SUPPORTING INFORMATION for

Novel transposon Tn*6433* variants accelerate the dissemination of *tet*(E) in *Aeromonas* in an aerobic biofilm reactor under oxytetracycline stresses

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Supporting materials and methods

Detection of tetracycline resistance genes by PCR screening

The presence of eight tetracycline resistance genes, including five efflux protein genes (*tet*(A), *tet*(C), *tet*(E), *tet*(G), *tet*(L)), two ribosomal protection protein genes *tet*(36) and *tet*(Q), and one inactivating enzyme gene *tet*(X) in the *Aeromonas* strains was determined by PCR screening. PCR primers are listed in Table S2. The PCR assays were conducted in 50 μL volume reactions using the ABI PCR System 9700 (ABI, USA). The PCR mixture contained 1×rTaq buffer, 1.25 U rTaq (Takara, Japan), 200 μM dNTP, 0.2 μM of each primer, ~10 ng of template DNA. The PCR conditions were performed with the following program: initial denaturation at 94 °C for 5 min; 30 cycles denaturation at 94 °C for 30 s, annealing at different temperatures for 30 s, elongation at 72 °C for 30 s, and then a final extension at 72 °C for 5 min.[1-3] Each sample was amplified in duplicate, and PCR products were checked by electrophoresis on a 1% (w/v) agarose gel in 1 × TAE buffer.

Whole genome sequencing

Eight *Aeromonas* strains were used for whole genome sequencing, including five *A. media* strains from OTC-0.1, OTC-1, OTC-5, OTC-25, and OTC-50 (T0.1-19, R1-18, T5-8, R25-3 and R50-22), and three *A. caviae* strains from OTC-25 (R25-2, R25-6 and T25-39).[4] Genomic DNA extractions were performed from 2 mL TSB cultures, incubated at 30 °C overnight with shaking at 150 rpm, using the Wizard® Genomic DNA Purification Kit (Promega Madison, USA). Genomic DNA was checked by electrophoresis on a 1% (w/v) agarose gel in 1 × TAE buffer, and quantified using the Quant-iT dsDNA HS assay (Invitrogen), following the manufacturers' instructions. All extracted DNA was sent to the Beijing Institute of Genomics (China). The SMRTbell™

libraries were prepared according to the manufacturer's instructions (Pacific Biosciences, Menlo Park, CA, USA) and sequenced on a PacBio RSII platform (Pacific Biosciences) using the C4 chemistry and P6 polymerase with average median depth of coverage 100×.[5] The raw sequences were then filtered and *de novo* assembled using the SMRT Analysis package (SMRTAnalysis 2.3.0) and hierarchical genome assembly process (HGAP3) workflow with standard parameters. Quiver was used to polish the full genomic DNA sequences to obtain high consensus accuracy.[6]

Detection of *tet*(E)-carrying MGEs by PCR screening

The PCR primers used in the present study are listed in Table S2. Specific primers were designed for insert IN-01 (*tet*(E)-carrying insertions into the *Aeromonas* chromosome by phage-like *int*), insert IN-02 (*tet*(E)-carrying insertion into the *Aeromonas* chromosome by a Tn*3* family transposon, named here as Tn*6433*), and plasmids (pAeca1-a and pAeca2 variants), respectively. One primer pair with the forward primer targeting the *tet*(E) gene and reverse primer targeting the *recD* gene was designed to test the presence of insert IN-01 in the *tet*(E)-positive isolates. Another set of primers based on the *tet*(E) gene and Tn*1722*-like/Tn*501*-like *tnpA* gene (the two genes shared 100% similarity), which belonged to the core region of the Tn*6433* variants, was used to detect the presence of the Tn*6433* variants in the *tet*(E)-positive isolates. Three sets of primers targeting the backbone genes (*repA*, *parA*, *traI*) of plasmids were used to detect the presence of the plasmids pAeca1-a and pAeca2 variants in the *Aeromonas* isolates without genome sequencing. To confirm the specificity of plasmid primers, three target genes *repA*, *parA* and *traI* were aligned against the NCBI database by BLASTX as shown in Tables S9 and S10. The amino acid sequences of *repA*, *traI* and *parA* genes of plasmid pAeca1-a showed 100% identity with *RepA*, *TraI* and *ParA* of

the recently discovered plasmids pS121-1a and pS121-1b harbored by *Aeromonas salmonicida*,[7] which were homologous of plasmid pAeca1-a. So the three target genes were specific to the plasmid pAeca1-a variants. As for the plasmid pAeca2, the *repA* gene exhibited 66% similarity to that of *Aeromonas salmonicida*, the *traI* 33% to that of *Xenorhabdus bovienii*, and the *parA* 39% to that of *Vibrio crassostreae*, respectively, indicating that the three set of primers are specific for pAeca2. Meanwhile, the criterion for the presence of the target plasmid is that all of the three sets of plasmid primers for each plasmid showed positive results simultaneously. At the same time, the annealing temperatures (52 ^{o}C, 55 ^{o}C, 58 ^{o}C) has been optimized to obtain sensitive responses for the three target genes *repA*, *parA* and *traI*, respectively, and the PCR detection for each strain was conducted after overnight cultivation to increase biomass.

Statistical analysis

The change in the abundance of *Aeromonas* was visualized using Origin 9.2.32 (OriginLab, USA). The Pearson x^2 test based on a 2×2 contingency table was also conducted by R 3.3.2 to compare of *tet*(E)-carrying MGEs at below-breakpoint and above-breakpoint concentrations of oxytetracycline.

Data availability

Complete genome sequences of the five *A. media* strains T0.1-19, R1-18, T5-8, R25-3 and R50-22 were deposited in the NCBI database with accession numbers ranging from CP038441 to CP038450, and complete genome sequences of the three *A. caviae* strains R25-2, R25-6, T25-39, with accession numbers CP039626-CP039630, CP025777, CP025705, and CP025706.[4] Other sequences of *Aeromonas* were also deposited in the NCBI database with accession numbers ranging from MK656323 to MK656385 for

16S rRNA, from MK675438 to MK675500 for the partial *gyrB* gene, from MK690452 to MK690454 for partial insert IN-01, from MK690457 to MK690459 for partial Tn*6433* variants, from MK690434 to MK690442 for partial *repA*, *parA*, and *traI* genes in plasmid pAeca1-a variants, from MK690443 to MK690451 for partial *repA*, *parA*, and *traI* genes in plasmid pAeca2 variants. Nucleotide sequences (16S rRNA genes of the 534 isolates, partial *tet*(E) gene of 21 isolates, partial *repA*, *parA*, and *traI* genes in plasmid pAeca1-a variants of *Microbacterium* sp. strain R50-10, partial *repA* and *parA* genes in plasmid pAeca2 variants of *Methyloversatilis discipulorum* strain R0.1-29) have been deposited in the GenBank database under accession numbers from MN330075 to MN330608, from MN332201 to MN332226.

