative ICE genomics: insights into the evolution of the SXT/R391 family of ICEs. *PLoS Genet.* 2009;5(12):e1000786. doi:10.1371/journal.pgen.1000786

19. Guedon G, Libante V, Coluzzi C, Payot S, Leblond-Bourget N. The obscure world of integrative and mobilizable elements, highly widespread plasmids that pirate bacterial conjugative systems. *Genes.* 2017;8(11):337. doi:10.3390/genes8110337

20. Carraro N, Rivard N, Cecarelli D, Colwell RR, Burlus V. IncA/C conjugative plasmids mobilize a new family of multidrug resistance islands in clinical *Vibrio cholerae* Non-O1/Non-O139 isolates from Haiti. *mBio.* 2016;7(4):e00509-0e00516. doi:10.1128/mBio.00509-16

21. Soliman AM, Shimamoto T, Nariya H, Shimamoto T. Emergence of *Salmonella* genomic island 1 variant SG1-W in a clinical isolate of *Providencia stuartii* from Egypt. *Antimicrob Agents Chemother.* 2019;63(1):e01793-e01718. doi:10.1128/AAC.01793-18

22. Luo X, Yin Z, Zeng L, et al. Chromosomal integration of huge and complex *bla*<sub>NDM</sub>-carrying genetic elements in Enterobacteriaceae. *Front Cell Infect Microbiol.* 2021;11:690799. doi:10.3389/fmicb.2021.690799

23. Peters JE. Targeted transposition with Tn7 elements: safe sites, mobile plasmids, CRISPR/Cas and beyond. *Mol Microbiol.* 2019;112(6):1635–1644. doi:10.1111/mmi.14383

24. Parks AR, Peters JE. Transposon Tn7 is widespread in diverse bacteria and forms genomic islands. *J Bacteriol.* 2007;189(5):2170–2173. doi:10.1128/JB.01536-06

25. Ramírez MS, Piheiro S, Centrón D. Novel insights about class 2 integrons from experimental and genomic epidemiology. *Antimicrob Agents Chemother.* 2010;54(2):699–706. doi:10.1128/AAC.01392-08

26. Zhang Y, Cao Y, Zhang L, Hikichi Y, Ohnishi K, The LJ. Tn7-based genomic integration is dependent on an attTn7 box in the glmS gene and is site-specific with monocopy in *Ralstonia solanacearum* species complex. *Mol Plant Microbe Interact.* 2021;34(7):720–725. doi:10.1094/MPMI-11-20-0325-SC

27. Ramírez MS, Quiroga C, Centrón D. Novel arrangement of a class 2 integron in two non-epidemiologically related isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2005;49(12):5179–5181. doi:10.1128/AAC.49.12.5179-5181.2005

28. Fu J, Zhang J, Yang L, et al. Precision methylation and *in vivo* methylation kinetics characterization of *Klebsiella Pneumoniae*. Genom Proteom Bioinform. 2021. doi:10.1016/j.gpb.2021.04.002

29. De Coster W, D’Hert S, Schultz DT, Cruts M, Van Broeckhoven C. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics.* 2018;34(15):2666–2669. doi:10.1093/bioinformatics/bty149

30. Ou D, Shen Y, Hu L, et al. Comparative analysis of KPC-2 encoding chimera plasmids with multi-replicon IncR:IncN1 or IncFlu<sub>ANTISEPTIC</sub>-Inc<sub>1703-KPC</sub>-IncN1. *Infect Drug Resist.* 2019;12:285–296. doi:10.2147/IDR.S189168

31. CLSI. *CLSI supplement M100*. In: *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

32. Hochhut B, Beaber JW, Woodgate R, Waldor MK. Formation of chromosomal tandem arrays of the SX7 element and R391, two conjugatively chromosomally integrating elements that share an attachment site. *J Bacteriol.* 2001;183(4):1124–1132. doi:10.1128/JB.183.4.1124-1132.2001

33. Botelho J, Schuenenburg H. The role of integrative and conjugative elements in antibiotic resistance evolution. *Trends Microbiol.* 2021;29(1):8–18. doi:10.1016/j.tim.2020.05.011

34. Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. *PLoS One.* 2015;10(5):e0123603. doi:10.1371/journal.pone.0123603

35. Partridge SR, Brown HJ, Stokes HW, Hall RM. Transposons Tn1666 and Tn21 and their integrons In4 and In2 have independent origins. *Antimicrob Agents Chemother.* 2001;45(4):1263–1270. doi:10.1128/AAC.45.4.1263-1270.2001

36. Liang Q, Fang X, Hu L, et al. Sequencing and genomic diversity analysis of IncHE5 plasmids. *Front Microbiol.* 2018;9:3318. doi:10.3389/fmicb.2018.03318

37. Desloges I, Taylor JA, Leclere J-M, et al. Identification and characterization of OmpT-like proteases in uropathogenic *Escherichia coli* clinical isolates. *MicrobiologyOpen.* 2019;8(11):e915. doi:10.1002/mbo3.915

