VIM-encoding Inc<sub>pSTY</sub> plasmids and chromosome-borne integrative and mobilizable elements (IMEs) and integrative and conjugative elements (ICEs) in <i>Pseudomonas</i>

Fangzhou Chen<sup>1,2,3†</sup>, Peng Wang<sup>1†</sup>, Zhe Yin<sup>1</sup>, Huiying Yang<sup>1</sup>, Lingfei Hu<sup>1</sup>, Ting Yu<sup>1</sup>, Ying Jing<sup>1</sup>, Jiayao Guan<sup>1</sup>, Jiahong Wu<sup>2*</sup> and Dongsheng Zhou<sup>1,3*</sup>

**Abstract**

**Background:** The carbapenem-resistance genes bla<sub>VIM</sub> are widely disseminated in <i>Pseudomonas</i>, and frequently harbored within class 1 integrons that reside within various mobile genetic elements (MGEs). However, there are few reports on detailed genetic dissection of bla<sub>VIM</sub>-carrying MGEs in <i>Pseudomonas</i>.

**Methods:** This study presented the complete sequences of five bla<sub>VIM-2/-4</sub>-carrying MGEs, including two plasmids, two chromosomal integrative and mobilizable elements (IMEs), and one chromosomal integrative and conjugative element (ICE) from five different <i>Pseudomonas</i> isolates.

**Results:** The two plasmids were assigned to a novel incompatibility (Inc) group Inc<sub>pSTY</sub>, which included only seven available plasmids with determined complete sequences and could be further divided into three subgroups Inc<sub>pSTY-1/2/3</sub>. A detailed sequence comparison was then applied to a collection of 15 MGEs belonging to four different groups: three representative Inc<sub>pSTY</sub> plasmids, two Tn<sub>6916</sub>-related IMEs, two Tn<sub>6918</sub>-related IMEs, and eight Tn<sub>6417</sub>-related ICEs and ten of these 15 MGEs were first time identified. At least 22 genes involving resistance to seven different categories of antibiotics and heavy metals were identified within these 15 MGEs, and most of these resistance genes were located within the accessory modules integrated as exogenous DNA regions into these MGEs. Especially, eleven of these 15 MGEs carried the bla<sub>VIM</sub> genes, which were located within 11 different concise class 1 integrons.

**Conclusion:** These bla<sub>VIM</sub>-carrying integrons were further integrated into the above plasmids, IMEs/ICEs with intercellular mobility. These MGEs could transfer between <i>Pseudomonas</i> isolates, which resulted in the accumulation and spread of bla<sub>VIM</sub> among <i>Pseudomonas</i> and thus was helpful for the bacteria to survival from the stress of antibiotics.

*Correspondence: jiahongw@gmc.edu.cn; dongshengzhou1977@gmail.com; zhouds@bmsac.cn
†Fangzhou Chen and Peng Wang contributed equally to this work
1 State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China
2 Basic Medical College, Guizhou Medical University, Guiyang 550025, China
Full list of author information is available at the end of the article
Introduction

VIM hydrolyzes nearly all β-lactam except for aztreonam and currently consists of 76 variants (https://www.ncbi.nlm.nih.gov/pathogens/refgene) [1]. The blaVIM genes are mostly found in Pseudomonas and Enterobacteriaceae and occur frequently within class 1 integrons with distinct gene cassette arrays (GCAs). The blaVIM-carrying integrons are always presented in various mobile genetic elements (MGEs) such as plasmids, integrative and conjugative elements (ICEs), and integrative and mobilizable elements (IMEs), enhancing the mobility and dissemination of blaVIM genes [2].

There are a wealth of sequenced blaVIM-carrying plasmids, which can be assigned to various incompatibility (Inc) groups such as IncA, IncC, IncN, IncP and IncL/M [3–7]. IMEs/ICEs are frequently located in bacterial chromosomes: ICEs encode self-conjugation modules and thus are able to transfer between cells [8], whereas IMEs needs a helper conjugative element to achieve intercellular mobility [9].

Our previous studies showed the detailed genetic characteristics of two novel carbapenemase-encoding MGEs: SIM-encoding plasmid pHN39-SIM [10] and blaVIM-4-containing ICE Tn6413 [7]. This follow-up study presented the complete sequences of five novel blaVIM-2/-4-carrying plasmids, which can be assigned to various incompatibility (Inc) groups such as IncA, IncC, IncN, IncP and IncL/M [3–7]. IMEs/ICEs are frequently located in bacterial chromosomes: ICEs encode self-conjugation modules and thus are able to transfer between cells [8], whereas IMEs needs a helper conjugative element to achieve intercellular mobility [9].