Figures

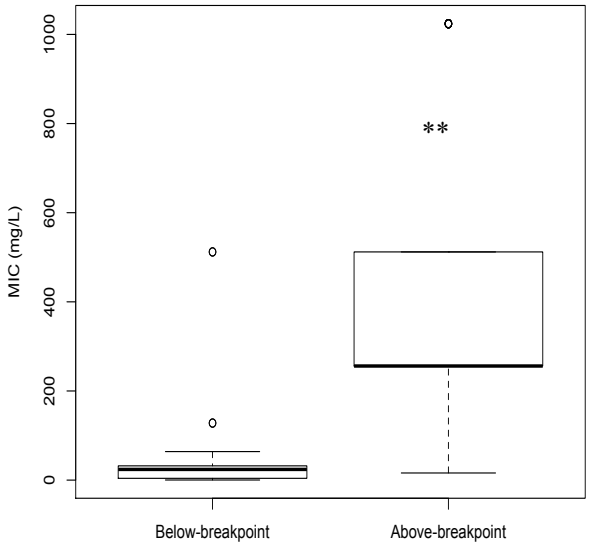

Figure S1. Changes to the MICs in *Aeromonas* below and above the oxytetracycline breakpoints. *P*-value was calculated using the Mann-Whitney *U* test (** *P*<0.001). Below-breakpoint stages included 0.1, 1, and 5 mg/L oxytetracycline, and above-breakpoint stages contained 25 and 50 mg/L oxytetracycline.

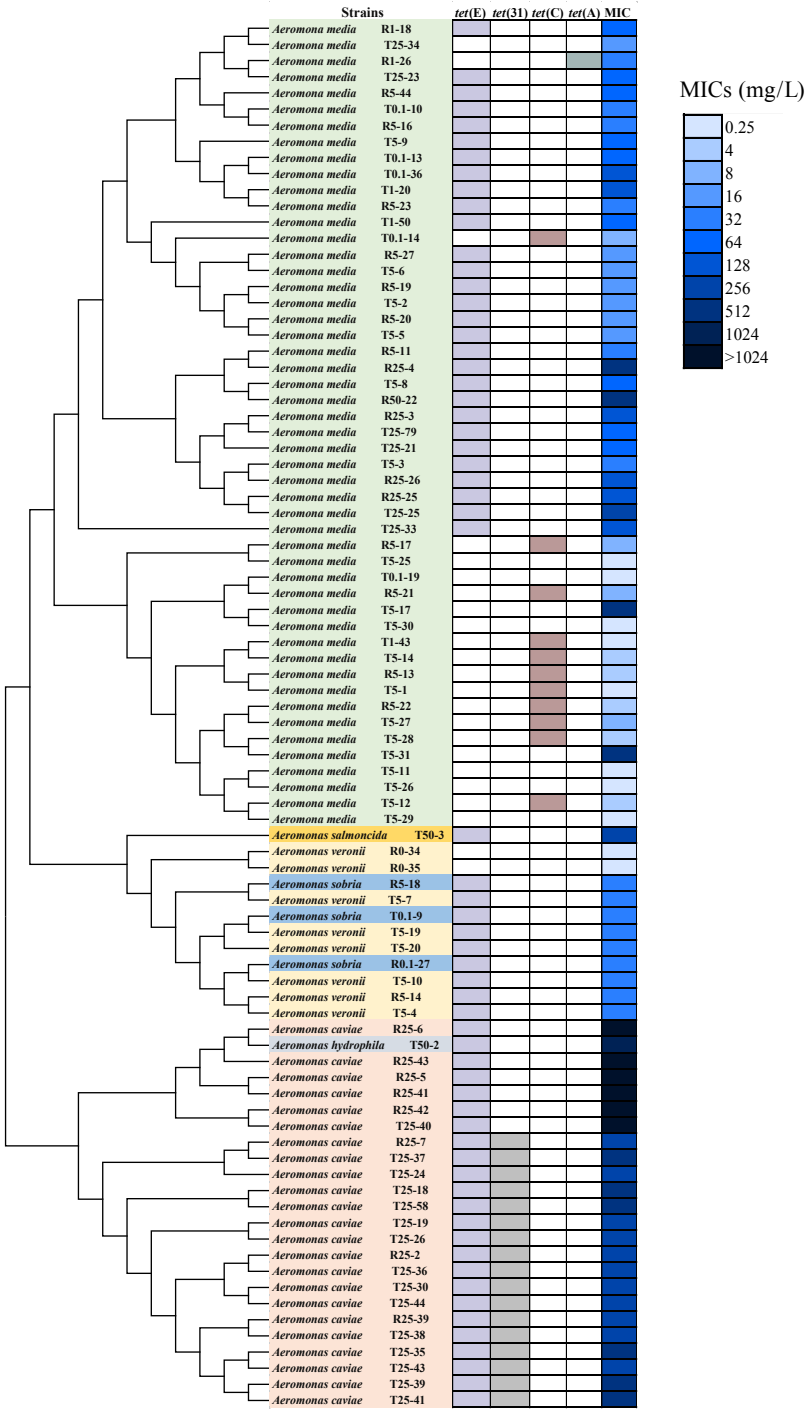

Figure S2. Dendrogram relationship of the *Aeromonas* strains isolated from the aerobic biofilm reactor. MICs to oxytetracycline of the 86 *Aeromonas* isolates were determined by broth microdilution, and the presence of the *tet* genes was detected by PCR screening. Isolates belonging to the same species are marked with the same color.

