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A clinical *Pseudomonas juntendi* strain with *bla*_{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*_{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 *accA4'* gene cassette by the Tn402-like type 1 integron (*Int11-bla*_{IMP-1}) to form *Int1886* before its capture by the Δ Tn4662a-carrying ICE 1276. The acquisition of *bla*_{IMP-1} confers carbapenem resistance to *P. juntendi* 18091276.

Conclusion: The formation of *bla*_{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*_{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) 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. junjendi* strain separated from a urine specimen of a patient in China in 2018, which was tentatively identified as *P. putida* by VITEK2 (bioMérieux), and the strain carried a carbapenem resistance gene *bla*_{IMP-1}. To our knowledge, this is the first report of clinical strain of *P. junjendi* carrying *bla*_{IMP-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, ceftazidime, 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/>; Bortolaia 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), *TnCentral* (<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.ezbiocloud.net/tools/ani>). A $\geq 95\%$ ANI cutoff was used to define bacterial species (Yoon 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 monteilii*, *Pseudomonas plecoglossicida*, 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

TABLE 1 Antimicrobial susceptibility of 18091276.

Antimicrobial type	Antimicrobial	MIC (µg/mL) ^a	SIR ^b
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
Colistin	Ampicillin-Sulbactam	>16/8	R
	Piperacillin-Tazobactam	8/4	S
	Colistin	1	NA
Sulfonamide	Trimethoprim-Sulfamethoxazole	>2/38	R
Chloramphenicol	Chloramphenicol	>16	R
Quinolones	Ciprofloxacin	≤0.5	S
	Levofloxacin	≤1	S
	Moxifloxacin	4	NA
Tetracycline	Tetracycline	4	S

^aMIC, minimum inhibitory concentration.

^bSIR, Susceptible (S), Intermediate (I), Resistant (R).

NA, not applicable.

TABLE 2 The information of *P. juntendi*.

