

Fig. S1. Comparison of the amino acid sequence of GES-47, -48, -50, and -54, GES-51, and GES-49 to those of GES-4, -5, and -6, respectively. The amino acids of the omega loop are underlined.

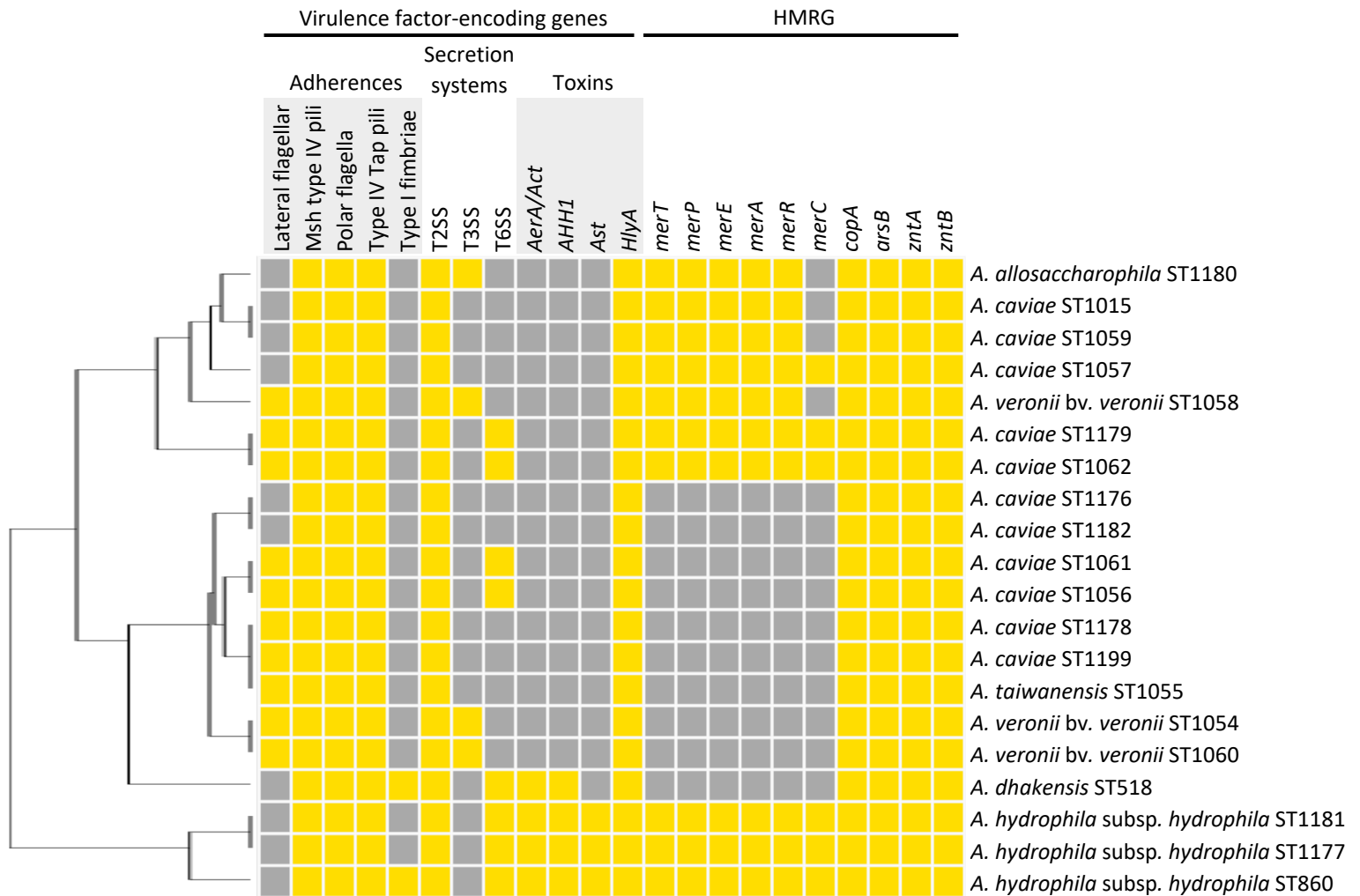


Fig. S2. Profiles of virulence factor-encoding genes and heavy metal resistance genes (HMRG) among 20 different lineage isolates of *Aeromonas* spp. Heat map, representing the presence (yellow) or absence (gray) of genes was generated using Morpheus software (<https://software.broadinstitute.org/morpheus/>).