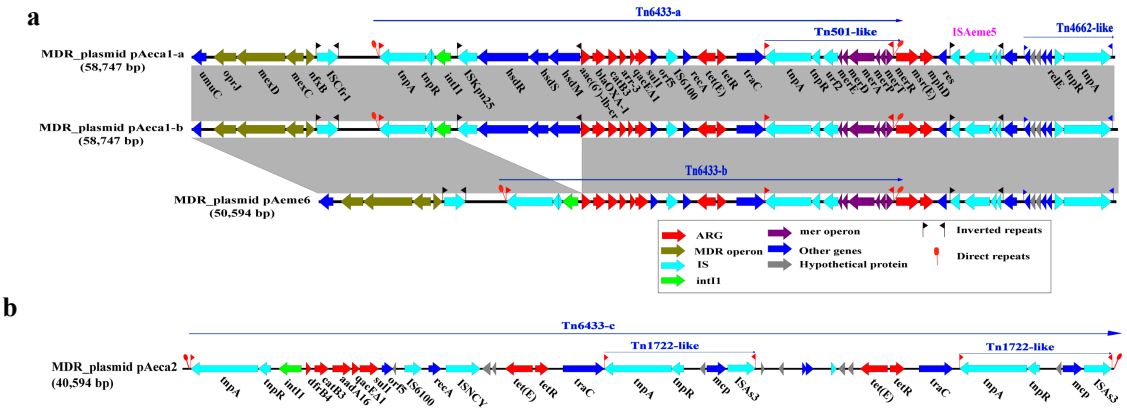

Figure S3. The multidrug resistance (MDR) region of four *tet*(E)-carrying plasmids. a, The MDR region of plasmid pAeca1-a, pAeca1-b, and pAeme6, began with the *umuC* gene, and ended with the Tn*4662*-like *tnpA*, including a *mexCD-oprJ* multidrug resistance operon, *ISCfr1*, Tn*6433-a*/Tn*6433-b*, macrolide resistance gene combination (*msr*(E) and *mphD*), *ISAeme5*, and a Tn*4662*-like transposon. The 38-bp inverted repeats of Tn*6433-a* and Tn*6433-b*, IRL (5'-GGGGAGCCCGCAGAATTCGGAAAAAATCGTACGCTAAG-3'), IRR (5'-GGGGTCGTCTCAGAAAACGGAAAATAAAGCACGCTAAG-3') are highlighted. The 5-bp direct sequence (5'-TAC/TTT-3') of Tn*6433* is directly adjacent to the IR. **b**, The MDR region of plasmid pAeca2 was a novel *tet*(E)-carrying transposon Tn*6433-c*, also a derivative of Tn*6433*, contained the core *tnpA* and *tnpR*, class 1 integron, the core passenger genes (*recA*, *tet*(E)-*tetR*, *traC*), two copies of Tn*1722*-like transposon. Tn*6433-c* was bounded by 38-bp IRs, IRL (5'-GGGGAGCCCGCAGAATTCGGAAAAAATCGTACGCTAAG-3'), IRR (5'-GGGGGAACCGCAGAATTCGGAAAAAATCGTACGCTAAG-3'), and further flanked by 5-bp DRs (5'-AATCA-3'). Genes and their corresponding transcription orientations are indicated by horizontal arrows. Vertical blocks between sequences indicate regions of shared similarity at the nucleotide level, shaded according to BLASTN (grey for matches in the same direction). Gene names are given when known.

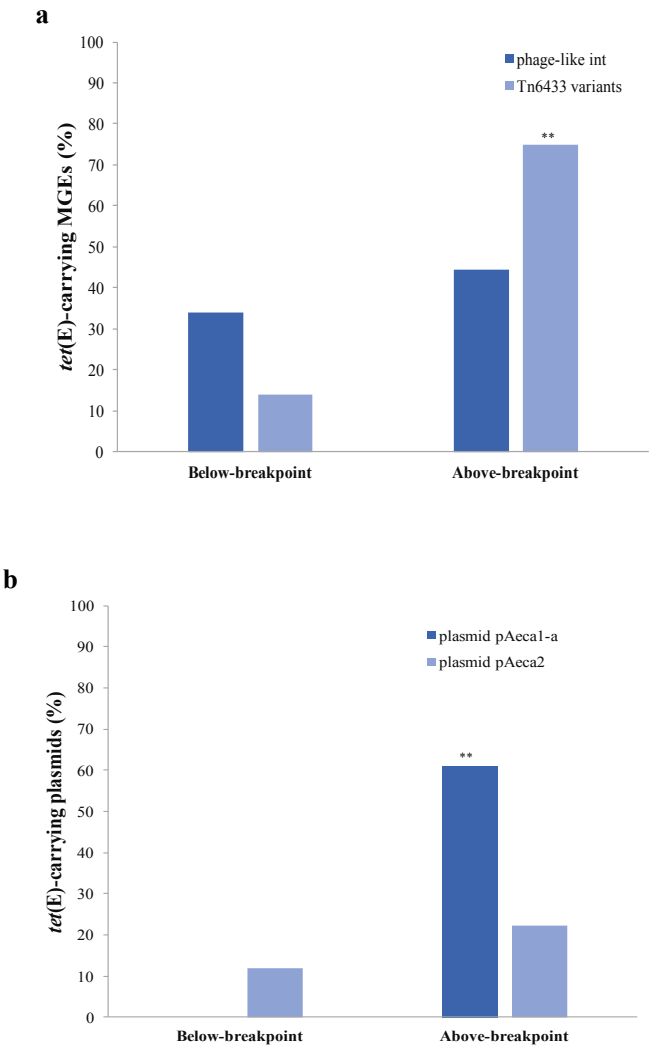

Figure S4. Comparison of *tet*(E)-carrying mobile genetic elements (MGEs) at below-breakpoint (BBP) (n=48) and above-breakpoint (ABP) (n=36) concentrations of oxytetracycline. a. *tet*(E)-carrying phage-like *int* and Tn*6433* variants in BBP and ABP groups. **b.** *tet*(E)-carrying plasmids pAeca1-a and pAeca2 in BBP and ABP groups. *P*-value was calculated by Pearson x^2 test based on a 2×2 contingency table of the numbers of *Aeromonas* isolates carrying and not carrying the MGEs (** $P<0.001$). BBP group included 0.1, 1, and 5 mg/L oxytetracycline, and ABP group contained 25 and 50 mg/L oxytetracycline.

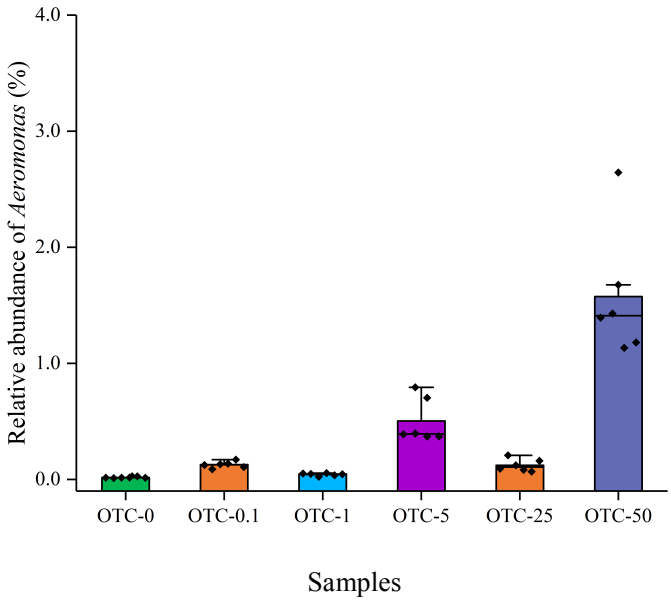

Figure S5. Changes in the relative abundance of *Aeromonas* at different oxytetracycline exposing stages in the biofilm reactor.

