Clinical and Microbiological Features of *Aeromonas hydrophila*-Associated Diarrhea

WILLIAM A. AGGER,^{1*} JAMES D. MCCORMICK,² AND MARC J. GURWITH³

Infectious Disease Section, Department of Internal Medicine, Gundersen Clinic, Ltd.,¹ and Microbiology Laboratory, La Crosse Lutheran Hospital,² La Crosse, Wisconsin 54601, and Infectious Disease Section, Michigan State University, East Lansing, Michigan 48824³

Received 13 August 1984/Accepted 19 November 1984

The prevalence of *Aeromonas hydrophila* in stool specimens from patients with diarrhea was studied during 18 months. *A. hydrophila* was found in 1.1% of patients with diarrhea and in none of 533 control patients ($P < 0.02$). Cases were detected 1.5 times more often during the summer months than the winter months, and most occurred in children less than 2 years of age. Clinical features included fever greater than 38°C (55%), abdominal cramps (35%), vomiting (25%), and duration of illness greater than 10 days (50%). Detection of *A. hydrophila* in stools was facilitated by the use of sheep blood agar with 15 µg of ampicillin per ml which was flooded with oxidase reagent after growth. A cytotoxin was produced by 62% of the isolates, and the cytotoxic strains showed positive results in a hemolysin assay and a lysine decarboxylase reaction.

The genus *Aeromonas* of the family *Vibrionaceae* contains gram-negative rods with single polar flagella (33). Important biochemical properties of this genus include a positive oxidase test result when grown on nonselective media (19, 26) and the ability to utilize carbohydrates fermentatively. Although taxonomic confusion exists among several species of the genus *Aeromonas*, it is agreed that the most common human isolate is *Aeromonas hydrophila* (11), which was not distinguished from *Aeromonas sobria* in this report. *A. hydrophila* is commonly isolated from fresh water (30) and estuaries (18).

Although considered an uncommon human pathogen, *A. hydrophila* has been reported to cause a variety of human infections, including cellulitis (23), wound infections (17), hepatobiliary infections (12), and septicemia, especially in immunocompromised individuals (21). However, the most commonly associated clinical infection has been diarrhea (2, 8, 20, 24, 28, 35). *A. hydrophila* has been isolated from diarrheal stool specimens in greater frequency than from control stools (4, 6, 15, 31, 36), and neutralizing antibodies have been demonstrated in the serum of convalescent patients with *Aeromonas*-associated diarrhea (16, 27).

The possible pathogenic mechanisms by which this organism may cause enteritis have been incompletely investigated. Many strains of *A. hydrophila* have been shown to possess virulence factors, including adherence to buccal cells via piliation (10), hemagglutination (3), and production of various exotoxins, including hemolysin (7). In addition, enterotoxigenic activity probably caused by a single toxin (9) has been demonstrated by the following methods: rabbit ileal loop assay (28), perfusion of rat jejunum in vivo (32), suckling mouse test (5), and cytotoxin assay (22, 37).

Despite these studies, *A. hydrophila* is not commonly sought from routine stool cultures, and enteritis caused by this organism has had few clinical evaluations. We, therefore, undertook this prospective study of patients with diarrhea. An easily prepared sheep blood agar containing 15 µg of ampicillin per ml (SB-A agar) was used to suppress

normal flora and allow direct observation of hemolysis and oxidase reactions to facilitate isolation of *A. hydrophila*.

MATERIALS AND METHODS

Clinical evaluation. Liquid stools from self-referred patients were studied over an 18-month period, from April 1981 through September 1982. During that period, 2,050 non-formed stool specimens from 1,821 patients were ordered for culture by private physicians of La Crosse Lutheran Hospital-Gundersen Clinic. Approximately 60% of the specimens were from outpatients. Twenty-four patients were associated with two discrete outbreaks of salmonellosis and were excluded from evaluation. In addition, throughout the period of study, 533 control specimens were obtained. Fifty of these were from hospitalized children, and the rest were from unselected patients in the outpatient internal medicine clinic. All control specimens were from individuals without diarrhea who had not received antibiotics within 2 weeks of specimen collection.