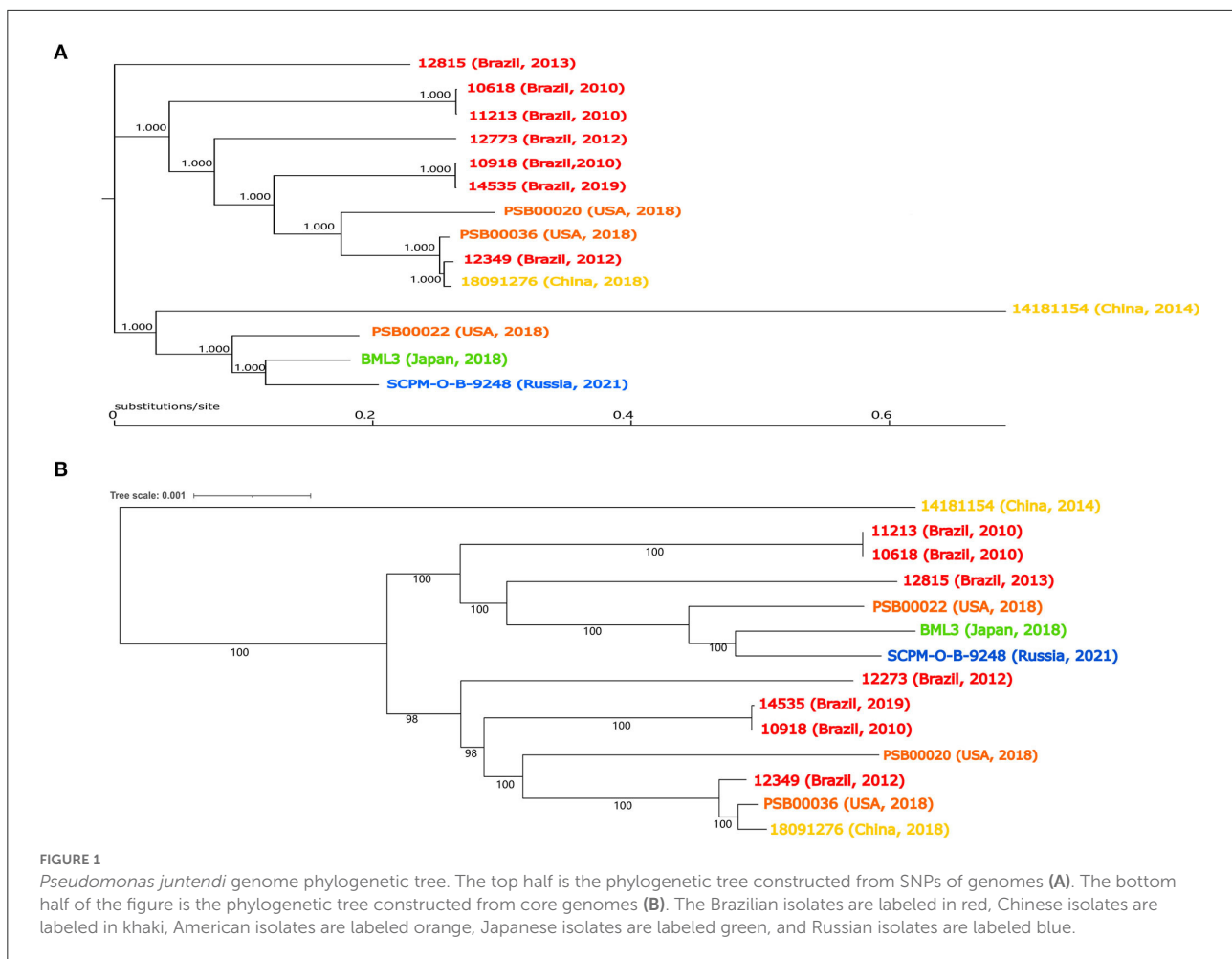
Strain	Source	Time	Country	Size (Mb)	GC content (%)	Resistance gene	Assembly ID
10918	Urine	2010	Brazil	6.09	62	<i>aadA1</i> , <i>aph(3')-VIa</i> , <i>aacA4'</i> , <i>sul1</i> , <i>bla_IMP-16</i> , <i>bla_{VIM-2}</i> , <i>bla_{OXA-129}</i>	GCA_014062235.1
10618	Blood	2010	Brazil	5.71	62.2	<i>bla_{VIM-2}</i> , <i>aacA4'</i> , <i>bla_{OXA-129}</i> , <i>sul1</i>	GCA_014062305.1
11213	Blood	2010	Brazil	5.70	62.2	<i>aacA4'</i> , <i>sul1</i> , <i>bla_{OXA-129}</i> , <i>bla_{VIM-2}</i>	GCA_014062275.1
12273	Urine	2012	Brazil	5.59	62.5		GCA_014062265.1
12349	Blood	2012	Brazil	5.64	62.3	<i>aacA4'</i> , <i>sul1</i> , <i>bla_IMP-16</i> , <i>bla_{VIM-2}</i>	GCA_014062185.1
12815	Urine	2013	Brazil	5.69	62.5	<i>sul1</i> , <i>aadA1</i> , <i>aacA4'</i> , <i>aph(3')-VIa</i> , <i>aadA1</i> , <i>bla_IMP-1</i>	GCA_014062135.1
14181154	Catheter	2014	China	5.61	62.3	<i>aacA4'</i> , <i>aadA1</i> , <i>bla_{OXA-21}</i> , <i>bla_IMP-1</i>	CP045554.1
18091276	Urine	2018	China	5.89	62.3	<i>aacA4'</i> , <i>bla_IMP-1</i>	This study
BML3	Sputum	2018	Japan	5.73	62.7		GCA_009932375.1
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	<i>aacA4'</i> , <i>sul1</i> , <i>bla_{VIM-2}</i> , <i>bla_IMP-16</i>	GCA_014062085.1
SCPM-O-B-9248	Sputum	2021	Russia	5.67	62.3		GCA_018138545.1

