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Clinical and Epidemiologic Aspects of Members of *Aeromonas* DNA Hybridization Groups Isolated from Human Feces

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Between June 1982 and May 1987 *Aeromonas* species were isolated from 208 of 34,311 (0.61%) fecal samples submitted to a Regional Public Health Laboratory in The Netherlands. *Aeromonas* isolates were found most frequently in summer and rarely in winter. Of 169 *Aeromonas* isolates that were available for further study, 19% were isolated from patients with a mixed infection, 5% from patients with underlying diseases, and 15% from patients who used medication that could predispose the intestinal tract to colonization with *Aeromonas* species. *Aeromonas* species that produced cytotoxins to Vero cells (cytotoxigenic) were found in hybridization groups 1 (11% of all isolates), 2 (1%), 3 (2%), and 8 (25%) and were identified phenotypically as *A. hydrophila* or *A. sobria*. *Aeromonas* species that did not produce cytotoxins to Vero cells (noncytotoxigenic) were found in hybridization groups 4 (57%) and 5A (4%) and were identified phenotypically as *A. caviae*. Distribution of *Aeromonas* species by age showed a predominance of noncytotoxigenic strains in children under the age of 5 years (46% of all noncytotoxigenic strains), while cytotoxigenic strains were mainly cultured from patients aged 50 years or older (54% of all cytotoxigenic strains). Significant correlations were found between cytotoxigenic strains and hospitalization, foreign travel, and contact with surface water. Cytotoxigenic strains were isolated significantly more often than noncytotoxigenic strains from patients with diarrhea, but in a multivariate analysis including age, previous medication, underlying disease, and foreign travel, this association was not significant.

An increasing number of studies report on the possible significance of some *Aeromonas* species as enteric pathogens (8–10, 15, 20, 21, 27, 31). However, the role of *Aeromonas* species in gastrointestinal disease remains controversial. The production of hemolysins, cytotoxins, and enterotoxins by these *Aeromonas* species has been considered as a virulence marker for enteropathogenicity (5, 6, 10, 17, 21, 26). The absence of outbreaks of *Aeromonas* species-associated diarrhea and the report that only 2 of 57 healthy volunteers developed diarrhea after oral administration of a high dose of enterotoxin-producing *Aeromonas* strains foster doubts as to the enteropathogenicity of *Aeromonas* species (30).

Problems in assessing the clinical significance of *Aeromonas* species have been greatly compounded by the inability to correctly identify *Aeromonas* species. Seven *Aeromonas* species have been identified on the basis of biochemical characteristics and polynucleotide sequence relatedness: *A. hydrophila*, *A. sobria*, *A. caviae*, *A. salmonicida*, *A. media*, *A. veronii*, and *A. schubertii* (1, 13, 14, 34). Several of these species are difficult or impossible to distinguish phenotypically (22; J. J. Farmer III, F. W. Hickman-Brenner, G. R. Fanning, M. J. Arduino, and D. J. Brenner, Abstr. Int. Workshop on Aeromonas, p. 1–2, 1986). In addition, some as yet unnamed DNA hybridization groups (HG) are phenotypically inseparable from named species (22).

Members of 5 of 11 hybridization groups recognized within the genus *Aeromonas* have been found in human feces, and these belong to groups 1 (genospecies, *A. hydrophila*), 2 (unnamed), 3 (*A. salmonicida*), 4 (*A. caviae*), 5 (*A. media*), and 8 (*A. veronii*) (15, 22). These reports serve to emphasize the problem of identifying *Aeromonas* species, since other studies indicate a high isolation rate of phenotypically identified *A. sobria* (HG 7) strains for human feces (8, 17, 27, 31).

We biochemically and genetically characterized *Aeromonas* strains recovered from human fecal samples that were submitted to the Public Health Laboratory in Tilburg, The Netherlands, over a 5-year period.

