Title Page

A novel mobile RND-type efflux pump gene cluster, *tmexC3D2-toprJ3*, confers tigecycline resistance in *Pseudomonas alcaligenes*

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Running Title: A novel *tmexC3D2-toprJ3* gene cluster in *P. alcaligenes*

Keywords: RND efflux pump, TMexCD-TOprJ, metallo-β-lactamase, IMP, class I integron, super-integron*, Pseudomonas alcaligenes*

Abstract

Tigecycline exhibits promising activity against multidrug-resistant gram-negative bacteria (MDR-GNB). However, mobile tigecycline resistance genes, such as *tmexCD-toprJ* encoding RND efflux pumps, have emerged. Here, we identified a novel *tmexC3D2-toprJ3* gene cluster in tigecycline- and carbapenem-nonsusceptible *Pseudomonas alcaligenes* isolates from hospital sewage in Japan in 2020. *tmexC3D2-toprJ3* and two copies of bla_{IMP-1} were located on the chromosome. This suggests that diverse *tmexCD-toprJ*-like genes have spread among MDR-GNB worldwide and further epidemiological genomic studies are needed.

Main Text

Tigecycline is considered a last-resort antimicrobial against infections caused by multidrug-resistant gram-negative bacteria (MDR-GNB). Recently, mobile tigecycline resistance genes, *tet*(X3), *tet*(X4), and other variants, *tet*(X5) to *tet*(X15), encoding flavin-dependent monooxygenases that catalyze tigecycline degradation have emerged (1-4). Furthermore, mobile tigecycline resistance gene clusters, *tmexCD1-toprJ1*, *tmexCD2-toprJ2*, and *tmexCD3-toprJ3*, encoding the resistance–nodulation–cell division (RND) efflux pumps that excrete multiple antimicrobials, including tetracyclines such as tigecycline, cephalosporins, fluoroquinolones, and aminoglycosides, have emerged (5-8).

Here, we report *Pseudomonas alcaligenes* isolates harboring a novel variant of *tmexCD-toprJ* along with two copies of a metallo-β-lactamase (MBL) gene, bla_{IMP-1}. *P. alcaligenes* is a gram-negative aerobic rod belonging to the bacterial family Pseudomonadaceae, of which members are common inhabitants of soil and water and are rare opportunistic human pathogens (9). *P. alcaligenes* has also been suggested to be a causative agent of secondary bacterial infection during COVID-19 pneumonia (10). However, little is known about the clinical importance of *P. alcaligenes*, mainly because of the difficulties in identifying and distinguishing this bacterium from closely related *Pseudomonas* species such as *P. aeruginosa*, *P. mendocina,* and *P. pseudoalcaligenes*, in medical settings.

Eight ceftriaxone-resistant isolates of *P. alcaligenes* were obtained from sewage water from a medical institution in Japan in 2020. Whole-genome sequence analysis of *P. alcaligenes* isolates using HiSeq X (Illumina) and the core genome phylogeny based on their draft genome sequences showed that

these isolates were phylogenetically very similar (Fig. S1). Moreover, all *P. alcaligenes* isolates harbored the same set of antimicrobial resistance (AMR) genes, including *tmexCD-toprJ*-like genes, bla_{IMP-1} (MBL gene conferring carbapenem resistance), *aac(6')-Ib-cr* (aminoglycoside resistance gene), *fosE* (fosfomycin resistance gene), *qacG2* (multidrug resistance gene), and *sul1* (sulfonamide resistance gene), suggesting that these isolates were clonally disseminated (Fig. S1).

One of the *P. alcaligenes* isolates, KAM426, was further sequenced using MinION [Oxford Nanopore Technologies (ONT)], and hybrid sequence analysis using both Illumina and ONT reads resulted in the circular complete chromosome sequence (4.68 Mb, accession no. AP024354). Average nucleotide identity (ANI) analysis revealed that KAM426 is 96.5% identical to *P. alcaligenes* strain NCTC 10367^T (type strain, accession no. UGUP00000000), and the isolate harbored *tmexCD-toprJ*-like genes along with two copies of bla_{IMP-1} on its chromosome (Fig. S2). Antimicrobial susceptibility testing showed that *P. alcaligenes* KAM426 was nonsusceptible to tigecycline and broad-spectrum β-lactams, including carbapenems. According to the broth dilution method based on CLSI 2020, the minimum inhibitory concentration (MIC) of tigecycline against KAM426 was 2 mg/L and was decreased to 1 mg/L in the presence of the efflux pump inhibitor 1-(1-naphthylmethyl)-piperazine (75 mg/L). According to the Etest (bioMérieux) based on to the manufacturer instructions, the MICs of imipenem and meropenem against KAM426 were 8 and >32 mg/L, respectively, and these were decreased to <1 and 0.19 mg/L, respectively, in the presence of the MBL inhibitor EDTA. These results

suggested that *tmexCD-toprJ*-like genes and bla_{IMP-1} are responsible for tigecycline and carbapenem resistance in this isolate.

