A clinical *Pseudomonas juntendi* strain with *bla*\textsubscript{IMP–1} carried by an integrative and conjugative element in China

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**Objective:** To precisely determine the species of a carbapenem-resistant *Pseudomonas* strain 1809276 isolated from the urine of a Chinese patient and analyze its integrative and conjugative element (ICE) 1276 formation mechanism.

**Methods:** Single-molecule real-time (SMRT) sequencing was carried out on strain 18091276 to obtain the complete chromosome and plasmid (pCN1276) sequences, and average nucleotide identity (ANI) was used for precise species identification. The ICEs in GenBank with the same integrase structure as ICE 1276 were aligned. At the same time, the transfer ability of *bla*\textsubscript{IMP–1} and the antibiotic sensitivity of *Pseudomonas juntendi* 18091276 were tested.

**Results:** This bacterium was *P. juntendi*, and its drug resistance mechanism is the capture of the accA\textsubscript{44} gene cassette by the TrnA\textsubscript{402} -like type 1 integron (IntI\textsubscript{1} - *bla*\textsubscript{IMP–1}) to form In1886 before its capture by the ΔTrn4662\textsubscript{a} -carrying ICE 1276. The acquisition of *bla*\textsubscript{IMP–1} confers carbapenem resistance to *P. juntendi* 18091276.

**Conclusion:** The formation of *bla*\textsubscript{IMP–1} -carrying ICE 1276, its further integration into the chromosomes, and transposition and recombination of other elements promote bacterial gene accumulation and transmission.

**Keywords**

*Pseudomonas juntendi*, integrative and conjugative, carbapenem-resistant, reorganization, *bla*\textsubscript{IMP–1}

**Introduction**

*Pseudomonas* sp. is a genus of varied and complex Gram-negative bacteria commonly isolated from soil and water, with a wide host diversity, including animals and plants. It consists of 254 species (List of Prokaryotic names with Standing in Nomenclature, LPSN; [www.bacterio.net](http://www.bacterio.net)) divided into three lineages and 13 groups based on the sequences of the
16S rRNA, gyrB, rpoB, and rpoD genes (Peix et al., 2018). Among them, several species of Pseudomonas sp. were regarded as important opportunistic pathogens affecting humans, including Pseudomonas aeruginosa, Pseudomonas asiatica, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas cepacia, Pseudomonas stutzeri, Pseudomonas maltophilia, and Pseudomonas putrefaciens (Tohya et al., 2019). Their capabilities of acquisition of exogenous genes lead to the prone of antibiotics resistance in this genus, including carbapenem antibiotics resistance, which enhances the difficulties of treatment. Pseudomonas sp. can acquire carbapenem resistance genes by integrons, which further can be captured by other mobile elements (such as plasmids, transposons, etc.), and then, these genes begin to spread among other bacteria.

In this study, we analyzed a P. juntendi strain separated from a urine specimen of a patient in China in 2018, which was tentatively identified as P. putida by VITEK2 (bioMerieux), and the strain carried a carbapenem resistance gene blaIMP−1. To our knowledge, this is the first report of clinical strain of P. juntendi carrying blaIMP−1 in a new gene context in China.

Materials and methods

Bacterial isolation and identification

Strain 18091276 was isolated from a urine specimen from a patient in a tertiary hospital in northeast China in 2018 and the species of the strain was determined by the part of the sequence of the 16S rRNA gene (Edwards et al., 1989). Then, a maximum likelihood (ML) evolutionary tree was constructed using MEGA 7.0 software to evaluate the sequence similarity of the 16S rRNA genes that were aligned and listed in the BLASTN program (top 100).

The minimum inhibitory concentration (MIC) of imipenem was determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines, and Escherichia coli ATCC 25922 was used as a control. MICs of amikacin, gentamicin, meropenem, cefazolin, cefazidime, cefotaxime, cefepime, aztreonam, ampicillin, piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, colistin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, levofloxacin, moxifloxacin, and tetracycline were tested by BD Phoenix-100.