MATERIALS AND METHODS

Isolation and phenotypic identification of *Aeromonas* strains. The Regional Public Health Laboratory in Tilburg, The Netherlands, serves an area with 600,000 people and receives specimens for bacteriological investigation from general practitioners as well as from four general hospitals. Between 1 June 1982 and 31 May 1987, we cultured all fecal samples (n = 34,311) submitted to our laboratory for *Aeromonas* identification. Specimens were diluted in 0.9% NaCl (1 g/10 ml), and 5 μl of this solution was inoculated onto sheep blood agar containing 10 μg of ampicillin per ml (10). After 48 h of incubation at 37°C, all oxidase- and catalase-positive gram-negative rods which reduced nitrates, fermented mannitol, and did not grow in broth containing 6% NaCl were identified further by using conventional biochemical methods. *Aeromonas* isolates were identified to the species level by using biochemical tests recommended by Popoff (34) and Janda et al. (17). Extracellular production of

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hemolysins to rabbit erythrocytes and cytotoxins to Vero cells was also determined (22).

Isolation of other enteropathogenic bacteria. Fecal samples were also cultured for *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter* species by plating the diluted feces onto salmonella-shigella agar (Oxoid Ltd., Hampshire, United Kingdom), deoxycholate-citrate agar (Oxoid), deoxycholate-citrate agar of Wauters (Oxoid) (G. Wauters, Ph.D. thesis, University of Louvain, Louvain, Belgium, 1970), Campy-Bap (Oxoid), bismuth sulfite agar (Oxoid), and in the enrichment selenite medium (E. Merck AG, Darmstadt, Federal Republic of Germany) as well as selenite malachite green medium (selenite medium plus 0.0007% malachite green). Inoculated deoxycholate-citrate agar, salmonella-shigella agar, bismuth sulfite agar, and selenite medium were incubated for 18 h at 37°C. Deoxycholate-citrate agar of Wauters plates were incubated for 48 h at 22°C. Selenite malachite green medium was held at 22°C for 72 h. Campy-Bap plates were held at 42°C in a microaerophilic atmosphere for 48 h. Bacteria were identified by conventional bacteriological methods. Microscopical examinations for parasitic protozoal infections were performed on fecal samples only when requested.

Fecal cytotoxins. To detect *Aeromonas* and *Clostridium difficile* cytotoxins, feces were diluted in Hanks balanced salt solution (0.5 g/3 ml) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) and were mixed by agitation in a Vortex mixer (The Vortex Manufacturing Co., Cleveland, Ohio). After centrifugation (2,500 × *g*, 20 min), the supernatant was decanted, filtered (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and transferred (0.2 ml) together with fresh tissue culture medium (0.8 ml) to monolayers of human embryonic lung fibroblasts and Vero cells. Cells were microscopically examined after 3, 16, and 48 h of incubation at 34°C. Fecal samples containing cytotoxic activity were twofold serially diluted from an initial dilution of 1:5. A toxin-neutralizing assay (*Clostridium sordellii* antitoxin; Wellcome Diagnostics, Dartford, England) was used to detect *C. difficile* toxin. Serum from a patient with a 1:640 neutralizing activity against *A. hydrophila* and *A. sobria* cytotoxins present in broth cultures filtrates was used in the neutralizing assay for *Aeromonas* cytotoxins in feces. Neutralization was done by incubating undiluted human serum or antitoxin with the filtered fecal sample for 1 h at 37°C.

DNA hybridization studies. A total of 142 human fecal *Aeromonas* strains were tested for DNA relatedness to *Aeromonas* strains representing the 11 DNA HG (3, 22; J. J. Farmer III et al., 1986).

Viral cultures. Viral cultures were done from fecal samples containing *Aeromonas* species by the use of Vero cells, Chang conjunctiva cells, and primary African green monkey kidney cells (Flow Laboratories Ltd., Irvine, Scotland). Viruses were identified by using characteristic cytopathic effects and virus-neutralizing antisera (25). An enzyme immunoassay (Abbott, Amstelveen, The Netherlands) was used to test for the presence of rotavirus in *Aeromonas* spp.-positive fecal samples. Electron microscopy was not performed.