Supplementary Tables

Table S1. Chemical composition of the synthetic municipal wastewater

Chemical substances	Concentration (mg/L)
Glucose	150
Starch	100
Sodium carboxymethyl cellulose	50
Tryptone	100
$(NH_4)_2SO_4$	126
KH_2PO_4	31
$NaHCO_3$	250
Trace elements stock solution*	10 mL/L
Oxytetracycline	0, 0.1, 1, 5, 25, 50 mg/L

*Trace elements stock solution: H_3BO_3, 50 mg/L; $MnSO_4 \cdot 4H_2O$, 40 mg/L; $ZnSO_4 \cdot 7H_2O$, 40 mg/L; $Na_2MoO_4 \cdot 4H_2O$, 20 mg/L; $CuSO_4 \cdot 5H_2O$, 10 mg/L; $CoCl_2$, 10 mg/L; KI, 10 mg/L.

Table S2. PCR primers for amplifying targets in tetracycline resistance genes, 16S rRNA, *gyrB*, insert IN-01, Tn*6433* variants, plasmids pAeca1-a and pAeca2.

Gene	Forward Primers (5'-3')	Reverse Primers (5'-3')	Annealing temp. (℃)	Amplicon length (bp)	Reference
tet(A)	GCTACATCCTGCTTGCCTTC	CATAGATCGCCGTGAAGAGG	61	210	[1]
tet(C)	GCGGGATATCGTCCATTCCG	GCGTAGAGGATCCACAGGACG	68	207	[2]
tet(E)	AAACCACATCCTCCATACGC	AAATAGGCCACAACCGTCAG	61	278	[1]
tet(G)	GCTCGGTGGTATCTCTGCTC	AGCAACAGAATCGGGAACAC	60	468	[1]
tet(L)	TCGTTAGCGTGCTGTCATTC	GTATCCCACCAATGTAGCCG	55	267	[1]
tet(36)	GCCAAAGTAGAGGATAGGAGTAAA	TCTATCGTAAGTCCCGCACC	56	326	This study
tet(Q)	AGAATCTGCTGTTTGCCAGTG	CGGAGTGTCAATGATATTGCA	63	169	[3]
tet(X)	CAATAATTGGTGGTGGACCC	TTCTTACCTTGGACATCCCG	55	468	[1]
16S rRNA gene	27F: AGAGTTTGATCCTGGCTCAG	1492R: GGTTACCTTGTTACGACTT	55	1500	[8]
gyrB	3F: TCCGGCGGTCTGCACGGCGT	14R: TTGTCCGGGTTGTACTCGTC	58	1130	[9]
IN-01	TCAGGTAGCACAGGGACAAT	TTCCACGAAACATCCAAAGA	55	2128	This study
Tn*6433*	AGCCTTTCCAACAAACTCCC	AACGCACGCTGTTCATCCTA	56	3641	This study
P1_*repA*	ATGAACAACAAGACAAACGAGG	CCTTTGAGACTGCTTACTCTGC	55	396	This study
P1_*parA*	CACTTTGGGCTCCGATACAC	CTTCGCTGGTTCAAGGTTCT	55	699	This study

P1_*traI*	CAATGAGCAAGTCCCACCAA	CAAGCATCAACCCCTCTGTA	55	566	This study
P2_*repA*	CTACCAGATGGGCGGTTTAT	TGTACCAGGATTCCTGACGG	55	535	This study
P2_*parA*	CTTGCCATCACCAACGAAAA	TGCCAGGATCTCCTTAACCAT	55	762	This study
P2_*traI*	AACCTGAAGGGACGTTATGC	TGATGCTGCCTTCTTGTTGA	55	829	This study

Table S3. Classification of the fifty most abundant bacterial species recovered from the biofilm reactor, against stepwise increased concentrations of oxytetracycline. Taxa were identified using 16S rRNA sequence analysis.

Genus or species	No. of isolates from						Total no. of isolates
	0 mg/L	0.1 mg/L	1 mg/L	5 mg/L	25 mg/L	50 mg/L	
Aeromonas media	0	5	5	29	10	1	50
Lactococcus chungangensis	2	3	4	15	11	7	42
Bacillus subtilis	13	1	7	4	5	0	30
Aeromonas caviae	0	0	0	0	23	0	23
Stenotrophomonas acidaminiphila	6	5	1	3	5	1	21
Bacillus cereus	4	1	4	5	4	2	20
Cloacibacterium normanense	11	0	3	3	3	0	20
Raoultella terrigena	0	0	2	3	2	13	20
Hydrogenophaga pseudoflava	0	0	2	8	9	0	19
Bacillus methylotrophicus	1	3	4	1	7	0	16
Pseudomonas sp.	0	0	0	0	2	13	15
Bacillus amyloliquefaciens	4	1	2	0	5	0	12
Comamonas koreensis	0	0	1	0	0	11	12
Xanthobacter flavus	0	3	6	0	4	0	13
Microbacterium oxydans	1	1	2	2	0	4	10
Bacillus sp.	4	2	2	1	0	0	9
Comamonas testosteroni	4	0	2	3	0	0	9
Gemmobacter fontiphilus	0	0	0	3	6	0	9
Lactococcus raffinolactis	2	3	0	4	0	0	9
Pseudoxanthomonas mexicana	4	0	1	0	2	2	9
Aeromonas veronii	2	0	0	6	0	0	8
Leucobacter chromiireducens	1	4	1	1	0	1	8
Paracoccus yeei	7	0	0	0	0	0	7
Pseudomonas putida	0	0	1	0	5	1	7
Acinetobacter johnsonii	0	0	1	5	0	0	6
Azonexus caeni	6	0	0	0	0	0	6
Gordonia neofelifaecis	0	2	2	2	0	0	6
Brevundimonas poindexterae	0	0	0	0	2	3	5
Shinella granuli	0	0	0	0	0	5	5
Brevundimonas faecalis	0	0	2	2	0	0	4

