

Identification of *Aeromonas* Strains to the Genospecies Level in the Clinical Laboratory

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One hundred thirty-three strains of *Aeromonas* (human, $n = 102$; animal, $n = 16$; environmental, $n = 15$) previously identified to the DNA group level by molecular methods were biochemically analyzed for 58 properties. On the basis of the use of between 9 and 16 selected tests, 132 of the 133 strains (99%) could be assigned to their correct hybridization group using this biochemical scheme. The results suggest a feasible approach for identifying aeromonads to genospecies level under appropriate conditions.

Recent studies using molecular approaches to analyze mesophilic and psychrophilic aeromonads have led to a more refined phylogeny for the genus *Aeromonas*. Analysis of the electromorphic variation at loci for selected cellular enzymes coupled with DNA-DNA hybridization studies indicate that the genus *Aeromonas* is composed of more than 12 species at the DNA level (12). Although some of these species have previously been assigned to recognized hybridization groups (HGs) (clusters, or DNA groups) by earlier taxonomic investigations (1, 9, 18), on the basis of recent systematic studies (5, 8, 10, 11, 22), several new species have been either accepted or recently proposed. These include *Aeromonas veronii* (HGs 8 and 10), *A. schubertii* (HG 12), *A. eucrenophila* (HG 6), *A. jandaiei* (HG 9), and *A. trota* (HG 13). Of the 13 currently published HGs (*Aeromonas* group 501 is not considered a separated HG herein), all have been recovered from clinical material, with the exception of *A. eucrenophila* and *A. sobria* (HG 7). Several epidemiological surveys that have genetically characterized more than 250 *Aeromonas* strains primarily isolated from human feces indicate that of the 11 HGs known to be recovered from clinical material, three (HGs 1, 4, and 8) account for more than 85% of all isolates (1, 17, 18). The reasons why these three HGs predominate in clinical material is unknown, although their predominance may be due either to their environmental frequency and distribution or to their relative pathogenicity for humans.

Despite these taxonomic accomplishments, the laboratory identification of aeromonads to the genospecies level is not a straightforward process. Over the past decade, most laboratories have gradually switched from identifying isolates as *A. hydrophila*, *A. hydrophila* complex, or *Aeromonas* spp. to *A. hydrophila*, *A. sobria*, or *A. caviae*. This change is with the understanding that these later species are actually phenospecies, that is, a collection of several genetically distinct groups that biochemically resemble one another but cannot unambiguously be separated from one another by phenotypic methods. Studies of the laboratory identification of aeromonads have, for the most part, utilized strains that were not genetically characterized or that have been identified with miniaturized kits or systems that often correlate poorly with conventional biochemicals (7, 20, 21). In this

article, we report the biochemical properties (by conventional methods) of 133 *Aeromonas* strains previously identified to the HG level and propose sets of useful phenotypic markers which aid in the identification of clinical isolates to the genospecies level.

MATERIALS AND METHODS

One hundred thirty-three *Aeromonas* strains were investigated in this study. Each strain had been previously assigned to a single HG (DNA group) by DNA-DNA hybridization studies against reference or type strains for each species as previously outlined by Kuijper et al. (18) and Altwegg et al. (1). The number of strains analyzed for each HG and their respective sources of isolation are given in Table 1. Reference strains included in this survey of 133 aeromonads are additionally listed in Table 2.

Biochemical studies. Each of the 133 *Aeromonas* strains was tested for 58 biochemical, morphologic, or growth properties. Of the 58 traits analyzed, 48 were part of a standard biochemical regimen utilized by the Microbial Diseases Laboratory in the identification of unknown enteric bacilli. Ten additional tests that have previously been shown or suggested to be useful in the identification of *Aeromonas* strains to the species level were added to this battery. These additional tests included production of a beta-hemolysin, elastase (15), and stapholysin (15); gluconate oxidation (4); ascorbate utilization (2); acid from D-mannose (15); susceptibility to 10 μg of ampicillin (8) and 30 μg of cephalothin (14); pyrazinamidase activity (6); and production of H_2S from cysteine-based agar (15). Ampicillin and cephalothin susceptibilities were read at 24 h, gluconate oxidation and pyrazinamidase activity were read at 48 h, and all other tests were read daily for 7 days. All properties were tested for reproducibility, and appropriate positive and negative controls were included. Unless otherwise specified, all tests were done at 35°C.

