Aeromonas Species in Septicemia: Laboratory Characteristics and Clinical Observations

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We retrospectively analyzed clinical and epidemiological data on and laboratory characteristics of 53 cases of aeromonas septicemia. Only four *Aeromonas* genomospecies (species defined by DNA relatedness) were associated with the 53 cases, with *Aeromonas hydrophila* (sensu stricto) predominating (47%). Nearly 60% of all *Aeromonas* isolates from blood fell into one of four somatic groups: serogroups O:11, O:16, O:18, and O:34. Unlike *Aeromonas*-associated gastroenteritis, septicemia did not peak in frequency during the warmer months but rather was most common in January through March, when ~40% of cases occurred. In vitro tests of the pathogenicity of 20 selected blood isolates of *Aeromonas* indicated that resistance to complement-mediated lysis, elevated levels of protease and hemolysin activity, and the ability to elaborate siderophores correlated with higher virulence. Species and serogroup designations also correlated with the degree of virulence. Susceptibility studies of 50 strains indicated that *A. hydrophila* was the most drug-resistant species and that *Aeromonas veronii* was the most susceptible. Susceptibility to first- and second-generation cephalosporins and carbenicillin was species-associated.

Members of the genus *Aeromonas* are responsible for a wide range of illnesses in humans, including gastrointestinal disorders and systemic infections [1]. Gastroenteritis, the most common clinical presentation associated with aeromonads, can range from mild enteritis to more fulminant forms resembling shigellosis and cholera-like diarrhea. Recent immunologic data from several investigations further support a role for these agents in gastrointestinal disease [2–4]. In addition, *Aeromonas* causes a variety of systemic infections, including endocarditis, osteomyelitis, and myonecrosis. By far the most serious and life-threatening complication of systemic aeromonad infection is aeromonas septicemia, in which fatality rates have exceeded 50% in selected surveys [1]. While the overall frequency of *Aeromonas* as a cause of gram-negative septicemia was exceedingly low (<0.15%) in one recent survey [5], organisms of this genus were among the leading causes of bacterial septicemia over a 16-month period in an Australian medical center [6] and caused between 2% and 3% of all septicemic episodes over a 4-year period in a Taiwan hospital [7]. Individuals predisposed to aeromonas septicemia include those with hepatic disorders, malignancies, and biliary obstructions, although an increasing number of cases have been reported in patients with no apparent immunologic or physiological deficits.

Although many case reports and surveys have dealt with aeromonas septicemia, most investigations have been hampered by the size of the sample studied and the number of parameters investigated [1, 8]. The genus *Aeromonas* has recently been expanded to include more than 15 known and 13 named species [9, 10]; most published studies on aeromonas septicemia predate these taxonomic changes [1]. Presently, it is unclear whether certain species and strains of *Aeromonas* are inherently more pathogenic than others and, if so, what markers or virulence characteristics are associated with increased pathogenicity. In this article we report on a number of clinical and laboratory characteristics associated with a large collection of mesophilic aeromonads involved in cases of human septicemia. The results suggest that a restricted number of *Aeromonas* species are associated with distinct clinical and laboratory characteristics.

Materials and Methods

Bacterial strains. Fifty-three strains of *Aeromonas* associated with documented cases of septicemia were studied. These strains, which originated from patients at 27 medical centers in California, Kentucky, Missouri, Nebraska, New York, Pennsylvania, Texas, Utah, and Washington, were collected over a 10-year period (1983–1993). Whenever possible, the patients' clinical and medical histories were obtained. All strains were maintained on extract agar slants at

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ambient temperatures during the course of these investigations; our previous (unpublished) studies had indicated that strains retain their phenotypic characteristics under these conditions for >6 months.

Biochemical investigations. Strains were initially identified to the genus level by standard biochemical criteria, including resistance to 10 μg and 150 μg of the vibriostatic agent O/129 and growth/no growth on nutrient agar containing no/6% NaCl [11]. For species determination, the recently published biochemical scheme of Abbott et al. [11] was used with the sole addition of the DL-lactate test, which helps distinguish *Aeromonas hydrophila* from *Aeromonas salmonicida* and *Aeromonas caviae* from *Aeromonas media* [12].