Chryseobacterium rhizoplanae	1	0	1	1	1	0	4
Delftia acidovorans	0	0	3	0	0	1	4
Microbacterium arabinogalactanolyticum	1	1	2	0	0	0	4
Paracoccus huijuniae	0	0	1	2	1	0	4
Pseudomonas plecoglossicida	0	0	0	0	4	0	4
Simplicispira limi	1	0	3	0	0	0	4
Sphingopyxis chilensis	0	0	0	1	0	3	4
Acidovorax facilis	1	0	1	0	0	1	3
Acidovorax wautersii	2	1	0	0	0	0	3
Aeromonas sobria	0	2	0	1	0	0	3
Bacillus anthracis	1	0	1	1	0	0	3
Bacillus tequilensis	0	0	0	2	1	0	3
Bacillus thuringiensis	2	0	0	0	1	0	3
Bacillus vallismortis	0	0	1	0	2	0	3
Chryseobacterium lactis	1	2	0	0	0	0	3
Flavobacterium hibernum	0	0	1	2	0	0	3
Hydrogenophaga electricum	0	0	0	0	3	0	3
Leucobacter denitrificans	0	1	1	1	0	0	3
Microbacterium lacticum	0	2	1	0	0	0	3
Pseudomonas migulae	0	0	3	0	0	0	3

Table S4. Recovery of *Aeromonas* species from the OTC-0, OTC-0.1, OTC-1, OTC-5, OTC-25, OTC-50 reactor samples.

Bacteria	OTC-0	OTC-0.1	OTC-1	OTC-5	OTC-25	OTC-50
A. caviae	0	0	0	0	23	0
A. media	0	5	5	29	10	1
A. hydrophila	0	0	0	0	0	1
A. salmonicida	0	0	0	0	0	1
A. sobria	0	2	0	1	0	0
A. veronii	2	0	0	6	0	0
Total *Aeromonas*	2	7	5	36	33	3

Table S5. Distribution of *tet*(E), Tn*6433*, plasmids pAeca1-a and pAeca2 in bacterial taxa isolated from the reactor (excluding *Aeromonas*).

Gene/MGEs	OTC-0	OTC-0.1	OTC-1	OTC-5	OTC-25	OTC-50	Hosts
tet(E)	9	2	9	0	1	0	*Bacillus*, *Comamonas*, *Lactococcus*, *Cloacibacterium*, *Pseudoxanthomonas*, *Paracoccus*, *Stenotrophomonas*, *Methyloversatilis*, *Shewanella*, *Pseudomonas*, *Microbacterium*, *Raoultella*, *Chryseobacterium*, *Xanthobacter*, *Hydrogenophaga*
pAeca1-a	0	0	0	0	0	1	*Microbacterium* sp.
pAeca2	0	0	1	0	0	0	*Methyloversatilis discipulorum*
Tn*6433*	0	0	0	0	0	0	ND

ND: not detected

Table S6. Summary of eight *Aeromonas* strains by whole genome sequencing.

Species	Strains	Genome	size (bp)	GC%	Genes	N50 (bp)
A. media	T0.1-19	Chr1	4,917,765	61.0	4,550	13,059
		pAeme1	2,785	58.9	3	
	R1-18	Chr1	4,743,402	60.9	4,077	15,780
	T5-8	Chr1	4,791,767	60.8	4,420	14,735
	R25-3	Chr1	4,683,582	60.8	4,510	14,646
		pAeme3	190,780	56.4	183	
		pAeme4	4,795	57.0	5	
	R50-22	Chr1	4,748,464	60.7	4,779	14,912
		pAeme5	198,927	56.3	186	
		pAeme6	199,818	53.2	199	
A. caviae	R25-2	Chr1	4,690,132	60.7	4,406	15,732
		pAeca1-a	228,716	53.9	209	
		pAsa8-b	95,589	53.5	121	
	T25-39	Chr1	4,650,143	60.7	4,250	15,959
		pAeca1-b	221,458	53.7	201	
		pAeca2	103,136	55.6	123	
	R25-6	Chr1	4,702,913	61.4	4,480	16,168
		pAeca3	10,471	56.0	11	

Table S7. Distribution of *tet*(E) in the genome of the eight *Aeromonas* strains.

Stages	*Aeromonas*	*tet*(E) gene copies		other ARGs
		chromosome	plasmid	
OTC-0.1	*A. media* T0.1-19	0	0	ND
OTC-1	*A. media* R1-18	1	0	*sul1*, *qacEΔ1, aadA16, catB3, dfrB4*
OTC-5	*A. media* T5-8	1	0	ND
OTC-25	*A. media* R25-2	2	0	ND
	A. caviae R25-6	1	0	ND
	A. caviae R25-2	0	1	*aac(6')-lb-cr, bla*$_{OXA-1}$*, catB3, arr-3, sul1, qacEΔ1, msr(E), mphD, strA, strB, sul2, floR, tet*(31)
	A. caviae T25-39	0	3	*aac(6')-lb-cr, bla*$_{OXA-1}$*, catB3, arr-3, sul1, qacEΔ1, msr(E), mphD, strA, strB, sul2, floR, tet*(31), *dfrB4*, *aadA16*
OTC-50	*A. media* R50-22	1	1	*aac(6')-lb-cr, bla*$_{OXA-1}$*, catB3, arr-3, sul1, qacEΔ1, msr(E), mphD*

ND: not detected

Table S8. Inverted repeats and direct repeats of the Tn*6433*, Tn*6433*-a, Tn*6433-b* and Tn*6433*-c transposons.

Transposon	IRL (5'-3')	IRR (5'-3')	DR (5'-3')
Tn*6433_A. media* R1-18_Chr	GGGGAGCTCGCAGAATTCGGAAAA AATCGTACGCTAAG	GGGGGAACCGCAGAATTCGGAAAAA ATCGTACGCTAAG	ATTTA
Tn*6433-c_A. caviae* T25-39 plasmid pAeca2	GGGGAGCCCGCAGAATTCGGAAAA AATCGTACGCTAAG	GGGGGAACCGCAGAATTCGGAAAAA ATCGTACGCTAAG	AATCA
Tn*6433-a_A. caviae* T25-39 plasmid pAeca1-b	GGGGAGCCCGCAGAATTCGGAAAA AATCGTACGCTAAG	GGGG**TCGT**C**T**CAGAA**AA**CGGAAAA**T** A**AA**G**C**ACGCTAAG	TAC/TTT
Tn*6433-a_A. caviae* R25-2 plasmid pAeca1-a	GGGGAGCCCGCAGAATTCGGAAAA AATCGTACGCTAAG	GGGG**TCGT**C**T**CAGAA**AA**CGGAAAA**T** A**AA**G**C**ACGCTAAG	TAC/TTT
Tn*6433-b_A. media* R50-22 plasmid pAeme6	GGGGAGCCCGCAGAATTCGGAAAA AATCGTACGCTAAG	GGGG**TCGT**C**T**CAGAA**AA**CGGAAAA**T** A**AA**G**C**ACGCTAAG	TAC/TTT

Table S9. Main backbone genes of plasmid pAeca1-a – Homology to database entries.

