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Phenotypic Markers Associated with Gastrointestinal *Aeromonas hydrophila* Isolates from Symptomatic Children

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Aeromonas hydrophila gastroenteritis was detected in 12 pediatric patients during a 5-month period. Chief complaints included bloody diarrhea, fever, vomiting, and abdominal pain. Severe symptoms in two patients necessitated hospitalization and supportive care. Phenotypic characteristics associated with enterotoxigenicity of *A. hydrophila* strains demonstrated that all 12 isolates were cytotoxic to HeLa cells and most were lysine decarboxylase positive (75%). A correlation existed between the presence of the five virulence-associated markers of two isolates of *A. hydrophila* and the severity of disease. Although the length and symptoms of gastroenteritis varied among all 12 patients, most had self-limiting diarrhea. The frequent occurrence of *A. hydrophila* gastroenteritis in pediatric patients warrants a greater appreciation of this agent as a significant cause of diarrhea, especially in summer.

Aeromonas hydrophila is one of several gram-negative fermentative bacteria belonging to the family *Vibrionaceae* currently receiving increased attention among medical scientists. A frequent inhabitant of natural aquatic environments throughout the United States (9), this oxidase-positive, motile bacterium, one of three species comprising the genus *Aeromonas* (17), has long been established as an important pathogen of fish, reptiles, and amphibia (10). Human infections have ranged from cellulitis in patients traumatized at seashores to bacteremia in immunocompromised individuals (5, 7, 20) in whom sepsis is thought to originate by antecedent colonization and infection of either the gastrointestinal tract (13) or wounds (1). Necrotic ecthyma gangrenosum lesions, often associated with *Pseudomonas aeruginosa*, have also been described (11). Other infrequent infections include endocarditis, osteomyelitis, meningitis, and urinary tract infections (5).

A. hydrophila wound and blood infections are frequently encountered, but gastrointestinal infections may actually be more common. The role of this microorganism in human gastrointestinal diseases, however, remains unresolved (15, 18, 21). In the United States, the fecal carriage rate of *A. hydrophila* in asymptomatic individuals appears to be extremely low or nonexistent (12, 16), whereas isolation from stool specimens has most often been associated with the diarrheic state. On the basis of biochemical parameters (e.g., biotype, hemolytic activity, and production of a membrane-damaging, eucaryotic cell-active cytotoxin), certain strains of *A. hydrophila* recovered from diarrhegenic patients have been implicated in gastroenteritis (GE) (2–4, 6).

Although several well-documented cases of *A. hydrophila* GE have been described in adults (14, 19), the overall importance of this bacterial species as a common cause of GE in pediatric patients is apparently not well appreciated. We describe here our recent experience with the recovery and characterization of *A. hydrophila* from pediatric patients presenting with GE.

MATERIALS AND METHODS

Bacterial strains. *A. hydrophila* isolates were identified by API 20E analysis (Analytab Products, Plainview, N.Y.). All stool specimens were also screened for other potential pathogens, including organisms of the genera *Salmonella, Shigella, Campylobacter*, and *Yersinia*. In addition to bacterial culture, several of these stool specimens containing *A. hydrophila* were simultaneously processed for intestinal parasites and detection of rotavirus antigen by enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill.). No other identifiable enteric pathogen was recovered in any specimen. Stools from asymptomatic patients cultured during this period (children or adults) were negative for *A. hydrophila*. Medical records of each patient with one or more positive stool cultures for *A. hydrophila* were reviewed for pertinent clinical information.

Phenotypic markers. Production of lysine decarboxylase and acetylmethylcarbinol (Voges-Proskauer) was detected by API 20E analysis. Hemolytic activity of individual *A. hydrophila* strains was detected by both plate and tube assays. Each isolate was radially streaked onto tryptic soy agar containing 5% sheep

TABLE 1. Data on patients with *A. hydrophila*-associated GE

Patient	Mo of isolation	Sex/age (yrs)	Isolates	Duration of illness (days)	Symptoms				
					Diarrhea	Vomiting	Abdominal pain	Blood in stool	Fever
1	7/81	F/<1	1	Unk[a]	+	−	−	−	−
2	8/81	F/4	1	10	+	+	+	−	−
3	8/81	F/4	1	1	+	+	−	+	+
4	8/81	M/2	1	12	+	−	−	−	+
5	8/81	M/6	1	2	+	+	+	−	+
6	8/81	F/1	1	2	+	+	−	−	+
7	9/81	F/<1	3	7	+	−	−	+	−
8	10/81	F/3	1	Unk	+	−	−	+	+
9	10/81	M/1	1	14	+	−	−	−	−
10	10/81	M/2	4	1	+	−	−	+	+
11	11/81	M/17	1	2	+	−	+	+	−
12	11/81	F/<1	1	1	+	+	−	−	+

[a] Unk, Unknown.

blood (GIBCO Diagnostics, Madison, Wis.) and incubated at 37°C for 48 h. Strains exhibiting hemolytic zones in excess of 2 mm from the streak inoculum were considered positive. In addition, each isolate was tested for cell-free hemolytic activity against rabbit erythrocytes in microtiter wells (2). Brain heart infusion broth cultures of each strain (18 h) were evaluated for relative growth (measured by optical density at 540 nm) before filtration through 0.45-μm Nalgene filters (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.). Hemolytic activity of cell-free supernatants was considered positive if dilutions of ≥1:16 of each supernatant yielded 50% hemolysis of the rabbit erythrocytes within 1 h of incubation at 37°C. Cytotoxicity of identical brain heart infusion filtrates of each test strain were determined on HeLa cell monolayers by previously described methods (3).