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 *bla_IMP* and *bla_{VIM}* and two Chinese strains carried *bla_IMP* (see Table 2 for strain information), showing that *P. juntendi* could acquire *bla_IMP* and *bla_{VIM}*, 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 IS*Psfu1* (IS5 family), and a complete In1886 (Tn402-like type 1 integron) carrying *bla_IMP-1* 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

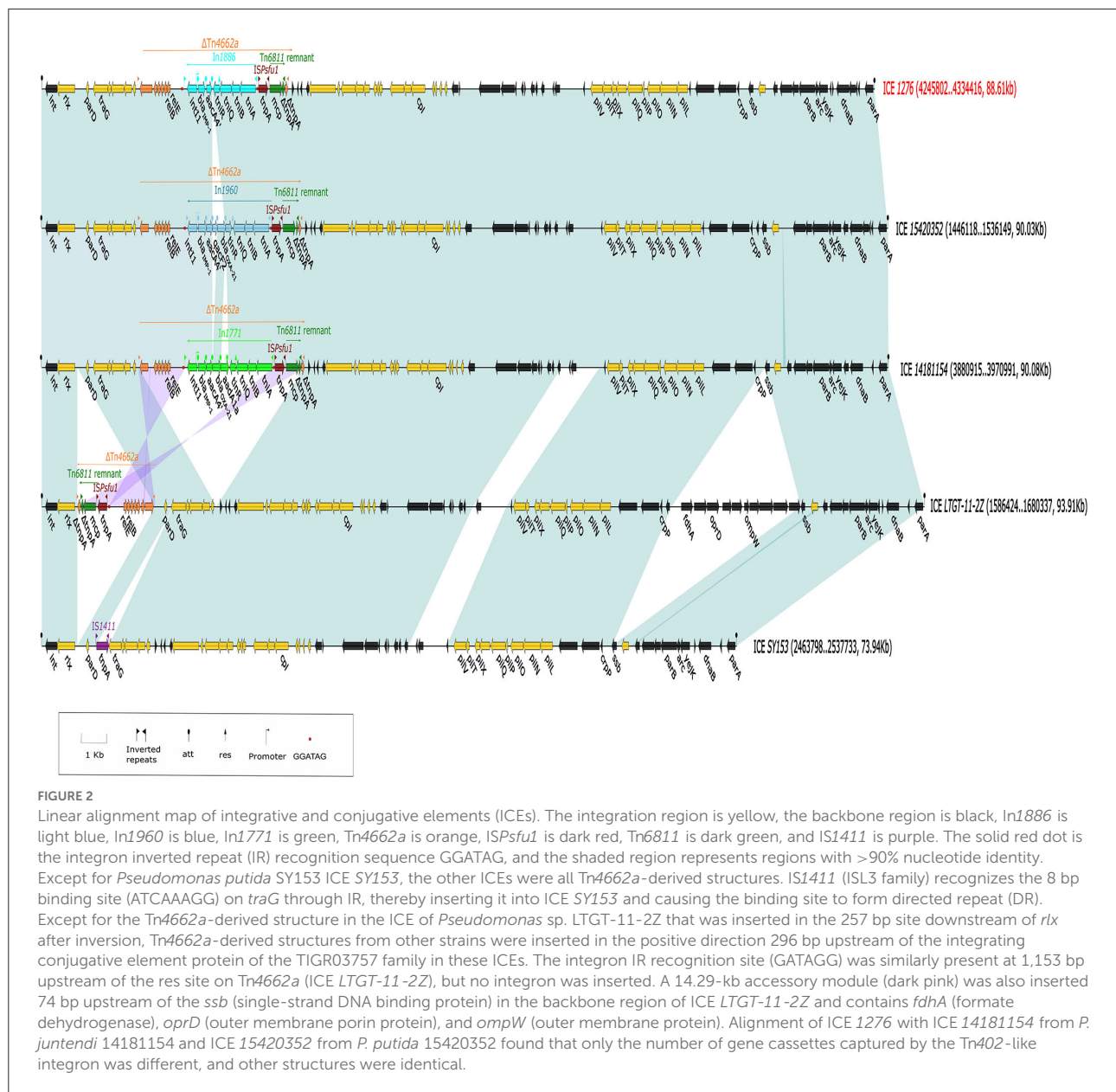


inserted at 74 bp upstream of *ssb* (single-strand DNA binding protein) in the backbone region of ICE *LTGT-11-2Z* (plant-derived *Pseudomonas sp.* LTGT-11-2Z). This ICE seemed to have caused the transfer of large fragments between *Pseudomonas* species under specific conditions.

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 *bla_{IMP-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-11-2Z* (Figure 2) indicated that, after *ISPsfu1* had undergone non-replicative transposition, *In1886* was inserted upstream of the *res_site_I* of Δ *Tn4662a* (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 *bla_{IMP-1}* were carried and transferred by type 1 integrons (Diene and Rolain, 2014). After the *Tn402* integron captures *bla_{IMP-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-bla_{IMP-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*, *bla_{OXA-21}*, *aadA1a*, *qacF2*, and *guc162* 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, *bla_{IMP-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 *tniABQR* 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
		GenBank	This study					
LTGT-11-2Z	Alhagi sparsifolia Shap.	<i>Pseudomonas sp.</i>	<i>Pseudomonas sp.</i>	2014	Xinjiang, China	6.07	61.7	CP033104.1
14181154	Homo sapiens, Catheter	<i>Pseudomonas sp.</i>	<i>P.juntendi</i>	2014	Hunan, China	5.61	62.3	CP045554.1
