

Characterization and antimicrobial susceptibility of motile aeromonads isolated from freshwater ornamental fish showing signs of septicaemia

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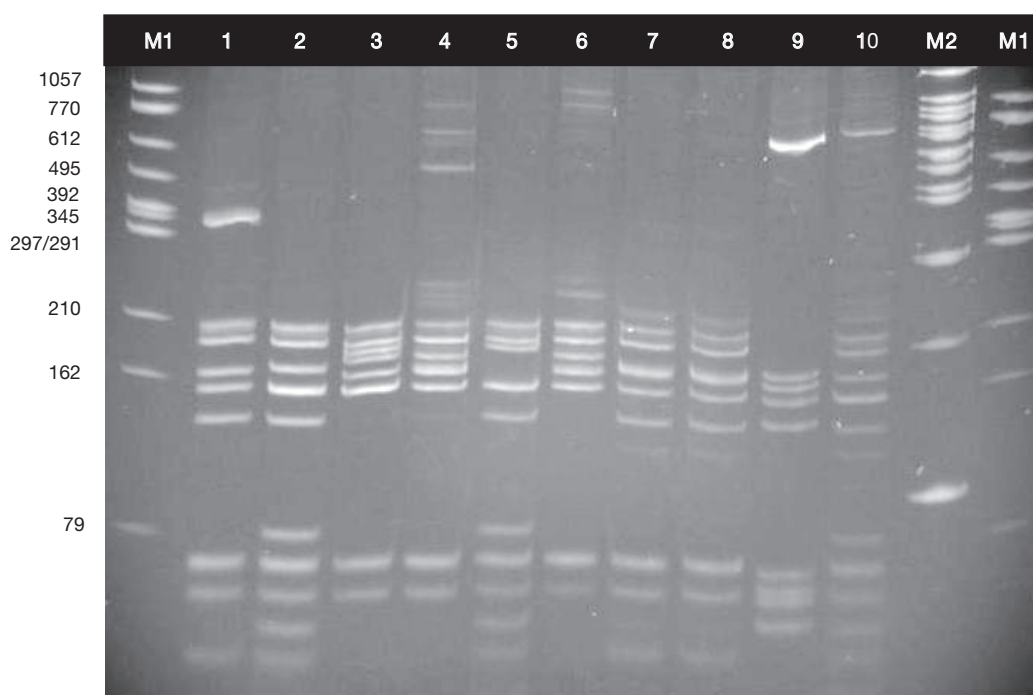


Fig. S1. Polyacrylamide gel showing 16S rDNA-RFLP patterns (*Alu*I and *Mbo*I). M1: OneSTEP Marker 5 (ϕ X174/*Hinc*II digest) (Wako, Nippongene); Lane 1: typical pattern of *Aeromonas hydrophila* (expected fragment sizes: 346, 207, 195, 165, 157, 138, 69, 66 bp); Lane 2: typical pattern of *A. veronii* (207, 195, 174, 158, 157, 138, 78, 69, 66 bp); Lane 3: typical pattern of *A. caviae* (207, 195, 188, 180, 165, 158, 157, 69, 66 bp); Lane 4: atypical pattern observed for *A. dhakensis*; Lane 5: typical pattern of *A. jandaei* (207, 195, 188, 158, 157, 138, 78, 69, 66 bp); Lane 6: typical pattern of *A. trola/enteropelogenes* (242, 207, 195, 180, 165, 158, 157, 69, 66 bp); Lanes 7–10: atypical patterns of *A. veronii*; M2: Gene Ladder 100 (Wako, Nippongene). Expected fragment sizes according to Borrell et al. (1997); fragments <60 bp were not considered