The coding sequences of *tmexCD-toprJ*-like genes in *P. alcaligenes* KAM426 (KAM426_19240, KAM426_19250, and KAM426_19260 in accession no. AP024354) were highly identical to those of *tmexCD1-toprJ1* in *Klebsiella pneumoniae* strain AH58I (accession no. MK347425) isolated from livestock in China in 2017 (5), those of *tmexCD2-toprJ2* in *Raoultella ornithinolytica* strain NC189 (accession no. MN175502) isolated from a human in China in 2018 (7), and those of *tmexCD3-toprJ3* in *Proteus mirabilis* strain RGF134-1 (accession no. CP066833) isolated from a pig in China in 2019 (8), respectively. The identities of the *tmexC*-like gene in KAM426 (KAM426_10690) compared with *tmexC1*, *tmexC2*, and *tmexC3* were 94.0% (1094/1164 nt), 94.6% (1101/1164 nt), and 98.6% (1148/1164 nt, the gene product was 98.7% identical to TMexC3 with five amino acid substitutions), respectively. For the *tmexD*-like gene in KAM426 (KAM426_10700), these identities were 96.5% (3025/3136 nt), 99.1% (3108/3135 nt, the gene product was perfect match to TMexD2), and 97.1% (3046/3136 nt), respectively. For the *toprJ*-like gene in KAM426 (KAM426_10710), the identities were 99.9% (1433/1434 nt), 99.9% (1417/1419 nt), and 100% (1434/1434 nt, the gene product was perfect match to TOprJ3), respectively. Thus, we designated *tmexCD-toprJ*-like genes in KAM426 as *tmexC3D2-toprJ3*.

The *nfxB*-like gene, which has been suggested to be involved in the expression of the *tmexCD-toprJ*-like gene (5), was found upstream *tmexC3D2-toprJ3* in *P. alcaligenes* KAM426 (KAM426_19230 in accession no.

AP024354), and *tmexC3D2-toprJ3* was flanked by the IS*5*/IS*1182* family transposase gene (Fig. 1A upper). Furthermore, the genomic region containing *nfxB* and *tmexC3D2-toprJ3* in KAM426 was surrounded by many putative mobile gene elements (MGEs), and this genomic region was not present in *P. alcaligenes* strain NEB 585 (accession no. CP014784) (Fig. 1A). NEB 585 was isolated from a water environment in the United States in 1989 and is the only other *P. alcaligenes* strain for which the complete chromosome sequence has been reported (11) other than KAM426. The genomic recombination regions in KAM426 and NEB 585 encoded a set of common genes (KAM426_19430 to KAM426_19470 in accession no. AP024354, and A0T30_13575 to A0T30_13555 in accession no. CP014784) (Fig. 1A), although their functions are unknown. The results suggest that KAM426 acquired *tmexCD-toprJ*-like genes, which confer resistance to multiple antimicrobials including tigecycline, via horizontal gene transfer (HGT) mediated by MGEs.

BLASTn analysis using megablast revealed that two *Pseudomonas* spp. strains in the NCBI database of Nucleotide collection (nr/nt) have the exact same sequence containing the *tmexC3D2-toprJ3* gene cluster along with *nfxB*. A $bla_{\text{DIM-2}}$-harboring *Pseudomonas* sp. strain, BJP69, isolated from a human in China in 2015 (12) carries *tmexC3D2-toprJ3* on its chromosome (accession no. CP041933) and a $bla_{\text{KPC-2}}$-harboring *P. aeruginosa* strain, NDTH9845, isolated from a human in China in 2018 carries *tmexC3D2-toprJ3* on plasmid pNDTH9845 (accession no. CP073081) (Fig. 1B). ANI analysis confirmed that BJP69 is 98.0% identical to *Pseudomonas juntendi* strain BML3^{T} (type strain, accession no. BLJG01000000) and that NDTH9845 is 99.2% identical to *P.*

aeruginosa DSM 50071^{T} (type strain, accession no. FUXR01000000). *tmexC3D2-toprJ3* was determined to be flanked by the IS*5*/IS*1182* family transposase gene in *P. aeruginosa* pNDTH9845, whereas no MGE was found upstream or downstream of *tmexC3D2-toprJ3* in *P. juntendi* BJP69 (Fig. 1B). Together with *P. alcaligenes* KAM426 in this study, these three *Pseudomonas* spp. strains harbor acquired carbapenemase genes, in addition to *tmexC3D2-toprJ3*, showing that they have accumulated clinically relevant AMR genes.