SY153	Homo sapiens, urine	<i>P. putida</i>	<i>P. putida</i>	2012	Sanya, China	5.60	62.0	CP062218.1
15420352	Homo sapiens, urine	<i>P. putida</i>	<i>P. putida</i>	2015	Hunan, China	6.17	61.6	CP045551.1
18091276	Homo sapiens, urine	<i>P.juntendi</i>	<i>P.juntendi</i>	2018	Changchun, China	5.89	62.3	This study

TABLE 4 Genetic contexts of In1886.

Strain	Country	Year	Source	Location	Structure	Genbank AN
<i>P. aeruginosa</i> PA15W	China	NA	Human	P	<i>Int1-bla_{IMP-1}-aacA4'-Tni402</i>	MN961673
<i>P. juntendi</i> 18091276	China	2018	Urine	C	<i>Int1-bla_{IMP-1}-aacA4'-Tni402-ISPsfu</i>	In this study
<i>Pseudomonas sp.</i> 14181154	China	2014	Catheter	C	<i>Int1-bla_{IMP-1}-aacA4'-bla_{OXA-21}-aadA1a-Tni402-ISPsfu</i>	CP045554.1
<i>P. putida</i> 15420352	China	2015	Urine	C	<i>Int1-bla_{IMP-1}-aacA4'-qacF2-bla_{OXA-21}-Tni402-ISPsfu</i>	CP045551.1
<i>K. pneumoniae</i> 2013050801	China	2013	Blood	P	<i>Int1-bla_{IMP-1}-guc162-aacA4'-aadA6-Tni402</i>	KT345947
<i>K. oxytoca</i> 7121	China	2014	Sputum	P	<i>Int1-bla_{IMP-1}-guc162-aacA4'-aadA6-Tni402</i>	KX784502
<i>P. aeruginosa</i> 97	Ghana, Western Africa	2015	Urine	C	<i>Int1-bla_{OXA-10}/aacA4-bla_{IMP-1}-Tni402</i>	CP031449
<i>P. aeruginosa</i> JUNP133	Japan	NA	NA	C	<i>Int1-bla_{OXA-10}/aacA4-bla_{IMP-1}-Tni402</i>	LC636409
<i>E. coli</i> MBL1-07 ^a	India	2008	NA	NA	<i>Int1-bla_{OXA-10}/aacA4-bla_{IMP-1}</i>	LC169568
<i>P. alcaligenes</i> KAM426	Japan	2020	wastewater	C	<i>Int1-fosE-aacA4'-3-aacA4'-3-bla_{IMP-1}-qacG8-qacEΔ1-sul1-Tni402</i>	AP024354

^aSince 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 *bla_{IMP-1}* through the ICE of Tn4662a. This provides a new vector and host for the horizontal transfer of *bla_{IMP-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.