Patients who had a positive culture for *A. hydrophila* were interviewed retrospectively concerning environmental exposures to sources such as spoiled food, surface water, or individuals with intestinal infections. In addition, symptoms and signs of each case were reviewed directly with the patient or the parent of the patient and from the records of the physician.

Microbiological evaluation. Stool specimens from patients with diarrhea were processed for *Salmonella*, *Shigella*, and *Campylobacter* spp. with MacConkey agar, Hektoen enteric, xylose-lysine-desoxycholate agar, *Campylobacter* spp.-selective agar, and gram-negative enrichment broth. Rotaviral evaluation was not done. In addition, stool specimens from these diarrheal patients and stools from controls were plated on SB-A agar. After 24 to 48 h of incubation at 37°C, these plates were flooded with 1% sodium dimethyl-*T*-phenylenediamine monohydrochloride (oxidase reagent). Oxidase-positive colonies were picked for subculture before total blackening had occurred, to ensure that viability was not lost. Reisolated organisms, not typical *Pseudomonas aeruginosa* by color or odor, were further evaluated by triple sugar

* Corresponding author.

FIG. 1. Incidence of *A. hydrophila*, *C. jejuni*, and *Salmonella enteritidis* gastroenteritis.

iron slants and API-20E commercial strips. In addition, all lysine decarboxylase reactions were confirmed by using a macrotube (Difco Laboratories, Detroit, Mich.), and all failed to grow in 1 and 6.5% sodium chloride broth.

SB-A agar was compared with plain sheep blood agar (SB agar), DNase-toluidine blue-ampicillin agar (DNTA agar; GIBCO Diagnostics, Madison, Wis.), and *Yersinia* agar (CIN agar; Difco) and has been recently reported to grow *A. hydrophila* frequently from clinical specimens (A. G. Helstad, E. Christenson, L. Dodge, and J. Archer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C152, p. 337). Comparison was done as described by von Graevenitz and Bucher (34). Ten stools from clinical specimens were plated to two SB agar plates and then incubated for 18 to 24 h at 35°C. After 24 h, one plate was flooded with oxidase reagent to be certain that test stools did not contain oxidase-positive organisms. The stools were diluted to a 0.5 McFarland standard and then further diluted (1:10) to an estimated final concentration of 10^7 colonies per ml.

An *A. hydrophila* isolate that initially grew on all four media was then diluted to a 0.5 McFarland standard, and further dilutions of 1:10, 1:100, and 1:1,000 were made. Equal portions of the normal stool dilution and the dilutions containing *A. hydrophila* were pipetted together, and then, by using a calibrated 0.001-ml loop, these mixtures (10^4 , 10^3 , and 10^2 organisms per ml) were streaked onto the CIN, DNTA, SB, and SB-A agars.

Testing for cytotoxin was performed by using HeLa cells as previously described (9). In addition, hemolysin was assayed by the method of Burke et al. (4). Finally, standard Kirby-Bauer disk diffusion susceptibility tests were performed with antibiotics which are commonly used for the treatment of gastroenteritis.

RESULTS

Clinical evaluation. *A. hydrophila* was isolated from stool specimens from 20 (1.1%) of 1,797 patients with diarrhea. No isolates of *A. hydrophila* were found in the 533 concurrently collected control samples ($\chi^2 = 6.0$; $P < 0.02$). Two

patients with *A. hydrophila* also had concurrent pathogens, either *Giardia lamblia* or *Campylobacter jejuni*, in their stool.

The frequency of *A. hydrophila* cases during warm months was 1.25 case per month, whereas during cold months it was 0.83 case per month. Although much less common than *C. jejuni*, which was found in 8.3% of diarrhea cases, *A. hydrophila* was only slightly less frequent than *Salmonella* sp. isolates, which were found in 1.6% of diarrhea cases, when two-point source epidemics totaling 24 cases of salmonellosis were excluded (Fig. 1). In the La Crosse area, shigellosis is uncommon; it accounted for only three (0.2%) of the diarrhea cases in this study. Age distribution, similar to that of *Salmonella* disease (1), revealed a greater number of cases in individuals less than 7 years of age and in those greater than 60 years of age (Fig. 2).