The integron-integrase IntI1 catalyzes site-specific recombination between the *attI1* and *attC* sites (13). The class 1 integron gene cassette consisting of *intI1* with the *attI1* site, *qacEΔ1* (disrupted form of *qacE*), and *sul1* in *P. alcaligenes* KAM426 (accession no. AP024354) contain several AMR genes, including *fosE*, two copies of *aac(6')-Ib-cr*, *bla*$_{IMP-1}$, and *qacG2* with their *attC* sites (Fig. 2A). The *bla*$_{IMP-1}$-containing integron gene cassette in KAM426 was found to be surrounded by many putative transposase genes, and this MGE-containing genomic region was not present in *P. alcaligenes* NEB 585 (accession no. CP014784) (Fig. 2A). The genomic region around ATP-dependent helicase genes (KAM426_37950 and KAM426_36180 in accession no. AP024354, and A0T30_05350 in accession no. CP014784) could be a hot spot for HGT (Fig. 2A), but there have been no reports to suggest this possibility to date.

The other copy of *bla*$_{IMP-1}$ was contained within a partial structure of the integron gene cassette consisting of two copies of *aac(6')-Ib-cr* followed by *bla*$_{IMP-1,}$ as described previously herein (Fig. 2A), in a different location in the chromosome of *P. alcaligenes* KAM426 (Fig. 2B). Interestingly, a comparison

between the *bla*$_{IMP-1}$-containing genomic region of *P. alcaligenes* KAM426 and the corresponding genomic region in *P. alcaligenes* NEB 585 revealed the presence of multiple copies of short repeated sequences (approximately 80 bp), which were identical to PARs (*Pseudomonas alcaligenes* repetitive DNAs) within the super-integron In*55044* in *P. alcaligenes* strain ATCC 55044 (accession no. AY038186) (14) (Fig. 2B).

The super-integron, which was first identified in *Vibrio cholerae*, is distinguished from conventional integrons in several respects, such as size and the nature of the genes contained within cassettes and contributes to the acquisition of AMR genes (15-17). The PARs were reported as recombination sites for the integrase gene (*intI*$_{Pac}$ in accession no. AY038186) in the super-integron in *P. alcaligenes* (14). The PARs in *P. alcaligenes* KAM426 and *P. alcaligenes* NEB 585 contained conserved sequences of inverted repeats (1L, 2L, 2R, and 1R) with a PAR signature and variable regions between inverted repeats 2L and 2R, as shown in *P. alcaligenes* ATCC 55044 (14) (Fig. S3). Although KAM426 and NEB 585 lacked the integrase gene flanking the PAR and most of the contained genes had no known function, these plastic genomic regions in both strains retained their evolutionary histories of gene acquisitions mediated by the super-integron. There was no PAR around *bla*$_{IMP-1}$ in KAM426, suggesting that the super-integron is not directly involved in the acquisition of *bla*$_{IMP-1}$, and this genomic region is likely one of the hot spots for HGT. KAM426 is thought to have incorporated *bla*$_{IMP-1}$ into the class 1 integron gene cassette first (Fig. 2A) and then incorporated the partial structure containing *bla*$_{IMP-1}$ into the other genomic region flanked by the super-integron (Fig. 2B), leading to a

high level of resistance by increasing the copy number of AMR genes.

Our study provides a glimpse into environmental bacteria that have been rapidly and silently becoming resistant to clinically relevant antimicrobials, including tigecycline and carbapenem, and highlights the importance of AMR monitoring using wastewater to detect a future clinical crisis before it happens. Furthermore, *P. alcaligenes* would be considered an important environmental reservoir that supplies AMR genes to other related *Pseudomonas* species that are more virulent and likely to cause nosocomial infections, such as *P. aeruginosa*.