Clinical and epidemiological data. For each *Aeromonas* isolate, clinical data were obtained, including symptoms of diarrhea, contact with surface water, underlying diseases, medication, and occurrence of diarrhea in the immediate environment of the patient. Follow-up fecal culture was also requested. Additional data were obtained on the severity and duration of diarrhea, consistency of the stools and number per day, presence of blood and mucus, occurrence of abdominal pain or cramps, the presence of nausea, vomiting, or fever, exposure to surface water, and recent travel. Daily charts were used to follow the clinical course of patients in St. Elisabeth Hospital (Tilburg, The Netherlands), as were discharge letters and questionnaires from patients in other hospitals.

Statistical analysis. Statistical analysis was performed with the chi-square test (including Yates' correction when necessary), the odds ratio test, and binomial 95 and 99% confidence intervals. To determine the relationships among four variables, a log-linear model using the Biomedical Computer Program (BMDP-4F) was used (7). A delta factor of 0.5 was added to each of the 24 cells in the log-linear analysis.

RESULTS

Aeromonas species were found in 208 of 34,311 samples, an isolation rate of 0.61%. Of 142 isolates characterized biochemically and by DNA relatedness, 87 (61%) were identified phenotypically as *A. caviae*, 29 (21%) were identified as *A. sobria*, and 26 (18%) were identified as *A. hydrophila*. By DNA relatedness, most strains were in DNA HG 4 (57%), 8 (25%), and 1 (11%), with a few strains in HG 5A (4%), 3 (2%), and 2 (1%).

Of strains identified phenotypically as *A. hydrophila*, 35% were in DNA HG that represent species other than *A. hydrophila*, and 10% of strains identified phenotypically as *A. sobria* were not confirmed by hybridization studies with the remaining 90% constituting a biogroup of *A. veronii* (HG 8). Of the strains identified phenotypically as *A. caviae*, 2% were in an HG that represents *A. veronii*. Because of the poor correlation between phenotypic identification of *Aeromonas* species and DNA HG, we chose to use the term cytotoxigenic for strains belonging to HG 1, 2, 3, and 8, since 96% of these strains produced cytotoxins to Vero cells, and the term noncytotoxigenic for groups 4 and 5A, of which 96% did not produce cytotoxins. Cytotoxigenic strains were identified phenotypically as *A. hydrophila* or *A. sobria*, and noncytotoxigenic strains were identified phenotypically as *A. caviae*. Cytotoxigenic strains differed phenotypically from the noncytotoxigenic strains in the production of gas and acetoin from glucose, production of H_2S, decarboxylation of L-lysine, and production of hemolysins to rabbit erythrocytes.

Enteropathogens isolated from fecal samples during this same 5-year period included *Campylobacter jejuni* (6.9%), *Salmonella* spp. (3.6%), *Shigella* spp. (0.33%), and *Yersinia enterocolitica* (0.67%). Isolated concomitantly with 32 (19%) of 169 *Aeromonas* isolates were 17 strains of *Salmonella*, 12 *C. jejuni*, 2 *C. difficile*, and 1 enterovirus. Enteropathogenic bacteria were isolated equally with cytotoxigenic and noncytotoxigenic *Aeromonas* strains.

A total of 84 fecal samples from patients with *Aeromonas*-associated diarrhea were examined for cytotoxic effects to Vero cells and to human lung fibroblasts. Human lung fibroblasts were used to detect cytotoxins of *C. difficile*. Samples from 2 children less than 1 year old, one with an *Aeromonas* strain from HG 8 and one with a strain from HG 4, contained *C. difficile* cytotoxins. Cytotoxic effects not due to *C. difficile* were found in fecal samples containing *Aeromonas* strains from HG 8 (6 of 14 patients), from HG 1 (2 of 12 patients), and from HG 4 (7 of 58 patients). Titers of fecal cytotoxic effects varied from 1:5 to 1:40. Cytotoxic effects were not removed by incubation of the filtered supernatants with *Aeromonas* cytotoxins neutralizing human serum.