RESULTS

Regardless of species (HG) designation, all *Aeromonas* strains exhibited a number of features in common, including oxidase, catalase, and nitrate positivity; production of acid from D-glucose and D-trehalose; failure to utilize malonate or mucate as the sole carbon source; inability to ferment adonitol, dulcitol, erythritol, inositol, and D-xylose; and

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TABLE 1. Composition and sources of *Aeromonas* strains used in this study

<i>Aeromonas</i> species	HG	No. of strains	No. of strains isolated from source		
			Human	Animal	Environment
<i>A. hydrophila</i>	1	22	17	2	3
Unnamed	2	9	4	3	2
<i>A. salmonicida</i>	3	7	5	1	1
<i>A. caviae</i>	4	15	14	1	0
<i>A. media</i>	5A, 5B	11	7	2	2
<i>A. eucrenophila</i>	6	7	0	2	5
<i>A. sobria</i>	7	2	0	2	0
<i>A. veronii</i> biotype <i>sobria</i>	8	18	15	3	0
<i>A. jandaei</i>	9	11	10	0	1
<i>A. veronii</i> biotype <i>veronii</i> ^a	10	5	5	0	0
Unnamed	11	2	1	0	1
<i>A. schubertii</i>	12	11	11	0	0
<i>A. trota</i>	13	13	13	0	0
Total		133	102	16	15

^a Ornithine decarboxylase-positive strains

growth in nutrient broth containing 0 and 3% NaCl. Test results for several other phenotypic properties were almost invariably positive (98 to 99%), such as those for motility, β-galactosidase activity, and resistance to 0/129; less than 2% of the strains tested hydrolyzed urea, degraded pectin, or produced acid from arabinose, D-raffinose, or D-amylgdalin. Thirty-six tests produced variable results, including those for production of indole, acetyl methyl carbinol, and phenylpyruvic acid; citrate, acetate, and ascorbate utilization; elaboration of deoxyribonuclease, elastase, hemolysin, stapholysin, and corn oil lipase; gelatin and esculin hydrolysis;

TABLE 2. *Aeromonas* reference strains used in this study

HG	Reference strains ^a
1ATCC 7966 (CDC 9079-79, Popoff 543)
2ATCC 14715 (CDC 9090-79), NCMB 1134, CDC 9533-76 (Popoff C218), CDC 9723-84
3CDC 434-84 (Popoff C316), CDC 9736-84
4ATCC 15468 (CDC 9083-79, Popoff 545), CDC 1399-77
5CDC 862-83 (Popoff C239), ATCC 33907 (CDC 9072-83), ATCC 35950, CDC 435-84 (Popoff C233)
6ATCC 23309 (CDC 859-83, Popoff C546), NCMB 74 (CDC 859-83), NCMB 73
7CIP 7433 (CDC 9538-76, ATCC 43979), CDC 9540-76 (Popoff 215)
8ATCC 9071 (CDC 9088-79), CDC 437-84 (Popoff C234)
9ATCC 49468 (CDC 787-80), CDC 1530-81, ATCC 49569, ATCC 49570, ATCC 49571, ATCC 49572 (CDC 1081-81), CDC 1531-81, CDC 2154-78, CDC 2156-78
10ATCC 35624 (CDC 1169-83), ATCC 35625 (CDC 1305-83), ATCC 35622 (CDC 140-84), ATCC 35623 (CDC 964-83), ATCC 35625 (CDC 1170-83)
11ATCC 35941 (CDC 1306-83), ATCC 35625 (CDC 1170-83)
12ATCC 43700 (CDC 2446-81), ATCC 43701 (CDC 9180-81), ATCC 43947 (CDC 463-83), ATCC 43945 (CDC 2508-86), CDC 1139-84, CDC 2446-85, CDC 2460-86, CDC 2561-87, CDC 2555-87
13ATCC 49657, ATCC 49658, ATCC 49661, ATCC 49660, ATCC 49659

^a Strain designations not in parentheses are for strains used in this study; those in parentheses are other designations by which the strains are known.

