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Aeromonas jandaei (Formerly Genospecies DNA Group 9 *A. sobria*), a New Sucrose-Negative Species Isolated from Clinical Specimens

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A large numerical taxonomy study conducted in 1988 of 165 mostly clinical *Aeromonas* strains from diverse geographic sources produced a cluster ($S = 84\%$, S_{SM}) of four sucrose-negative strains that included the DNA definition strain for DNA group 9 *A. sobria* (CDC 0787-80). These four strains, together with five additional strains received in 1989, were subjected to DNA-DNA hybridization (hydroxyapatite, ^{32}P, 60 and 75°C), and all eight strains were closely related to the ninth labeled DNA group 9 definition strain CDC 0787-80 (73 to 86% relatedness at 60°C and 68 to 80% relatedness at 75°C; percent divergence, 2.0 to 3.5). Type strains and DNA definition strains for all other established *Aeromonas* species were only 35 to 72% related (60°C) to CDC 0787-80. We propose the name *Aeromonas jandaei* for this highly related group of nine strains, formerly known as DNA group 9 *A. sobria*. The type strain was designated ATCC 49568 (CDC 0787-80). The nine strains were examined at 36°C and were found to be resistant to 0/129 (vibriostatic agent) and uniformly positive for oxidase, gas production from glucose, indole, lysine decarboxylase, arginine dihydrolase, *o*-nitrophenyl-β-D-galactopyranoside, motility (25°C), nitrate reduction, citrate utilization, hemolysis on sheep blood agar, and growth in Trypticase soy broth with no added NaCl. They all fermented D-glucose, D-mannitol, and mannose but did not ferment sucrose, cellobiose, L-arabinose, inositol, salicin, or D-sorbitol. They were uniformly negative for esculin and urea hydrolysis, elastase production, ornithine decarboxylation, and the string test. The antibiogram of *A. jandaei* resembled that of other aeromonads (resistance to ampicillin and cephalothin), but it differed from most other aeromonads because of resistance to a single dilution of colistin and differed from clinical *A. veronii* biogroup sobria (formerly *A. sobria*) by its nearly uniform resistance to cephalothin. The esculin-, sucrose-, and cellobiose-negative and colistin-resistant profile distinguished *A. jandaei* from other *Aeromonas* species. These *A. jandaei* strains were isolated from blood (two strains), wounds (two strains), diarrheal stools (four strains), and a prawn (one strain). The blood and wound isolates, in particular, suggest that there is a possible clinical significance for this species and justify identification of and further research on this group of motile aeromonads.

Aeromonads are oxidase-positive, polar flagellated, glucose-fermenting, facultatively anaerobic, gram-negative rods that are resistant to 0/129 (vibriostatic agent) and autochthonous to aquatic environments worldwide (9, 20). Although they currently reside in the family *Vibrionaceae*, Colwell et al. (6) proposed in 1986 that aeromonads constitute a new family, *Aeromonadaceae*, based on 5S rRNA cataloging, 5S rRNA sequencing, and RNA-DNA hybridization data.

There are currently eight recognized phenotypically distinct genospecies distributed among 12 DNA hybridization groups (1). The genus consists of both psychrophiles and mesophiles that are found in soil and aquatic environments and that produce a diverse spectrum of diseases among both warm- and cold-blooded animals (14).

The species *Aeromonas sobria* was first proposed by Popoff and Veron (26) in 1976 and encompasses four DNA hybridization groups: DNA group 7 (which includes the type strain CIP 7433), DNA group 8 (which includes all clinical *A. sobria* isolates hybridized to date but has been proposed as a biogroup of *A. veronii*), DNA group 10, *A. veronii* biogroup veronii (which is phenotypically distinct from but genotypically identical to DNA group 8) (1, 10, 26), and the rare unnamed DNA group 9 aeromonads (7).

The purpose of this study was to establish the taxonomic position of and proper designation for the DNA group 9 aeromonads by using a suitable number of phenotypically and genotypically related strains. Additionally, by examination of case studies, antimicrobial susceptibility patterns, and phenotypic markers, the possible clinical significance of this group of aeromonads was investigated.

MATERIALS AND METHODS

Bacterial strains. The nine strains of *A. jandaei* that were studied are listed in Table 1. All strains were frozen in 2 ml of Trypticase soy broth with 10% glycerol (Remel, Lenexa, Kans.), maintained at −70°C and subsequently subcultured onto Trypticase soy agar slants (BBL Microbiology Systems, Cockeysville, Md.), and incubated overnight at 36 ± 1°C. Unless stated otherwise, all analyses were performed at 36 ± 1°C.