Nucleotide Sequences

The complete genome sequence of *P. alcaligenes* KAM426 has been deposited at GenBank/EMBL/DDBJ under the accession number AP024354. Draft genome sequences of *P. alcaligenes* KAM428, KAM429, KAM430, KAM432, KAM434, KAM435, and KAM436 have been deposited at GenBank/EMBL/DDBJ under the accession numbers BPMN00000000, BPMO00000000, BPMP00000000, BPMQ00000000, BPMR00000000, BPMS00000000, and BPMT00000000, respectively.

Funding

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Transparency declarations

None to declare.

Legends

Fig. 1. The *tmexC3D2-toprJ3* gene cluster in *Pseudomonas alcaligenes* KAM426. (A) Genetic context of the *tmexC3D2-toprJ3* gene cluster in *P. alcaligenes* KAM426 and its surrounding genomic region (the region between 1,977,208 and 2,027,209 nt in accession no. AP024354), and structural comparison with the corresponding genomic region in *P. alcaligenes* NEB 585 (the region between 2,990,265 and 2,915,762 nt in accession no. CP014784). (B) Structural comparison of the *tmexC3D2-toprJ3* gene cluster in *P. alcaligenes* KAM426 (the region between 1,995,475 and 2,006,928 nt in accession no. AP024354) with that in the chromosome of *P. juntendi* BJP69 (the region between 3,340,041 and 3,349,533 nt in accession no. CP041933) and in plasmid pNDTH9845 of *P. aeruginosa* NDTH9845 (the region between 225,014 and 215,640 nt in accession no. CP073081). The strain names of *Pseudomonas* spp., along with the country and year in which bacteria were isolated, are shown. *tmexC3D2-toprJ3* genes (TRG), other AMR genes (ARG), mobile gene elements (MGE), and other genes (Other) are highlighted in red, yellow, light blue, and gray, respectively. Sequence identity is shown as a color scale with the indicated percentages. Linear comparisons of sequences were performed using BLASTn and visualized with Easyfig (http://mjsull.github.io/Easyfig/).

Fig. 2. Two copies of bla_{IMP-1} genes in *Pseudomonas alcaligenes* KAM426. (A) Genetic context of the class I integron gene cassette containing bla_{IMP-1} in *P. alcaligenes* KAM426 and its surrounding genomic region (the region between 3,757,827 and 4,033,855 nt in accession no. AP024354), and structural

238 comparison with the corresponding genomic region in *P. alcaligenes* NEB 585
239 (the region between 1,159,724 and 1,200,667 nt in accession no. CP014784).
240 (B) Genetic context of the partial integron gene cassette containing the other
241 bla_{IMP-1} gene in *P. alcaligenes* KAM426 and its surrounding genomic region (the
242 region between 2,181,279 and 2,188,592 nt in accession no. AP024354), and
243 structural comparison with the corresponding genomic regions in *P. alcaligenes*
244 NEB 585 (the region between 2,758,939 and 2,768,635 nt in accession no.
245 CP014784) and in *P. alcaligenes* ATCC 55044 (super-integron In*55044* in
246 accession no. AY038186). The strain names of *P. alcaligenes*, along with the
247 country and year in which bacteria were isolated, are shown. bla_{IMP-1} genes
248 (CRG), other AMR genes (ARG), mobile gene elements (MGE), other genes
249 (Others), and *P. alcaligenes* repetitive DNA (PAR) are highlighted in red, yellow,
250 light blue, gray, and khaki green, respectively. Sequence identity is shown as a
251 color scale with the indicated percentages. Linear comparisons of sequences
252 were performed using BLASTn and visualized with Easyfig
253 (http://mjsull.github.io/Easyfig/).
254
255 Fig. S1. Core genome phylogeny constructed by Roary v3.13.0
256 (https://github.com/sanger-pathogens/Roary) with minimum percentage identity
257 for BLASTp=70% and RAxML v8.2.4
258 (https://github.com/stamatak/standard-RAxML), with 1,000 bootstraps using
259 ceftriaxone-resistant *Pseudomonas alcaligenes* isolates in this study and
260 reference strains of *P. alcaligenes* and *P. aeruginosa* (NCTC 10367^T and NEB
261 585 for *P. alcaligenes*, and PAO1 for *P. aeruginosa*). *P. aeruginosa* was used as

the outgroup. Bar lengths represent the number of substitutions per site in the core genome. AMR genes shown in color were detected by ResFinder v4.1 (https://cge.cbs.dtu.dk/services/ResFinder) with the customized AMR gene database, including known *tmexCD-toprJ* genes. Genome assembly status (complete or draft genome sequence, contig numbers if draft), sizes, and accession numbers are shown.