Clinical features of *A. hydrophila*-associated diarrhea are shown in Table 1. Only one of the cases was associated with recognized contaminated surface water, but this case had

FIG. 2. Age distribution of *A. hydrophila* diarrhea.

TABLE 1. Clinical features of 20 patients with *Aeromonas* sp.-associated diarrhea

Feature	No. (%) of patients in which feature was manifested
Fever	
38 to 39°C.....	6 (30)
>39°C.....	5 (25)
Vomiting	
1 to 2 days.....	3 (15)
>2 days.....	2 (10)
Abdominal pains	
Cramps.....	7 (35)
Palpation.....	6 (30)
Blood in stool	
Hematochezia ^a	1 (5)
Occult blood ^b	4 (33)
Length of diarrhea	
3 to 10 days.....	10 (50)
>10 days.....	10 (50)
Hospitalization.....	6 (30)

^a Concurrent *C. jejuni* infection.^b Only 12 patients were tested for this feature.

concurrent *G. lamblia* infection. Vomiting was a feature in *A. hydrophila*-associated gastroenteritis in only 25% of cases. The diarrhea continued longer than 10 days in 50% of cases but was not associated with tenesmus or hematochezia except in one case with concurrent *C. jejuni* infection. Four patients had occult blood in their stools. Mild abdominal pain or tenderness was noted in 65% of cases, and fever

occurred in 55%. Six cases required hospitalization because of dehydration or persistent diarrhea.

Microbiological evaluation. In the comparison of the four different media with the 10 test stools mixed with *A. hydrophila*, the DNTA, SB, and SB-A agars grew *A. hydrophila* in all dilutions. However, colonies on the SB-A agar were oxidase positive, whereas on the DNTA agar, five of the test samples required subculturing to an SB agar before the oxidase test became reactive. Plain SB agar without ampicillin did not suppress normal flora adequately to show hemolysis and allow for direct oxidase testing in the majority of the test plates. The CIN agar was not as sensitive as the other two media, with 40% of samples not supporting the growth of *A. hydrophila*, and, as was observed with the DNTA agar, colonies on CIN agar did not consistently have a positive oxidase reaction.

In all 20 specimens with *A. hydrophila*, this organism grew in large numbers. In 18 of 20 of these, *A. hydrophila* was found in large numbers on the SB-A agar only. Colonies were easily identified due to frequent beta-hemolysis and oxidase positivity (Fig. 3). Seventy-five percent of the isolates were hemolytic. In one specimen, *A. hydrophila* was found in large numbers on all primary plates, including the SB-A agar. From only one specimen was the organism detected only on an XLD plate, due to nonlactose fermentation, and not found on the SB-A agar.

Sixteen isolates that remained viable were later assayed for both cytotoxin, by using HeLa cells, and hemolysin, by using rabbit erythrocytes. Of these tested strains, 10 (62%) were cytotoxic and 6 (37%) were noncytotoxic. Cytotoxin was found in all isolates that were lysine decarboxylase positive and in none that were lysine decarboxylase negative. All cytotoxic strains produced hemolysin in titers of greater than 1:4, and all noncytotoxic strains were nonhemolytic. A positive Voges-Proskauer reaction occurred in 4 of 10 (40%) of cytotoxic strains and in none that were noncytotoxic. Most of the cytotoxic strains (9 of 10) were arabinose

FIG. 3. *A. hydrophila* colonies on SB-A agar. (Oxidase reagent has been added, and *A. hydrophila* stand out as black colonies with haloes of hemolysis.)

negative, whereas 4 of 6 noncytotoxic strains were arabinose positive. A similar observation has been made by Burke et al. (6), who correlated enterotoxigenicity with biotypes of *Aeromonas* species.