Serogrouping. The somatic antigen designation for each *Aeromonas* strain was determined by the serogrouping scheme of Sakazaki and Shimada [13]. This scheme establishes 44 recognized and 49 provisional serogroups within this genus. A provisional serogroup is one that appears to recognize a new somatic antigen but has not yet been firmly established by rigorous challenge experiments.

Mouse pathogenicity. The relative pathogenicity of selected *Aeromonas* isolates from blood was evaluated in female Swiss-Webster mice by previously described methods [14]. The LD_{50} of each strain was calculated according to the method of Reed and Muench [15] and then converted to a $\log_{10}$ value.

Protease assay. Selected *Aeromonas* strains were inoculated into a protease-based medium containing 0.5% sucrose, as previously described [16]. These preparations were orbitally shaken (100 rpm, 35°C) in an environmental shaker (New Brunswick Scientific, Edison, NJ) for 18 hours. At the end of the incubation period, the amount of growth was assessed turbidimetrically at 610 nm. A 1-mL sample of this suspension was microfuged (model E, Beckman Instruments, Fullerton, CA). A 200-μL aliquot of the cell-free supernatant was then mixed in a disposable tube containing 15 mg of hide powder azure (Sigma Chemical, St. Louis) and 4.8 mL of 50 m*M* Tris (pH, 7.8). The mixture was incubated for 30 minutes at 35°C, after which the amount of dye liberated by proteolytic activity was determined spectrophotometrically at 595 nm; a positive control (pronase E) and a negative control (no enzyme) were included in each run. One unit of protease activity was defined as that quantity of protease causing an increase in OD_{595} absorbance of 0.001 OD units relative to total growth (OD_{610}). Total protease activity (total number of units) for each strain was expressed as U/mL/h.

Hemolytic assay. The β-hemolytic activity of selected *Aeromonas* strains was evaluated in a fashion similar to that used for protease activity. In brief, 4- to 6-hour tryptone cultures of each strain were used to seed a 125-mL flask containing 25 mL of brain-heart infusion broth. Cultures were orbitally shaken for 18 hours at 35°C in an environmental shaker (100 rpm). Resultant growth was measured spectrophotometrically (610 nm), after which a 1-mL portion of the suspension was microfuged as has already been described. A 200-μL portion of the cell-free supernatant was mixed with 1.8 mL of a 1% (vol/vol) solution of sheep erythrocytes suspended in a buffer containing 0.02 *M* KH_2PO_4, 0.06 *M* Na_2HPO_4, and 0.12 *M* NaCl [17]. This mixture was incubated for 30 minutes and then centrifuged for the removal of cells. The amount of hemoglobin released was determined at 540 nm. A positive control (saponin lysed) and a negative control (no enzyme) were included with each run. One unit of hemolysin was defined as that quantity of enzyme required to increase absorbance by 0.001 OD units; hemolytic activity for each strain was expressed as U/mL/h divided by relative growth at 610 nm.

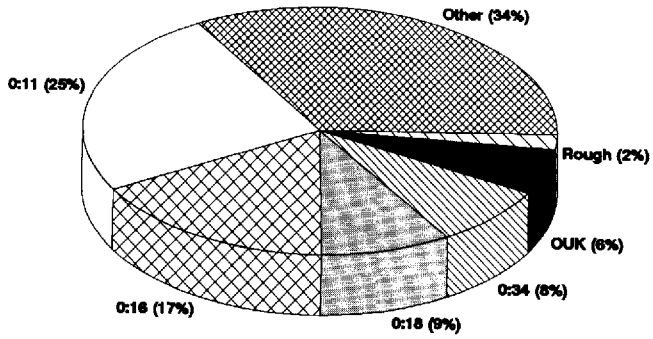

Figure 1. Serogroup distribution of 53 septicemia-associated strains of *Aeromonas*. OUK = unknown somatic antigen group; "Other" = antigenic groups other than the four major serogroups listed.

Miscellaneous assays. A number of phenotypic properties thought to be associated with the pathogenicity of *Aeromonas* were tested in studies with specific strains. These properties included susceptibility to 65% pooled human serum, agglutination in 0.2% acriflavine, and elaboration of siderophore activity on chrome azurol S (CAS) agar [18, 19].

Antimicrobial susceptibility studies. The susceptibility profile of septicemia-associated *Aeromonas* strains was determined with a MicroScan Neg Combo Type 4 panel (MicroScan, West Sacramento, CA). Strains grown on blood agar were suspended and inoculated into individual panels according to the manufacturer's instructions. Susceptibility results were recorded at 18 hours.