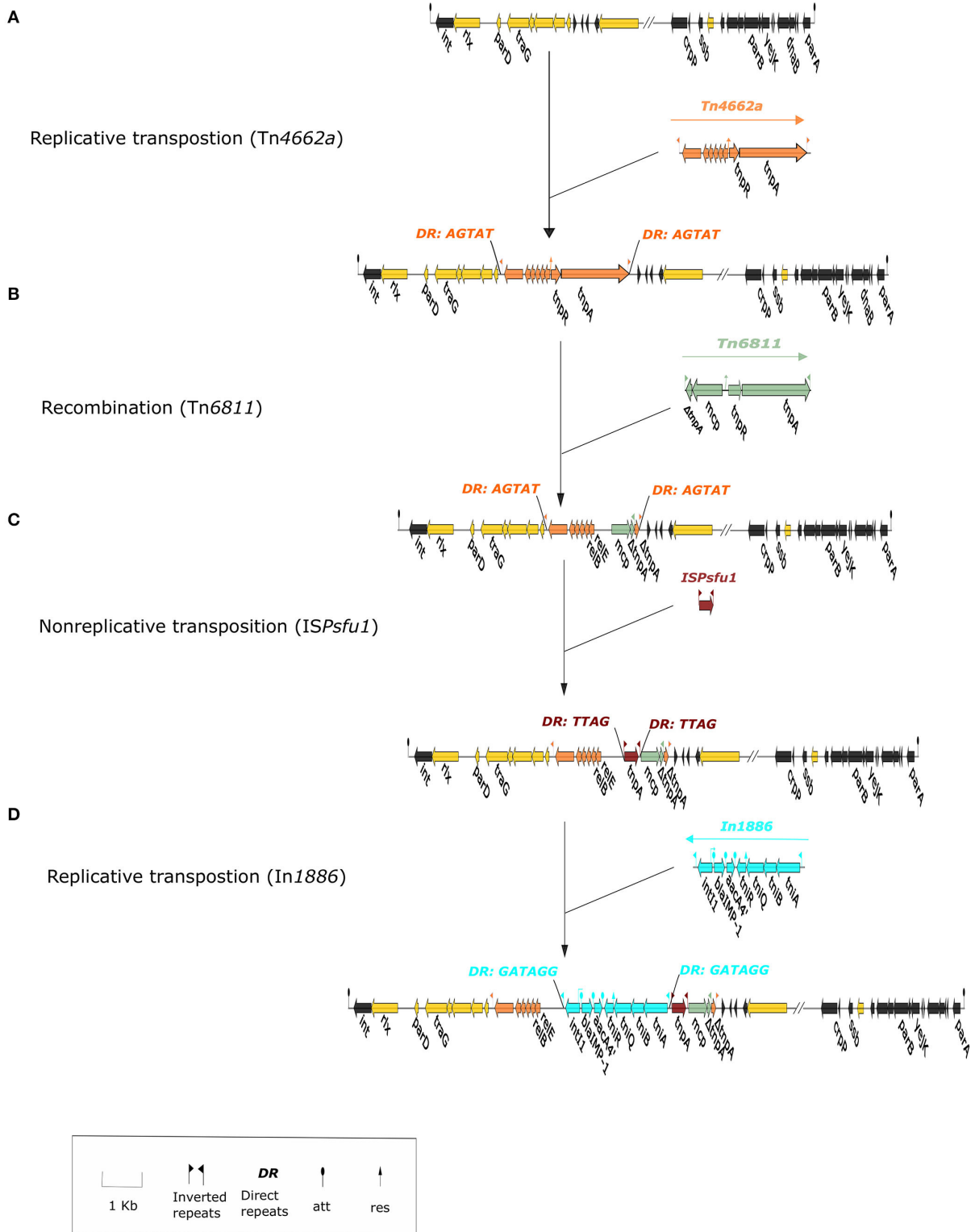