Phenotypic analysis. All of the standard identification tests (Table 2) were performed by either conventional methods (16, 19) with media and biochemicals obtained from Remel or by rapid identification with the API 20E system (Analytab Products, Plainview, N.Y.). Media for ascorbate utilization

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TABLE 1. *Aeromonas* DNA group 9 strains studied

A. jandaei strain	Case no.	ATCC no.[a]	Location of sender	Source	Other clinical information
CDC 0787-80	1	49568^T	Oregon	Stool	Male with diarrhea and dehydration
AS-14	2	49569	New York	Blood	71-yr-old male with multiple myeloma and diabetes
WRII/4658	3	49570	Maryland	Wound, below left eye	Riflegun-imposed BB injury that became infected after subject swam in a fresh H_2O pond
NMRI-6	4	49571	Maryland	Wound, leg	Navy diver with leg wound infection with two *Aeromonas* spp. from Anacostia River, Washington, D.C.
AS-206	5		New York	Stool	<1-yr-old female with gastroenteritis
AS-235	6		New York	Blood	96-yr-old male with acute erythroleukemia
CDC 1081-81 (AS-167)	7	49572	Hawaii	Prawn	Isolated from water off Honolulu
CDC 1530-81	8		Georgia	Stool	Two isolates from the same patient in Children's Hospital
CDC 1531-81 (AS 171)	9		Georgia	Stool	

[a] ATCC, American Type Culture Collection, Rockville, Md.

TABLE 2. Biochemical reactions of nine *A. jandaei* strains

Test	Cumulative % positive on day:				Reaction for ATCC 49568^T (CDC 0787-80)[a]
	1	2	3	4	
Indole production	100				+
Voges-Proskauer	78	78	89		+
Citrate[b]	100				+
H_2S on triple sugar iron	0	0	0		−
H_2S on modified GCF	11	56	89		+
Urea hydrolysis[b]	0				−
Lysine (Moeller's)	100				+
Arginine (Moeller's)	100				+
Ornithine (Moeller's)	0	0	0		−
Motility (25°C)	100				+
Gelatin hydrolysis[b]	89				+
D-Glucose:					
Acid	100				+
Gas	100				+
Acid from:					
Amygdalin[b]	11				−
L-Arabinose			0		−
Cellobiose			0		−
Inositol[b]	0				−
D-Mannitol	100				+
Mannose	100				+
Melibiose	36	56	56		+
L-Rhamnose[b]	0				−
Salicin	0	0	0		−
D-Sorbitol[b]	0				−
Sucrose			0		−
Gluconate oxidation		78			−
Ascorbate utilization		67			+
Elastase production	0	0	0	0	−
Esculin hydrolysis	0	0	0		−
Nitrate reduction	100				+
Oxidase	100				+
ONPG test[b,c]	100				+
String test	11				−
Sensitivity to 0/129	0				−
Hemolysis on sheep blood	34	100			+

[a] −, Negative at end of appropriate incubation period; +, positive at 24 h or after appropriate incubation period.
[b] API 20E test result.
[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

and H_2S production from GCF (gelatin-cysteine-thiosulfate) were prepared as described previously (8, 29).

DNA relatedness analysis. DNA-DNA hybridization was conducted in duplicate by using the batch hydroxyapatite thermal elution procedure (2, 3). DNA from the definition strain for DNA group 9 aeromonads (CDC 0787-80) was nick translated with ^{32}P (NEK-004; Dupont, NEN Research Products, Boston, Mass.) and reacted with unlabeled DNA from the other eight phenotypically similar strains as well as reference strains for the other *A. sobria* groups at both the optimal reassociation temperature of 60°C and the stringent incubation temperature at 75°C. Relatedness was expressed as the relative binding ratio and as the divergence in the melting temperature (Table 3). The relative binding ratio was calculated as a percentage to enable determination of similarity between strains. Relative binding ratio values of 70% (for reactions at 60°C) and 55% (for reactions at 75°C) were considered to be the cutoff values for DNA relatedness. A divergence in melting temperature of 5°C was considered the cutoff value for DNA relatedness.