Fig. S2. Circular representation of the chromosome of *Pseudomonas alcaligenes* KAM426 (accession no. AP024354) harboring *tmexC3D2-toprJ3*, along with two copies of bla_{IMP-1} (shown in Figs. 1, 2A, and 2B), isolated in Japan in 2020. This was visualized with the CGView server (http://cgview.ca). Gray, green, purple, black, red, yellow, cyan, light green, and orange indicate coding sequences (CDS), GC skew+, GC skew-, GC content, tigecycline or carbapenem resistance genes (TRG/CRG), other AMR genes (ARG), mobile gene elements (MGE), type IV secretion system (T4SS)-associated genes, and type VI secretion system (T6SS)-associated genes, respectively. T4SS- and T6SS-associated genes were detected by TXSScan v1.0.5 (https://research.pasteur.fr/en/tool/txsscan-models-and-profiles-for-protein-secretion-systems/).

Fig. S3. Alignment of *Pseudomonas alcaligenes* repetitive DNAs (PARs) in *P. alcaligenes* strains. One PAR in *P. alcaligenes* ATCC 55044 (super-integron In*55044* in accession no. AY038186), two PARs in *P. alcaligenes* KAM426 (accession no. AP024354), and 11 PARs in *P. alcaligenes* NEB 585 (accession

286 no. CP014784) are shown. The multiple alignment comparison was performed
287 and visualized using MAFFT v7 (https://mafft.cbrc.jp/alignment/software/). The
288 PAR signature sequence and variable region are shown. Open boxes and
289 arrows represent consensus sequences of inverted repeats (1L, 2L, 2R, and 1R),
290 as described previously (14).
291

References

1. Fang LX, Chen C, Cui CY, Li XP, Zhang Y, Liao XP, Sun J, Liu YH. Emerging High-Level Tigecycline Resistance: Novel Tetracycline Destructases Spread via the Mobile Tet(X). **Bioessays.** 2020 42(8):e2000014.

2. Gasparrini AJ, Markley JL, Kumar H, Wang B, Fang L, Irum S, Symister CT, Wallace M, Burnham CD, Andleeb S, Tolia NH, Wencewicz TA, Dantas G. Tetracycline-inactivating enzymes from environmental, human commensal, and pathogenic bacteria cause broad-spectrum tetracycline resistance. **Commun Biol.** 2020 3(1):241.

3. Cheng Y, Chen Y, Liu Y, Guo Y, Zhou Y, Xiao T, Zhang S, Xu H, Chen Y, Shan T, Xiao Y, Zhou K. Identification of novel tetracycline resistance gene *tet*(X14) and its co-occurrence with *tet*(X2) in a tigecycline-resistant and colistin-resistant *Empedobacter stercoris*. **Emerg Microbes Infect.** 2020 9(1):1843-1852.

4. Li R, Peng K, Xiao X, Wang Y, Wang Z. Characterization of novel IS*Aba1*-bounded *tet*(X15)-bearing composite transposon Tn6866 in *Acinetobacter variabilis*. **J Antimicrob Chemother.** 2021 in press.

5. Lv L, Wan M, Wang C, Gao X, Yang Q, Partridge SR, Wang Y, Zong Z, Doi Y, Shen J, Jia P, Song Q, Zhang Q, Yang J, Huang X, Wang M, Liu JH. Emergence of a Plasmid-Encoded Resistance-Nodulation-Division Efflux Pump Conferring Resistance to Multiple Drugs, Including Tigecycline, in *Klebsiella pneumoniae*. **mBio.** 2020 Mar 3;11(2):e02930-19.

6. Hirabayashi A, Ha VTT, Nguyen AV, Nguyen ST, Shibayama K, Suzuki M. Emergence of a plasmid-borne tigecycline resistance in *Klebsiella pneumoniae* in Vietnam. **J Med Microbiol.** 2021 70(3). doi: 10.1099/jmm.0.001320.