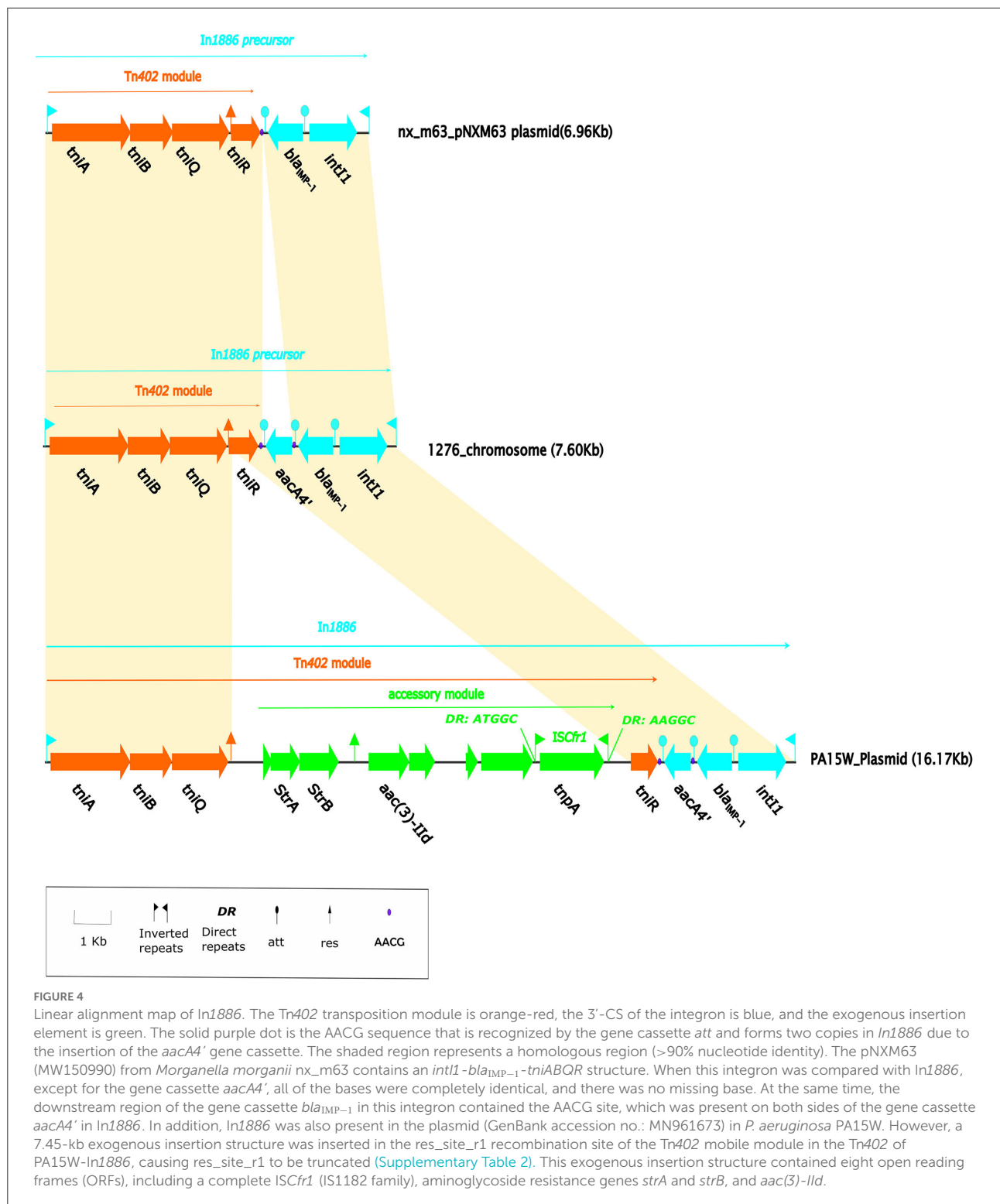