Aeromonas isolates were found throughout the year but were most common in the months June through September

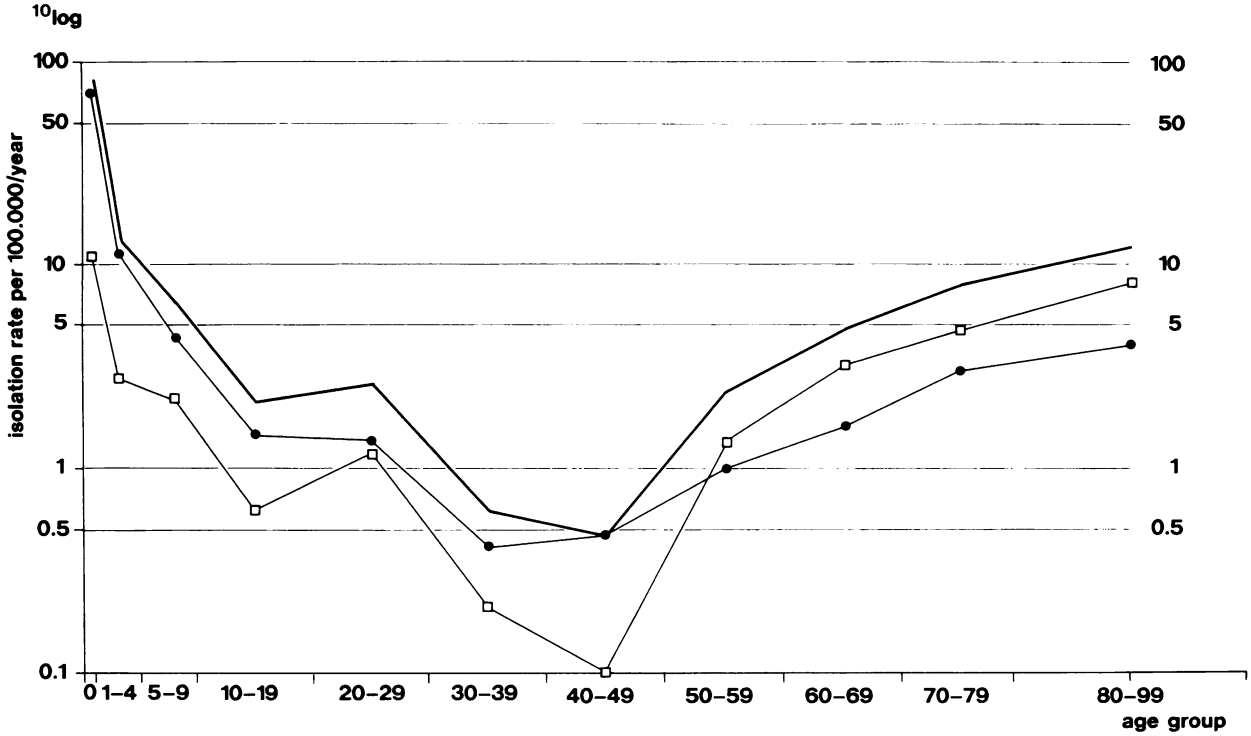

FIG. 1. Age- related occurrence of *Aeromonas* isolations. (The Tilburg region had a total population of 600,000 between 1982 and 1987.) Symbols: ——, cytotoxigenic and noncytotoxigenic strains; ●, noncytotoxigenic strains; □, cytotoxigenic strains.

(for months 6, 7, and 8, the expected percentage of *Aeromonas* isolates was 25.1%; the observed percentage was 40.3% [99% confidence interval, 31.2 to 49.2%]). The lowest incidence occurred in the winter months (for months 12, 1, and 2, the expected percentage was 24.7%; the observed percentage was 13% [99% confidence interval, 7.6 to 20.1%]).

Distribution of *Aeromonas* species by age (Fig. 1) showed a predominance of noncytotoxigenic strains in children under the age of 5 years (46% of all noncytotoxigenic strains), while cytotoxigenic strains were mainly cultured from patients aged 50 years or older (54% of all cytotoxigenic strains; Table 1). In all age groups, diarrhea was more frequent in patients with cytotoxigenic strains than in patients with noncytotoxigenic strains. *Aeromonas* strains in individuals with and without diarrhea were distributed equally among males and females in all age groups.