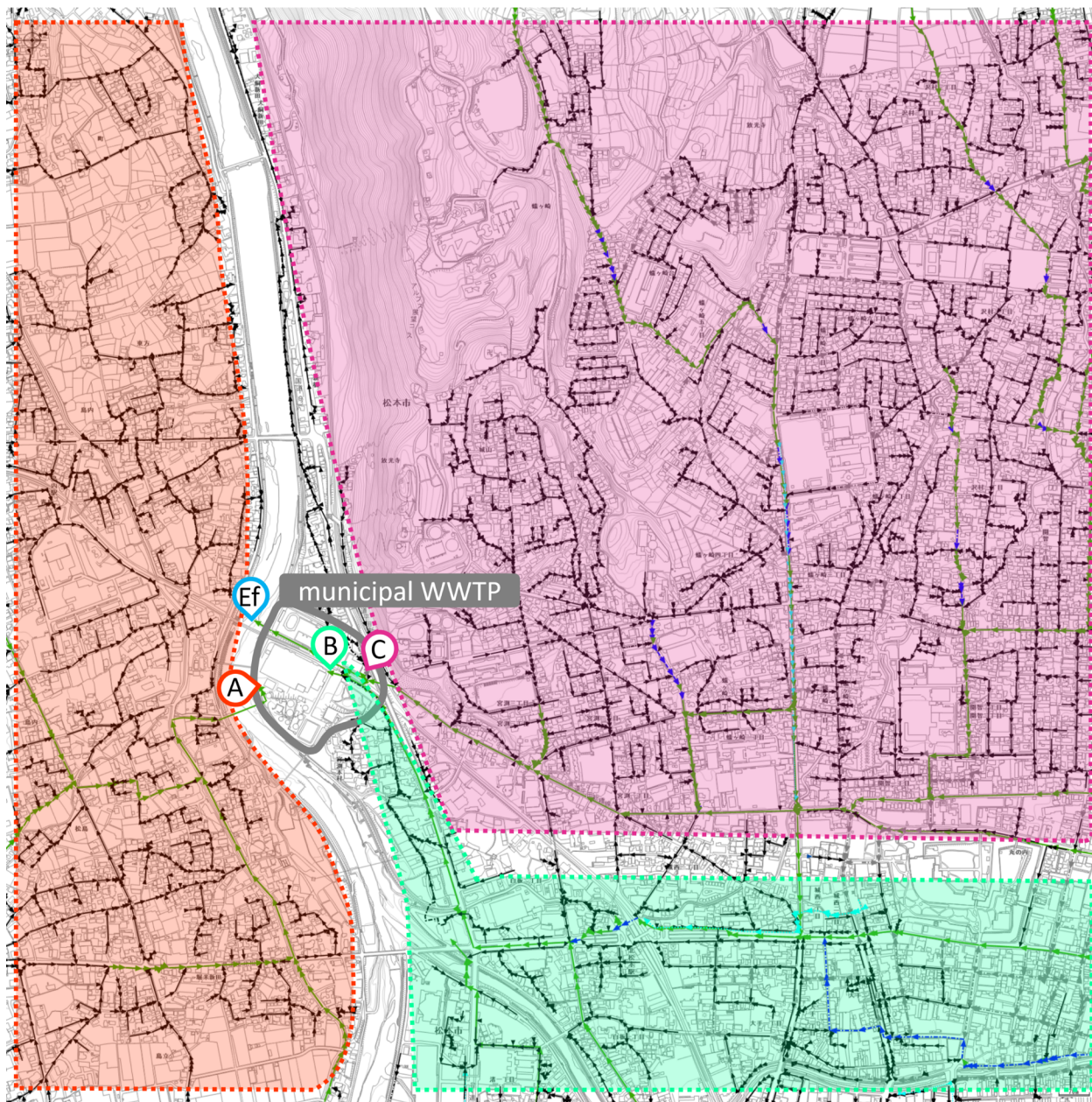


Fig. S3. Sampling points at the municipal wastewater treatment plant located in Matsumoto City, Nagano Prefecture, Japan. A, inlet A receiving separated sewer line in the western area of the city (orange); B, inlet B receiving combined sewer line in the southeastern area of the city (green); C, inlet C receiving combined sewer line in the northeastern area of the city (pink); Ef, effluent outlet.