Materials and methods

Bacterial strains and identification

Five clinical isolates P. aeruginosa SE5443, P. putida 716811 and 159349, and P. monteilii J20133 and 918607 were collected from four different Chinese public hospitals (Additional file 1: Table S1). The 16S rRNA genes and the blaVIM genes were detected as described previously [7].

Genomic DNA extraction, 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) [11]. The paired-end short Illumina reads were used to correct the long PacBio reads utilizing proovread [12], and then the corrected PacBio reads were assembled de novo utilizing SMARdenovo (https://github.com/ruanjue/smartdenovo).

Sequence annotation and comparison

Genome sequences were annotated by the Rapid Annotation using Subsystem Technology (RAST) [13] combined with BLASTP/BLASTX/BLASTN [14], Domain (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), and RefSeq database [15]. Annotation of resistance genes, MGEs, and other features were carried out using the online databases including CARD [16], ResFinder [17], ISfinder [18], INTEGRALL [19], and Tn Number Registry [20]. Multiple and pairwise sequence comparisons were performed using BLASTN. Gene organization diagrams were drawn using Inkscape 1.0 (https://inkscape.org/en/).

Phylogenetic analysis

Indicated nucleotide sequences were aligned using Clustal Omega 1.2.2 [21], and then maximum-likelihood phylogenetic trees were constructed from aligned sequences using MEGA X 10.1.8 [22] with a bootstrap iteration of 1000.

Conjugal transfer

Each indicated blaVIM-carrying MGE was transformed from its wild-type isolate into rifampin-resistant P. aeruginosa ATCC 27853 or PAO1, through conjugal transfer or electroporation experiments. Three milliliters of overnight cultures of each donor and recipient bacteria were mixed together, harvested, and resuspended in 80 mL 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 26 °C or 37 °C for 12 h to 18 h. Bacteria were washed from filter membrane and spotted on BHI plates, for selecting a bla_vim-carrying transconjugant. 1500 mg/mL rifampin (for ATCC 27853 or PAO1), together with 4 mg/mL meropenem (for bla_vim) was used as transconjugant selection.

**Phenotypic assays and multi-locus sequence typing (MLST)**

Bacterial antimicrobial susceptibility was tested by BioMérieux VITEK 2, and interpreted as per the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines [23]. Activity of Amber class A/B/D carbapenemases was determined with a modified CarbaNP test [24]. The sequence types (STs) of *Pseudomonas* isolates were identified according to the online *Pseudomonas* MLST schemes (https://pubmlst.org/organisms).

**Results**

**Identification of three STs for *Pseudomonas* isolates**

Three different STs, namely ST129, ST17, and ST639, were identified from the five *Pseudomonas* isolates (Additional file 1: Table S1), whose complete genome sequences were determined in this work. *P. putida* 716811 and 159349 belonged to ST129 and ST17, respectively. *P. aeruginosa* SE5443 belonged to ST639. ST129 was a novel ST of *P. putida*. The two *P. monteilii* isolates J20133 and 918607 could not be assigned with any ST types due to lack of *P. monteilii* MLST scheme.

**Proposal of a novel group of IncpSTY plasmids**

Two bla_vim-carrying plasmids pJ20133-VIM and p716811-VIM were identified from the complete genome sequences of the two *Pseudomonas* isolates J20133 and 716811 (Additional file 1: Table S1). A novel IncpSTY group was proposed from a total of seven available fully sequenced single-replicon plasmids (Additional file 1: Table S2; last accessed August 10th 2020) that included the above two plasmids together with five additional ones from GenBank, because these seven plasmids harbored not only homologous repA (replication initiation protein) genes together with its iterons (Fig. 1) but also similar backbone gene organizations (Fig. 2). Since the Inc group of plasmids could be divided according to the homology of repA genes [25–27], a phylogenetic tree (Fig. 1) was constructed based on the repA sequences of these seven plasmids, showing that these seven plasmids...
Fig. 2 Linear comparison of the three IncSTY plasmids pSTY, pJ20133-VIM, and p716811-VIM. Genes are denoted by arrows. Genes, mobile elements and other features are colored based on function classification. Light blue shading regions denote nucleotide identity > 80%. Red characters represent accessory modules. Numbers in brackets indicate nucleotide positions within corresponding plasmids.
could be divided into three separately clustering subgroups Inc<sub>STY</sub>-1/2/3. As shown by pairwise comparison of repA sequences, plasmids within each subgroup showed ≥ 95% identity, while those from different subgroups displayed ≤ 95% but ≥ 82% identity (Additional file 1: Table S3). pSTY [28], p716811-VIM, and pHN39-SIM [10] were the first sequenced plasmids of Inc<sub>STY</sub>-1, Inc<sub>STY</sub>-2, and Inc<sub>STY</sub>-3, respectively, and thus were identified as the references of the corresponding Inc<sub>STY</sub> subgroups.