316 7. Wang CZ, Gao X, Yang QW, Lv LC, Wan M, Yang J, Cai ZP, Liu JH. A Novel
317 Transferable Resistance-Nodulation-Division Pump Gene Cluster,
318 *tmexCD2-toprJ2*, Confers Tigecycline Resistance in *Raoultella ornithinolytica*.
319 **Antimicrob Agents Chemother.** 2021 65(4):e02229-20.
320 8. Wang Q, Peng K, Liu Y, Xiao X, Wang Z, Li R. Characterization of
321 TMexCD3-TOprJ3, an RND-Type Efflux System Conferring Resistance to
322 Tigecycline in *Proteus mirabilis*, and Its Associated Integrative Conjugative
323 Element. **Antimicrob Agents Chemother.** 2021 65(7):e0271220.
324 9. Suzuki M, Suzuki S, Matsui M, Hiraki Y, Kawano F, Shibayama K. Genome
325 Sequence of a Strain of the Human Pathogenic Bacterium *Pseudomonas*
326 *alcaligenes* That Caused Bloodstream Infection. **Genome Announc.** 2013
327 1(5):e00919-13.
328 10. Gaibani P, Viciani E, Bartoletti M, Lewis RE, Tonetti T, Lombardo D,
329 Castagnetti A, Bovo F, Horna CS, Ranieri M, Viale P, Re MC, Ambretti S. The
330 lower respiratory tract microbiome of critically ill patients with COVID-19. **Sci**
331 **Rep.** 2021 11(1):10103.
332 11. Morgan RD, Luyten YA, Johnson SA, Clough EM, Clark TA, Roberts RJ.
333 Novel m4C modification in type I restriction-modification systems. **Nucleic**
334 **Acids Res.** 2016 44(19):9413-9425.
335 12. Jiang X, Yin Z, Yuan M, Cheng Q, Hu L, Xu Y, Yang W, Yang H, Zhao Y, Zhao
336 X, Gao B, Dai E, Song Y, Zhou D. Plasmids of novel incompatibility group
337 IncpRBL16 from *Pseudomonas* species. **J Antimicrob Chemother.** 2020
338 75(8):2093-2100.

13. Gillings MR. Integrons: past, present, and future. **Microbiol Mol Biol Rev.** 2014 78(2):257-77.

14. Vaisvila R, Morgan RD, Posfai J, Raleigh EA. Discovery and distribution of super-integrons among pseudomonads. **Mol Microbiol.** 2001 42(3):587-601.

15. Mazel D, Dychinco B, Webb VA, Davies J. A distinctive class of integron in the *Vibrio cholerae* genome. **Science.** 1998 280(5363):605-8.

16. Rowe-Magnus DA, Guerout AM, Ploncard P, Dychinco B, Davies J, Mazel D. The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. **Proc Natl Acad Sci U S A.** 2001 98(2):652-7.

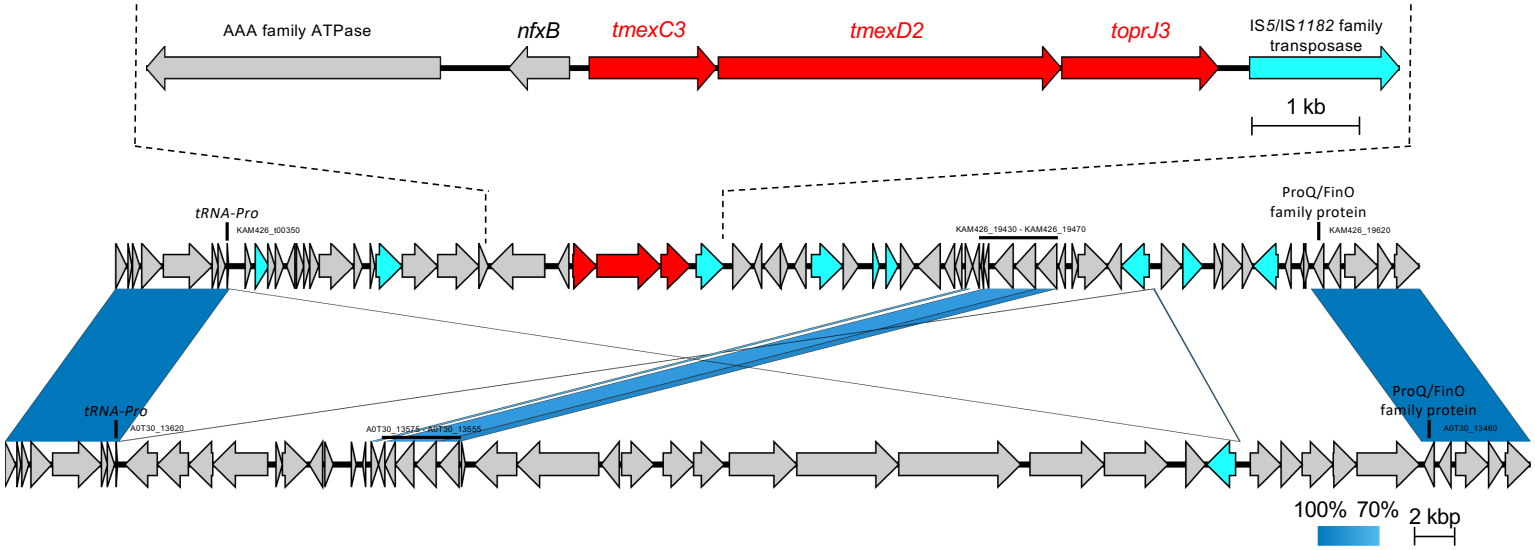
17. Rowe-Magnus DA, Guerout AM, Mazel D. Bacterial resistance evolution by recruitment of super-integron gene cassettes. **Mol Microbiol.** 2002 43(6):1657-69.