FIGURE 3
 The formation process of integrative and conjugative element (ICE) 1276. The figure shows the ICE 1276 structure as a local structure. The integration region of ICE 1276 is yellow, the backbone region is black, In1886 is light blue; (A) Tn4662a was recombined in ICE 1276 through replicative transposition and produces bilateral directed repeat sequence (DRs) (AGTAT); (B) Tn6811 and Tn4662a underwent recombination to form the Δ Tn4662a- Δ Tn6811 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 Δ Tn4662a- Δ Tn6811 through non-replicative transposition and produced bilateral DRs (TTAG); (D) In1886 was inserted upstream of the *res_site_I* in Δ Tn4662a through replicative transposition and produced bilateral DRs (GATAGG).



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, JJ, 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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References

- Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., et al. (2020). CARD: antibiotic resistance surveillance with the Comprehensive Antibiotic Resistance Database. *Nucl. Acids Res.* 48, D517–D525. doi: 10.1093/nar/gkz935
- Boratyn, G. M., Camacho, C., Cooper, P. S., Coulouris, G., Fong, A., Ma, N., et al. (2013). BLAST: a more efficient report with usability improvements. *Nucl. Acids Res.* 41, W29–33. doi: 10.1093/nar/gkt282
- Bortolaia, V., Kaas, R. F., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., et al. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *J. Antimicrobial Chemother.* 75, 3491–3500. doi: 10.1093/jac/dkaa345
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., et al. (2015). RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci. Rep.* 5:8365. doi: 10.1038/srep08365
- Brovedan, M. A., Marchiaro, P. M., Diaz, M. S., Faccione, D., Corso, A., Pasteran, F., et al. (2021). *Pseudomonas putida* group species as reservoirs of mobilizable Tn402-like class 1 integrons carrying blaVIM-2 metallo- β -lactamase genes. *Infect Genet Evol.* 96:105131. doi: 10.1016/j.meegid.2021.105131
- CLSI (2020). *Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA: Clinical and Laboratory Standards Institute.
- Diene, S. M., and Rolain, J. M. (2014). Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. *Clin. Microbiol. Infect.* 20, 831–838. doi: 10.1111/1469-0691.12655
- Edwards, U., Rogall, T., Blöcker, H., Emde, M., and Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucl. Acids Res.* 17, 7843–53. doi: 10.1093/nar/17.19.7843
- Gillings, M. R. (2017). Class 1 integrons as invasive species. *Curr. Opin. Microbiol.* 38, 10–15. doi: 10.1016/j.mib.2017.03.002
- Grindley, N. D., and Reed, R. R. (1985). Transpositional recombination in prokaryotes. *Annu. Rev. Biochem.* 54, 863–896. doi: 10.1146/annurev.bi.54.070185.004243
- Johnson, C. M., and Grossman, A. D. (2015). Integrative and Conjugative Elements (ICEs): what they do and how they work. *Annu. Rev. Genet.* 49, 577–601. doi: 10.1146/annurev-genet-112414-055018
- Kaas, R. S., Leekitcharoenphon, P., Aarestrup, F. M., and Lund, O. (2014). Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS ONE* 9:e104984. doi: 10.1371/journal.pone.0104984
- Labbate, M., Case, R. J., and Stokes, H. W. (2009). The integron/gene cassette system: an active player in bacterial adaptation. *Methods Mol. Biol.* 532, 103–125. doi: 10.1007/978-1-60327-853-9_6
- Liu, M., Li, X., Xie, Y., Bi, D., Sun, J., Li, J., et al. (2019). ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. *Nucl. Acids Res.* 47, D660–D665. doi: 10.1093/nar/gky1123
- Mizuno, S., Ogawa, M., Yano, H., Kasahara, K., and Mikasa, K. (2020). Molecular and epidemiological characteristics of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates in Japan. *mSphere* 5, e00490–e00420. doi: 10.1128/mSphere.00490-20
- Morimoto, Y., Tohya, M., Aibibula, Z., Baba, T., Daida, H., Kirikae, T., et al. (2020). Re-identification of strains deposited as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas putida* in GenBank based on whole genome sequences. *Int. J. Syst. Evol. Microbiol.* 70, 5958–5963. doi: 10.1099/ijsem.0.004468