RESULTS

Over a 5-month period (July to November 1981), 14 patients seen at The Mount Sinai Hospital for diarrheal disease had *A. hydrophila* isolated from their stool cultures. Of these, 12 (86%) were pediatric patients residing in the East Harlem area whose chief presentation was GE (Table 1). The age distribution of these 12 individuals ranged from 2 months to 17 years with a mean age of 2 years. Stool specimens were often loose and watery, foul smelling, and tinged greenish yellow. Bowel movements ranged from 1 to 12 daily; several of the patients had had chronic diarrhea lasting 10 days to 2 weeks before seeking medical care. Additional symptoms included fever (75%), blood in the stool (42%), vomiting (42%), and abdominal pain (25%). On the basis of clinical findings, 2 of these 12 patients (no. 7 and no. 10) were provisionally diagnosed as having *Salmonella* GE and were admitted because of the severity of symptoms. Stool cultures of these two patients grew *A. hydrophila* on multiple occasions but were negative for other enteric pathogens, intestinal parasites, and rotavirus (Rotazyme; Abbott Laboratories). Of the remaining 10 children, only 2 received antibiotics (ampicillin, penicillin, trimethoprim-sulfamethoxazole); the rest received only supportive care and did not return for additional follow-up visits referable to their initial symptoms. Stool cultures of these 10 patients did not reveal other known enteric pathogens.

The 12 *A. hydrophila* isolates were tested for virulence-associated characteristics. All 12 strains were cytotoxic to HeLa cells, as evi-

TABLE 2. Virulence-associated properties of *A. hydrophila* isolates

Patient	Properties of *A. hydrophila*				
	Biotype		Hemolysis		Cytotoxicity[c]
	Lysine decarboxylase	Voges-Proskauer	Sheep blood agar[a]	Tube[b]	
1	−	−	−	−	+
2	+	−	−	−	+
3	+	−	+	−	+
4	−	−	−	−	+
5	+	+	−	−	+
6	+	−	−	−	+
7	+	+	+	17	+
8	+	+	−	−	+
9	+	+	+	−	+
10	+	+	+	>100	+
11	+	−	−	−	+
12	−	−	−	−	+

[a] Hemolysis of sheep erythrocytes extending 2 mm from edge of colonial growth after 48 h of incubation at 37°C.

[b] Cell-free supernatant was titrated against 1% fresh rabbit erythrocytes. Numbers show relative hemolytic activity (ratio of 50% hemolysis to optical density at 540 nm).

[c] Cytotoxicity of cell-free supernatant on HeLa cells.

denced by the rounding, vacuolization, and crenation of these cells (Table 2). The absorption of trypan blue (final concentration, 0.04%) upon its addition to the HeLa cell culture indicated cell death and confirmed the cytotoxic nature of the *A. hydrophila* isolates. Of these strains, 75% possessed two or more of the five factors associated with enterotoxigenicity and virulence. These included positive reactions for lysine decarboxylase (75%), Voges-Proskauer (42%), and the production of hemolysins on sheep blood agar (33%). Culture filtrates of the *Aeromonas* isolates from the two hospitalized patients hemolyzed rabbit erythrocytes to a ≥1:16.

DISCUSSION

The results of this study strongly suggest that *A. hydrophila* is an important cause of GE, especially in pediatric patients. Evidence supporting this contention is drawn from several avenues. No other enteric, viral, or parasitic pathogen that was tested for could be isolated or identified in the stool specimens of these patients. During the same time period, the recovery of *A. hydrophila* from the stool specimens of diarrheic patients ranked behind *Salmonella* spp. and *Campylobacter* spp. but ahead of *Shigella* spp. and rotavirus, although the frequencies and the total number of samples were not taken into account. In both patients admitted with protracted GE and from whom *A. hydrophila* was isolated, remission of symptoms was associated with the subsequent disappearance of *A. hydrophila* from their stool specimens.

The 12 *A. hydrophila* isolates all possessed at least one of the phenotypic markers associated with enteropathogenic strains; 9 isolates had multiple-associated virulence markers. Of striking importance, the *A. hydrophila* isolates from the two hospitalized children proved the most virulent as judged by possession of all five in vitro virulence-associated factors.

Since most of the children seen in the emergency room did not return for follow-up examination, it appears that *A. hydrophila*, in most instances, produces a self-limiting disease, as previously suggested (5), although symptoms may persist for up to 2 weeks. Antimicrobial therapy is apparently not indicated. However, in a small fraction of these children, severe GE does occur which may require hospitalization and supportive care, as with two of our patients. The degree of severity of infection may be related to the age and health status of the host, socioeconomic factors, and the particular biotype of the infecting *A. hydrophila* strain. Since blood was seen in the stools of several of these patients, it appears that certain *A. hydrophila* strains are invasive as well as enterotoxigenic. Patients infected with such strains may potentially show severe symptoms. The seeming increase in the incidence of *A. hydrophila*-associated GE during the summer may well reflect acquisition of the microorganism from the aquatic environment. Inoculum densities, virulence of a strain, and host factors may all contribute to the expression of virulence as manifested by GE. Clinicians and microbiologists should be aware that this microorganism may be present as an infectious cause of diarrhea so that its disease spectrum and epidemiology can be more readily defined. As a final observation, we note that recently Gracey and co-workers (8) have isolated *A. hydrophila* from 10% of the fecal specimens of Australian children with diarrhea (as opposed to 0.4% in age-matched controls).

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