growth in KCN broth; lysine and ornithine decarboxylase, arginine dihydrolase, and pyrazinamidase activities; pigment production at 25°C; H₂S formation in gelatin-cysteine-thio-sulfate medium; gluconate oxidation; susceptibility to cephalothin and ampicillin; and acid production from arabinose, rhamnose, cellobiose, lactose, maltose, sucrose, glycerol, mannitol, sorbitol, α-methylglucoside, salicin, melibiose, and mannose fermentation. Of these, we selected nine tests as a primary battery in the identification of aeromonads to the genospecies level. These tests were chosen because they are commonly included in most gram-negative fermenter identification schemes and are, in many instances, part of commercial microidentification panels, although results may differ using these later systems. The results of testing these 133 *Aeromonas* strains against this biochemical battery are listed in Table 3. With this nine-test scheme, aeromonads could be broken down into the *A. hydrophila* complex (HGs 1, 2, and 3) and the *A. caviae* group (HGs 4 and 5) on the basis of results of lysine decarboxylase and Voges-Proskauer tests; on the basis of aerogenicity, the ornithine decarboxylase-positive species (HGs 10 and 11) and the individual genospecies *A. eucrenophila* (HG 6), *A. sobria* (HG 7), *A. veronii* biotype *sobria* (HG 8), and *A. jandaei* (HG 9) could be differentiated. Two strains of *A. trota* that were anaerogenic and failed to produce acid from D-mannitol could not be unambiguously separated from *A. schubertii* by using this biochemical scheme.

To separate individual species comprising the *A. hydrophila* complex (HGs 1 to 3), seven tests that provided the greatest discriminatory value were chosen (Table 4). *A. hydrophila* strains (sensu stricto) were salicin positive and sorbitol negative, and most failed to produce acid from lactose and rhamnose. Most HG 2 strains, on the other hand, were rhamnose positive and salicin, sorbitol, and elastase negative; this group additionally failed to oxidize gluconate. *A. salmonicida* strains were lactose and salicin positive, and two-thirds of the strains tested fermented D-sorbitol, a trait previously noted by Altwegg and colleagues (1) to be significantly associated with HG 3 strains. Acid from salicin, elastase activity, and gluconate oxidation collectively distinguished all *A. hydrophila* strains from isolates of HG 2. Elastase activity was exclusively associated with the *A. hydrophila* complex (HGs 1 and 3), as no other strain of any other HG was found to elaborate this enzyme. A comparable situation existed for stapholytic activity; 50 to 85% of strains from HGs 1 to 3 produced this enzyme, while stapholysin was not detected in any other strain belonging to HGs 4 to 13.

To separate strains that phenotypically resemble *A. caviae* (HGs 4 to 6), a combination of seven additional tests were found to be of use (Table 5). Although *A. eucrenophila* is aerogenic and could be potentially identified by using the characteristics listed in Table 3, we found gas production by this species in Durham tubes to be extremely weak compared with that by aerogenic *A. hydrophila* or *A. veronii* and could, therefore, be potentially missed. Key characteristics that aided in the separation of these genospecies included citrate utilization, glycerol fermentation, and production of H₂S (weak) from cysteine-based media as previously outlined by Schubert and Hegazi (22). *A. caviae* and *A. media* could most easily be separated by using D-mannose, hemolysis, and pyrazinamidase activity. One strain genetically identified as *A. caviae* (HG 4) that was mannose positive and pyrazinamidase negative could be misidentified as *A. media* even by using this supplementary scheme. Unlike the *A. hydrophila* complex, out of the 58 characteristics screened,

TABLE 3. Selected biochemical properties of *Aeromonas* HGs

Test ^a	HG ^b												
	1	2	3	4	5	6	7	8	9	10	11	12	13
LDC	100	50	67	0	0	0	100	100	100	100	50	82	100
ODC	0	0	0	0	0	0	0	0	0	100	100	0	0
ADH	100	88	67	100	91	86	0	100	100	0	0	91	100
Esculin	95	100	100	93	91	100	0	0	0	100	50	0	8
Gas (glucose)	91	75	50	0	0	100	50	89	100	80	50	0	69
VP	91	75	67	0	0	0	0	94	91	100	0	18	0
Arabinose	86	100	100	100	100	86	0	17	0	0	0	0	0
Mannitol	95	100	100	100	100	100	100	100	100	100	100	0	69
Sucrose	100	100	100	100	100	71	100	100	0	100	100	0	23

^a Abbreviations: LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; VP, Voges-Proskauer.

^b Each number indicates percentage of isolates positive for each indicated trait.

no unique markers were associated with the *A. caviae* complex.

For the ornithine decarboxylase-positive species (HGs 10 and 11), separation could be based on acetyl methyl carbinol production (Table 3), as HG 11 is Voges-Proskauer negative. However, only two strains belonging to HG 11 are known to exist, and any conclusions regarding this group are extremely tentative, at best. For separation of *A. schubertii* from *A. trota*, five additional tests were found to be helpful in species assignment (Table 6). With the exception of corn oil lipase, these characteristics as differential traits in identifying aeromonads to the species level have been previously described (8, 10).