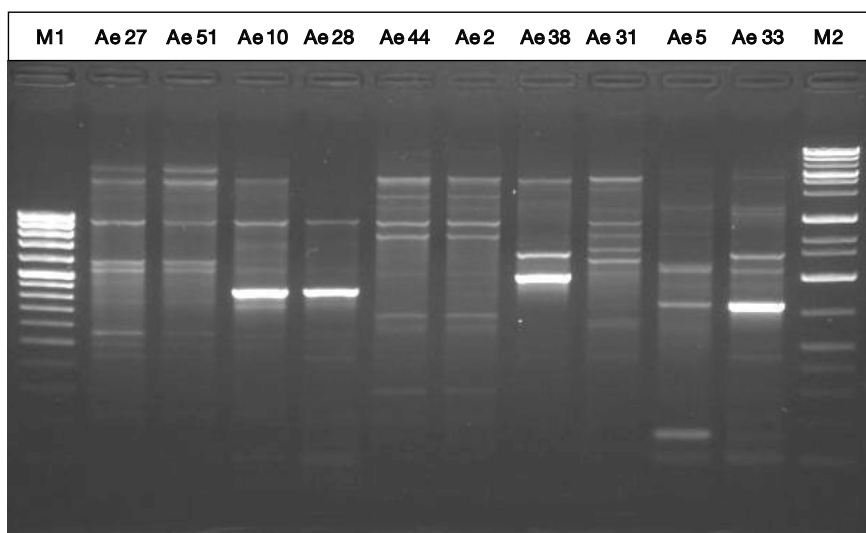


Fig. S2. ERIC-PCR fingerprints of some of the *Aeromonas* isolates used in the present study. The numbers at the top (Lanes 2–11) are the isolate numbers listed in Table S1. Lane M1: OneSTEP Ladder 100 (Wako, Nippongene), Lane M2: Gene Ladder Wide 1 (Wako, Nippongene)

Table S1. GenBank accession numbers for the *rpoD/gyrB* gene sequences of *Aeromonas* isolates used in the present study

Isolate ref. no.	GenBank accession number		Ae 26	AB828752	AB829137
	<i>rpoD</i>	<i>gyrB</i>			
Ae 1	AB828727	AB829112	Ae 27	AB828753	AB829138
Ae 2	AB828728	AB829113	Ae 28	AB828754	AB829139
Ae 3	AB828729	AB829114	Ae 29	AB828755	AB829140
Ae 4	AB828730	AB829115	Ae 30	AB828756	AB829141
Ae 5	AB828731	AB829116	Ae 31	AB828757	AB829142
Ae 6	AB828732	AB829117	Ae 32	AB828758	AB829143
Ae 7	AB828733	AB829118	Ae 33	AB828759	AB829144
Ae 8	AB828734	AB829119	Ae 34	AB828760	AB829145
Ae 9	AB828735	AB829120	Ae 35	AB828761	AB829146
Ae 10	AB828736	AB829121	Ae 36	AB828762	AB829147
Ae 11	AB828737	AB829122	Ae 37	AB828763	AB829148
Ae 12	AB828738	AB829123	Ae 38	AB828764	AB829149
Ae 13	AB828739	AB829124	Ae 39	AB828765	AB829150
Ae 14	AB828740	AB829125	Ae 40	AB828766	AB829151
Ae 15	AB828741	AB829126	Ae 41	AB828767	AB829152
Ae 16	AB828742	AB829127	Ae 42	AB828768	AB829153
Ae 17	AB828743	AB829128	Ae 43	AB828769	AB829154
Ae 18	AB828744	AB829129	Ae 44	AB828770	AB829155
Ae 19	AB828745	AB829130	Ae 45	AB828771	AB829156
Ae 20	AB828746	AB829131	Ae 46	AB828772	AB829157
Ae 21	AB828747	AB829132	Ae 47	AB828773	AB829158
Ae 22	AB828748	AB829133	Ae 48	AB828774	AB829159
Ae 23	AB828749	AB829134	Ae 49	AB828775	AB829160
Ae 24	AB828750	AB829135	Ae 50	AB828776	AB829161
Ae 25	AB828751	AB829136	Ae 51	AB828777	AB829162
			Ae 52	AB828778	AB829163
			Ae 53	AB828779	AB829164

Table S2. GenBank accession numbers for the *rpoD*/*gyrB* gene sequences of *Aeromonas* reference strains used in the phylogenetic analysis