Results

The 53 strains of *Aeromonas* isolated from cases of septicemia were serogrouped according to previously established criteria [13]. Overall, 92% of strains were typable by this method; the exceptions were one rough isolate and three strains that did not fall into any of the established or provisional serogroups (figure 1). Of the 49 typable strains, 45 (92%) fell into recognized serogroups, while only four (8%)

Table 1. *Aeromonas* species and serogroups associated with septicemia.

Aeromonas species (*n*)	No. of strains in indicated predominant *Aeromonas* serogroup				Total percentage of strains in four predominant serogroups
	O:11	O:16	O:18	O:34	
A. hydrophila (25)	6	4	4	4	72
A. caviae (15)	0	5	1	0	40
A. veronii (11)	7	0	0	0	64
A. jandaei (2*)	0	0	0	0	. . .

* These strains were identified as *Aeromonas sobria* in a previously published study [20].

belonged to provisionally established serogroups. The 49 typable strains represented 19 different serogroups, four of which were responsible for 62% of all cases of septicemia (i.e., for 31 of 50 cases). Serogroup O:11 accounted for 13 cases, serogroup O:16 for nine cases, serogroup O:18 for five cases, and serogroup O:34 for four cases. Isolates from only three other serogroups (O:3, O:23, and O:25) were recovered on more than one occasion (two instances each).

Although there are now 13 named species within the genus *Aeromonas*, only four species were found to be associated with cases of septicemia (table 1). The species most commonly identified was *A. hydrophila* (sensu stricto), which accounted for just over 47% of all septicemic episodes; next were *A. caviae* (28%), *Aeromonas veronii* (21%; formerly referred to clinically as *Aeromonas sobria*), and *Aeromonas jandaei* (4%; included as part of the *A. sobria* group before its recognition as a separate and distinct species [21]). In a search for a specific association between individual species and serogroups, the four most common serogroups were analyzed for frequency distribution. A majority of the blood isolates of *A. hydrophila* (72%) and *A. veronii* (64%) were found to belong to the four most prevalent serogroups; in fact, *A. veronii* was associated with only one of these four serogroups (O:11). *A. caviae* was more heterogeneous, being found in almost half (42%) of all the septicemia-associated serogroups identified in this study. Only *A. hydrophila* was linked with serogroup O:34. Each of the other three most common serogroups was represented by more than one genomospecies. However, there was an association between each of these somatic antigens and specific *Aeromonas* genomospecies (*A. hydrophila:* O:34, O:18, O:16, O:11; *A. caviae:* O:16; *A. veronii:* O:11).

Aeromonas septicemia was most common among middle-aged and elderly men (table 2), who typically presented with signs of bacterial sepsis (fever, chills, nausea, and vomiting). Ninety-eight percent of patients were immunocompromised, with cancer as the most frequent underlying disease (table 3). Malignancies associated with aeromonas septicemia most often involved hematologic dyscrasias ($n = 5$); next in frequency were cancer of the colon ($n = 3$), the lung ($n = 3$), the pancreas ($n = 2$), the liver ($n = 1$), the bladder ($n = 1$), and unspecified sites ($n = 2$). Slightly fewer than one-third of patients were simultaneously positive for *Aeromonas* at body sites other than blood (table 3). The most common sites involved were wounds (seven instances); catheters (two instances); and urine, pleural fluid, peritoneal fluid, abdominal fluid, bile, gall bladder, and liver (one instance each). Overall mortality was ~30%.

In addition, we looked for any distinct trends associated with the three major *Aeromonas* species involved in septicemia (excluding *A. jandaei*). The most striking associations were noted with *A. caviae*. Patients infected with *A. caviae* tended to have cancer as an underlying condition more often than those infected with either *A. hydrophila* ($P < .02$) or *A. veronii* ($P < .05$). Moreover, polymicrobic septicemia was more common among individuals infected with *A. caviae* than among patients infected with either *A. hydrophila* or *A. veronii* ($P < .005$). The group of patients with *A. hydrophila* septicemia was younger than that with *A. veronii* septicemia ($P < .02$).

