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Aeromonas aquariorum Is Widely Distributed in Clinical and Environmental Specimens and Can Be Misidentified as *Aeromonas hydrophila*▽†

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Genotypic characterization of 215 *Aeromonas* strains (143 clinical, 52 environmental, and 20 reference strains) showed that *Aeromonas aquariorum* (60 strains, 30.4%) was the most frequently isolated species in clinical and water samples and could be misidentified as *Aeromonas hydrophila* by phenotypic methods.

The genus *Aeromonas* consists of oxidase-positive, Gram-negative rods that are motile by polar flagella. *Aeromonas* species are inhabitants of aquatic environments and can also be found in foods, soil, and the microflora of fish (8, 13, 17). In the present study, we analyzed the nucleotide sequences derived from the *gyrB* and *rpoD* genes to characterize a collection of 20 reference, 143 clinical, and 52 environmental *Aeromonas* strains, of which more than 50% comprised *A. hydrophila*, as previously identified by a conventional biochemical scheme (1, 3). Clinical samples included wound (54 samples), stool (33 samples), blood (33 samples), and 23 miscellaneous specimens. Environmental specimens were collected from water (44 samples), fish (7 samples), and crab (1 sample).

DNA was extracted by the method described by Coenye and LiPuma (4), and amplification and sequencing of the *gyrB* and *rpoD* genes were performed as previously described (15, 18). Nucleotide sequences were determined with an ABI 3130XL analyzer (Applied Biosystems). The nucleotide sequences of *gyrB* and *rpoD* were independently aligned by the CLUSTAL_X program, version 1.8 (16). Genetic distances were obtained using Kimura's (7) two-parameter model, and concatenated trees were constructed by the neighbor-joining method (14) with the MEGA program (9).

Sequence dissimilarity percentages were calculated using the ClustalW and MEGA5 software. Nucleotide sequencing showed that *A. aquariorum* was the most frequently isolated aeromonad, with 60 strains (30.7%) clustering with *A. aquariorum* (CECT 7289) (Fig. 1), followed by *A. veronii* bv. *sobria* (49 strains, 25.1%), *A. hydrophila* (38 strains, 19.4%), and *A. caviae* (36 strains, 18.4%). In clinical specimens, *A. aquariorum* was most prevalent in wounds (22 strains, 40.7%) and water samples (24 strains, 54.5%), but it was less frequently isolated from feces (4 strains, 12.1%) and blood (3 strains, 9.0%) samples, while only 2 (28.5%) strains were recovered from fish (Table 1).

Biochemically, *A. aquariorum* could be clearly differentiated from *A. hydrophila* by its inability to produce acid from L-arabinose, while most strains utilized citrate (93%) or produced alkylsulfatase (73%). In contrast, all *A. hydrophila* strains tested produced acid from L-arabinose and were less likely to utilize citrate (26%) or produce alkylsulfatase (3%). *A. aquariorum* differed from *A. caviae* by a positive Voges-Proskaeur (VP) reaction (95% of *A. aquariorum* specimens), decarboxylation of lysine (95% positive), and production of gas from glucose (90%), while *A. caviae* was usually negative in all these tests. *A. aquariorum* could be differentiated from *A. veronii* bv. *sobria* by its ability to utilize DL-lactate (78% versus 2%) and production of staphylolysin (82% versus 0%) (3).

The genus *Aeromonas* has long been recognized to contain strains that are difficult to differentiate from one another, particularly when identification is based on phenotypic methods alone (1). Housekeeping genes such as *rpoD* and *gyrB* perform essential functions in bacteria and, unlike 16S rRNA, are single-copy genes where horizontal transfer seldom occurs (15, 17). The intraspecies dissimilarity derived from the combination of these two genes (approximately 1,294 bp) ranged from 0.4% to 3.5% between the type species and the wild strains identified as *A. aquariorum*, consistent with the position of these isolates as shown in Fig. 1. The sequence divergence of strain 213 ranged from 0.4% to 4.2%, suggesting that this isolate needs further investigation. Interspecies dissimilarity ranged from 19.1% between *A. molluscorum* and *A. diversa* to 1.4% between *A. encheleia* and *Aeromonas* sp. HG11, confirming the close relationship that exists between the latter species (see the supplemental material).