Species	GenBank accession number	
	<i>rpoD</i>	<i>gyrB</i>
<i>A. allosaccharophila</i> CECT 4199	HQ442825	HQ442733
<i>A. aquariorum</i> CECT 7289	HQ442798	HQ442712
<i>A. bestiarum</i> CECT 4227	HQ442854	HQ442683
<i>A. bivalvium</i> CECT 7113	HQ442817	HQ442703
<i>A. cavernicola</i> MDC 2508	HQ442864	HQ442702
<i>A. caviae</i> CECT 838	HQ442790	HQ442748
<i>A. diversa</i> CECT 4254	HQ442805	HQ442756
<i>A. encheleia</i> CECT 4253	HQ442777	HQ442651
<i>A. enteropelogenes</i> CECT 4255	HQ442822	HQ442718
<i>A. eucrenophila</i> CECT 4224	HQ442770	HQ442657
<i>A. fluvialis</i> CECT 7401	FJ603453	FJ603455
<i>A. hydrophila</i> CECT 839	HQ442791	HQ442746
<i>A. jandaei</i> CECT 4228	HQ442840	HQ442736
<i>A. media</i> CECT 4232	HQ442785	HQ442709
<i>A. molluscorum</i> CECT 5864	HQ442812	HQ442671
<i>A. piscicola</i> CECT 7443	HQ442859	HQ442690
<i>A. popoffii</i> CECT 5176	HQ442853	HQ442693
<i>A. rivuli</i> DSM 22539 (CECT 7518)	FJ969433	FJ969434
<i>A. salmonicida</i> CECT 894	HQ442843	HQ442680
<i>A. sanarelli</i> CECT 7402	FJ807275	FJ807277
<i>A. schubertii</i> CECT 4240	HQ442809	HQ442755
<i>A. simiae</i> CIP 107798	HQ442811	HQ442758
<i>A. sobria</i> CECT 4245	HQ442867	HQ442698
<i>A. taiwanensis</i> CECT 7403	FJ807271	FJ807272
<i>A. tecta</i> CECT 7082	HQ442762	HQ442662
<i>A. veronii</i> CECT 4257	HQ442833	HQ442728
<i>A. veronii</i> CECT 5761	HQ442831	HQ442729
<i>A. veronii</i> CECT 4246	HQ442829	HQ442724

Table S3. Sequences of PCR primers used to amplify the virulence genes and the integrase genes of *Aeromonas* isolates, expected size of PCR products and their references

Gene	Nucleotide sequence (5' to 3')	Product size (bp)	Reference
Aerolysin/hemolysin	aer-f: CCTATGGCCTGAGCGAGAAG aer-r: CCAGTTCAGTCCCACCACT	431	Soler et al. (2002)
Serine protease	serine-f: CACCGAAGTATTGGGTCAGG serine-r: GGCTCATGCGTAACTCTGGT	350	Soler et al. (2002)
DNase	exu-f: (A/G)GACATGCACAACCTCTTCC exu-r: GATTGGTATTGCC(C/T)TGCAA(C/G)	323	Soler et al. (2002)
Cytotoxic enterotoxin (<i>act</i>)	act F: AGAAGGTGACCACCACCAAGAACA act R: AACTGACATCGGCCTTGAATC	232	Nawaz et al. (2010)
Cytotoxic enterotoxin (<i>ast</i>)	ast F: TCTCCATGCTTCCCTTCCACT ast R: GTGTAGGGATTGAAGAAGCCG	331	Nawaz et al. (2010)
Cytotoxic enterotoxin (<i>alt</i>)	alt F: TGACCCAGTCCTGGCACGGC alt R: GGTGATCGATCACCACCAGC	442	Nawaz et al. (2010)
Flagellin	fla F: TCCAACCGTYTGACCTC fla R: GMYTGGTTGCGRATGGT	608	Nawaz et al. (2010)
Integrase 1	Int1 F: GGGTCAAGGATCTGGATTTCG Int1 R: ACATGGGTGTAATCATCGTC	483	Mazel et al. (2000)
Integrase 2	Int2 F: CACGGATATGCGACAAAAGGT Int2 R: GTAGCAAACGAGTGACGAAATG	788	Mazel et al. (2000)

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