Aeromonas gastroenteritis has been shown by several investigators to have a higher incidence during the summer months, when increased aeromonad concentrations in freshwater habitats presumably lead to exposure to higher infectious doses [22, 23]. To assess whether aeromonas septicemia had a similar seasonal pattern, we analyzed the month of isolation for 37 strains of *Aeromonas* recovered from cases of septicemia. As shown in figure 2, the greatest number of cases of aeromonas septicemia occurred during a 3-month interval from January through March ($n = 14$, 38%), while the three individual months with the highest number of recorded cases were March ($n = 6$, 16%), July ($n = 5$, 14%), and November ($n = 5$, 14%). Surprisingly, only 41% of all cases took place in the warmer months of May through September.

Twenty strains of *Aeromonas* recovered from the blood were evaluated further for a number of in vitro markers po-

Table 2. Demographic characteristics of patients with aeromonas septicemia.

Aeromonas species	Characteristic for patients with species isolated*		
	Age range in y	Mean age in y	Male-to-female ratio
A. hydrophila	21–88 (17)	56.3 (17)	1.5 (20)
A. caviae	0–83 (11)	50.3 (11)	1.4 (12)
A. veronii	52–84 (7)	68.3 (7)	1.3 (7)
A. jandaei	71–96 (2)	83.5 (2)	NA† (2)
All *Aeromonas*	0–96 (37)	58.2 (37)	1.6 (41)

* Values in parentheses represent the number of patients with the indicated isolate for whom demographic information was available.

† NA = not applicable; *A. jandaei* was recovered from males only.

Table 3. Clinical characteristics of patients with aeromonas septicemia.

Characteristic	No. (%) of patients with indicated isolate			
	A. hydrophila	*A. caviae*	*A. veronii*	All *Aeromonas* isolates*
Presenting diagnosis†				
Sepsis	10 (63)	9 (82)	4 (57)	25 (69)
Hepatobiliary disease	2 (13)	1 (9)	1 (14)	4 (11)
Cellulitis/myonecrosis	4 (25)	0 (. . .)	0 (. . .)	4 (11)
Pancreatitis	0 (. . .)	1 (9)	1 (14)	2 (6)
Peritonitis	0 (. . .)	0 (. . .)	1 (14)	1 (3)
Proctosigmoiditis	1 (6)	0 (. . .)	0 (. . .)	1 (3)
Fever of unknown origin	0 (. . .)	0 (. . .)	1 (14)	1 (3)
Total	16	11	7	36
Underyling disease				
Cancer	5 (36)	9 (82)	1 (20)	17 (53)
Cirrhosis	2 (14)	0 (. . .)	1 (20)	4 (13)
Biliary disease	0 (. . .)	2 (18)	1 (20)	3 (9)
Renal disease	2 (14)	1 (9)	0 (. . .)	3 (9)
Diabetes	2 (14)	0 (. . .)	1 (20)	4 (13)
Chronic heart disease	1 (7)	0 (. . .)	0 (. . .)	1 (3)
Inflammatory bowel disease	1 (7)	0 (. . .)	0 (. . .)	1 (3)
Trauma	1 (7)	0 (. . .)	0 (. . .)	1 (3)
Total	14	11	5	32
Septicemia				
Monomicrobic	17 (89)	3 (25)	6 (100)	28 (72)
Polymicrobic	2 (11)	9 (75)	0 (. . .)	11 (28)
Additional positive sites	8 (42)	4 (33)	1 (17)	13 (33)
Total	19	12	6	39
Mortality	6 (40)	2 (20)	1 (14)	10 (29)

* Includes *A. jandaei* strains ($n = 2$).
† Some patients presented with multiple symptoms or underlying diseases.

tentially associated with pathogenicity (table 4). According to previously established criteria [14] based on intraperitoneal inoculation of Swiss-Webster mice, 10 of these strains fell into the high-virulence category (LD_{50}, $<10^7$ cfu), two strains fell into the moderate-virulence category (LD_{50}, 1×10^7 to 3.2×10^7 cfu), and the remaining eight strains were designated as belonging to the low-virulence category (LD_{50} $>3.2 \times 10^7$ cfu). The 10 highly virulent strains were compared with the strains exhibiting moderate or low pathogenicity with regard to a number of potential virulence-associated properties. Highly pathogenic strains tended to belong to the genomospecies *A. hydrophila* or *A. veronii* as opposed to *A. caviae* ($P < .005$). A majority (69%) of strains tested from the four major serogroups previously found to be associated with aeromonas septicemia (O:11, O:16, O:18, and O:34) were in the highly virulent category ($P < .025$); the only exceptions to this rule were four strains of serogroup O:16, two of which fell into the moderately virulent category (table 4). High-virulence strains were more resistant to complement-mediated lysis of fresh serum ($P < .01$) and produced more protease ($P < .001$) and hemolysin ($P < .001$) than did strains of moderate or low pathogenicity; moreover, the high-virulence strains elaborated detectable siderophore activity on CAS agar ($P < .05$). No significant difference in the ability to agglutinate in the presence of acriflavine was noted among virulence groups.