Sequencing and sequence assembly

Bacterial genomic DNA was extracted from the strain 18091276 using the UltraClean Microbial Kit (Qiagen, NW, Germany) and sequenced by a PacBio RSII sequencer (Pacific Biosciences, CA, USA). The reads were assembled de novo by using SMARTdenovo (http://github.com/ruanjue/smartdenovo).

Sequence annotation and comparison

Rast 2.0 (Brettin et al., 2015) and BLASTP/BLASTN (Boratyn et al., 2013) searches were used to predict open reading frames (ORFs), online databases CARD (https://card.mcmaster.ca; Alcock et al., 2020), and ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder; Bortolai et al., 2020) to find out resistance genes, and ISfinder (https://www.is.biotoul.fr/; Lastest Database Update 2021-9-21; Varani et al., 2011), TrnCentral (https://tncentral.proteininformationresource.org/), and ICEberg 2.0 (http://db-mml.sjtu.edu.cn/ICEberg/; Liu et al., 2019) were used to find out the mobile elements. Pairwise sequence comparisons were carried out by BLASTN. Gene organization diagrams were drawn by Inkscape 1.0 (http://inkscape.org/en/).

Bacterial precise species identification and evolutionary tree construction

Bacterial precise species identification was performed using the pair-wise average nucleotide identity (ANI) analysis between strain 18091276 and the reference genome (http://www.ncbi.nlm.nih.gov/tools/ani). A ≥95% ANI cutoff was used to define bacterial species (Toon et al., 2017). CSI Phylogeny (https://cge.cbs.dtu.dk/services/CSIPhylogeny/; Kaas et al., 2014) calls and filters single nucleotide polymorphisms (SNPs) of strain 18091276, does site validation, and infers a phylogeny based on the concatenated alignment of the high-quality SNPs. In addition, the MUSCLE software program was used to align multiple single-copy core-encoded proteins identified by the core-/pan-genome analysis. The aligned sequences were then subjected to phylogenetic analysis using the TreeBeST (Version 1.9.2) program, a neighbor-joining tree reconstruction algorithm, and 1,000 bootstrap replicates (Nandi et al., 2010).

Conjugation experiments

Conjugation experiments were performed as described previously (Mizuno et al., 2020). Briefly, strain 18091276 was used as a donor and sodium azide-resistant E.coli J53 as a recipient. Donor and recipient strains were cultured overnight at 37°C separately. Then, 3 ml of 18091276 culture was mixed up with an equal volume of E.coli J53 culture. The mixed cells were harvested by centrifugation for 3 min at 12,000 × g, washed with 3 ml of lysogeny broth (LB), and resuspended in 150 μl of LB. The mixture was spotted on a 1 cm² hydrophilic nylon membrane filter (Millipore) with a 0.45-μm pore size, which was...
then placed on an LB agar plate and then incubated for mating at 37°C for 6 h. The cells were recovered from the filter membrane and spotted on LB agar containing 100 µg/ml sodium azide and 4 µg/ml imipenem for selecting carbapenem-resistant E. coli transconjugant.

Nucleotide sequence accession numbers

The complete sequence of 18091276 has been submitted to GenBank under the accession number CP091311.

Results and discussion

After the 18091276 strain was cultured overnight at 37°C, 2 mm round protruding colonies with smooth and regular edges, non-fusion growth, non-pyocyanin production, and the absence of metallic sheen were found on brain heart infusion agar (imipenem concentration: 4 µg/ml).