CDS	Position	Similarity (%)	Host	Classification	GenBank Accession No.
repA	1-582	100	*Aeromonas salmonicida*	Plasmid replication	WP_088813916
yqaJ	9341-10345	38	*Thiorhodovibrio* sp.	Plasmid maintenance	WP_009146551.1
cobS	10412-11725	31	*Methylomonas koyamae*	Plasmid maintenance	WP_064041570.1
dnaG	16074-17510	42	*Yersinia pestis*	Plasmid maintenance	EDR32692.1
kfrA	21667-22797	33	*Gallaecimonas pentaromativorans*	Plasmid maintenance	WP_050660063.1
traI	136248-137510	100	*Aeromonas salmonicida*	Conjugal transfer	WP_088813968.1
traD	145870-148014	100	*Aeromonas salmonicida*	Conjugal transfer	WP_088813978.1
stbB	174128-174805	38	*Pectobacterium carotovorum*	Plasmid maintenance	WP_102119080.1
parA	175698-176927	100	*Aeromonas salmonicida*	Plasmid maintenance	WP_088814017.1
parB	176927-177925	100	*Aeromonas salmonicida*	Plasmid maintenance	WP_088814018.1
dnaN	182262-183416	30	*Lysobacter* sp.	Plasmid maintenance	WP_103073895.1
parB	185403-183700	100	*Aeromonas salmonicida*	Plasmid maintenance	WP_088814027.1
pulE	202551-204071	100	*Aeromonas salmonicida*	Conjugal transfer	WP_088814045.1
pulF	204068-205105	99	*Aeromonas salmonicida*	Conjugal transfer	WP_088814046.1
pulG	206207-207127	67	*Aeromonas allosaccharophila*	Conjugal transfer	WP_082034755.1
traK	212708-213640	100	*Aeromonas salmonicida*	Conjugal transfer	WP_088814066.1
traC	215567-218539	99	*Aeromonas salmonicida*	Conjugal transfer	WP_088814070.1
traU	219196-220272	94	*Aeromonas*	Conjugal transfer	WP_103828353.1
traG	222739-224262	100	*Aeromonas salmonicida*	Conjugal transfer	WP_088814080.1
traG	224259-225734	25	*Pseudomonas psychrotolerans*	Conjugal transfer	WP_058772429.1

Table S10. Main backbone genes of plasmid pAeca2 – Homology to database entries.

CDS	Position	Similarity (%)	Host	Classification	GenBank Accession No.
repA	1-927	66	*Aeromonas salmonicida*	Plasmid replication	ASD49212.1
parA	2078-2887	39	*Vibrio crassostreae*	Plasmid maintenance	WP_048667599.1
parB	2884-4308	38	*Vibrio crassostreae*	Plasmid maintenance	WP_048667598.1
dndB	6228-7787	34	*Mycobacterium asiaticum*	Plasmid maintenance	WP_065155000.1
radC	9982-10434	54	*Xanthomonas arboricola*	Plasmid maintenance	WP_047127309.1
traD	22176-24125	42	*Yersinia kristensenii*	Conjugal transfer	WP_087795769.1
traI	24249-28892	33	*Xenorhabdus bovienii*	Conjugal transfer	WP_051870683.1
traG	32390-36283	37	*Yersinia enterocolitica*	Conjugal transfer	WP_012291377.1
traH	36457-37620	52	*Yersinia enterocolitica*	Conjugal transfer	WP_012291383.1
traF	39045-39221	54	*Yersinia frederiksenii*	Conjugal transfer	WP_087770275.1
trbC	39497-40197	54	*Enterobacter cloacae*	Conjugal transfer	WP_063840916.1
traU	40246-41794	71	*Yersinia frederiksenii*	Conjugal transfer	WP_087770274.1
ydgT	41904-42119	45	*Escherichia coli*	Conjugal transfer	WP_096293187.1
chpB	88409-88756	66	*Photorhabdus asymbiotica*	Plasmid maintenance	WP_065822344.1
mazE	88756-89007	54	*Candidatus Hamiltonelladefensa*	Plasmid maintenance	WP_015873810.1
traN	90332-91117	60	*Escherichia coli*	Conjugal transfer	WP_038998997.1
traN	92161-94146	53	*Yersinia ruckeri*	Conjugal transfer	WP_080748422.1
traC	94155-96644	55	*Pantoea ananatis*	Conjugal transfer	WP_089529226.1
traV	96646-97185	51	*Yersinia enterocolitica*	Conjugal transfer	WP_050127664.1
traB	97316-98425	78	*Yersinia enterocolitica*	Conjugal transfer	CFV32186.1
traK	98428-99210	55	*Salmonella enterica*	Conjugal transfer	WP_079895007.1
traE	99214-99870	35	*Citrobacter* sp.	Conjugal transfer	WP_079939726.1
traL	99884-100081	38	*Desulfuromonas* sp.	Conjugal transfer	WP_066723377.1

Table S11. The number of shared and unique MGEs (Tn*6433*, plasmids pAeca1-a and pAeca2 variants) among *Aeromonas* strains.

Stages	Tn*6433*	pAeca1-a	pAeca2	Tn*6433* and pAeca1-a	Tn*6433* and pAeca2	Tn*6433*, pAeca1-a and pAeca2
OTC-0.1	0	0	1	0	0	0
OTC-1	1	0	0	0	0	0
OTC-5	6	0	5	0	0	0
OTC-25	25	20	7	20	7	6
OTC-50	2	2	1	2	1	1

Table S12. Distribution of *tet*(E), Tn*6433*, plasmids pAeca1-a and pAeca2 in bacterial taxa isolated from the reactor.

This table is available as a supplementary dataset; Supplementary Table S12.xlsx.

Table S13. Hosts and associated MGEs of the *tet*(E) gene.