38. Misra R, McKenzie GJ, Yi L, Lee CA, Craig NL. Characterization of the Tns7-attTn7 complex that promotes site-specific insertion of Tn7. *Mol DNA.* 2010;1(1):18. doi:10.1186/1759-8753-1-18

39. McGrath BM, Pembroke JT. Detailed analysis of the insertion site of the mobile elements R997, pMERPH, R392, R705 and R391 in *E. coli* K12. *FEMS Microbiol Lett.* 2004;237(1):19–26. doi:10.1016/j.femsle.2003.03.018

40. Li X, Du Y, Du P, et al. SXT/R391 integrative and conjugative elements in *Proteus* species reveal abundant genetic diversity and multidrug resistance. *Sci Rep.* 2016;6:37372. doi:10.1038/srep37372

41. Sarkar A, Morita D, Ghosh A, et al. Altered integrative and conjugative elements (ICEs) in recent *Vibrio cholerae* O1 isolated from cholera cases, Kolkata, India. *Front Microbiol.* 2019;10:2072. doi:10.3389/fmi.2019.02072

42. Fang Y, Wang Y, Li Z, et al. Distribution and genetic characteristics of SXT/R391 integrative conjugative elements in *Shewanella* spp. from China. *Front Microbiol.* 2018;9:920. doi:10.3389/fmicb.2018.00920

43. Carraro N, Poulain D, Burlus V. Replication and active partition of integrative and conjugative elements (ICEs) of the SXT/R391 family: the line between ICEs and conjugative plasmids is getting thinner. *PLoS Genet.* 2015;11(6):e1005298. doi:10.1371/journal.pgen.1005298

44. Wozniak RAF, Waldor MK. A toxin-antitoxin system promotes the maintenance of an integrative conjugative element. *PLoS Genet.* 2009;5(3):e1000439. doi:10.1371/journal.pgen.1000439

45. Oliveira PH, Touchon M, Rocha EPC. The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. *Nucleic Acids Res.* 2014;42(16):10618–10631. doi:10.1093/nar/gku734

46. Huguet KT, Gonnell M, Doublet B, Cloeckaert A. A toxin antitoxin system promotes the maintenance of the IncAC-mobilizable *Salmonella* genomic island 1. *Sci Rep.* 2016;6(1):32285. doi:10.1038/srep32285

47. Peters JE, Craig NL. Tn7: smarter than we thought. *Nat Rev Mol Cell Biol.* 2001;2(11):806–814. doi:10.1038/35099006

48. Sun F, Zhou D, Sun Q, et al. Genetic characterization of two fully sequenced multi-drug resistant plasmids pP10164-2 and pP10164-3 from *Lecierca adecarboxylata*. *Sci Rep.* 2016;6(1):33982. doi:10.1038/srep33982
49. Xie L, Wu J, Zhang F, et al. Molecular epidemiology and genetic characteristics of various blaper genes in Shanghai, China. *Antimicrob Agents Chemother*. 2016;60(6):3849–3853. doi:10.1128/AAC.00258-16

50. Chen L, Hu H, Chavda KD, et al. Complete sequence of a KPC-producing IncN multidrug-resistant plasmid from an epidemic *Escherichia coli* sequence type 131 strain in China. *Antimicrob Agents Chemother*. 2014;58(4):2422–2425. doi:10.1128/AAC.02587-13

51. Stanisich VA, Bennett PM, Richmond MH. Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in *Pseudomonas aeruginosa*. *J Bacteriol*. 1977;129(3):1227–1233. doi:10.1128/jb.129.3.1227-1233.1977

52. L’Abée-Lund TM, Sørum H. Functional Tn5393-like transposon in the R plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *Salmonicida* isolated in Norway. *Appl Environ Microbiol*. 2000;66(12):5533–5535. doi:10.1128/AEM.66.12.5533-5535.2000

53. Wrighton CJ, Strike P. A pathway for the evolution of the plasmid NTP16 involving the novel kanamycin resistance transposon Tn4352. *Plasmid*. 1987;17(1):37–45. doi:10.1016/0147-619X(87)90006-0

54. Wang L, Fang H, Feng J, et al. Complete sequences of KPC-2-encoding plasmid p628-KPC and CTX-M-55-encoding p628-CTXM coexisted in *Klebsiella pneumoniae*. *Front Microbiol*. 2015;6:838. doi:10.3389/fmicb.2015.00838

55. Heffron F, Sublett R, Hedges RW, Jacob A, Falkow S. Origin of the TEM-beta-lactamase gene found on plasmids. *J Bacteriol*. 1975;122(1):250–256. doi:10.1128/jb.122.1.250-256.1975

56. Galimand M, Sabtcheva S, Courvalin P, Lambert T. Worldwide disseminated armA aminoglycoside resistance methylase gene is borne by composite transposon Tn1548. *Antimicrob Agents Chemother*. 2005;49(7):2949–2953. doi:10.1128/AAC.49.7.2949-2953.2005

57. Poirel L, Bonnin RA, Boulanger A, Schrenzel J, Kaase M, Nordmann P. Tn125-related acquisition of blaNDM-like genes in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2012;56(2):1087–1089. doi:10.1128/AAC.05620-11

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