Our results revealed that, when used independently, sequences of both genes led to comparable identification, suggesting that *gyrB* and *rpoD* were reliable equivalent markers for the taxonomic discrimination of *Aeromonas* species. Phylogenetic trees generated from the *rpoD* and *gyrB* sequences (results not shown) were comparable to that derived from a partial *rpoB* gene sequence (10) except that the former sequences consistently placed *A. molluscorum* well within the center of the trees, while it was placed in a more distant position when the tree was constructed from the *rpoB* sequences alone.

Results obtained in the present study may help to explain the

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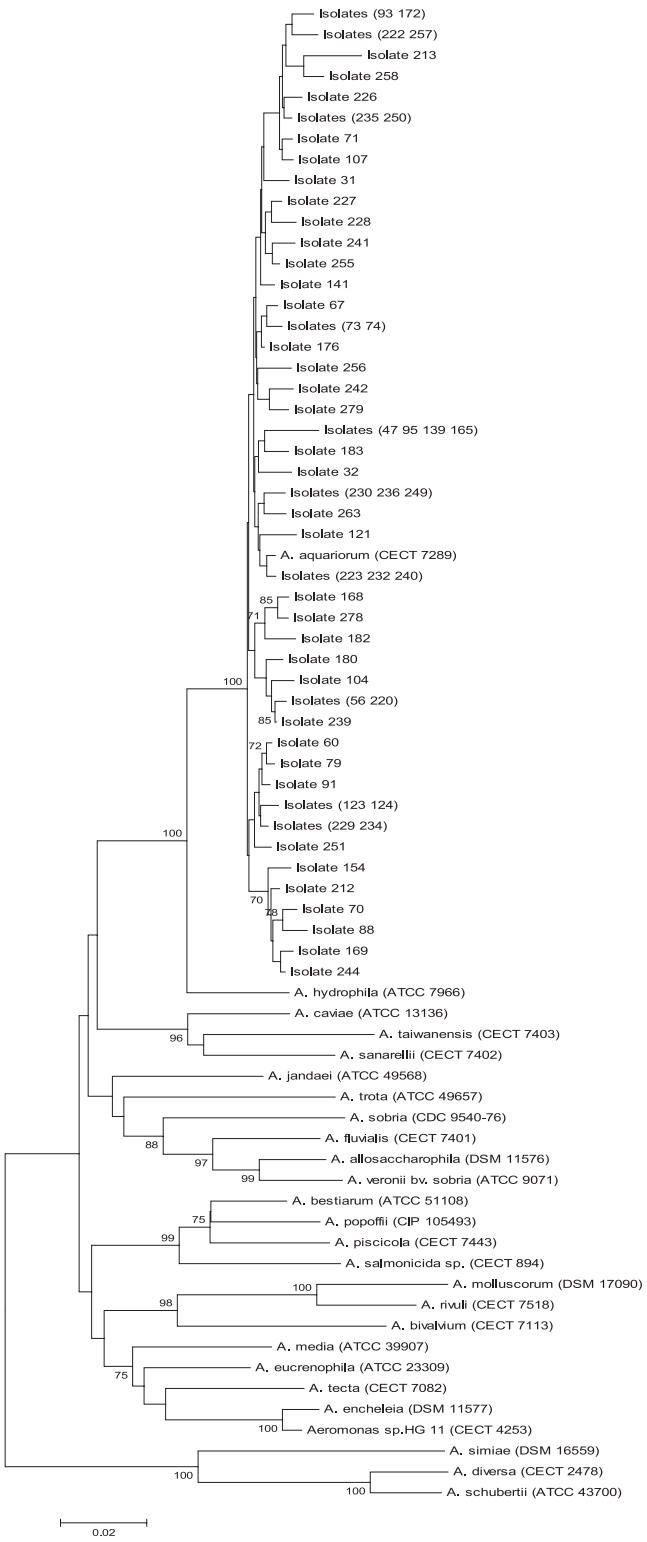