Antimicrobial susceptibility testing by standard Kirby-Bauer disk diffusion showed all 16 isolates to be sensitive to nalidixic acid, tetracycline, trimethoprim-sulfamethoxazole, and sulfamethoxazole. All were resistant to ampicillin, penicillin, and carbenicillin, whereas only 31% were sensitive to cephalothin. Similar antibiotic patterns have been reported elsewhere in greater detail (14).

DISCUSSION

In this study, *A. hydrophila* was found only in stool specimens from patients with diarrhea. The organism was present in large numbers, and no isolates were found in control specimens. Others have reported a low or negative frequency of *A. hydrophila* from normal stools (6, 15, 31, 36). In contradistinction, Echeverria et al. found *A. hydrophila* in normal subjects nearly as frequently as in patients with gastroenteritis (13). These divergent results may be related to geographic location, season of collection, and to the microbial media used for isolation. The sensitivity and specificity varied when different media were compared by von Graevenitz and Bucher (34).

SB-A agar was chosen in this study because it is a practical medium that is easy to prepare. This medium not only inhibits many of the enteric flora but also allows direct observation of both hemolysis and oxidase reactions from the primary plate. In these latter characteristics, we found it to be very practical in comparison with the DNTA media. Despite a recent report of *A. hydrophila* isolates growing on CIN agar (Helstad et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1983), we found this medium to be not only less sensitive (40% false-negative results) but also less practical due to an initial negative oxidase reaction. If SB-A agar had not been used on the diarrheal stool samples from the clinical cases, only 2 of the 20 isolates would have been found. Thus, we strongly recommend inclusion of a selective plate if *A. hydrophila* is sought from patients with diarrhea.

Clinically, *A. hydrophila*-associated diarrhea was not distinct from other types of diarrhea commonly encountered in United States practice. Most cases were not severe, but six patients had diarrhea severe enough or diagnostically difficult enough to require hospitalization. We expected to find an association of this illness with exposure to surface water, but the only such patient had concurrent giardiasis. We have theorized that contaminated water or foods processed with contaminated water are the source of *A. hydrophila* intestinal infections. Similar to other bacterial enteric pathogens, *A. hydrophila*-associated diarrhea was seen more often in summer months. In this series, the disease was unlike shigellosis and more like toxigenic diarrheas in that no cases of tenesmus or hematochezia occurred except in one patient who had a concurrent *C. jejuni* infection. This observation is divergent from the recent report by Burke et al. (4), who found that 25% of the children tested had macroscopic blood in their stool.

Cytotoxic activity was demonstrable in only 62% of isolates. This was invariably associated with a positive lysine decarboxylase reaction. A similar relationship was noted by Cumberbatch et al. (9). Whether the toxin-negative strains possess other mechanisms capable of causing enteritis or whether they are commensals is not yet known. However, other possible pathogenic markers have been identified (7),

and it is possible that like non-group O1 *Vibrio cholerae* (25) and other *Vibrio* spp. (29), *A. hydrophila* has other mechanisms capable of producing diarrhea.

In conclusion, our study confirms the work of others who have shown that *A. hydrophila* is found in association with diarrhea more often than in normal controls. In addition, a majority of strains produced a cytotoxin, and the presence of this toxin usually correlates closely with a hemolysin and a positive lysine decarboxylase reaction. We feel that SB-A agar is an inexpensive and practical way to culture stools for this organism. However, confirmation that the organism is an enteric pathogen should be sought by further epidemiological studies and studies in animal models and volunteers. If this organism is confirmed as a pathogen, antibiotic therapy such as trimethoprim-sulfamethoxazole or tetracycline could be studied to determine whether antibiotics can alter the length of illness. Cost considerations might well warrant such a study, as *A. hydrophila*-associated diarrhea lasted longer than 10 days in 50% of our patients and was related to hospitalization in six cases.

ACKNOWLEDGMENTS

We thank James E. Glasser and Jay Grimes for their helpful discussions.

This study was supported by the Gundersen Medical Foundation, Ltd., and the Infectious Disease Research Institute of Michigan.