Strain 18091276 was identified by the BD Phoenix-100 identification system and VITEK 2 as P. putida, and Table 1 shows its drug resistance spectrum. The similarity of the 16S rRNA sequence of this bacterium and P. putida, Pseudomonas montelii, Pseudomonas plecoglossica, and P. juntendi in the P. putida group was more than 99% and the strain was identified to be in the P. putida group (Supplementary Figure 1). After single-molecule real-time (SMRT) sequencing (basic information about SMRT sequence results was provided in Supplementary Table 1), it was found that the ANI value of strain 18091276 was more than 95% with the reference strain P. juntendi BML3 (GCA_009932375.1), and this strain was confirmed to be P. juntendi (ANI value of P. juntendi 18091276 were provided in Supplementary Table 2A). In 2019, Tohya M. was the first to carry out a systematic identification of P. juntendi and officially named this species (Tohya et al., 2019). The analysis of all 14 strains that were annotated as P. juntendi in GenBank (cutoff date was November 2021; Table 2) found that the clinical isolates before 2018 were from Brazil, and the isolates in China, the USA, Japan, and Russia only appeared after 2018. Although P. juntendi 18091276 and 14181154 were all isolated in China, they had a far phylogenetic relationship (Figure 1). According to the SNPs and core-genome phylogenetic tree, P. juntendi 18091276 was the closest relative to P. juntendi 12349 (Brazil, 2012) and P. juntendi PSB00036 (the USA, 2018; Figure 1). We speculate

| Antimicrobial type | Antimicrobial | MIC (µg/mL)a | SIRb |
|--------------------|---------------|--------------|------|
| Aminoglycosides    | Amikacin      | ≤8           | S    |
|                    | Gentamicin    | 4            | S    |
| β-lactam           | Imipenem      | 32           | R    |
|                    | Meropenem     | >8           | R    |
|                    | Cefazolin     | >16          | R    |
|                    | Ceftazidime   | >16          | R    |
|                    | Cefotaxime    | >32          | R    |
|                    | Cefepime      | >16          | R    |
|                    | Aztreonam     | 16           | I    |
|                    | Ampicillin    | >16          | R    |
|                    | Piperacillin  | 8            | S    |
|                    | Amoxicillin-Clavulanate | >16/8     | R    |
|                    | Ampicillin-Sulbactam | >16/8     | R    |
|                    | Piperacillin-Tazobactam | 8/4      | S    |
| Colistin           | Colistin      | 1            | NA   |
| Sulfonamide        | Trimethoprim-Sulfamethoxazole | >2/38    | R    |
| Chloramphenicol    | Chloramphenicol | >16         | R    |
| Quinolones         | Ciprofloxacin | ≤0.5         | S    |
|                    | Levofoxacin   | ≤1           | S    |
|                    | Moxifloxacin  | 4            | NA   |
| Tetracycline       | Tetracycline  | 4            | S    |

aMIC, minimum inhibitory concentration.
bSIR, Susceptible (S), Intermediate (I), Resistant (R).
NA, not applicable.
that *P. juntendi* 18091276 might be transferred from the USA or Brazil through international food (animal- and plant-based) trade and travel. The identification method at the time was not precise, many *P. juntendi* isolates were wrongly identified as *P. putida* or other species in the *P. putida* group (Morimoto et al., 2020), resulting in fewer epidemiological data and whole-genome data of this strain in other countries. ResFinder screening found that, out of the 14 *P. juntendi* strains, six Brazilian strains carried the carbapenem resistance genes *blaIMP* and *blaVIM* and two Chinese strains carried *blaIMP* (see Table 2 for strain information), showing that *P. juntendi* could acquire *blaIMP* and *blaVIM*, like *P. aeruginosa* and *P. putida*, and develop carbapenem resistance, and it is potentially harmful.