Hosts	Genetic element with *tet*(E)	GenBank Accession no.	Reference
Aeromonas hydrophila	plasmid pMCR5_045096	CP028567.1	–
Aeromonas hydrophila	chr	CP014774.1	[10]
Aeromonas media	chr	CP007567.1	[11]
Aeromonas salmonicida	plasmid pAsa4	CP000645.1	[12]
Aeromonas salmonicida	plasmid pAsa4b	KT033469.1	–
Aeromonas salmonicida	plasmid pS121-1a	CP022170.1	[7]
Aeromonas hydrophila *Aeromonas sobria*	plasmid pAHH01	JN315882.1	[13]
Aeromonas veronii	chr	CP033604.1	–
Aeromonas caviae	unknown	unknown	[14]
Aeromonas hydrophila, *Aeromonas caviae*	unknown	unknown	[15]
Aliivibrio salmonicida	plasmid pRVS1-224	CP034238.1	–
Enterobacter cloacae	–	KY971306.1	–
Escherichia coli	plasmid pSL1456	Y19116.1	[16]
Vibrio salmonicida	plasmid pRVS1	AJ289103.1	[17]

Table S14. Host, location, genetic structure, sources of transposons with 99% nucleotide sequence similarity to Tn*6433 tnpA*.

Strains	Location	Genetic structure	Source of isolation	Accession NO.
Klebsiella pneumoniae strain QL24	plasmid pKPC-QL24	*tnpA-tnpR*	Clinical isolates	MH263653
Klebsiella pneumoniae subsp. pneumoniae strain SCKP020079	plasmid pKPC2_020079	*tnpA-tnpR*	Hospital	CP029381
Klebsiella pneumoniae strain L388	plasmid pKPC-L388	*tnpA-tnpR*	Hospital	CP029225
Klebsiella pneumoniae strain TP-P16	plasmid pKPC_P16	*tnpA-tnpR*	_	KY689238
Klebsiella pneumoniae subsp. pneumoniae strain KP4898	plasmid pIncAC-KP4898	*tnpA-tnpR-intI1-dfrA14-IS6100-IS91-qnrA1-lysR-hypA-sul1-orf5-tniB delta3-tniA*	Hospital	KY882285
Klebsiella pneumoniae	plasmid pHS091147	*tnpA-tnpR*	Clinical isolates	KX236178
Klebsiella pneumoniae strain HK02-026	plasmid pHK02-026	*tnpA-tnpR-urf2-intI1-dfrA12-aadA2-qacE delta1-sul1-orf5*	Clinical isolates	KY751926
Klebsiella pneumoniae strain	plasmid pA324-IMP	*tnpA-tnpR-mcp*	_	MF344566

A324				
Klebsiella pneumoniae strain 1068	plasmid p1068-KPC	*tnpA-tnpR*	_	MF168402
Klebsiella pneumoniae strain 447	plasmid p447-IMP	*tnpA-tnpR-bla-IMP-8-aac(6')-lb-tniR-tniQ-tniB-tniA-tonB-IS903B-blaCTX-M-14-IS1R-ISEcp1-mcp*	_	KY978631
Klebsiella pneumoniae strain JS187	plasmid p187-1	*tnpA-tnpR-urf2-merE-merD-merA-merC-merP-IS1R*	Hospital	CP025467
Klebsiella oxytoca strain pKOX3	plasmid P2-OXA	*tnpA-tnpR-urf2-merE-merD-merA-merC-merP-merT-merR*	Patient urine	KY913898
Escherichia coli strain ECOR 6	plasmid: RCS91_p.	*tnpA-tnpR-mcp*	_	LT985299
Escherichia coli strain 513	plasmid: RCS30_p	*tnpA-tnpR-tonB-IS903B-ISEcp1-blaCTX-M-14-IS903B-ISEcp1-mcp*	_	LT985224
Escherichia coli	plasmid pSCE516-4	*delta tnpA-eamA-tet(A)-mcp-relaxase-tnpA-tnpR-intI1-dfrA12-aadA2-cmlA-aadA1-qacE delta1 IS256-tetM-IS26-intI1-delta tnpA-IS26-aac(3')-lld-IS26-ISEc57-lysR-floR-virD2-IS91-IS15D-sul3-mef(B)-IS26-aph(3')-la-IS26-tniA-urf2-merE-merD-merA-merC-merP-merT-merR*	Chicken	KX023259
Escherichia coli strain MS8345	plasmid pMS8345A	*tnpA-tnpR-intI1-aadA2-qacE delta1-sul1-tetX-rteC-insA*	Respiratory secretions	CP025402

Escherichia coli strain HS13-1	plasmid pHS13-1-IncF	*delta tnpA-eamA-tet(A)-tetR-relaxase-tnpA-tnpR-mcp*	faecal sample	CP026494
Escherichia coli strain H17	plasmid pH17-2	*tnpA-tnpR*	Hospital	CP021195
Escherichia coli	plasmid pEC16I_1	*tnpA-tnpR-mcp*	Patients	KU932032
Escherichia coli plasmid pCOV4 clone COV4_c1	plasmid pCOV4 clone COV4_c1	*tnpA-tnpR-intI1-dfrA1-aadA2-bla-CTX-M-14-Tn2 tnpR-Tn2 tnpA*	French broilers	MG648859
Escherichia coli strain 1106	plasmid p1106-IncFIC	*tnpA-tnpR-mcp*	Food	MG825372
Escherichia coli strain 1943	plasmid p54	*tnpA-tnpR-intI1-dfrA1-catB3-aadA2-Tn3 tnpA-Tn3 tnpR-blaCTX-M-14-mcp*	Veterinary clinical care setting	CP023360
Escherichia coli strain 14EC047	plasmid p14EC047b	*tnpA-tnpR-intI1-dfrA1-aadA2-cmlA-aadA1-qacE delta1-IS256-sul1-mef(B)-IS15DIV*	Clinical patient	CP024157
Escherichia coli strain 144	plasmid 134q	*tnpA-tnpR-intI1-dfrA1-IS26-Tn2 delta tnpR-blaCTX-M-14-IS26-sul1-strA-strB-IS26-tniA-urf2-merE-merD-merA-merC-merP-merT-merR*	Veterinary clinical care setting	CP023363
Enterobacter cloacae strain	plasmid unnamed2	*tnpA-tnpR-padR-chrA-IS4321R-orf5-sul1-qacE delta1-aac(3')-ib-intI1*	_	CP027606