**Comparison of three Inc<sub>STY</sub> plasmids pSTY, pJ20133-VIM and p716811-VIM**

A detailed comparative genomics analysis was applied to three representative Inc<sub>STY</sub> plasmids including the Inc<sub>STY</sub>-1 plasmids pSTY and pJ20133-VIM and the Inc<sub>STY</sub>-2 plasmid p716811-VIM. The Inc<sub>STY</sub>-3 plasmid pHN39-SIM was not included herein because it had been detailed characterized in our previous study [10]. These three characterized plasmids varied in size from about 243 to 321 kb with predicted ORFs from 255 to 282 (Table 1). The modular structure of each plasmid was divided into the backbone, and the accessory modules that were acquired DNA regions and inserted at different sites of the backbone (Fig. 2). pSTY, pJ20133-VIM, and p716811-VIM had > 80% nucleotide identity across > 66% of their backbone sequences, while pSTY and pJ20133-VIM shared > 74% of their backbones with > 95% nucleotide identity (Additional file 1: Table S4). These three plasmids shared the key Inc<sub>STY</sub> backbone markers: repA together with its iterons, parABC for partition, and multiple conjugal transfer genes (Fig. 2). A 47.9-kb backbone regionorf246–to–orf375 was presented in pSTY, but it was deleted from pJ20133-VIM and p716811-VIM due to insertion of an 11.5-kb Tn7-fam family transposon remnant (see below) and a 21.6-kb orf282–to–orf237 region, respectively, at the same backbone site. The orf282–to–orf237 region carried IS1491, mvaT (DNA-binding domain of bacterial xenogeneic silencer MvaT), five unnamed genes (encoding nucleoid-associated protein, predicted NTPase, AAA family ATPase, endonuclease, and putative transposase, respectively), and eight genes encoding hypothetical proteins.

pSTY, pJ20133-VIM, and p716811-VIM had totally different profiles of accessory modules (Table 1 and Fig. 2): (i) a 59.7-kb paa (phenylacetic acid degradation) region carrying paa locus and mer (mercury resistance) locus, Tn6734, Tn6603a, and ISPsp16 in pSTY; (ii) a 67.2-kb MDR (multi-drug resistance) region, the 11.5-kb Tn7-family transposon remnant, IS2000:Pae13-like intron, ISPmo2, ISPsp22, ISPmo3, and ISPmo5 in pJ20133-VIM; and (iii) a 29.6-kb MDR region, Tn6735, orf282–to–orf237 region, IS1491, and ISPsp2 in p716811-VIM.

The integration of the 59.7-kb paa region into pSTY led to truncation of orf246–to–orf846 region (as originally observed in pJ20133-VIM) into a 138-bp orf846 remnant (Fig. 2). A presumed prototype paa region was terminally bracketed by two copies of Tns5563 [29]; the right copy was intact while the left copy was truncated due to insertion of Tn6481 in the 59.7-kb paa region from pSTY (Additional file 1: Fig. S1). This 59.7-kb paa region still contained a complete set of phenylacetic acid degradation genes (Additional file 1: Fig. S1). Tn6734 was an IME containing a phenol degradation gene locus (Additional file 1: Fig. S2a). Tn6603a was a Tn3-family unit transposon containing an oxidative stress defense gene osmC (Additional file 1: Fig. S2b). The 67.2-kb MDR

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**Table 1** Major features of the three Inc<sub>STY</sub> plasmids analyzed

| Plasmid   | Accession number | Total length (bp) | Total number of ORFs | Mean G + C content (%) | Length of backbone (bp) | Accessory modules | bla<sub>VIM</sub> | Host bacterium                  |
|-----------|------------------|------------------|----------------------|------------------------|-------------------------|-------------------|----------------|-------------------------------|
| pSTY      | CP003962         | 321,653          | 355                  | 56.8                   | 210,612                 | paa region       | None            | Pseudomonas taiwanensis        |
| pJ20133-VIM | MN310371       | 255,073          | 298                  | 56.5                   | 168,325                 | MDR region       | bla<sub>VIM</sub>2          | P. monteilii J20133           |
| p716811-VIM | MN310372       | 243,028          | 262                  | 55.4                   | 163,184                 | MDR region       | bla<sub>VIM</sub>2          | P. putida 716811              |

The Inc<sub>STY</sub>-1 reference plasmid pSTY [28] from GenBank, and the Inc<sub>STY</sub>-1 plasmid pJ20133-VIM plus the Inc<sub>STY</sub>-2 reference plasmid p716811-VIM sequenced in this study were included in a detailed sequence comparison.
region from pJ20133-VIM contained In58, Tn5046 and ΔTn512, a 3.6-kb Tn4662α remnant, and several IS elements (Additional file 1: Fig. S3).