LITERATURE CITED

- Blaser, M. J., and R. A. Feldman. 1981. *Salmonella* bacteremia: reports to the Centers for Disease Control, 1968-1979. *J. Infect. Dis.* 143:743-746.
- Bpat, P., S. Shanthakumari, and D. Rajan. 1974. The characterization and significance of *Plesiomonas shigelloides* and *Aeromonas hydrophila* isolated from an epidemic of diarrhea. *Indian J. Med. Res.* 62:1051-1060.
- Burke, V., M. Cooper, J. Robinson, M. Gracey, M. Lesmana, P. Echeverria, and J. M. Janda. 1984. Hemagglutination patterns of *Aeromonas* spp. in relation to biotype and source. *J. Clin. Microbiol.* 19:39-43.
- Burke, V., M. Gracey, J. Robinson, D. Peck, J. Beaman, and C. Bundell. 1983. The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. *J. Infect. Dis.* 148:68-74.
- Burke, V., J. Robinson, H. M. Atkinson, and M. Gracey. 1982. Biochemical characteristics of enterotoxigenic *Aeromonas* spp. *J. Clin. Microbiol.* 15:48-52.
- Burke, V., J. Robinson, J. Beaman, M. Gracey, M. Lesmana, R. Rockhill, P. Echeverria, and J. M. Janda. 1983. Correlation of enterotoxigenicity with biotype in *Aeromonas* spp. *J. Clin. Microbiol.* 18:1196-1200.
- Champsaur, H., A. Andreumont, D. Mathieu, E. Rottman, and P. Auzepy. 1982. Cholera-like illness due to *Aeromonas sobria*. *J. Infect. Dis.* 145:248-254.
- Chatterjee, B. D., and K. N. Neogy. 1972. Studies on *Aeromonas* and *Plesiomonas* species isolated from cases of choleraic diarrhea. *Indian J. Med. Res.* 60:520-524.
- Cumberbatch, N., M. J. Gurwith, C. Langston, R. B. Sack, and J. L. Brunton. 1979. Cytotoxic enterotoxin produced by *Aeromonas hydrophila*: relationship of toxigenic isolates to diarrheal disease. *Infect. Immun.* 23:829-837.
- Daily, O. P., S. W. Joseph, J. C. Coolbaugh, R. I. Walker, B. R. Merrell, D. M. Rollins, R. J. Seidler, R. R. Colwell, and C. R. Lissner. 1981. Association of *Aeromonas sobria* with human infection. *J. Clin. Microbiol.* 13:769-777.
- Davis, W. A., J. G. Kane, and V. F. Garagusi. 1978. Human *Aeromonas* infections: a review of the literature and a case of endocarditis. *Medicine* 57:267-275.
- DeFronzo, R. A., G. F. Murray, and W. C. Maddrey. 1973. *Aeromonas* septicemia from hepatobiliary disease. *Dig. Dis. Sci.* 18:323-331.