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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>

- Nandi, T., Ong, C., Singh, A. P., Boddey, J., Atkins, T., Sarkar-Tyson, M., et al. (2010). A genomic survey of positive selection in *Burkholderia pseudomallei* provides insights into the evolution of accidental virulence. *PLoS Pathog.* 6:e1000845. doi: 10.1371/journal.ppat.1000845
- Peix, A., Ramírez-Bahena, M. H., and Velázquez, E. (2018). The current status on the taxonomy of *Pseudomonas* revisited: an update. *Infect. Genet. Evol.* 57, 106–116. doi: 10.1016/j.meegid.2017.10.026
- Rowland, S. J., Boocock, M. R., Burke, M. E., Rice, P. A., and Stark, W. M. (2020). The protein-protein interactions required for assembly of the Tn3 resolution synapse. *Mol. Microbiol.* 114, 952–965. doi: 10.1111/mmi.14579
- Tohya, M., Watanabe, S., Teramoto, K., Shimojima, M., Tada, T., Kuwahara-Arai, K., et al. (2019). *Pseudomonas juntendi* sp. nov., isolated from patients in Japan and Myanmar. *Int. J. Syst. Evol. Microbiol.* 69, 3377–3384. doi: 10.1099/ijsem.0.003623
- Varani, A. M., Siguier, P., Gourbeyre, E., Charneau, V., and Chandler, M. (2011). ISsaga: an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. *Genome Biol.* 12:R30. doi: 10.1186/gb-2011-12-3-r30
- Walsh, T. R., Toleman, M. A., Poirel, L., and Nordmann, P. (2005). Metallo-beta-lactamases: the quiet before the storm? *Clin. Microbiol. Rev.* 18, 306–325. doi: 10.1128/CMR.18.2.306-325.2005
- Xiang, G., Lan, K., Cai, Y., Liao, K., Zhao, M., Tao, J., et al. (2021). Clinical molecular and genomic epidemiology of *Morganella morganii* in China. *Front. Microbiol.* 12:744291. doi: 10.3389/fmicb.2021.744291
- Yano, H., Miyakoshi, M., Ohshima, K., Tabata, M., Nagata, Y., Hattori, M., et al. (2010). Complete nucleotide sequence of TOL plasmid pDK1 provides evidence for evolutionary history of IncP-7 catabolic plasmids. *J. Bacteriol.* 192, 4337–4347. doi: 10.1128/JB.00359-10
- Yoon, S. H., Ha, S. M., Lim, J. M., Kwon, S. J., and Chun, J. (2017). A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 110, 1281–1286. doi: 10.1007/s10482-017-0844-4
- Zhu, Z., Yang, H., Yin, Z., Jing, Y., Zhao, Y., Fu, H., et al. (2021). Diversification and prevalence of the quinolone resistance *crpP* genes and the *crpP*-carrying Tn6786-related integrative and conjugative elements in *Pseudomonas aeruginosa*. *Virulence* 12, 2162–2170. doi: 10.1080/21505594.2021.1962160
- Zong, Z. (2014). The complex genetic context of *bla*_{PER-1} flanked by miniature inverted-repeat transposable elements in *Acinetobacter johnsonii*. *PLoS ONE* 9:e90046. doi: 10.1371/journal.pone.0090046