FIG. 1. Concatenated neighbor-joining phylogenetic tree showing the position of *A. aquariorum* strains derived from the *rpoD* and *gyrB* sequences.

biochemical and genotypic heterogeneity previously observed in *A. hydrophila* (6, 11). Correct identification occurred in only 35 (33.6%) out of 104 strains phenotypically identified as *A. hydrophila*, while the remaining strains were reidentified as *A. aquariorum* (54 strains [51.9%]), *A. veronii* bv. *sobria* (14 strains [13.4%]), and *A. bestiarum* (1 strain [1.2%]) by molecular analysis.

The distribution of *A. aquariorum* strains in clinical and water samples found in the present study contradicts the long-standing notion that *A. caviae*, *A. hydrophila*, and *A. veronii* bv. *sobria* represent the most frequently isolated aeromonads (>85% in clinical specimens) (2, 6, 12). The number of *A. hydrophila* strains reclassified into several different species after genotypic characterization indicates that accurate identification of aeromonads requires molecular methods. Furthermore, our results concurred with those of Soler et al. (15), who suggested that the combined analysis of more than one target improved the resolving power and the ability to differentiate between closely related species.

In summary, accurate identification of *Aeromonas* to the species level using only biochemical tests is still unreliable and must include a genotypic method. *Aeromonas* of clinical or environmental interest should be presumptively reported as an *Aeromonas* species, and the strain should be sent to a reference center for final identification if appropriate molecular facilities are not available locally. Finally, our results are consistent with those of Figueras et al. (5), who suggested that *A. aquariorum* is globally distributed and can be confused with *A. hydrophila.*

Nucleotide sequence accession numbers. The GenBank/EMBL accession numbers for the *rpoD*/*gyrB* gene sequences, respectively, are as follows: *A. allosaccharophila* DSM 11576, FN773342/FN813470; *A. aquariorum* CECT 7289, FN773316/FN691767; *A. bestiarum* ATCC 51108, FN773317/FN065556; *A. bivalvium* CECT 7113, FN773318/FN691768; *A. caviae* ATCC 13136, FN773319/FN691769; *A. diversa* CECT 4254, AY169345/AY101806; *A. encheleia* DSM 11577, FN773320/FN796740; *A. eucrenophila* ATCC 23309, FN773321/FN706557; *A. fluvialis* CECT 7401, FJ603453/FJ603455; *A. hydrophila* ATCC 7966, FN773322/FN706558; *A. jandaei* ATCC 49568, FN773323/FN706559; *A. media* ATCC 33907, FN773324/FN706560; *A. molluscorum* DSM 7090, FN773325/FN706561; *A. piscicola* CECT 7443, FM999969/FM999963; *A. popoffii* CIP 105493, FN773336/FN706562; *A. rivuli* CECT 7518, FJ969433/FJ969434; *A. salmonicida* CECT 894, AY169327/AY101773; *A. sanarelli* CECT 7402, FJ472929/FJ607277; *A. schubertii* CECT 4240, AY169336/AY101772; *A. simiae* DSM 16559, DQ41159/FN706563; *A. sobria* CDC 9540-76, FN773345/FN706564; *A. taiwanensis* CECT 7403, FJ474928/FJ807272; *A. tecta* CECT 7082, FN773337/FN796745; *A. trota* ATCC 49657, FN773339/FN796746; *A. veronii* bt. *sobria* ATCC 9071, FN773340/FN796747. Accession numbers for the *rpoD* gene sequence (wild strains) are *A. allosaccharophila*, FN773344; *A. aquariorum*, FR675826 to FR675856, FR675886 to Fr675894, FR681589 to FR681596, FN77333, FN773334, FN796724 to FN796728, FN796733, FN808217, FR682782; *A. bestiarum*, FN773343; *A. caviae*, FN796729, FR681906 to FR681925, FR682010 to FR682023; *A. hydrophila*, FN795730, FR681805 to FR681811, FR681865 to FR681892; *A. jandaei*, FN773326, FN773327, FR682798; *A. media*, FN773332, FR682799, FR682800; *A. salmonicida*, FN773330, FN773331; *A. schubertii*, FR865967; *A. veronii* bt. *sobria*, FR682024, FN773335,