Fig. 1

A

P. alcaligenes
KAM426
(1,977,208 - 2,027,209 nt)
Japan, 2020

P. alcaligenes
NEB 585
(2,990,265 - 2,915,762 nt)
USA, 1989



B

P. alcaligenes
KAM426
(1,995,475 - 2,006,928 nt)
Japan, 2020

P. juntendi
BJP69
(3,340,041 - 3,349,533 nt)
China, 2015

P. aeruginosa
pNDTH9845
(225,014 - 215,640 nt)
China, 2018

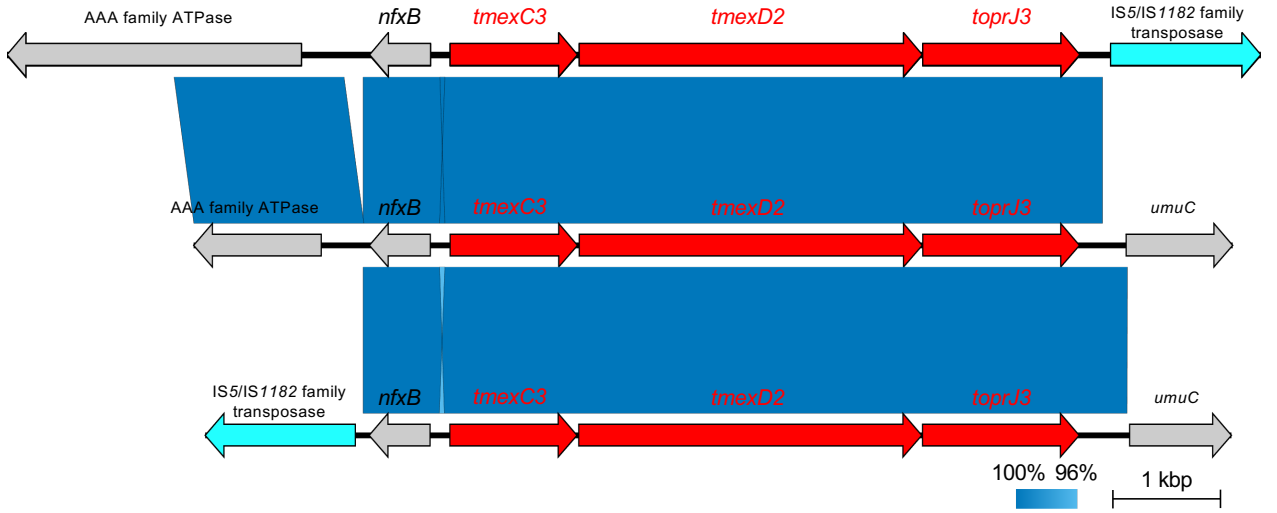
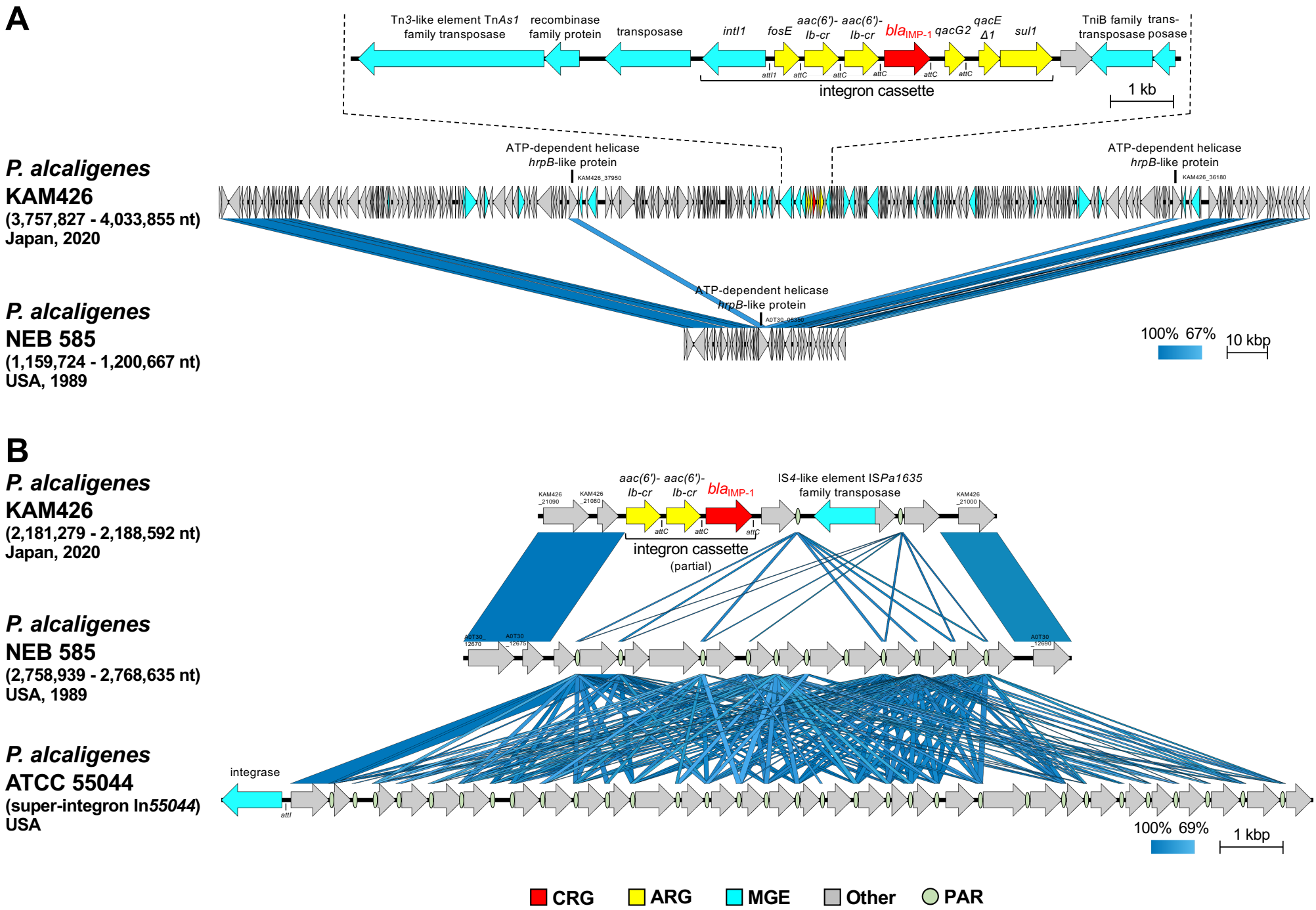


Fig. 2


A
Tn3-like element TnAs1 family transposase
recombinase family protein
transposase
intI1
fosE
aac(6')-Ib-cr
aac(6')-Ib-cr
blaIMP-1
qacG2
qacE Δ1
sul1
TniB family trans-transposase posase
attI1
attC
integron cassette
1 kb
P. alcaligenes
KAM426
(3,757,827 - 4,033,855 nt)
Japan, 2020
ATP-dependent helicase hrpB-like protein
KAM426_37950
KAM426_36180
P. alcaligenes
NEB 585
(1,159,724 - 1,200,667 nt)
USA, 1989
A0T30_05350
100% 67%
10 kbp
B
P. alcaligenes
KAM426
(2,181,279 - 2,188,592 nt)
Japan, 2020
KAM426_21090
KAM426_21080
KAM426_21000
IS4-like element ISPa1635 family transposase
integron cassette
(partial)
P. alcaligenes
NEB 585
(2,758,939 - 2,768,635 nt)
USA, 1989
A0T30_12670
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A0T30_12690
P. alcaligenes
ATCC 55044
(super-integron In55044)
USA
integrase
attI
100% 69%
1 kbp
CRG
ARG
MGE
Other
PAR