Control reactions, in which labeled DNA was incubated in the absence of unlabeled DNA, were included, and the label-only control values were subtracted for all reactions before the relative binding ratio was calculated.

Antimicrobial susceptibility. MICs were determined for 17 antimicrobial agents in a 96-well microdilution plate (MicroScan Gram Negative Panel 7; Baxter Healthcare Corp., MicroScan Division, West Sacramento, Calif.). Panels were inoculated according to the instructions of the manufacturer and incubated for 24 h at 36°C. After incubation, the MIC for each strain against each antimicrobial agent was determined and recorded both manually and with an AutoScan-4 reader, with computer-assisted analysis done by an IBM PS/2 model 60 computer, which provided interpretations in accordance with guidelines of the National Committee for Clinical Laboratory Standards (24, 25). In addition to the 17 antimicrobial agents listed in Table 4, the panel also tested for a "growth" or "no growth" response against a single dilution of colistin of 4 μg/ml.

RESULTS AND DISCUSSION

DNA hybridization results. By DNA hybridization (Table 3), all eight strains of esculin-, sucrose-, and cellobiose-negative *A. jandaei* were closely related to the DNA group 9 definition strain CDC 0787-80 (ATCC 49568^T) under both the optimal and stringent conditions. The type strain ATCC

TABLE 3. DNA relatedness of the type strain of *A. jandaei* to other strains of *A. jandaei* and other strains of *Aeromonas* and members of the family *Vibrionaceae*

Source of unlabeled DNA	% Relatedness to labeled DNA of *A. jandaei* ATCC 49568^T		
	60°C	dT_m[a]	75°C
A. jandaei (DNA group 9)			
CDC 0787-80 (ATCC 49568^T)	100	0	100
CDC 1530-81	85	2.0	76
CDC 1531-81 (AS 171)	86	2.7	75
CDC 1081-81 (AS 167)	84	2.3	80
NMRI-6	83	3.5	77
WRII/4658	80	3.4	78
AS-14	73	2.0	68
AS-206	83	2.6	77
AS-235	82	2.8	76
A. sobria (DNA group 7) CDC 9538-76 (CIP 7433^T)	53	7.7	32
A. veronii biogroup sobria (DNA group 8)			
CDC 9080-79 (ATCC 9071)	54	5.3	38
CDC 0437-84	72	7.9	46
A. veronii biogroup veronii (DNA group 10) CDC 1169-83 (ATCC 35624^T)	58	5.7	40
A. hydrophila (DNA group 1) CDC 9079-79 (ATCC 7966^T)[b]	51	9.8	ND[c]
A. salmonicida subsp. *salmonicida* (DNA group 3) CDC 9701-84 (ATCC 33658^T)[b]	47	11.3	ND
A. caviae/punctata (DNA group 4) CDC 9083-79 (ATCC 15468^T)[b]	39	10.3	ND
A. media (DNA group 5b) CDC 9072-83 (ATCC 33907^T)	40	10.9	ND
A. eucrenophila (DNA group 6) CDC 0859-83 (ATCC 23309^T)[b]	47	11.0	ND
A. schubertii (DNA group 12) CDC 2446-81 (ATCC 43700^T)[b]	35	12.6	ND
Vibrio cholerae CDC 9060-79	3	ND	ND

[a] dT_m, Difference in melting temperature between each homologous and heterologous DNA complex.
[b] Data from Fanning et al. (7), with permission of the authors.
[c] ND, Not determined.

TABLE 4. In vitro susceptibilities of nine *A. jandaei* isolates to 17 antimicrobial agents

Antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
Amikacin	4–8	4 (S)	8 (S)
Ampicillin	≥16	≥16 (R)	≥16 (R)
Cefazolin	4–≥16	≥16 (R)	≥16 (R)
Cefoxitin	≤2–4	≤2 (S)	4 (S)
Ceftazidime	≤2–4	≤2 (S)	≤2 (S)
Ceftriaxone	≤4	≤4 (S)	≤4 (S)
Cefuroxime	≤2–8	≤2 (S)	4 (S)
Cephalothin	≤8–≥16	≥16 (R)	≥16 (R)
Ciprofloxacin	≤1	≤1 (S)	≤1 (S)
Gentamicin	≤1–2	2 (S)	2 (S)
Imipenem	2–≥8	≥8 (R)	≥8 (R)
Piperacillin	≥64	≥64 (R)	≥64 (R)
Tetracycline	≤4	≤4 (S)	≤4 (S)
Ticarcillin-K clavulanate	≤8–64	16 (S)	64 (MS)
Ticarcillin	32–≥64	≥64 (R)	≥64 (R)
Tobramycin	≤1–2	≤1 (S)	2 (S)
Trimethoprim-sulfamethoxazole	≤0.5/9.5	≤0.5/9.5 (S)	≤0.5/9.5 (S)

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively; S, R, and MS, susceptible, resistant, and moderately susceptible, respectively, based on guideline M100-2S of the National Committee for Clinical Laboratory Standards (25).