According to the previous grouping scheme of Tn7-family transposons [30], the phylogenetic tree was constructed based on the nucleotide sequences of tnsA, which encoded the endonuclease responsible for excision of Tn7-family transposons. A phylogenetic analysis of Tn6735, the 11.5-kb Tn7-family transposon remnant, Tn6922, and Tn6921, together with additional 12 representative sequenced Tn7-family transposons from GenBank based on the tnsA genes, indicated that the first two belonged to a novel Tn6735 subfamily and an unnamed subfamily (it had no intact sequenced transposon), respectively (Fig. 3). Similar to Tn7 [31], both Tn6735 (carrying pyrimidine biosynthesis genes) and the 11.5-kb Tn7-family transposon remnant encoded the core transposition determinants (endonuclease TnsA, transposase TnsB, regulator TnsC, target-site selection protein TnsD, and TnsB-binding sites), and the later one still encoded an additional target-site selection protein TnsE (Additional file 1: Fig. S4).

The 29.6-kb MDR region from p716811-VIM has a primary structure of a P.p.I3-like intron with interruption by Tn6740 that carried bla<sub>VIM-2</sub>, aacA4, qnrVC1, and mer locus (Additional file 1: Fig. S5). As a derivant of the IS66-family transposon Tn5042 [32], Tn6740 retained the whole Tn5042 sequence with integration of a 21.2-kb region that comprised mer-containing Tn3-family unit transposon Tn5563 [29], qnrVC1, and bla<sub>VIM-2</sub>-carrying In1722 (see below) (Additional file 1: Fig. S5).

Collection of 12 chromosome-borne IMEs/ICEs for sequence comparison

Three bla<sub>VIM</sub>-carrying chromosomal MGEs Tn6917, Tn6918 and Tn6953 were identified from the complete genome sequences of the three Pseudomonas isolates 918607, 159349, and SE5443 (Additional file 1: Table S1). A detailed sequence comparison was applied to a total of 12 chromosomal MGEs belonging to three groups:

![Fig. 3](image-url)
two related IMEs Tn6916 and Tn6917; two related IMEs Tn6916 and Tn6919; and eight related ICEs Tn6417, Tn6413, Tn6953, Tn6454, Tn6455, Tn6456, Tn6457 and Tn6458 (Table 2; last accessed August 10th 2020). Tn6917 was the only bla\_VIM-carrying one belonging to Tn6916-related IMEs in GenBank. Tn6919 was the only one (in addition to Tn6918) belonging to Tn6918-related IMEs in GenBank, and it did not carry bla\_VIM. The seven Tn6417-related ICEs were selected from GenBank because Tn6417 was used as reference and the rest six ones Tn6413, Tn6954, Tn6955, Tn6956, Tn6957 and Tn6958 carried bla\_VIM genes. Similar to the above Inc\_ASTY plasmids, the modular structure of each IME/ICE was divided into the backbone and the accessory modules.

### Comparison of two related IMEs Tn6916 and Tn6917

Tn6916 (a 51.0-kb prototype IME initially identified in \textit{P. monteilii} FDAARGOS\_171 [33]) and Tn6917 were integrated at the same site downstream of the \textit{P. monteilii} chromosomal gene \textit{dinG} (ATP-dependent DNA helicase), and they shared the core backbone markers \textit{attL} (attachment site at the left end), \textit{int} (integrase), and \textit{attR} (attachment site at the right end); in addition, the \beta-lactam resistance gene \textit{ampC} was considered as a backbone component of these two IMEs because no associated MGEs were identified for this resistance gene (Fig. 4). Tn6916 thereby had no accessory modules, but \textit{bla}\_VIM-carrying In528 (see below) was integrated at a site upstream of \textit{eamA} (transcription regulator) and identified as the sole accessory module in Tn6917 (Fig. 4).