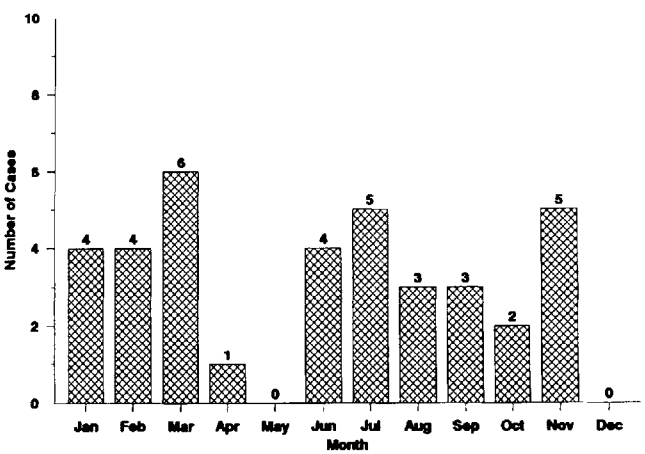

Figure 2. Distribution of 37 strains of *Aeromonas* recovered from blood, by month of isolation.

We then determined the susceptibility profile for 50 septicemia-associated *Aeromonas* isolates designated as *A. hydrophila*, *A. veronii*, or *A. caviae* (table 5). All strains tested were susceptible to ciprofloxacin, norfloxacin, and trimethoprim-

Table 4. Pathogenic properties of selected septicemia-associated strains of *Aeromonas*.

Strain no.	Species	Serogroup	Acr	CAS	Rsk*	ProAct	HAct	LD_{50}
184	*A. hydrophila*	O:34	−	+	+0.9	2.9	2.0	5.50
211	*A. hydrophila*	O:18	−	+	+0.8	3.5	7.8	6.33
106	*A. hydrophila*	O:18	−	+	+0.9	3.6	0.1	6.39
186	*A. hydrophila*	O:16	−	++	+0.9	3.8	6.7	6.51
39	*A. veronii*	O:11	−	+	+0.8	2.8	0	6.59
180	*A. veronii*	O:11	−	+	+0.5	2.9	0.1	6.71
145	*A. hydrophila*	O:34	−	+	+0.9	3.2	0	6.80
351	*A. hydrophila*	O:18	+	++	+0.8	1.4	0	6.92
127	*A. veronii*	O:6	−	+	+1.9	2.8	0.1	6.98
342	*A. hydrophila*	O:11	+	++	+0.3	3.6	0.1	6.99
88	*A. hydrophila*	O:16	−	+++	−4.0	3.7	8.2	7.43
207	*A. caviae*	O:16	+	+	0.6	0.1	0	7.46
366	*A. caviae*	O:16	+	−	−0.4	<0.1	0	7.52
360	*A. hydrophila*	O:16	−	+	+0.9	3.2	2.8	7.73
362	*A. caviae*	O:3	+	+	+0.5	0.8	0	7.79
219	*A. caviae*	O:3	+	−	−5.7	0.2	0	7.90
315	*A. veronii*	O:33	−	+	+0.8	2.9	0	7.95
34	*A. caviae*	O:47	−	+	+0.8	0.8	0	8.29
119	*A. caviae*	O:23	+	−	−2.1	<0.1	0	8.79
361	*A. hydrophila*	O:78	−	−	−0.6	<0.1	0	9.19

NOTE. Abbreviations: Acr = agglutination in 0.2% acriflavine; CAS = siderophore production on chrome azurol S agar (zone sizes: + = 1–3 mm; ++ = 3–5 mm; +++ = >5 mm); Rsk = resistance to serum killing; ProAct = protease activity ($\times 10^3$ U/mL/h); HAct = hemolytic activity ($\times 10^3$ U/mL/h); LD_{50} = 50% lethal dose ($\log_{10}$ in Swiss-Webster mice [14]).