The chromosome of *P. juntendi* 18091276 contained an 88.61 kb integrative and conjugative element (ICE) that was named ICE 1276. ICE 1276 contained *attL* and *attR* produced from recombination, a complete integrase gene (*int*), the F-type IV secretion system responsible for the ICE transfer, the conjugation gene *cpl*, and *rlx* responsible for autonomous rolling circle replication (Johnson and Grossman, 2015). ICE 1276 did not contain the cleavage enzyme *xis* or the origin of replication *oriT*. *crpP* (quinolone resistance gene) was also found in the backbone region of ICE 1276. *crpP* was mainly present in the ICEs of *Pseudomonas aeruginosa* and could increase ciprofloxacin resistance (Zhu et al., 2021). However, *P. juntendi* 18091276 did not possess quinolone resistance. In addition, a 15.96-kb accessory module was discovered in ICE 1276, and this structure had the closest phylogenetic relationship with Tn4662a (a Tn3 family transposon) in plasmid pDK1 (NC_014124.1; coverage: 58%, identity: 98.65%). Moreover, it had the same 38 bp inverted repeat sequence (IRs) as Tn4662a, and a 5-bp (AGTAT) directed repeat sequence (DRs) was generated during insertion (detailed information is shown in Supplementary Table 3). Tn4662a was first found on a plasmid pDK1 carried by *P. putida* HS (Yano et al., 2010). In contrast to Tn4662a (pDK1), the insertion structure of *P. juntendi* 18091276 also contained a Tn6811 remnant (Tn3 family), a single copy insertion element IS541 (IS5 family), and a complete Tn1886 (Tn402-like type 1 integron) carrying *blaIMP* and *aacA4*'. However, the *res_site_II*, *res_site_III*, *tnpR*, and *tnpA* sequences in Tn4662a (ICE 1276) were partially deleted, which should have a Tn4662a-derived structure (Figure 2).

Until November 2021, only 4 ICES with the same integrase as ICE 1276 were indexed in GenBank (Table 3 shows the strain information). Interestingly, these ICE 1276-carrying strains were *Pseudomonas* sp. from China. ANI calculator was used to precisely analyze the identified *Pseudomonas* sp. 14181154 and LTGT-11-2Z. The ANI value of *Pseudomonas* sp. 14181154 and the reference strain *P. juntendi* BML3 was more than 95% (ANI value in Supplementary Table 2B), so *Pseudomonas* sp. 14181154 was identified as *P. juntendi* (Figure 1) shows the phylogenetic relationship with other *P. juntendi* strains, but the ANI of LTGT-11-2Z and all *Pseudomonas* species were lower than 95%. Therefore, LTGT-11-2Z was only identified as *Pseudomonas* sp. All ICES were obtained from *Pseudomonas* sp. chromosomes, and *E. coli* transconjugants were not obtained after repeated conjugation experiments. Except for the exogenous insertion sites, the remaining structure of the ICES was nearly identical. Another exception is that a 14.29 kb accessory module was