A strong association was observed (Table 2) between the presence of cytotoxigenic *Aeromonas* strains in human feces and clinical symptoms of gastroenteritis or enterocolitis, if other variables for age and predisposing factors were not included in the statistical analysis (see log-linear analysis). Of 48 patients with cytotoxigenic *Aeromonas* strains, 44 had diarrhea, which differed significantly from the percentage of noncytotoxigenic strains isolated from patients with diarrhea ($\chi^2 = 7.95$, $P < 0.005$). Information from questionnaires completed by 55 (33%) of 169 patients studied is shown in Table 3.

Two weeks after primary culture, all repeat fecal cultures ($n = 38$) were negative for *Aeromonas* strains (23 noncytotoxigenic and 15 cytotoxigenic strains).

Cytotoxigenic strains were more frequently present in fecal samples of patients with traveler's diarrhea than were noncytotoxigenic strains ($\chi^2 = 4.26$, $P < 0.05$) (Tables 1 and 2). Of 10 patients with traveler's diarrhea, seven traveled to

TABLE 1. Relation between age, predisposing factors, enterotoxin, and diarrhea[a]

Predisposing factor	No. of patients ($n = 48$) with cytotoxigenic strains by age (yr [%])[b]				No. of patients ($n = 89$) with noncytotoxigenic strains by age (yr [%])[b]			
	0 (8%)	1–49 (38%)	>50 (54)	Total (100)	0 (29)	1–49 (54)	>50 (17)	Total (100)
None	1/1	6/8 (75)	13/14 (93)	20/23 (87)	15/22 (68)	21/37 (57)	11/11 (100)	47/70 (67)
Medication	1/1	2/3	9/9 (100)	12/13 (92)	4/4	5/7 (71)	2/2	11/13 (85)
Underlying disease	0/0	2/2	3/3	5/5 (100)	0/0	1/1	1/2	2/3 (67)
Foreign travel	2/2	5/5 (100)	0/0	7/7 (100)	0/0	3/3	0/0	3/3 (100)
Total	4/4 (100)	15/18 (83)	25/26 (96)	44/48 (92)	19/26 (73)	30/48 (63)	14/15 (93)	63/89 (71)

[a] Predisposing factors (underlying disease, medication, and foreign travel) were more common in patients with cytotoxigenic strains (52%) than noncytotoxigenic strains (21%) ($\chi^2 = 13.51$, $P = 0.001$).

[b] The fraction represents the number of patients with diarrhea divided by the total number of patients. Percentages of patients with diarrhea are in parentheses.

TABLE 2. Characteristics of individuals with cytotoxigenic and noncytotoxigenic *Aeromonas* strains[a]

Characteristic	No. (%) of patients (n = 137) with strain type in group		
	Cytotoxigenic (n = 48)	Noncytotoxigenic (n = 89)	Odds ratio (χ^2)[b]
Diarrhea	44 (92)	63 (71)	4.54 (7.95)
Hospitalization	10 (21)	8 (9)	2.66 (3.83)
Underlying diseases	5 (10)	3 (3)	3.33 (1.68*)
Foreign travel	7 (15)	3 (3)	4.89 (4.26*)
Contact with surface water	10 (21)	3 (3)	7.54 (9.13*)
Prior medication	13 (27)	13 (14)	2.17 (3.16)
Penicillin derivates	8 (17)	10 (11)	
Antacids	2 (4)	1 (1)	
Immunotherapy	3 (6)	2 (2)	

[a] Individuals with mixed infections were excluded.
[b] *, Including Yates' correction.

The Netherlands from countries in southern Europe, two from countries in Africa, and one from Sri Lanka.

The occurrence of cytotoxigenic *Aeromonas* strains in feces was strongly associated with contact with surface water (Table 2), such as swimming, surfing, or fishing during the week preceding the illness. All patients who recently had contact with surface water and whose feces contained *Aeromonas* strains had diarrhea.

Ten patients with diarrhea associated with cytotoxigenic strains were hospitalized because of their symptoms; in two of these patients, *Aeromonas* strains were also isolated from blood (Table 2). Of the 10 patients, 7 had an underlying illness: an 82-year-old male with prostatic carcinoma with metastases; a 75-year-old male with amyotrophic lateral sclerosis (who subsequently died from a combination of cardiac asthma, bronchopneumonia, and ascending cholangitis); an 87-year-old male with pneumonia treated with amoxycillin; a 64-year-old female with cervical carcinoma stage IIb treated with radical hysterectomy and radiation therapy; a 67-year-old male with chronic bronchitis, rheumatoid arthritis, and hypertension; a 78-year-old female with senile dementia, diabetes mellitus (type II), and hypertension; and a 22-year-old male with rectal adenocarcinoma. Six of the hospitalized patients recovered after antibiotic treatment. In three patients, conservative therapy alone resulted in clinical improvement. Diarrhea generally showed an acute onset and disappeared within 1 week of hospitalization.