DISCUSSION

The identification of *Aeromonas* isolates to the genospecies level in the clinical laboratory is fraught with numerous difficulties, including a complicated taxonomy and the need to use many nonconventional biochemical assays that are often time-consuming to make and difficult to interpret and that require a long incubation period before final results can be recorded. Furthermore, strains that possess biochemical properties that are atypical for a given genospecies are commonly encountered in the clinical laboratory. Such strains do not easily lend themselves to accurate species identification by using either a limited number of phenotypic properties or a dichotomic scheme. Equally frustrating is the fact that the most commonly used commercial identification systems in the United States fail to identify *Aeromonas* strains to the species level, and, in some instances, cannot distinguish aeromonads from phenotypically similar *Vibrio* species, such as *Vibrio fluvialis* (12, 19).

Despite these problems, advances have recently been made; genetic investigations primarily based on DNA-DNA hybridization results have defined the major taxons within the genus, proposed new *Aeromonas* species, and detailed the frequency of isolation of various HGs from clinical material (1, 9, 12). In the present investigation, we have been able to correctly identify 132 of 133 (99.2%) *Aeromonas* isolates to the genospecies level by using a series of biochemical tests listed in Tables 3 to 6. The only isolate which could not be assigned to its correct HG by using this format was a previously mentioned *A. caviae* strain that was mannose positive and resembled *A. media*. By using this biochemical format, a minimum of nine tests (Table 3) and a maximum of 16 traits were required to identify individual *Aeromonas* genospecies or HGs. With the exception of a few tests (elastase, corn oil lipase, H₂S from cysteine), most biochemicals used in this investigation are commonly found in most laboratories of moderate or larger size in either conventional or commercial form. Although we did not compare the sensitivities and specificities of different formulations of individual biochemical tests used in this study, one recent investigation suggests that many of these conventional and commercial formulas for phenotypic tests used in identifying aeromonads are roughly equivalent (7). It is interesting that only 1 of the 58 traits analyzed in this study was genospecies specific, that being D-sorbitol fermentation by *A. salmonicida* (HG 3), although several other traits were phenospecies specific. Such results preclude at present the use of only a few biochemicals or a dichotomous scheme to accurately identify aeromonads to the species level. The use of a group of biochemical reactions to identify aeromonads to the species level will also help remedy the potential

TABLE 4. Biochemical separation of the *A. hydrophila* complex^a

Test	% of isolates with positive result		
	<i>A. hydrophila</i>	HG 2	<i>A. salmonicida</i>
Acid from:			
D-Rhamnose	27	75	0
D-Sorbitol	0	0	67
Salicin	95	0	100
Lactose	23	12	100
Gluconate oxidation	68	0	0
Elastase	73	0	67
Phenylpyruvic acid	64	12	67

^a Based on the analysis of the 38 strains of HGs 1, 2, and 3 as listed in Table 1.

TABLE 5. Biochemical separation of *A. caviae*, *A. media*, and *A. eucrenophila*^a

Test	% of isolates with positive result		
	<i>A. caviae</i>	<i>A. media</i>	<i>A. eucrenophila</i>
Citrate utilization	100	82	0
Acid from:			
Glycerol	73	91	0
D-Mannose	27	100	100
H ₂ S from cysteine	0	0	71
Hemolysis	0	45	86
Pyrazinamidase	80	18	100
Phenylpyruvic acid	93	73	29

^a Based on the analysis of 33 strains of HGs 4, 5, and 6 as listed in Table 1.

TABLE 6. Biochemical separation of *A. schubertii* and *A. trota*^a

Test	% of isolates with positive result	
	<i>A. schubertii</i>	<i>A. trota</i>
Corn oil lipase	100	0
Acid from cellobiose	0	100
H ₂ S from cysteine	0	62
Cephalothin susceptibility	73	0
Ampicillin susceptibility	0	100

^a Based on the analysis of 25 strains of HGs 12 and 13.

misidentification of atypical strains by relying on a group of phenotypic traits and not one or two selected biochemical properties.