* Gain (+) or loss (−) in viable progeny (cfu, $\log_{10}$) after 120 minutes of exposure to 65% pooled human serum.

sulfamethoxazole; all but one strain was also susceptible to cinoxacin, tetracycline, and chloramphenicol. More than 80% of the aeromonads tested were susceptible to the second-generation cephalosporins cefuroxime, cefotetan, and cefonicid and to the third-generation cephalosporins ceftriaxone and cefixime. Resistance to the first-generation cephalosporin cephalothin and to the second-generation cephalosporin cefaclor was widespread; the β-lactamase inhibitor combination amoxicillin/clavulanate inhibited more than 80% of the strains tested. A strikingly different susceptibility profile was noted when species designation was taken into account (table 5). Strains of *A. veronii* were more suscep-

Table 5. Susceptibility of *Aeromonas* species to selected antimicrobial agents.

	MIC_{90} in μg/mL (% of strains susceptible)			
Drug	Total (n = 50)	*A. hydrophila* (n = 24)	*A. caviae* (n = 15)	*A. veronii* (n = 11)
Ampicillin	>128 (6)	>128 (0)	128 (13)	128 (9)
Carbenicillin	>128 (30)	>128 (17)	>128 (67)	>128 (9)
Amoxicillin/clavulanate	16/8 (82)	16/8 (75)	>16/8 (80)	8/4 (100)
Cephalothin	>16 (32)	>16 (21)	>16 (13)	16 (82)
Cefaclor	>16 (42)	>16 (33)	>16 (27)	8 (91)
Cefuroxime	16 (88)	8 (92)	>16 (72)	≤2 (100)
Cefotetan	32 (88)	32 (88)	>32 (80)	≤4 (100)
Cefonicid	>16 (80)	>16 (71)	>16 (80)	≤2 (100)
Ceftriaxone	8 (94)	8 (92)	8 (93)	≤4 (100)
Cefixime	>2 (86)	>2 (83)	>2 (80)	≤0.25 (100)
Ciprofloxacin	≤0.5 (100)	≤0.5 (100)	≤0.5 (100)	≤0.5 (100)
Norfloxacin	≤4 (100)	≤4 (100)	≤4 (100)	≤4 (100)
Cinoxacin	≤16 (98)	≤16 (96)	≤16 (100)	≤16 (100)
Tetracycline	≤1 (98)	≤1 (96)	≤1 (100)	≤1 (100)
Trimethoprim-sulfamethoxazole	≤0.5/9.5 (100)	≤0.5/9.5 (100)	≤0.5/9.5 (100)	≤0.5/9.5 (100)
Chloramphenicol	≤8 (98)	≤8 (96)	≤8 (100)	≤8 (100)

tible to cephalothin ($P < .001$) and cefaclor ($P < .001$) than were strains of *A. hydrophila* or *A. caviae; A. veronii* was also more susceptible to cefonicid than was *A. hydrophila* ($P < .05$). *A. caviae* was more susceptible to carbenicillin than were the other two species ($P < .001$). Overall, *A. veronii* was the species most susceptible to the agents tested, while *A. hydrophila* was the most resistant. The quinolones were most active overall against the 50 strains tested in vitro.

Discussion

Although there are 13 named species within the genus *Aeromonas*, only four (*A. hydrophila*, *A. caviae*, *A. veronii*, and *A. jandaei*) were found in this study to be associated with cases of septicemia. In addition to these species, only *Aeromonas schubertii* has previously been associated with septicemia [24]. Similarly, in our study, only four of 93 established or provisional serogroups of *Aeromonas* were found to be involved in a majority (60%) of septicemic episodes. The limited number of species and serogroups associated with septicemia suggests that both the environmental frequency (with *A. hydrophila*, *A. caviae*, and *A. veronii* predominating clinically) and the pathogenic potential of a given species or strain play a key role in the ability to cause septicemia. Previous studies characterizing the pathogenic potential of various *Aeromonas* genomospecies in mice indicate that—overall—*A. jandaei*, *A. hydrophila*, *A. schubertii*, and *A. veronii* are the four most pathogenic species within the genus [14].