**Table 2** The information of *P. juntendi*.

| Strain   | Source | Time | Country | Size (Mb) | GC content (%) | Resistance gene | Assembly ID               |
|----------|--------|------|---------|-----------|----------------|----------------|--------------------------|
| 10918    | Urine  | 2010 | Brazil  | 6.09      | 62             | *aadA1*, *aph(3’)-Vla*, *aacA4’, *sul1*, *blaIMP*-2, *blaVIM*-2, *blaOXA-129* | GCA_04602235.1 |
| 10618    | Blood  | 2010 | Brazil  | 5.71      | 62.2           | *blaIMP*-2, *aacA4’, *blaOXA-129*, *sul1* | GCA_04602305.1 |
| 11213    | Blood  | 2010 | Brazil  | 5.70      | 62.2           | *aacA4’, *sul1*, *blaIMP* | GCA_04602275.1 |
| 12273    | Urine  | 2012 | Brazil  | 5.59      | 62.5           |                      | GCA_04602265.1 |
| 12349    | Blood  | 2012 | Brazil  | 5.64      | 62.3           | *aacA4’, *sul1*, *blaIMP*-16, *blaVIM*-2 | GCA_04602185.1 |
| 12815    | Urine  | 2013 | Brazil  | 5.69      | 62.5           | *sul1*, *aadA1*, *aacA4’, *aph(3’)-Vla*, *blaIMP* | GCA_04602135.1 |
| 14181154 | Catheter | 2014 | China   | 5.61      | 62.3           | *aacA4’, *aadA1*, *blaOXA-129*, *blaIMP* | CP045554.1 |
| 18091276 | Urine  | 2018 | China   | 5.89      | 62.3           | *aacA4’, *blaIMP*-1 | This study               |
| BML3     | Sputum | 2018 | Japan   | 5.73      | 62.7           |                      | GCA_00993275.1 |
| PSB00022 | Urine  | 2018 | USA     | 5.77      | 62.1           |                      | GCA_016009075.1 |
| PSB00036 | Urine  | 2018 | USA     | 5.42      | 62.4           |                      | GCA_016337345.1 |
| PSB00020 | Sputum | 2018 | USA     | 6.20      | 62.2           |                      | GCA_016009085.1 |
| 14535    | Blood  | 2019 | Brazil  | 6.07      | 62.1           | *aacA4’, *sul1*, *blaIMP* | GCA_04602085.1 |
| SCPMS-O-B-9248 | Sputum | 2021 | Russia  | 5.67      | 62.3           |                      | GCA_018188545.1 |
inserted at 74 bp upstream of ssb (single-strand DNA binding protein) in the backbone region of ICE LTGT-11-Z (plant-derived Pseudomonas sp. LTGT-11-Z). This ICE seemed to have caused the transfer of large fragments between Pseudomonas species under specific conditions.

As the downstream region of Tn4662a is truncated by Tn6811, it is unable to carry out normal replicative transposition (Grindley and Reed, 1985), but it still contains an intact res_site_I (Supplementary Table 4A) and can recombine with In1886 and ISPsfu1 (Grindley and Reed, 1985; Brovedan et al., 2021) (Figure 2). IRs and DRs were absent at both ends of ISPsfu1-In1886 and did not constitute a composite transposon (Zong, 2014). Alignment with 10 Tn402-like integrons containing blaIMP−1 and aacA4’ gene cassettes that were indexed before November 2021 (Table 4 shows the strain information) found that except for In1960, In1771, and In1886 located in ICEs, the ISPsfu1 element was not inserted upstream or downstream of the remaining integrons (regardless of whether it is on the plasmid or chromosome) and there were two independent insertion processes. The Tn4662a-driven structure in ICE LTGT-11-Z (Figure 2) indicated that, after ISPsfu1 had undergone non-replicative transposition, In1886 was inserted upstream of the res_site_I of ΔTn4662a (Figure 3 shows the formation process of ICE 1276).

IMP-1 was first discovered in a P. aeruginosa strain from Japan (Walsh et al., 2005) and is now the most widely circulating IMP enzyme in Southeast Asia (Walsh et al., 2005). Up until November 2021, 86 IMP family members were indexed in GenBank, and most blaIMP−1 were carried and transferred by type 1 integrons (Diene and Rolain, 2014). After the Tn402 integron captures blaIMP−1, Tn402 possesses the self-transfer capability and can be integrated into transposons or res sites on plasmids to expand its transmission range (Gillings, 2017). In 2014, pNXM63 (MW150990) from Morganella morganii nx_m63 that was isolated in a hospital in China contained a Tn402-like type 1 integron (intI1-blaIMP−1; Xiang et al., 2021). Based on the repeat sequence (AACG) on both sides
of the gene cassette, it can be deduced that In1886 is a structure formed after the integron on pNXM63 captured the aacA4’ gene cassette (Figure 4). The Tn402-like transposon module at the 3’ CS end of In1886 could capture other drug resistance genes. An example is In1886 in the PA15W plasmid in P. aeruginosa (GeneBank accession no.: MN961673). However, as this In1886 IScfr1 fragment mediated the insertion of strA-strB-aac(3)-IId to the Tn402 recombination site on PA15W-In1886, it resulted in the truncation of the res_site_r1 site (Figure 4. Supplementary Table 4B), leading to irreversible recombination (Rowland et al., 2020). In1886 can also capture aadA6, blaOXA−21, aadA1a, qacF2, and gus162 gene cassettes through recombination at specific sites to obtain a series of derived structures (Labbate et al., 2009; Table 4). This integron and its derived structures were mostly present on Pseudomonas sp. chromosomes and were also discovered in K. pneumoniae and K. oxytoca plasmids (Table 4).