### Comparison of two related IMEs Tn6918 and Tn6919

Tn6918 (a 70.49-kb prototype IME initially found in \textit{P. putida} 159,349) and Tn6919 were integrated at a site downstream of the \textit{P. putida} chromosomal gene tRNA\textsuperscript{Thr}, and they shared the core backbone markers \textit{attL}, \textit{int}1, \textit{int}2, and \textit{attR} (Fig. 5). There were at least three major modular differences in the backbone of Tn6919 relative to Tn6918: i) inversion of \textit{orf}1965, ii) interruption of \textit{orf}549 and \textit{orf}2007 due to insertion of \textit{ΔIS}\textit{Ppu13} and \textit{ISP}en2, respectively, and iii) replacement of 3’-terminal 22.8-kb \textit{orf}270–to–\textit{orf}213 region by 3.4-kb \textit{orf}255–to–\textit{orf}282 region (Fig. 5). The IS\textit{Pai}122–\textit{mer} region was integrated at the same site of the backbones of Tn6918 and Tn6919 (Fig. 5), and it was composed of IS\textit{Pai}122 and a \textit{mer}-carrying \textit{ΔTn5041}-like element (Additional file 1: Fig. S6). Tn6918 and Tn6919 acquired \textit{bla}\_VIM-carrying In1770 (see below) and \textit{strAB} region, respectively, which were inserted at the same site upstream of \textit{orf}555 (Fig. 5). The \textit{strAB} region

### Table 2: Major features of the 12 chromosome-borne IMEs and ICEs analyzed

| Category            | MGE     | Accession number | Host bacterium | Country | \textit{bla}\_VIM | Nucleotide position in the chromosome | Refs. |
|---------------------|---------|------------------|----------------|---------|-----------------|--------------------------------------|-------|
| Tn6916-related IMEs | Tn6916  | CP014062         | \textit{P. monteilii} FDAARGOS\_171 | USA     | None            | Not applicable                       | [33]  |
|                     | Tn6917  | CP043395         | \textit{P. monteilii} 918,607 | China   | \textit{bla}\_VIM\_2 | 2624899.2625699                      | This study |
| Tn6918-related IMEs | Tn6918  | CP045553         | \textit{P. putida} 159,349 | China   | \textit{bla}\_VIM\_2 | 813783.814583                        | This study |
|                     | Tn6919  | CP016634         | \textit{P. putida} IEC33019 | Brazil  | None            | Not applicable                       | —     |
| Tn6417-related ICEs | Tn6417  | CP013993         | \textit{P. aeruginosa} DHS01 | France  | None            | Not applicable                       | [7]   |
|                     | Tn6413  | CP030075         | \textit{P. aeruginosa} 6762 | China   | \textit{bla}\_VIM\_4 | 409196.409996                        | [7]   |
|                     | Tn6953  | CP046405         | \textit{P. aeruginosa} SES443 | China   | \textit{bla}\_VIM\_2 | 490654.4907348                       | This study |
|                     | Tn6954  | KY623658         | \textit{Alcaligenes faecalis} GZAF3 | Palestine | \textit{bla}\_VIM\_2 | 63643.64443                         | [36]  |
|                     | Tn6955  | MF168944         | \textit{P. aeruginosa} HSV3483 | Portugal | \textit{bla}\_VIM\_2 | 62615.63415                         | [3]   |
|                     | Tn6956  | MF168946         | \textit{P. aeruginosa} FFUP\_PS\_CBS | Portugal | \textit{bla}\_VIM\_2 | 93931.94731                         | [3]   |
|                     | Tn6957  | CP043328         | \textit{P. aeruginosa} CUG31971 | Sweden  | \textit{bla}\_VIM\_4 | 5251865.5252665                     | –     |
|                     | Tn6958  | CP048039         | \textit{A. faecalis} MUB14 | Poland  | \textit{bla}\_VIM\_2 | 659107.659907                       | [37]  |

A collection of 12 fully sequenced chromosome-borne MGEs, which included all the four Tn6916- or Tn6918-related IMEs and the eight Tn6417-related ICEs composed of Tn6417 itself plus all the six \textit{bla}\_VIM-carrying ones, were included in a detailed sequence comparison.
Fig. 4 Comparison of the two-related IMEs Tn6916 and Tn6917. Genes are denoted by arrows. Genes, mobile elements and other features are colored based on their functional classification. Shading regions denotes of nucleotide identity ≥ 95%. Numbers in brackets indicate nucleotide positions within the chromosomes of strains FDAARGOS_171 and 918607, respectively. The accession number of Tn6916 [33] for reference is CP014062.
contained a cryptic unit transposon Tn6920, a strAB-carrying unit transposon Tn6921, a 3.6-kb Tn4662a remnant, and several IS elements (Additional file 1: Fig. S7). Tn6920 and Tn6921 had the core transposition tniABQ−res−tniR modules with the highest sequence identities to Tn5053 [34] and Tn5058a [32], respectively, and thus they belonged to the Tn5053 subfamily of Tn7 family (Fig. 3).