AR_0093				
Enterobacter cloacae strain ECIY2402	plasmid pKPC2_ECIY2402	*tnpA-tnpR*	_	KY399972
Enterobacter cloacae strain Encl-30969cz	plasmid pEncl-30969cz	*tnpA-tnpR-intI1-aadA2-aac(6')-lb-aadA2-qacE delta1-sul1-orf5-IS1326-ISPa38-tniB-tinA-urf2-IS5075-ISPa38-IS5075-qnrB-sapA-IS26-mcp*	_	MG049738
Citrobacter freundii strain Cfr-31816cz	plasmid pCfr-31816cz	*tnpA-tnpR-intI1-aac(6')-lb-blaCTX-M-14-catB3-arr-3-qacE delta1-sul1-orf5*	_	MF497780
Alcaligenes faecalis strain GZAF1	plasmid pGZAF1_VIM	*tnpA-tnpR-intI1-IS881-intI1-blaVIM-4-qacE-ISPa21-qacE delta1-sul1-orf5-tniB-tniA-urf2-merE-merD-merA-merC-merP-merT-merR-IS1R-IS26-mcp*	Patient	KY623659
Proteus mirabilis strain T21	plasmid pT211	*tnpA-tnpR*	Sputum	CP017083
Leclercia sp. LSNIH1	plasmid pLEC-000f	*tnpA-tnpR-PAP2-zantA-merR-IS26-kexD-IS26*	Hospital	CP026170
Escherichia coli strain CFSAN061770	plasmid pEGY1-MCR-1	*delta tnpA-emaA-tetA-mcp-relaxase-tnpA-tnpR-intI1-aadA2-serB-aadA2-cmlA-aadA1-qacE delta1-IS256-sul1*	Raw milk cheese	CP023143
Klebsiella pneumoniae strain Kpn-35786cz	plasmid pKpn-35786cz	*tnpA-tnpR-intI1-aac(6')-lb-blaCTX-M-14-catB3-arr-3-qacEdelta1-sul1-orf5*	Hospital	MG557998
Klebsiella pneumoniae subsp. pneumoniae strain	plasmid pNDM5_020046	*tnpA-tnpR-intI1-sul1-orf5*	_	CP028781

SCKP020046				
Klebsiella pneumoniae strain BR	plasmid pWSZBR	*tnpA-tnpR-IS26-Tn3 tnpR-ISKpn27-blaCTX-M-14-ISKpn6-IS26-Tn2 delta tnpA-IS26-aac(6')-lb-blaCTX-M-14-catB3-arr-3-dfrA1-aadA2-qacE delta1-sul1-IS91-qnrA3-mphA-nanR-tetR-IS6100-urf2-merE-merD-merA-merC-merP-merT-merR*	_	CP015991
Klebsiella pneumoniae strain AR_0098	plasmid tig00000002	*tnpA-tnpR-intI1-aadA2-serB-aadA2-cmlA-aadA1-qacE delta1-IS406-ISKpn26-sul1*	_	CP020110
Klebsiella variicola strain WCHKP19	plasmid pKPC2_020019	*tnpA-tnpR-ISKpn6-blaCTX-M-14-ISKpn27-Tn3 tnpR-Tn3 tnpA*	_	CP028554
Kluyvera cryocrescens strain BO64W	plasmid pKC-BO-N1-VIM	*tnpA-tnpR-intI1-dfrA1-IS6100-tetR-nanR*	Rectal swab	MG228427
Proteus mirabilis strain T18	plasmid pT18	*tnpA-tnpR*	Urine	CP017086
Proteus mirabilis strain PmPHI	genomic island	*tnpA-tnpR-IS26-Tn5393 tnpA-Tn5393 tnpR-IS1133-strA-strB*	Stools	MF490433
Proteus mirabilis strain T21	plasmid pT212	*tnpA-tnpR-mcp*	Sputum	CP017084
Pseudomonas fragi strain NMC25	plasmid unnamed2	*tnpA-tnpR-mcp*	Meat	CP021134
Pseudomonas fragi strain NMC25	plasmid unnamed3	*tnpA-tnpR-mcp*	Meat	CP021135

Raoultella ornithinolytica strain 23141	plasmid p23141-2	*tnpA-tnpR*	_	MF788070
Salmonella enterica subsp. Enterica serovar Enteritidis strain 81-1607	plasmid pSE81-1607-1,	*tnpA-tnpR-mcp*	Human	CP018649
Salmonella enterica subsp. Enterica serovar Enteritidis strain 95-0621	plasmid pSE95-0621-1	*eamA-tetA-tetR-tnpA-tnpR-blaCTX-M-14-Tn2 tnpR-Tn2 tnpA*	Human	CP018662
Vibrio parahaemolyticus	plasmid pVPS129	*tnpA-tnpR-intI1-aac(6')-lb-yoeB-yefM-catB3-qacE delta1-sul1*	_	KY014464
Salmonella enterica subsp. Enterica serovar Enteritidis strain 77-2980	Chromosome	*tnpA-tnpR-mcp*	Human	CP018644.1
Escherichia coli strain DSM 103246	Chromosome	*tnpA-tnpR-mcp*	Chicken carcass	CP019944.1
Escherichia coli strain 127	Chromosome	*tnpA-tnpR-mcp*	Veterinary clinical care setting	CP023377.1
Aeromonas veronii strain X11	Chromosome	*tnpA-tnpR-mcp*	Megalobrama amblycephala	CP024930.1

Aeromonas sp. ASNIH5	Chromosome	*delta tnpA-eamA-tetA-tetR-tnpA-tnpR-intI1-dfrA1-aadA2-sul1-orf5-chrA-padR-mphA-IS26-aph(3')-la-IS26-tniA-IS5D*	Hosptial	CP026122.1
Aeromonas hydrophila subsp. hydrophila strain WCHAH045096	Chromosome	*delta tnpA-eamA-tetA-tetR-relaxase-tnpA-tnpR-urf2-intI1-aadA1-sul1-orf5-IS91-catB3-sul1-orf5-IS1326-ISAs29-IS1326-IS1353-tniB-tniA-urf2-merE-merD-merA-merC-merP-merT-merR*	Sewage	CP028568.1
Photobacterium damselae strain Phdp Wu-1	plasmid plas1	*tnpA-tnpR-mcp-IS15-blmS-nimC-IS15-mcp*	heart blood	CP018299
Escherichia coli strain Eco-36682cz	plasmid pEco-36682cz	*tnpA-tnpR-urf2-intI1-aac(6')-lb-bla*$_{OXA-1}$*-catB3-arr-3-qacE delta1-sul1-orf5-chrA-padR-IS6100-mphA-IS26-xerC-ISPsy42*	_	MG557999

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