TABLE S1. Measurement results of temperature and pH at sampling points

Sample	Sampling point (latitude/longitude)	Parameter	Sampling date (month/year)					Average \pm SD	
			Dec-20	Feb-21	Apr-21	Jun-21	Aug-21		Oct-21
Influent A	Inlet A (36°14'38.0"N 137°57'02.9"E)	Temperature	16°C	14°C	16°C	20°C	23°C	22°C	18.5 \pm 3.4
		pH	7.53	7.55	7.51	7.31	7.23	7.29	7.4 \pm 0.1
Influent B	Inlet B (36°24'30.3"N 137°95'21.0"E)	Temperature	17°C	13°C	17°C	20°C	22°C	21°C	18.3 \pm 3.0
		pH	7.47	7.41	7.29	7.36	7.37	7.06	7.3 \pm 0.1
Influent C	Inlet C (36°14'35.2"N 137°57'12.0"E)	Temperature	18°C	13°C	18°C	21°C	24°C	23°C	19.5 \pm 3.7
		pH	7.53	7.29	7.28	7.09	7.19	7.17	7.3 \pm 0.1
Effluent	36°14'38.0"N 137°57'02.9"E	Temperature	17°C	14°C	18°C	21°C	25°C	22°C	19.5 \pm 3.6
		pH	7.22	6.84	6.95	6.71	7.01	7.06	7.0 \pm 0.2
River water (1.35 km upstream from the effluent outlet)	36°13'54.7"N 137°57'11.0"E	Temperature	11°C	9°C	10°C	17°C	18°C	15°C	13.3 \pm 3.5
		pH	7.6	7.4	8.1	5.9	7	6	7.0 \pm 0.8
River water (0.56 km downstream from the effluent outlet)	36°14'56.0"N 137°57'04.7"E	Temperature	11°C	9°C	11°C	17°C	19°C	16°C	13.8 \pm 3.7
		pH	7.4	7.2	7.8	6	6.8	6	6.9 \pm 0.7

TABLE S2. Minimum inhibitory concentrations (MICs) of 25 antimicrobials against 75 CPOs in this study