Therefore, blaIMP−1 can be recombined upstream of res_site_I in Tn4662a using a Tn402-like integron as a medium to achieve transfer from other species to P. juntendi 18091276. After that, large fragments are transferred between Pseudomonas sp. through ICEs. The Tn4662a-derived structures in these ICEs contain the intact res_site_I sequence. It can also continue recombination with other elements. In addition, it was also found that, in addition to capturing gene cassettes, the res_site_r1 on miniABQR at 3’ CS can also undergo
TABLE 3 The information of strains carrying ICEs.

| Strain         | Source       | Species identification       | Year | Country        | Size (Mb) | GC content (%) | Assembly ID   |
|----------------|--------------|------------------------------|------|----------------|-----------|----------------|---------------|
| LTGT-11-2Z     | Alhagi sparsifolia Shap. | *Pseudomonas sp.* | 2014 | Xinjiang, China | 6.07      | 61.7           | CP033104.1    |
| 14181154       | Homo sapiens, Catheter | *Pseudomonas sp.*          | 2014 | Hunan, China   | 5.61      | 62.3           | CP04554.1     |
| SY153          | Homo sapiens, urine | *P. putida*                 | 2012 | Sanya, China   | 5.60      | 62.0           | CP062218.1    |
| 15420352       | Homo sapiens, urine | *P. putida*                 | 2015 | Hunan, China   | 6.17      | 61.6           | CP045551.1    |
| 18091276       | Homo sapiens, urine | *P. juntendi*               | 2018 | Changchun, China | 5.89     | 62.3           | This study    |

TABLE 4 Genetic contexts of In1886.

| Strain         | Country | Year | Source | Location | Structure | Genbank AN |
|----------------|---------|------|--------|----------|-----------|------------|
| *P. aeruginosa* PA15W | China   | NA   | Human  | P        | Intl1-*blaIMP−1*-*aacA4*-*Tni402* | MN961673    |
| *P. juntendi* 18091276 | China   | 2015 | Urine  | C        | Intl1-*blaIMP−1*-*aacA4*-*Tni402* | In this study |
| *Pseudomonas sp.* 14181154 | China   | 2014 | Catheter | C    | Intl1-*blaIMP−1*-*aacA4*-*blaOXA−21*-*aadA1*-*Tni402* | CP045554.1 |
| *P. putida* 15420352 | China   | 2015 | Urine  | C        | Intl1-*blaIMP−1*-*aacA4*-*qacF2*-*blaOXA−21*-*aadA1*-*Tni402* | CP045551.1 |
| *K. pneumoniae* 2013050801 | China   | 2013 | Blood  | P        | Intl1-*blaIMP−1*-*guc162*-*aadA6*-*Tni402* | KT345947    |
| *K. oxytoca* 7121 | China   | 2014 | Sputum | P        | Intl1-*blaIMP−1*-*guc162*-*aadA4*-*aadA6*-*Tni402* | KX784502    |
| *P. aeruginosa* 97 | Ghana, Western Africa | 2015 | Urine  | C        | Intl1-*blaIMP−1*-*aacA4*-*blaOXA−21*-*Tni402* | CP031449    |
| *P. aeruginosa* JUNP133 | Japan   | NA   | NA     | C        | Intl1-*blaIMP−1*-*aacA4*-*blaOXA−21*-*Tni402* | LC36409     |
| *E. coli* MBL1-07+ | India   | 2008 | NA     | NA       | Intl1-*blaIMP−1*-*aacA4*-*blaOXA−21*-*Tni402* | LC169568    |
| *P. alcaligenes* KAM426 | Japan   | 2020 | wastewater | C | Intl1-*foxE*-*aacA4*-*blaIMP−1*-*qacG8*-*qacEAl-delt1*-*Tni402* | AP024354    |

*Since the uploaded of this strain was only an integrator sequence, unable to determine the mobile genetic elements upstream and downstream.