Comparison of eight Tn6417-related ICEs

Tn6417 was a 108.2-kb reference ICE [7] initially found in *P. aeruginosa* DHS01 [35]. The eight related ICEs Tn6417, Tn6413, Tn6953, Tn6954 (GZAF3_G1) [36], Tn6955 (ICE6440) [3], Tn6956 (ICE6441) [3], Tn6957, and Tn6958 [37] shared the core backbone markers attL/attR (these sequences showed somewhat differences but shared a core motif ’CCTTCTGCGGCTCCA’), int, cpl (coupling protein), rlx (relaxase), and a F (TivF)-type type IV secretion system gene set (mating pair formation). However, the backbones of these eight ICEs varied in size from 67.8 to 95.1 kb and had at least four major modular differences: (i) presence of unique orf672, orf306, pilL1-to-orf381 region, and orf3336-to-orf2514 region in Tn6953; (ii) presence of two highly similar regions...
lysR2–to–orf1068 and orf348–to–orf1188 in Tn6417 and Tn6413, respectively; (iii) absence of smc–to–orf3006 region from Tn6955; and (iv) interruption of orf2280 owing to insertion of intron E.c.15 in Tn6954 and Tn6955 (Fig. 6). The first six ICEs were integrated into the *P. aeruginosa* or *A. faecalis* chromosomal tRNAGly gene, while the last two ones into the *P. aeruginosa* chromosomal gene *mhpC* (methyl ester carboxylesterase) and orf1203 (putative DNA-binding protein), respectively.

Each ICE carried a single accessory module, and thus there were the resulting eight different accessory modules *bla*VIM-2-carrying In1779 (see below), Tn6532, Tn6403, Tn6959, a 15.2-kb Tn6346-related region, Tn6960, Tn6961, and Tn6962 from the eight ICEs; the first one was inserted into *ftsk* (cell division protein), while all the other seven ones were integrated at a site upstream of orf582 and identified as the derivatives of Tn6346 (Fig. 7). Tn6346 was a prototype Tn3-family unit transposon originally identified in *Achromobacter* spp. AO22 [38] and manifested as a hybrid of the core transposition module *tnpAR–res* from Tn5051 and the *mer* region from Tn501 (Fig. 7). The seven Tn6346 derivatives differed from Tn6346 in two major aspects: (i) insertion of IS1071 at the same position within *tnpA* in all these seven Tn6346 derivatives, and furthermore insertion of ISPa91 at another position within *tnpA* in only Tn6960; and (ii) insertion of different concise class 1 integrons In159, In127, In1365, In56, In1879, In1853, and In58.
Fig. 7 (See legend on previous page.)
into \textit{urf2} in these seven Tn6346 derivatives, sometimes leading to truncation of surrounding regions (Fig. 7). The \textit{bla}_{VIM} genes were found in the last five integrons (see below) rather than the first two (Additional file 1: Fig. S8).

**Comparison of eleven \textit{bla}_{VIM}-carrying integrons**

The \textit{bla}_{VIM} Genes were found in the two plasmids p20133-VIM and p716811-VIM (Table 1) and the nine chromosomal IMEs/ICEs Tn6917, Tn6918, Tn6413, Tn6953, Tn6454, Tn6455, Tn6456, Tn6457 and Tn6458 (Table 2). The 11 corresponding local \textit{bla}_{VIM} genetic environments manifested as 11 different class 1 concise integrons, and three of them contained \textit{bla}_{VIM}-4 while the other nine carried \textit{bla}_{VIM}-2 (Fig. 8). A complete class 1 concise integron contained IRi (inverted repeat at the integrase end), 5'-conserved segment (5'-CS: \textit{int1}–\textit{att1}), GCA, 3'-CS (\textit{qacED1–sul1–orf5–orf6}), \textit{tni}_{m6402} (Tn402 core transposition module \textit{tni}_{ABQ–res–tniR}), and IRt (inverted repeat at the \textit{tni} end). Except for In1443\texttimes\textit{tni}_{6613} with terminal truncations, all the other tens had the terminal IRi/IRt pairs. Insertions or truncations occurred in 5'-CS or 3'-CS or \textit{tni}_{m6402} of all these 11 integrons, especially including In1779 that showed the longest modular structure because of integration of a \textit{mer}-containing \textit{Tn3} family prototype unit transposon Tn6758. In56\texttimes\textit{tni}_{6606} had the shortest GCA that contained only \textit{bla}_{VIM}-2, while all the other 10 integrons had the multiple-gene GCAs harboring \textit{bla}_{VIM}-2 plus one or more additional antibiotic resistance genes.