TABLE 1. Distribution of *Aeromonas* spp. in clinical and environmental specimens

Species	No. of specimens (% of total)	No. (%) of clinical specimens					No. (%) of environmental specimens			
		Wound	Stool	Blood	Miscellaneous[a]	Total	Water	Fish	Crab	Total
A. allosaccharophila	1 (0.5)		1 (3.0)			1 (0.6)				
A. aquariorum	60 (30.7)	22 (40.7)	4 (12.1)	3 (9.0)	5 (27.1)	34 (23.8)	24 (54.5)	2 (28.5)		26 (50.0)
A. bestiarum	1 (0.5)			1 (3.0)		1 (0.6)				
A. caviae	36 (18.4)	5 (9.2)	11 (33.3)	11 (32.2)	7 (30.4)	34 (23.8)	1 (2.2)	1 (14.2)		2 (3.8)
A. hydrophila	38 (19.4)	16 (29.6)	2 (6.0)	7 (21.2)	8 (34.7)	33 (23.0)	4 (9.0)	1 (14.2)		5 (9.6)
A. jandaei	3 (1.5)						2 (4.5)	1 (14.2)		3 (5.7)
A. media	3 (1.5)		1 (3.0)	1 (3.0)		2 (1.3)		1 (14.2)		1 (1.9)
A. salmonicida	2 (1.0)	1 (1.8)				1 (0.6)			1	1 (1.9)
A. schubertii	1 (0.5)	1 (1.8)				1 (0.6)				
A. veronii bv. *sobria*	49 (25.1)	9 (16.7)	14 (42.4)	10 (30.3)	3 (13.0)	36 (25.1)	12 (27.2)	1 (14.2)		13 (25.0)
Aeromonas sp.	1 (0.5)						1 (2.2)			1 (1.9)
Total	195	54	33	33	23	143	44	7	1	52

[a] Miscellaneous specimens include *A. aquariorum* from bone chip (1), urine (2), sputum (1), and unknown (1) specimens, *A. caviae* from dialysis fluid (1), peritoneal fluid (1), bile (2), T-tube tip (1), and unknown (2) specimens, *A. hydrophila* from tissue (1), sputum (2), biliary shunt (1), bile (1), pancreatic cyst (1), drain fluid (1), and mortuary (1) specimens, and *A. veronii* bv. *sobria* from sputum (2) and appendix (1) specimens.

FR682572 to FR682581, FR682762 to FR682794, FR682796, FR682797; *Aeromonas* sp., FN773335. Accession numbers for the *gyrB* gene sequence (wild strains) are *A. allosaccharophila*, FN691770; *A. aquariorum*, FR675858 to FR675872, FR676941 to FR676960, FR681574 to FR681588, FN796734 to FN796739, FN706555, FN796752, FN691766; *A. bestiarum*, FN691771; *A. caviae*, FR682025 to FR682044, FR682131 to FR682140, FR682504 to FR682507, FR685963 to FR865964; *A. hydrophila*, FN796741, FR681597 to FR681606, FR681735 to FR681761; *A. jandaei*, FR682555, FN796742, FN796743; *A. media*, FN691772, FR682802, FR682803; *A. salmonicida*, FR682801, FN796744; *A. schubertii*, FN691774; *A. veronii* bt. *sobria*, FN691773, FN796749, FN796750, FR682508 to FR682554; *Aeromonas* sp., FN691773.

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