TABLE 3. Results of questionnaires from patients with *Aeromonas* species in their feces[a]

Characteristic	No. (%) of patients with strain type in group	
	Cytotoxigenic (n = 23)	Noncytotoxigenic (n = 32)
Median age (variation)	42 (2 mo–86 yr)	23 (3 days–75 yr)
Gastroenteritis	22 (96)	24 (75)
Acute onset	14 (61)	12 (38)
Nausea and vomiting	12 (52)	11 (34)
Abdominal pain or cramps	16 (70)	16 (50)
Fever	7 (30)	9 (28)
Frequency of diarrhea (per day)		
1–2 stools	3 (13)	14 (44)
2–10	15 (65)	13 (41)
>10	4 (17)	5 (16)
Unknown	1 (4)	0
Consistency of feces		
Watery diarrhea	13 (57)	11 (34)
Mucous diarrhea	7 (30)	8 (25)
Semisolid stool	3 (13)	13 (41)
Bloody stool	3 (13)	5 (16)
>2-Wk duration of diarrhea	11 (48)	8 (25)
Antibiotic treatment because of diarrhea	4 (17)[b]	3 (9)[c]

[a] Individuals with mixed infections were excluded.
[b] All patients were cured of diarrhea within 1 week of antibiotic treatment. Two patients received co-trimoxazole, and two others were treated with amoxycillin.
[c] One patient was cured of diarrhea by antibiotic treatment with co-trimoxazole. Another patient temporarily improved after treatment with co-trimoxazole, but diarrhea recurred, although subsequent fecal cultures were *Aeromonas* species negative. No information was available from the third patient.

TABLE 4. DNA HG of *Aeromonas* species in relation to clinical symptoms

DNA HG (n)	No. (%) of patients in HG with:		
	Gastroenteritis (n = 87)	No symptoms (n = 27)	Mixed infections (n = 27)
1 (15)	11 (13)	2 (7)	2 (7)
2 (1)			1 (4)
3 (2)	2 (2)		
4 (81)	43 (50)	20 (74)	18 (67)
5A (6)	3 (3)	3 (12)	
8 (36)	28 (32)	2 (7)	6 (22)

None of the eight hospitalized patients with noncytotoxigenic strains was known to have an underlying illness. Blood cultures were not done. Seven of the hospitalized patients were under 6 months of age, representing 29% of all patients younger than 6 months of age with noncytotoxigenic *Aeromonas* strains (χ^2 = 12.88, $P < 0.0005$). One patient had acute watery diarrhea, whereas the seven others suffered from chronic gastrointestinal complaints. Reflecting the mild course of the gastrointestinal disease, only two patients received antibiotic treatment. All eight hospitalized patients recovered uneventfully, and after 2 weeks, repeat fecal cultures were always negative.

The relationship between DNA HG and clinical symptoms of gastrointestinal infection is shown in Table 4. It was assumed that *Aeromonas* strains were not the causative agents in cases of mixed infection. Strains from HG 3 (100%), 8 (78%), and 1 (73%) were isolated more frequently from patients with *Aeromonas* species-associated gastroenteritis than from healthy individuals or from patients with mixed infections. Strains from HG 4 (53%) and 5A (50%) were isolated approximately equally from patients with *Aeromonas* species-associated gastroenteritis, from healthy individuals, and from patients with mixed infections. The single strain from HG 2 was isolated from a patient with a mixed infection.