The present study also suffers from several limitations. First, for several HGs (notably HGs 7 and 11), only a couple of strains are known to exist, and reliable markers which aid in the recognition of such DNA groups are presently not available. Second, in this investigation, we did not test *A. salmonicida* strains (HG 3) that originated from fish. These strains are biochemically distinct (nonmotile and indole negative) from HG 3 isolates recovered from clinical material (3), and preliminary studies indicate that fish isolates are sorbitol negative, unlike human strains (Table 4). Finally, only 23% of the *Aeromonas* strains studied in the current investigation were from nonhuman sources. While we did not see any unusual differences in phenotypic patterns between human and nonhuman strains belonging to a given DNA group (e.g., HG 1 or 2), in view of the above-noted differences observed among HG 3 strains, more variability in biochemical properties of other HGs may be seen when larger numbers of environmental isolates are tested.

A decade ago, *Aeromonas* strains were identified only to the phenospecies level. It is now entirely possible to accurately identify most aeromonads to the genospecies level (HG or DNA group) by using one or more biochemical schemes. This advance makes identification for the clinical laboratory easier than the previously utilized molecular approaches of DNA-DNA hybridization and multilocus enzyme electrophoresis. Table 7 lists three feasible approaches to identify aeromonads in the clinical laboratory. Which identification scheme a clinical laboratory should use is a relevant issue. However, with the mounting evidence supporting the role of aeromonads in gastroenteritis (16), differences in pathogenicity observed among isolates of different genospecies (13), and the potential of certain HGs to cause

TABLE 7. Potential approaches for final identification of *Aeromonas* spp. in the clinical laboratory

Identification level	No. of biochemicals required ^a	No. of groups (species) distinguished	Final identification
genus	8-11	0	<i>Aeromonas</i> spp.
phenospecies	13-18	3	<i>A. hydrophila</i> <i>A. sobria</i> <i>A. caviae</i>
genospecies	19-24	11	<i>A. hydrophila</i> <i>A. salmonicida</i> <i>A. media</i> ^b

^a Range in number of biochemicals required based on scheme and to what DNA group the isolate belongs to.

^b *A. caviae*, *A. eucrenophila*, *A. sobria*, *A. jandaei*, *A. schubertii*, *A. veronii* (biotypes *sobria* and *veronii*), and *A. trota*.

significant morbidity and mortality via extraintestinal infections in healthy and immunocompromised individuals (10, 17) under select circumstances, identification of aeromonads to the genospecies level may be justified. It presently seems appropriate that reference or research laboratories identify *Aeromonas* isolates to the genospecies level since biochemical methods are now available, and such results will extend our current knowledge of the epidemiology and pathogenesis of those species (12). Larger clinical laboratories should at a minimum identify aeromonads to the phenospecies level since certain invasive and enterotoxigenic properties correlate with these designations; if clinical cases of *Aeromonas* infection are to be published, identification to the genospecies level seems warranted. For small laboratories, identification to genus level only is appropriate with isolates sent to larger centers for further workup, if needed. Further studies documenting the environmental frequency and association of individual *Aeromonas* genospecies with infectious processes in both animals and humans will help to define the need and relevance of such identification schemes in the future.

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REFERENCES

1. Altwegg, M., A. G. Steigerwalt, R. Altwegg-Bissig, J. Luthy-Hottenstein, and D. J. Brenner. 1990. Biochemical identification of *Aeromonas* genospecies isolated from humans. *J. Clin. Microbiol.* **28**:258-264.
2. Arduino, M. J., F. W. Hickman-Brenner, and J. J. Farmer III. 1988. Phenotypic analysis of 132 *Aeromonas* strains representing 12 DNA hybridization groups, abstr. P-3, p. 37. Abstr. 2nd Int. Workshop *Aeromonas Plesiomonas* 1988.
3. Austin, D. A., D. McIntosh, and B. Austin. 1989. Taxonomy of fish associated *Aeromonas* spp., with the description of *A. salmonicida* subsp. *smithia* subsp. nov. *Syst. Appl. Microbiol.* **11**:277-290.
4. Burke, V., J. Robinson, H. M. Atkinson, and M. Gracey. 1982. Biochemical characteristics of enterotoxigenic *Aeromonas* spp. *J. Clin. Microbiol.* **15**:48-52.
5. Carnahan, A., G. R. Fanning, and S. W. Joseph. 1991. *Aeromonas jandaei* (formerly genospecies DNA group 9 *A. sobria*), a new sucrose-negative species isolated from clinical specimens. *J. Clin. Microbiol.* **29**:560-564.
6. Carnahan, A., L. Hammontree, L. Bourgeois, and S. W. Joseph. 1990. Pyrazinamidase activity as a phenotypic marker for several *Aeromonas* spp. isolated from clinical specimens. *J. Clin. Microbiol.* **28**:391-392.
7. Carnahan, A. M., S. Behram, and S. W. Joseph. 1991. Aerokey II: a flexible key for identifying clinical *Aeromonas* species. *J. Clin. Microbiol.* **29**:2843-2849.
8. Carnahan, A. M., T. Chakraborty, G. R. Fanning, D. Verma, A. Ali, J. M. Janda, and S. W. Joseph. 1991. *Aeromonas trota* sp. nov., an ampicillin-susceptible species isolated from clinical specimens. *J. Clin. Microbiol.* **29**:1206-1210.
9. Fanning, G. R., F. W. Hickman-Brenner, J. J. Farmer III, and D. J. Brenner. 1985. DNA relatedness and phenotypic analysis of the genus *Aeromonas*, abstr. C-116, p. 319. Abstr. 85th Annu. Meet. Am. Soc. Microbiol. 1985. American Society for Microbiology, Washington, D.C.
10. Hickman-Brenner, F. W., G. R. Fanning, M. J. Arduino, D. J. Brenner, and J. J. Farmer III. 1988. *Aeromonas schubertii*, a new mannitol-negative species found in human clinical specimens. *J. Clin. Microbiol.* **26**:1561-1564.
11. Hickman-Brenner, F. W., K. L. MacDonald, A. G. Steigerwalt, G. R. Fanning, D. J. Brenner, and J. J. Farmer III. 1987. *Aeromonas veronii*, a new ornithine decarboxylase-positive