49568 was sufficiently distant from the other established *A. sobria*-like reference strains *A. sobria*^{T} and *A. veronii* (*A. veronii* biogroups sobria and veronii) to reinforce the validity of DNA group 9 as originally proposed by Fanning et al. (7). Their original data for DNA hybridization between CDC 0787-80 and previously established *Aeromonas* species are presented in Table 3.

Designation of *A. jandaei*. We propose the name *A. jandaei* for the nine strains listed in Table 1. The species name (pronounced jan.dae'i) sp. nov., is derived from the surname of J. Michael Janda, an American microbiologist who has contributed greatly to our knowledge of *Aeromonas* species, in particular, their biotypes and clinical significance and the relative pathogenicities of mesophilic members (11–16). The species name is treated as a modern (neo) Latin genitive noun and was derived after consultation with T. O. MacAdoo, Virginia Polytechnic Institute and State University, Blacksburg. The ending "ei" is appropriate for a male name ending in the vowel "a" and has been proposed and accepted as such in the latest revision of Appendix 9, Orthography, of the *International Code of Nomenclature of Bacteria* (21, 22).

The type strain of the species is designated ATCC 49568 (CDC 0787-80), with a complete description of the species given in Tables 2 and 5. The unique profile of *A. jandaei* is as follows: negative esculin hydrolysis, negative fermentation of sucrose and cellobiose, and resistance to a specific dilution of colistin (4 μg/ml). In previous taxonomic work in 1984, Turnbull et al. (28) noted the presence of a small asaccharolytic group of aeromonads that were sucrose and esculin negative, and even earlier work in 1981 on antimicrobial susceptibility by Richardson et al. (27) established the nearly universal susceptibility of most *Aeromonas* species to colistin.

A. jandaei resides in the genus *Aeromonas* because it is an oxidase-positive, glucose-fermenting, motile gram-negative rod that is resistant to 0/129, is string test negative, reduces nitrate to nitrite, and does not require NaCl for growth. It is nearly uniformly resistant to ampicillin, cefazolin, cephalothin, imipenem, piperacillin, and ticarcillin (Table 4).

Presumptive identification. Rapid identification with the API 20E strip and data base yields an identification of "*Aeromonas hydrophila* complex" with an identification level of "very good" to "excellent," but with the comment that the negative reaction for sucrose fermentation is considered atypical for their data base. The four different profile numbers generated for the nine strains in this study were 7247144, 7247104, 7246144, and 7247145. Additional presumptive markers for *A. jandaei* were a positive Voges-Proskauer reaction (89%), positive H_2S production from GCF (89%), resistance to cephalothin (78%), and utilization of ascorbate (67%).

Clinical significance of *A. jandaei* strains. *A jandaei* was isolated from specimens of diverse geographic origins, including wounds, blood, stools, and a prawn (Table 1). The isolation of these strains, excluding the environmental prawn isolate, suggests that there is a possible clinical significance for this species, and four case studies are given below with references for those that have been published previously.

(i) Case 1, blood (isolate AS-14) (12). The patient was a

TABLE 5. Differentiation of *A. jandaei* from other *Aeromonas* species

Characteristic	*A. jandaei*	*A. veronii* biogroup sobria[a]	*A. veronii* biogroup veronii	*A. schubertii*	*A. hydrophila*	*A. caviae*
Esculin hydrolysis	−[b]	−	+[c]	−	+	+
Acid from:						
Sucrose	−	+	+	−	+	+
Arabinose	−	−	−	−	V[d]	+
Cellobiose	−	V[e]	+	−	−	+
Salicin	−	−	+	−	V[d]	+
Elastase production	−	V	−	−	+	−
Resistance to cephalothin	+	−	−	−	+	+
Resistance to colistin[f]	+[d]	−[d]	−[d]	−[d]	V[d]	−[d]
Gas production from glucose	+	+	+	−	+	−
Voges-Proskauer	+	+	+	V	+	−
Lysine decarboxylase	+	+	+	+	+	−
Arginine dihydrolase	+	+	−	+	+	+
Indole production	+	+	+	−	+	+
Mannitol production	+	+	+	−	+	+
H_2S from modified GCF	+[d]	+[d]	+[d]	−[d]	+[d]	−[d]
Hemolysis on sheep blood	+	+	+	+	+	−