Clinically, a number of differences were observed among the three major species of *Aeromonas* involved in septicemia. Strains of *A. caviae* were more often isolated from patients with polymicrobic septicemia and severe underlying diseases (e.g., cancer). This fact, coupled with their overall low pathogenic potential in mice (table 4), suggests that members of this species are inherently less pathogenic and supports our previous findings in this area [14, 20, 25]. In contrast, cases of *A. hydrophila* and *A. veronii* septicemia were most often monomicrobic and associated with less serious underlying diseases; the association of *A. hydrophila* septicemia with the highest mortality suggested a more pathogenic role for this species. While differences in the susceptibility of *Aeromonas* genomospecies to antimicrobial agents were noted—particularly with regard to *A. veronii*, which was susceptible to both first- and second-generation cephalosporins (table 5)—such antibiogram patterns are unlikely to account for differences in observed mortality attributable to *Aeromonas* species since septicemia is most likely to be empirically treated with agents that cover all aeromonad groups. The date of isolation of many of the aeromonads from the blood did not coincide with the seasonal peak of diarrheal disease associated with this genus (i.e., the summer months; figure 2); this finding suggests that the infectious dose may not be as critical to the development of aeromonas septicemia as it is to that of gastroenteritis and that the virulence characteristics of the infecting strain and the physiological and immunologic status of the host may be more important in aeromonas septicemia.

The factors that regulate the pathogenicity of aeromonads are not presently known. The data presented in table 4 indicate that most *Aeromonas* isolates from the blood are of moderate to high pathogenicity in animal models and belong to the genomospecies *A. hydrophila* or *A. veronii*. These data are consistent with previous findings on the virulence of these two genomospecies relative to that of members of the *A. caviae* complex [14]. However, the least virulent strain analyzed was an *A. hydrophila* isolate. Factors that clearly separated this strain and others with LD_{50} values of $>3 \times 10^7$ cfu from more pathogenic isolates were susceptibility to complement-mediated lysis and decreased production of protease and hemolysin. Both of the latter factors have been implicated as key extracellular activities associated with pathogenicity in infections of both animals and humans [10, 26]. Some virulent strains, however (i.e., strains 39, 145, and 351), did not express detectable amounts of hemolytic activity in broth assays although they were hemolytic on blood agar plates. This observation suggests either that the quantitative expression of individual factors is not critical or that other determinants are also involved in the regulation of pathogenicity. We have previously demonstrated that typical strains of *A. hydrophila* and *A. veronii* belonging to serogroup O:11 possess an additional surface layer, termed the S layer [27]. While this material is not lethal by itself, coinfection of S-layer material with nonpathogenic S layer–negative *Aeromonas* strains enhances its infectivity in mice upon intraperitoneal challenge; thus this material may have antiphagocytic activity [28]. Additional factors may be present in invasive strains but may not have been previously reported. Clearly, most of the *Aeromonas* strains associated with low pathogenicity in mice were negative for or produced only low levels of several virulence-associated characteristics—an observation similar to that made previously in an analysis of *A. schubertii* strains [16]. This finding suggests that, like that of most gram-negative bacteria, the pathogenicity of aeromonads is polygenic in nature, not restricted to a single factor.

The collective results of this survey serve as the basis for the first large-scale analysis of clinical and epidemiological data on aeromonas septicemia and on the laboratory characteristics of the *Aeromonas* strains involved. From the data presented, it is clear that significant differences exist in the disease presentation, virulence characteristics, and susceptibility profiles of the three major *Aeromonas* species involved in septicemia. Whether specific strains have an enhanced ability to cause serious extraintestinal disease is unknown, although data on both serogroup distribution and pathogenicity in mice support the hypothesis that they do. The key factors regulating invasion from either the gastrointestinal tract or wounds have not yet been identified, but it seems

likely that both hemolysins and proteases are critical in this process; proteases have been shown to play an analogous role in infections due to *Pseudomonas aeruginosa* disseminating from wounds [29]. Other cell-associated factors, such as the possession of an S layer and the endotoxic effects of specific lipopolysaccharide chemotypes, are probably important in increasing the invasive and lethal effects of specific *Aeromonas* strains. Further studies aimed at identifying the contributory role of such factors in the disease process through genetic manipulation seem appropriate.

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