- species that may cause diarrhea. *J. Clin. Microbiol.* **25**:900-906.
12. Janda, J. M. 1991. Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with the genus *Aeromonas*. *Clin. Microbiol. Rev.* **4**:397-410.
 13. Janda, J. M., and R. P. Kokka. 1991. The pathogenicity of *Aeromonas* strains relative to genospecies and phenospecies identification. *FEMS Microbiol. Lett.* **90**:29-34.
 14. Janda, J. M., and M. R. Motyl. 1985. Cephalothin susceptibility as a potential marker for the *Aeromonas sobria* group. *J. Clin. Microbiol.* **22**:854-855.
 15. Janda, J. M., M. Reitano, and E. J. Bottone. 1984. Biotyping of *Aeromonas* isolates as a correlate to delineating a species-associated disease spectrum. *J. Clin. Microbiol.* **19**:44-47.
 16. Jiang, Z. D., A. C. Nelson, J. J. Mathewson, C. D. Ericsson, and H. L. DuPont. 1991. Intestinal secretory immune response to infection with *Aeromonas* species and *Plesiomonas shigelloides* among students from the United States in Mexico. *J. Infect. Dis.* **164**:979-982.
 17. Kokka, R. P., J. M. Janda, L. S. Oshiro, M. Altwegg, T. Shimada, R. Sakazaki, and D. J. Brenner. 1991. Biochemical and genetic characterization of autoagglutinating phenotypes of *Aeromonas* associated with invasive and noninvasive disease. *J. Infect. Dis.* **163**:890-894.
 18. Kuijper, E. J., A. G. Steigerwalt, B. S. C. I. M. Schoenmakers, M. F. Peeters, H. C. Zanen, and D. J. Brenner. 1989. Phenotypic characterization and DNA relatedness in human fecal isolates of *Aeromonas* spp. *J. Clin. Microbiol.* **27**:132-138.
 19. Overman, T. L., J. F. Kessler, and J. P. Seabolt. 1985. Comparison of API 20E, API Rapid E, and API Rapid NFT for identification of members of the family *Vibrionaceae*. *J. Clin. Microbiol.* **22**:778-781.
 20. Pazzaglia, G., J. R. Escalante, R. B. Sack, C. Rocca, and V. Benavides. 1990. Transient intestinal colonization by multiple phenotypes of *Aeromonas* species during the first week of life. *J. Clin. Microbiol.* **28**:1842-1846.
 21. Renaud, F., J. Freney, J.-M. Boeufgras, D. Monget, A. Sedailan, and J. Fleurette. 1988. Carbon substrate assimilation patterns of clinical and environmental strains of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* observed with a micromethod. *Zentralbl. Bakteriol. Hyg. A* **269**:323-330.
 22. Schubert, R. H. W., and M. Hegazi. 1988. *Aeromonas eucrenophila* species nova *Aeromonas caviae*, a later and illegitimate synonym of *Aeromonas punctata*. *Zentralbl. Bakteriol. Mikrobiol. Hyg. A* **268**:34-39.