Bacterial species	Strain	Sequence types	Carbapenemases	Others	MIC (μg/mL)																								
					Ampicillin	Piperacillin	β-LAM	TZP	Ceftazidime	Ceftiofur	Ceftazidime	Cefepime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime	Ceftazidime		
<i>Aeromonas</i>	10CC9	1180	GES-24	TEM-1B	>16	>64	>16-8	64-4	>16	>4	4	>4	>4	16	8	8	16	>16	2	4	>64	16	4	8	32	>9.5-0.5	8	0.5	
	4C99	057	GES-5		>16	>64	>16-8	32-4	>16	>4	8	>4	8	>4	8	>4	>32	>16	2	0.5	0.5	32	16	4	32	>38-2	>0.25	0.5	
<i>Aeromonas</i>	6B8C	1178	GES-5	MOX	>16	>64	>16-8	>64-4	>16	>4	16	>4	16	32	>16	>32	>16	16	0.5	1	32	16	4	32	>9.5-0.5	1	0.5		
	6CC6	1178	GES-5	MOX	>16	>64	>16-8	64-4	>16	>4	8	>4	16	>16	>32	>16	8	>4	16	8	2	4	16	8	32	>38-2	0.5	2	
<i>Aeromonas</i>	10B8C	1059	GES-5	MOX+VEB-1	>16	32	>16-8	8-4	>16	>4	>16	>4	>16	>4	>32	8	>32	>16	>16	0.5	0.5	16	2	32	>38-2	>0.25	1		
	2CC7	1059	GES-5	MOX+VEB-1	>16	>64	>16-8	>64-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	>16	4	4	32	32	32	>38-2	0.5	2		
<i>Aeromonas</i>	4CC12	1059	GES-5	MOX+VEB-1	>16	64	>16-8	32-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	>16	4	4	16	16	4	>38-2	>0.25	>0.25		
	10CC10	1176	GES-5	MOX+VEB-1	>16	64	>16-8	16-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	>16	1	2	16	4	32	>38-2	>0.25	0.5		
<i>Aeromonas</i>	6BC6	1199	GES-24		>16	32	>16-8	>4-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>38-2	1	2		
	10BC6	1179	GES-24		>16	>64	>16-8	64-4	>16	>4	16	>4	16	>4	32	>16	>32	>16	0.5	0.5	0.25	32	2	32	>38-2	1	8		
<i>Aeromonas</i>	6CC5	1062	GES-24, IMP-1		>16	>64	>16-8	32-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	4	>8	>64	32	8	32	>38-2	0.5	1		
	2BC3	1056	GES-48		>16	16	>16-8	8-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>9.5-0.5	1	0.5		
<i>Aeromonas</i>	4BC2	1056	GES-48		>16	>64	>16-8	32-4	>16	>4	16	>4	16	>4	>32	>16	>32	>16	1	0.25	32	1	32	8	>9.5-0.5	0.5	0.5		
	6BC4	1056	GES-48		>16	>64	>16-8	16-4	>16	>4	8	>4	32	>4	32	>4	32	>4	0.5	0.5	32	2	32	8	>9.5-0.5	0.5	0.5		
<i>Aeromonas</i>	8BC3	1056	GES-48		>16	64	>16-8	16-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>38-2	4	2		
	10BC4	1056	GES-48		>16	64	>16-8	16-4	>16	>4	8	>4	32	>4	32	>4	32	>4	0.5	0.5	32	16	16	16	>38-2	4	4		
<i>Aeromonas</i>	2CC8	1056	GES-48		>16	16	>16-8	16-4	>16	>4	8	>4	16	>4	16	>4	16	>4	>32	>16	>0.25	0.5	32	2	>64	19-1	1		
	4CC5	1056	GES-48		>16	16	>16-8	16-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>38-2	2	1		
<i>Aeromonas</i>	6CC12	1056	GES-48		>16	>64	>16-8	32-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>9.5-0.5	1	0.5		
	8CC4	1182	GES-48		>16	>64	>16-8	32-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	4	>0.25	0.25	16	4	16	>38-2	0.5	1		
<i>Aeromonas</i>	10CC8	1056	GES-48		>16	32	>16-8	16-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>9.5-0.5	0.5	2		
	6BC7	1061	IMP-10		>16	8	>16-8	>4-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	2	>8	>64	>64	4	2	>38-2	0.5	4		
<i>Aeromonas</i>	12BC1	1015	GES-5		>16	32	>16-8	32-4	>16	>4	8	>4	16	>4	32	>16	>32	>16	1	2	32	4	32	8	>9.5-0.5	0.5	2		