To study the relationship between cytotoxigenic strains with diarrhea more carefully, the data from Tables 1 and 2 were separated into 24 categories for a log-linear analysis (6.2 samples per cell, including a delta factor of 0.5) with four variables: age (0, 1 to 49, and >50 years), predisposing factors (present and absent), cytotoxigenic strains (positive and negative), and diarrhea (present and absent). The significant association between cytotoxigenic strains and presence of diarrhea was absent in the multivariate analysis (χ^2 = 1.43, P = 0.23), apparently due to influence of two confound-

ing factors, age and predisposition. Correlations remained significant between diarrhea and age ($\chi^2 = 6.68$, $P = 0.04$), between cytotoxigenicity and age ($\chi^2 = 16.01$, $P = 0.000$), and between predisposing factors and cytotoxigenicity ($\chi^2 = 7.20$, $P = 0.002$).

DISCUSSION

Aeromonas species were isolated from 208 (0.61%) of 34,311 fecal samples submitted to the Public Health Laboratory in Tilburg, The Netherlands, an isolation rate comparable to those in France (0.67%) and Denmark (0.18%), but significantly lower than those in United Kingdom (3.8%), the United States (7.4%), and Austria (>10%) (4, 10, 23, 28, 31). The reported rates may reflect actual differences or may be influenced by the selection of patients and differences in culture media. Various selective media have been compared for isolation of *Aeromonas* species from human feces, and sheep blood agar plates with ampicillin appear the most sensitive (18, 29, 31). Therefore, it is unlikely that our culture method was responsible for a low isolation rate.

The nomenclature of *Aeromonas* species is quite confusing and may have given rise to discrepancies in clinical interpretation of isolates from human feces due mainly to the inability to distinguish phenotypically among the DNA HG (genospecies). We therefore identified *Aeromonas* strains by DNA relatedness to type and reference strains of the 11 known named and unnamed *Aeromonas* species and were able to associate each DNA HG with clinical symptoms. *Aeromonas* strains isolated from human feces were restricted to DNA HG 1 to 4, 5A, and 8. HG 1 represents *A. hydrophila*, HG 2 is unnamed, HG 3 is *A. salmonicida* but is identified as *A. hydrophila* in clinical laboratories, and HG 4 is *A. caviae*. HG 5 contains two subgroups, both of which contain strains of *A. media* which would be identified phenotypically as *A. caviae*. HG 8 contains strains that are genetically *A. veronii* but are identified phenotypically as *A. sobria*, and HG 7, which is *A. sobria*, was not found in human feces in this or a previous study (15).

Some *Aeromonas* species produce a variety of extracellular substances, including hemolysins, cytotoxins, and enterotoxins (26). These have been considered as virulence markers for enteropathogenicity (5, 6, 35, 37). We found strains from HG 1, 2, 3, and 8 to produce cytotoxins to Vero cells. Subsequently, these strains were classified as cytotoxigenic strains. Noncytotoxigenic strains have not been considered to be enteropathogenic, but enteropathogenic mechanisms other than toxin production have not been studied extensively (24, 32). In our study, noncytotoxigenic strains were identified phenotypically as *A. caviae* and were found in HG 4 and 5A.

To define the clinical significance of *Aeromonas* strains in human feces, we compared the clinical data from patients with cytotoxigenic or noncytotoxigenic species in their feces. Because of the low isolation rate of *Aeromonas* strains, a matched control group of healthy individuals was not included. Of 48 patients from whom cytotoxigenic strains were isolated, 44 (92%) had gastroenteritis which showed an acute onset and persisted for more than 2 weeks in 48% of the patients. Diarrhea was generally characterized as watery or mucous, with a frequency of 2 to 10 stools per day. Of 89 noncytotoxigenic isolates, 63 (71%) were from patients with mild diarrhea, which was generally characterized as semisolid stools, with a frequency of 1 or 2 or 2 to 20 stools per day. Only 25% of these patients had diarrhea for more than 2 weeks.

The presence of watery diarrhea in 57% of patients with cytotoxigenic strains suggests enterotoxin involvement. We could not demonstrate *Aeromonas*-specific cytotoxins in fecal samples, despite the presence of cytotoxic effects in 8 (31%) of 26 fecal samples containing cytotoxigenic strains. It is possible that cytotoxic enterotoxins of other enteropathogenic bacteria caused diarrhea in these patients, especially since stools from 7 (12%) of 56 patients with noncytotoxigenic strains in their feces also produced fecal cytotoxic effects. It is also possible that either our cytotoxin-neutralizing assay is not sufficiently sensitive to detect fecal *Aeromonas* cytotoxins or the cytotoxic effects are nonspecific. We did not investigate the presence of cytotonic toxins in fecal samples, although *Aeromonas* enterotoxins have also been described as cytotonic (6, 35).