[a] Formerly *A. sobria*.
[b] −, Negative, i.e., positive for ≤30% of isolates.
[c] +, Positive for ≥70% of isolates.
[d] Data from Carnahan et al. (4).
[e] Variable, positive for 31 to 69% of isolates.
[f] 4 μg/ml (MicroScan Gram Negative Panel 7).

71-year-old male with adult-onset diabetes mellitis. Five months prior to this admission he had been diagnosed as having multiple myeloma and had been treated for this condition with combinations of vincristine, cytoxan, and prednisone. During the course of his chemotherapy his leukocyte count had fallen to 1,900 and he had had several septic episodes with *Enterococcus faecalis* and *Escherichia coli*. In August 1981 he presented to the emergency room with acute mental deterioration and a fever of 102°F (39°C); laboratory findings included a leukocyte count of 1,300, an hematocrit of 22, and a blood pressure of 150/60. The impression on admission was gram-negative sepsis. Blood cultures drawn on two separate occasions yielded *Aeromonas* species. The patient was treated with cephalothin and tobramycin but became lethargic, tachypneic, and hypotensive and died 7 days after admission.

(ii) Case 2, wound (isolate WRII/4658) (17). A 10-year-old male who swam in a freshwater pond after an injury had been inflicted below the left eye presented at a hospital emergency room for cellulitis of the left lacrimal sac. Subsequent cultures revealed both *A. veronii* biogroup veronii and *A. jandaei*. Surgery and treatment with cefotaxime and trimethoprim-sulfamethoxazole resulted in a complete recovery.

(iii) Case 3, wound (isolate NMRI-6) (18). Briefly, case 3 was a U.S. Navy diver who had an infection caused simultaneously by two different species of *Aeromonas* (*A. hydrophila* and *A. jandaei*). The infection was of the soft tissue of the leg and was secondary to a puncture wound sustained during diver training operations in the Anacostia River near Washington, D.C.

(iv) Case 4, blood (isolate AS-235) (12). Case 4 was a 96-year-old male who had been diagnosed as having acute myelogenous leukemia 3 years previously by bone marrow biopsy. He had received no chemotherapy but had been transfused with erythrocytes and platelets periodically for episodes of epistaxis. In July 1984 he presented to the hematology clinic with a 1-day history of shaking, chills, and fever to 101°F (38°C). Laboratory findings included a leukocyte count of 3,200, a platelet count of 25,000, and a hemoglobin level of 7.9%. Blood cultures were drawn because of the apparent septic episode and revealed *Aeromonas* species. The patient was treated with tobramycin and cefamandole for 16 days with no recurrence of fever and was discharged 3 weeks after admission.

The motile aeromonads represent a diverse group of potentially pathogenic organisms whose taxonomy has clearly been in a constant state of flux. An explanation of the mode of pathogenesis, particularly in relation to gastroenteritis, awaits either the development of a successful animal model or the completion of a successful human volunteer trial.

Nonetheless, the species of *A. hydrophila*, *A. veronii* (*A. veronii* biogroups veronii and sobria), *A. schubertii*, and even *A. caviae* have been delineated as distinct phenotypic species to be considered by clinical microbiologists. Some investigators have suggested in the literature an association between a particular taxonomic species and specific disease syndromes, i.e., the significance of *A. veronii* biogroup sobria in bacteremia, *A. schubertii* in traumatic wound infections, and *A. caviae* in cases of pediatric gastroenteritis (5, 12, 23).

In this report we described the phenotypic and genotypic distinction of a previously rare DNA group of aeromonads and proposed *A. jandaei* sp. nov. based on these nine strains. This new species appears to have important clinical significance and warrants identification by clinical microbiologists and further investigation by infectious disease researchers. Only by accurate and complete biotyping of clinical aeromonad isolates can we achieve an understanding of the ecology, epidemiology, and pathogenesis of these enigmatic aquatic microorganisms.

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