**Summary of newly identified or designated MGEs**

There were eight newly identified MGEs, including two IMEs Tn6917 and Tn6918, one ICE Tn6953, two unit transposons Tn6735 and Tn6740, and three integrons In1770, In1772, and In1779. Additional 13 MGEs (IMEs: Tn6734, Tn6916, Tn6919, and Tn6957; unit transposons: Tn6920, Tn6921, Tn6959, Tn6960, Tn6961, and Tn6962; IS elements: \textit{ISP}m02, \textit{ISP}m03, \textit{ISP}m05) were newly designated but with previously determined sequences. The three previously designated ICES GZAF3\texttimes\textit{G1}, ICE6440, and ICE6441 were renamed as the standard Tn designations Tn6954, Tn6955, and Tn6956, respectively.

**Transferability and antimicrobial susceptibility**

p20133-VIM, p716811-VIM, and Tn6953, which were selected to represent the \text{Inc}\textsubscript{pSTY-1} and \text{Inc}\textsubscript{pSTY-2} plasmids and the Tn6417-related ICES, respectively, could be transferred from the relevant wild-type isolates into ATCC 27853 or PAO1 through conjugation, generating the transconjugants PAO1/p20133-VIM, PAO1/p716811-VIM, and ATCC 27853/Tn6953 respectively. All these wild-type and transconjugant strains had the Ambler class B carbapenemase activity (data not shown) and were resistant to imipenem and meropenem with minimal inhibitory concentration (MIC) values \(\geq 16\) mg/L (Additional file 1: Table S5), owing to production of VIM enzyme.

**Discussion**

The present work identifies a novel group of \text{Inc}\textsubscript{pSTY} plasmids, which can be further divided into the three subgroups \text{Inc}\textsubscript{pSTY-1/2/3}. A detailed genetic dissection analysis is applied to a collection of 15 \textit{Pseudomonas} MGEs, including three \text{Inc}\textsubscript{pSTY} plasmids, two Tn6916-related IMEs, two Tn6918-related IMEs, and eight Tn6417-related ICES. All these IMEs/ICEs were located within the bacterial chromosomes. At least 22 genes for resistance to seven different categories of antibiotics and heavy metals are identified within these 15 MGEs (Additional file 1: Table S6). Eleven of these 15 MGEs carry the \textit{bla}_{VIM} genes, which are all located within GCAs of concise class 1 integrons. For 10 of these integrons, the \textit{bla}_{VIM} genes were presented together with other resistance genes, especially including those for resistance to β-lactams, aminoglycosides, trimethoprim, and rifampicin. These \textit{bla}_{VIM}-carrying integrons were further integrated into the above plasmids, IMEs and ICES.

Confirmed by conjugal transfer experiments herein, \text{Inc}\textsubscript{pSTY} Plasmids and Tn6417-related ICES have the ability to transfer from one cell to another cell. These two groups MGEs could transfer autonomously by utilizing self-encoded conjugation gene sets [39, 40]. However, the conjugal transfer of Tn6916- and Tn6918-related IMEs are nonautonomous due to lack of essential conjugation genes encoding Cpl and type IV secretion system, and thereby its intercellular transfer is relied on the help of other conjugative elements [40].

\textit{bla}_{VIM} genes are widely disseminated in \textit{Pseudomonas} [41–43], \textit{Enterobacteriaceae} [5, 44, 45], \textit{Providence} [46, 47], \textit{Achromobacter} [48], \textit{Acinetobacter} [49, 50], \textit{Aeromonas} [51], and \textit{Morganella} [52]. Currently, at least 183 different \textit{bla}_{VIM}-carrying integrons [19, 53, 54] have been identified, and they either directly integrate into the chromosomes or reside as the inner-components of various MGEs such as plasmids, unit transposons, ICES and IMEs [3, 55–57].
These results indicate that \textit{bla}_{VIM} genes have evolved to reside diverse MGEs with the intra- or inter-cellular mobility, which will promote the wide dissemination of \textit{bla}_{VIM} genes. 