13. Echeverria, P., N. R. Blacklow, L. B. Sanford, and G. C. Cukor. 1981. Travelers' diarrhea among American Peace Corps volunteers in rural Thailand. *J. Infect. Dis.* **143**:767-771.
14. Fass, R. J., and J. Barnishan. 1981. In vitro susceptibilities of *Aeromonas hydrophila* to 32 antimicrobial agents. *Antimicrob. Agents Chemother.* **19**:357-358.
15. Geizer, E., K. Kopecky, and E. Aldova. 1966. Isolation of *Aeromonas shigellois* in children. *J. Hyg. Epidemiol.* **10**:190-194.
16. Gurwith, M., C. Bourque, E. Cameron, G. Forrest, and M. Green. 1977. Cholera-like diarrhea in Canada: report of a case associated with enterotoxigenic *Escherichia coli* and a toxin-producing *Aeromonas hydrophila*. *Arch. Intern. Med.* **137**:1461-1464.
17. Hanson, P. G., J. Standridge, F. Jarrett, and D. G. Maki. 1977. Fresh water wound infection due to *Aeromonas hydrophila*. *J. Am. Med. Assoc.* **238**:1053-1054.
18. Hazen, T. C., C. B. Fliermans, R. P. Hirsch, and G. W. Esch. 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl. Environ. Microbiol.* **36**:731-738.
19. Hunt, L. K., T. L. Overman, and R. B. Otero. 1981. Role of pH in oxidase variability of *Aeromonas hydrophila*. *J. Clin. Microbiol.* **13**:1054-1059.
20. Janda, J. M., E. J. Bottone, C. V. Skinner, and D. Calcaterra. 1983. Phenotypic markers associated with gastrointestinal *Aeromonas hydrophila* isolates from symptomatic children. *J. Clin. Microbiol.* **17**:588-591.
21. Ketover, B. P., L. S. Young, and D. Armstrong. Septicemia due to *Aeromonas hydrophila*: clinical and immunologic aspects. *J. Infect. Dis.* **127**:284-289.
22. Ljungh, Å., M. Popoff, and T. Wadström. 1977. *Aeromonas hydrophila* in acute diarrheal disease: detection of enterotoxin and biotyping of strains. *J. Clin. Microbiol.* **6**:96-100.
23. McCracken, A. W., and R. Barkely. 1972. Isolation of *Aeromonas* species from clinical sources. *J. Clin. Pathol.* **25**:970-975.
24. Mohieldin, M. S., M. Gabr, A. El-Hefny, S. S. Mahmoud, and A. Abdallah. 1966. Bacteriological and clinical studies in infantile diarrhea. *J. Trop. Pediatr.* **11**:88-94.
25. Morris, J. G., H. G. Miller, R. Wilson, B. R. Davis, I. K. Wachsmuth, C. F. Riddle, H. G. Wathen, M. A. Pollard, and P. A. Blake. 1981. Nongroup O1 *Vibrio cholerae* gastroenteritis in the United States. *Ann. Intern. Med.* **94**:656-658.
26. Overman, T. L., R. F. D'Amato, and K. M. Tomfohrde. 1979. Incidence of "oxidase-variable" strains of *Aeromonas hydrophila*. *J. Clin. Microbiol.* **9**:244-247.
27. Rosner, R. 1964. *Aeromonas hydrophila* as the etiologic agent in a case of severe gastroenteritis. *Am. J. Clin. Pathol.* **42**:402-404.
28. Sanyal, S. C., S. J. Singh, and P. C. Sen. 1975. Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *J. Med. Microbiol.* **8**:195-198.
29. Shandera, W. X., J. M. Johnston, B. R. Davis, and P. A. Blake. 1983. Disease from infection with *Vibrio mimicus*: a newly recognized *Vibrio* species. *Ann. Intern. Med.* **99**:169-171.
30. Shotts, E. B., Jr., and R. Rimler. 1973. Medium for the isolation of *Aeromonas hydrophila*. *Appl. Microbiol.* **26**:550-553.
31. Shread, P., T. J. Donovan, and J. V. Lee. 1981. A survey of incidence of *Aeromonas* in human faeces. *Soc. Gen. Microbiol.* **8**:184-185.
32. Thelen, P., V. Burke, and M. Gracey. 1978. Effects of intestinal microorganisms on fluid and electrolyte transport in the jejunum of the rat. *J. Med. Microbiol.* **11**:463-470.
33. Popoff, M. 1984. *Aeromonas*, p. 545. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
34. von Graevenitz, A., and C. Bucher. 1983. Evaluation of differential and selective media for isolation of *Aeromonas* and *Plesiomonas* spp. from human feces. *J. Clin. Microbiol.* **17**:16-21.
35. von Graevenitz, A., and A. H. Mensch. 1968. The genus *Aeromonas* in human bacteriology: report of 30 cases and review of the literature. *N. Engl. J. Med.* **278**:245-248.
36. von Graevenitz, A., and L. Zinterhofer. 1970. The detection of *Aeromonas hydrophila* in stool specimens. *Health Lab. Sci.* **7**:124-127.
37. Wadstrom, T., A. Ljungh, and B. Wretling. 1976. Brief reports: enterotoxin, haemolysin, and cytotoxic protein in *Aeromonas hydrophila* from human infections. *Acta Pathol. Microbiol. Scand. Sect. B* **84**:112-114.