P, Plasmid.

C, Chromosome.

NA, not applicable.

Further recombination, resulting in the production of multidrug resistance. Regarding different hosts, ICE 1276-like integration elements can also undergo recombination with exogenous genes through ssb to carry out adaptive evolution.

**Conclusion**

This study was the first to report a clinical isolate of *P. juntendi* in China. At the same time, it was found that this bacterium can capture Tn402-like type 1 integron containing *blaIMP−1* through the ICE of Tn4662a. This provides a new vector and host for the horizontal transfer of *blaIMP−1*. Hence, there is a need to improve bacterial identification methods and drug resistance monitoring in opportunistic pathogens in hospitals. At the same time, the ICE 1276-Tn4662a-In1886 structure and its mutant-derived structures should be closely monitored, particularly its epidemiology in China.
The formation process of integrative and conjugative element (ICE) 1276. The figure shows the ICE 1276 structure as a local structure. The integration region of ICE 1276 is yellow, the backbone region is black, In1886 is light blue. (A) Tn4662a was recombined in ICE 1276 through replicative transposition and produces bilateral directed repeat sequence (DRs) (AGTAT). (B) Tn68II1 and Tn4662a underwent recombination to form the ΔTn4662a-ΔTn68II1 structure; the truncated part of Tn4662a includes tnpA, complete deletion of tnpR, and the recombination sites res_site II and res_site III. (C) ISPsfu1 was inserted into ΔTn4662a-ΔTn68II1 through non-replicative transposition and produced bilateral DRs (TTAG). (D) In1886 was inserted upstream of the res_site I in ΔTn4662a through replicative transposition and produced bilateral DRs (GATAGG).
FIGURE 4
Linear alignment map of In1886. The Tn402 transposition module is orange-red, the 3’-CS of the integron is blue, and the exogenous insertion element is green. The solid purple dot is the AACG sequence that is recognized by the gene cassette att and forms two copies in In1886 due to the insertion of the aacA4 gene cassette. The shaded region represents a homologous region (>90% nucleotide identity). The pNXM63 (MW150990) from Morganella morgani nx_m63 contains an intI1-bla<sup>imp</sup>-1-TnABQR structure. When this integron was compared with In1886, except for the gene cassette aacA4, all of the bases were completely identical, and there was no missing base. At the same time, the downstream region of the gene cassette bla<sup>imp</sup>-1 in this integron contained the AACG site, which was present on both sides of the gene cassette aacA4 in In1886. In addition, In1886 was also present in the plasmid (GenBank accession no.: MN961673) in P. aeruginosa PA15W. However, a 7.45-kb exogenous insertion structure was inserted in the res_site_r1 recombination site of the Tn402 mobile module in the Tn402 of PA15W-In1886, causing res_site_r1 to be truncated (Supplementary Table 2). This exogenous insertion structure contained eight open reading frames (ORFs), including a complete ISCH1 (IS1162 family), aminoglycoside resistance genes strA and strB, and aac(3)-IId.
Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, CP091311.

Author contributions

All strains were provided by China-Japan Union Hospital, Jilin University, PC, XG, JC, JL, and LZhu conceived, directed, and carried out the study. ML, GL, JI, YW, XZ, and BJ prepared samples for sequence analysis. JG, SS, and LZhe acquired samples and analyzed the data. All authors have read and approved the final manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.929800/full#supplementary-material

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