Until now, only seven \textit{Inc}_{PSTY} plasmids are identified in six \textit{Pseudomonas} isolates and one \textit{Stenotrophomonas rhizophila} isolate, which come from China \((n=5)\), Japan \((n=1)\) and Germany \((n=1)\) (Additional file 1: Table S2). Only six \textit{Tn6916}-related IMEs and two \textit{Tn6918}-related IMEs are detected in eight \textit{Pseudomonas} isolates, two of which are sequenced in this study. The six \textit{Tn6916}-related IMEs come from China \((n=4)\), USA \((n=1)\) and Japan \((n=1)\), while the two \textit{Tn6918}-related IMEs from China \((n=1)\) and Brazil \((n=1)\). These observations indicate that these three group MGEs are mainly prevalent in \textit{Pseudomonas} isolates and meanwhile still not widely disseminated in multiple geographic regions. By contrast, \textit{Tn6417}-related ICEs are frequently identified in \textit{Pseudomonas} \([7, 58, 59]\) and also occasionally in \textit{Bordetella}, \textit{Alcaligenes}, \textit{Klebsiella pneumoniae}, \textit{Achromobacter xylosoxidans}, \textit{Morganella morganii}, \textit{Aeromonas caviae}, \textit{Casimicrobium huifangae}, and \textit{Delftia acidovorans}. \textit{Tn6417}-related ICEs have been already disseminated worldwide in a plenty of bacterial species.

**Conclusion**

The four groups of \textit{bla}_{VIM}-carrying MGEs characterized in this study have a wide range of hosts including \textit{Pseudomonas}, and they are able to transfer across different bacterial species. Integration of \textit{bla}_{VIM} genes into these MGEs contributes to the accumulation and distribution of \textit{bla}_{VIM} genes and enhances the ability of bacteria to survive under selection pressure of carbapenems especially in hospital settings. Moreover, these four groups of MGEs display a high-level diversification in modular structures, which have complex mosaic natures and carry a large number of drug resistance genes (particularly in the MDR regions in these MGEs). This study provides a deeper insight into the genetic diversification and evolution of VIM-encoding MGEs in \textit{Pseudomonas}.

**Abbreviations**

MGE: Mobile genetic element; ICE: Integrative and conjugative element; IME: Integrative and mobilizable element; GCA: Gene cassette array; Inc: Incompatibility; RAST: Rapid Annotation using Subsystem Technology; BHI: Brain Heart Infusion; CLSI: Clinical and Laboratory Standards Institute; MLST: Multi-locus Sequence Typing; MDR: Multi-drug resistance; IRi: Inverted repeat at the integrase end; CS: Conserved segment; IRT: Inverted repeat at the \textit{tri} end; MIC: Minimal inhibitory concentration.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12941-022-00502-w.

**Additional file 1:** Fig S1. Organization of \textit{pao} region from \textit{pSTY} Fig S2. Organization of \textit{Tn6734} and \textit{Tn66620} from \textit{pSTY} Fig S3. Organization of MDR region from \textit{pJ20133-VIM}, and comparison with related regions. Fig S4. Organization of two \textit{Tn7}-family elements, and comparison with related region. Fig S5. Organization of MDR region from \textit{p716811-VIM}, and comparison with related regions. Fig S6. Organization of \textit{ISPba122} – \textit{mer} region from \textit{Tn6918} and \textit{Tn6919}; and comparison with related region. Fig S7. Organization of \textit{strAB} region from \textit{Tn6919}, and comparison with related regions. Fig S8. Organization of \textit{In159} and \textit{In127} from \textit{Tn6532} and \textit{Tn6643} respectively, and comparison with related region. Table S1. Major features of the five \textit{Pseudomonas} isolates sequenced in this study. Table S2. List of all the seven \textit{Inc}_{PSTY} single-replicon plasmids. Table S3. Pairwise comparison of \textit{rep\textsubscript{PSTY}} sequences from the seven \textit{Inc}_{PSTY} plasmids using BLASTN. Table S4. Pairwise comparison of backbone sequences from the three \textit{Inc}_{PSTY} plasmids using BLASTN. Table S5. Antimicrobial drug susceptibility profiles. Table S6. Resistance genes in the 15 mobile genetic elements (MGEs) analyzed.

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**Authors’ contributions**

DZ and JW conceived the study and designed experimental procedures. FC, HY, LH and TY performed the experiments. FC, JJ, and JG analyzed the data. ZY and PW contributed to reagents and materials. FC and HY wrote the original draft. DW and JW reviewed the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The complete sequences of plasmids \textit{pJ20133-VIM} and \textit{p716811-VIM}, and of the chromosomes of strains \textit{SES443}, \textit{918607}, and \textit{159349} were submitted to GenBank under accession numbers MN310371, MN310372, CP046405, CP043395, and CP045553 respectively.

**Declarations**

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

**Author details**

1. State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China. 2. Basic Medical College, Guangzhou Medical University, Guciarc 550025, China. 3. Guangzhou Medical University, Guangzhou 511436, China.

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