We found two patients with *Aeromonas* bacteremia caused by cytotoxigenic *Aeromonas* strains. One of these patients suffered from an underlying illness (prostatic carcinoma with metastases), and severe diarrhea was present before bacteremia occurred in both patients. The occurrence of *Aeromonas* bacteremia caused primarily by cytotoxigenic strains in patients with underlying illnesses has also been described (16, 19, 36, 38). In most cases the gastrointestinal tract was suspected as the source of the bacteremia, and patients were neutropenic due to hematological malignancies or treatment with immunosuppressive drugs.

Aquatic environments favor *Aeromonas* species and could serve as a source of transmission for human infections. In The Netherlands, *Aeromonas* species are isolated most often from surface water during summer months when the water temperature fluctuates between 15 and 25°C. Cytotoxigenic strains are isolated more often from surface water than are noncytotoxigenic strains (12). The number of cytotoxigenic *Aeromonas* strains in surface water varies up to 10^3 CFU/ml depending on the geographic location. In our study, recreational contact with surface water, for example by swimming, fishing, or surfing, was more frequent in patients with cytotoxigenic strains than with noncytotoxigenic strains. Of patients with fecal cytotoxigenic *Aeromonas* strains, 21% had contact with surface water in the week before the onset of diarrhea, in contrast to 3% of patients with noncytotoxigenic strains. Recreational contact with surface water may also play a role in the significance of *Aeromonas* species as a cause of traveler's diarrhea, as shown in 7 of 10 patients with this condition. In our study, 10 of 169 (6%) patients with *Aeromonas* (mainly cytotoxigenic) strains in their feces and 10 of 78 (13%) patients with diarrhea had traveler's diarrhea. In the study of George et al. (9), recent travel abroad preceded *Aeromonas* species-associated diarrhea in 4 of 80 (5%) patients. The significance of *Aeromonas* species as a cause of traveler's diarrhea was also shown by Gracey et al. (11) and Pitarangsi et al. (33).

It is possible that the presence of *Aeromonas* species in the intestinal tract reflects the occurrence of an opportunistic pathogen attacking an intestine predisposed to infection by a lack of (local) immunity, by an enteric pathogen, by medication, by an underlying disease, by an alteration of the normal enteric flora, or by nutritional factors. This hypothesis is supported by the finding that 66 of 169 (39%) *Aeromonas* isolates were from patients who had either a mixed infection or an underlying disease or who used prior medication that could predispose the gastrointestinal tract to colonization with *Aeromonas* strains. The age-related occurrence of *Aeromonas* species-associated diarrhea suggests that host immunity to *Aeromonas* species is acquired at an early age but may be lost in later life, since fecal *Aeromonas*

strains were frequently found in children less than 1 year of age (28% of all noncytotoxigenic isolates) and in adults aged 50 years or older (54% of all cytotoxigenic isolates). Hospitalized patients with cytotoxigenic strains were predominantly elderly people with an underlying illness, whereas most hospitalized patients with noncytotoxigenic strains were younger than 6 months of age. In young children, it is difficult to dismiss the possibility that noncytotoxigenic strains are pathogenic (2).

Initial analysis of our results suggested a significant association between cytotoxigenic strains and clinical symptoms of diarrhea. Log-linear analysis showed that the positive correlation between cytotoxigenic strains and the presence of diarrhea was not significant, due to interaction with two variables, age and predisposing factors. Although these variables have been recognized by other investigators, multivariate analysis was never used to study relationships among them. Our results suggest that *Aeromonas* species-associated diarrhea cannot be attributed solely to known virulence properties of the bacteria but that it is also strongly associated with host factors. This might explain why only 2 of 57 healthy volunteers developed diarrhea after oral administration of high doses of *Aeromonas* strains (30).

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