	8BC4	1015	GES-5		>16	>64	>16-8	64-4	>16	>4	16	>4	16	>4	32	>16	>32	>16	1	4	16	8	8	4	>9.5-0.5	>0.25	8		
<i>Aeromonas</i>	10BC1	1015	GES-5		>16	>64	>16-8	64-4	>16	>4	>16	>4	>16	>4	32	>16	>32	>16	1	4	64	8	8	4	>9.5-0.5	>2	4		
	6CC8	1182	GES-6		>16	>64	>16-8	16-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	2	2	32	32	8	4	>9.5-0.5	0.5	>0.25		
<i>Aeromonas</i>	10CC4	518	GES-24		>16	16	>16-8	8-4	>16	>4	4	>4	>4	>4	>32	>16	>32	>16	8	4	4	>64	64	4	>38-2	>64	4		
<i>Aeromonas hydrophila</i> subsp. <i>hydrophila</i>	8CC6	1177	GES-24		>16	>64	>16-8	32-4	>16	>4	16	>4	32	>4	32	>16	>32	>16	>8	>8	>64	>64	4	2	>38-2	8	4		
	8CC5	860	GES-24, IMP-1		>16	>64	>16-8	>64-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	4	4	>8	>64	>64	4	>38-2	8	4		
<i>Aeromonas</i>	6CC13	1181	GES-5		>16	>64	>16-8	64-4	>16	>4	8	>4	8	>4	>16	>32	>16	>32	>16	>8	8	>64	>64	4	>38-2	>64	2		
<i>Aeromonas taiwanensis</i>	2BC2	1055	GES-5		>16	32	>16-8	16-4	>16	>4	16	>4	16	>4	16	>4	32	>16	>0.25	>0.12	64	2	32	8	>9.5-0.5	1	0.5		
	4BC3	1055	GES-5		>16	>64	>16-8	64-4	>16	>4	16	>4	16	>4	32	>16	>32	>16	4	2	2	>64	8	4	>9.5-0.5	0.5	1		
<i>Aeromonas veronii</i> bv. <i>veronii</i>	12BC2	1054	GES-5		>16	32	>16-8	>4-4	>16	>4	2	4	2	2	2	2	2	2	2	2	2	2	2	2	>38-2	0.5	1		
	2BC1	1054	GES-5		>16	16	>16-8	>4-4	>16	>4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	>38-2	2	2		
<i>Aeromonas</i>	2CC6	1058	GES-24		>16	64	>16-8	8-4	>16	>4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	>38-2	2	0.5		
	6BC9	1060	GES-49		>16	>64	>16-8	>64-4	>16	>4	>16	>4	>16	>4	16	>4	32	>16	2	1	>64	32	8	4	>38-2	1	4		
<i>Kluyvera cryocrescens</i>	6BC3	-	GES-6		>16	64	>16-8	16-4	>16	>4	16	>4	4	4	4	4	4	4	4	4	4	4	4	4	>9.5-0.5	0.5	1		
<i>Citrobacter</i>	8BC2	110	GES-5		>16	16	>16-8	8-4	>16	>4	2	>4	2	>4	2	>4	2	>4	2	>4	2	>4	2	2	>9.5-0.5	1	1		
<i>Citrobacter braakii</i>	4CC11	22	GES-24	CTX-M-3+TEM-1B	>16	>64	>16-8	>64-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	16	8	1	32	1	>8	>38-2	>0.25	1		
	2CC2	116	IMP-1	CTX-M-3+TEM-1B	>16	>64	>16-8	16-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	>16	4	8	>64	32	>8	>9.5-0.5	4	0.5		
<i>Citrobacter</i>	12CC1	116	IMP-1	CTX-M-3+TEM-1B	>16	>64	>16-8	8-4	>16	>4	>16	>4	>16	>4	>32	>16	>32	>16	16	4	8	>64	32	>8	>9.5-0.5	1	0.5		
<i>Citrobacter portucalensis</i>	10C7	166	GES-51		>16	64	>16-8	16-4	>16	>4	4	>4	2	>4	2	>4	2	>4	2	>4	4	32	32	2	>9.5-0.5	>0.25	0.5		
<i>Enterobacter cloacae</i> subsp. <i>cloacae</i>	8BC1	1821	GES-4		>16	32	>16-8	>4-4	>16	>4	8	4	2	>4	2	>4	2	>4	2	>4	2	>4	2	2	>9.5-0.5	2	2		
	10BC2	1821	GES-4		>16	32	>16-8	8-4	>16	>4	16	>4	2	>4	2	>4	2	>4	2	>4	2	>4	2	2	>9.5-0.5	1	1		
<i>Enterobacter kobei</i>	6CC1	1822	GES-4		>16	16	>16-8	8-4	>16	>4	8	4	2	>4	2	>4	2	>4	2	>4	2	8	4	32	8	>9.5-0.5	32	2	
<i>Enterobacter</i>	6BC5	520	GES-54		>16	32	>16-8	16-4	>16	>4	16	>4	2	>4	2	>4	2	>4	2	>4	2	2	2	2	>9.5-0.5	>0.25	0.5		
<i>Enterobacter roggenkampii</i>	4CC4	1059	GES-4		>16	32	>16-8																						