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Typing of *Aeromonas* Strains by DNA Restriction Endonuclease Analysis and Polyacrylamide Gel Electrophoresis of Cell Envelopes

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The outer membrane protein (OMP) composition (OMP typing) of 46 fecal *Aeromonas* strains from hybridization groups (HGs) 1 (*A. hydrophila*; *n* = 10), 4 (*A. caviae*; *n* = 16), and 8 (*A. veronii*; *n* = 20) were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a phenotypic typing method. Almost every isolate of HG-1 and HG-8 had a unique OMP profile, in contrast to isolates of HG-4, which were separated into five different OMP types. It was possible to recognize HGs 1, 4, and 8 by OMP profiles. Twenty-three *Aeromonas* strains from HGs 1 (*n* = 5), 4 (*n* = 10), and 8 (*n* = 8) were tested by whole-cell DNA restriction endonuclease analysis (REA) as a genetic typing method. All strains tested by REA (with *Sma*I) had different DNA digestion patterns. Although additional DNA-rRNA hybridization analyses with *Sma*I and 16S and 23S rRNAs from *Escherichia coli* showed a reduction in the number of restriction bands to 8 to 13 hybridized fragments, the discriminative value was less when compared with that obtained by REA. The individual differences found by REA were used to analyze whether patients remained colonized by the same *Aeromonas* strain. Of 11 patients with diarrhea, 2 had a different isolate on repeat culture. In addition, one of nine tested fecal samples contained two *Aeromonas* isolates with different REA patterns. These results indicate that during diarrheal disease the intestinal tract may be colonized simultaneously with different *Aeromonas* isolates.

Bacteria belonging to the genus *Aeromonas* are gram-negative, waterborne bacteria which have been isolated from lakes, rivers, swimming pools, tap water, and other watery sources (8, 21, 25). Human disease is often related to water exposure, as in the case of water-contaminated wound infections and aspiration pneumonia (3, 5, 20, 22, 29–31). The presence of *Aeromonas* species in the water supply of hospitals has been considered as a possible source of hospital-acquired infections (1, 2, 16, 17, 19, 20, 29). Cookson et al. (2) have reported a cluster of four cases of septicemia caused by *Aeromonas* in immunocompromised patients, but the source of this clustering remained unclear. In a period of 5 weeks, Mellersh et al. (16) isolated *Aeromonas* strains from 19 patients (one patient had two isolates) shortly after they underwent an operation. Eleven of the isolates colonized the respiratory tract, but three isolates were associated with pneumonia. Other isolates were from wounds (*n* = 3), high vaginal swabs (*n* = 2), and urine (*n* = 1). A common source for these infections was not found. However, these examples indicate that discriminative typing methods are required to investigate the epidemiology of *Aeromonas*-related hospital infections.

An increasing number of studies report the possible significance of *Aeromonas* strains as enteric pathogens, and some studies suggest that enteropathogenic *Aeromonas* strains are acquired by drinking untreated natural water (6, 18). Recently, we completed a 5-year study of the clinical and bacteriological significance of various *Aeromonas* species isolated from human fecal samples. We were interested in using a typing method to determine whether the intestinal tract of patients with *Aeromonas*-associated diarrhea is simultaneously colonized with different *Aeromonas* strains. Analysis of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) profiles from cell envelopes as a phenotypic typing method and analysis of restriction patterns of chromosomal DNA as a genetic typing method have been valuable for investigating the epidemiology of disease outbreaks caused by a variety of bacterial pathogens. This prompted us to study the usefulness of these typing methods for *Aeromonas* species. Since restriction endonuclease analysis (REA) does not provide easily interpretable results, the number of restriction fragments was reduced by a DNA-rRNA hybridization with rRNA from *Escherichia coli* as a broad-spectrum nucleic acid probe (4, 28).

MATERIALS AND METHODS

Bacterial strains. *Aeromonas* strains were isolated from fecal samples by plating the samples on sheep blood agar plates containing 10 μg of ampicillin per ml. *Aeromonas* strains were phenotypically identified by the criteria of Popoff (21) and Janda et al. (9). The strains were tested for DNA relatedness to reference strains of 11 *Aeromonas* DNA hybridization groups (HGs) as described previously (10). HG-10 (*A. veronii* ATCC 35624^T) is genetically the same as HG-8 (phenotypically *A. sobria* ATCC 9071); strains belonging to one of these HGs were identified as *A. veronii* (10). Strains were maintained at −70°C in Proteose Peptone (Difco Laboratories, Detroit, Mich.) broth supplemented with 15% (vol/vol) glycerol.

A total of 46 *Aeromonas* strains isolated from patients with *Aeromonas*-related diarrhea were used for outer membrane protein (OMP) typing, 23 were used for REA typing, and 18 were used for rRNA typing. Twenty-three strains from primary and repeat fecal cultures of samples from 11 patients with *Aeromonas*-related diarrhea were examined both by OMP and REA typing: 2 patients had isolates of HG-4, 5 patients had isolates of HG-8, 2 patients had isolates of HG-1, and the HGs of 2 other patients were not determined. Three to five different colonies isolated from stool specimens of nine patients with *Aeromonas*-associated diar-

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rhea were also included: five fecal cultures had *Aeromonas* isolates of HG-4, two samples contained isolates of HG-5A, and two samples contained isolates of HG-1 or HG-2.

Isolation of cell envelopes. The isolation of cell envelopes was performed as described previously (13, 14). *Aeromonas* strains were grown to the late logarithmic phase in 30 ml of brain heart infusion broth. The cells were harvested, washed with 40 mM sodium phosphate–0.1 M NaCl (pH 7.4), and suspended in 50 mM Tris hydrochloride (pH 7.8). The suspended cells were cooled in an ice bath and sonically disrupted by four 15-s exposures (sonifier, cell disrupter B15; Branson Sonic Power Co., Danbury, Conn.). Unbroken cells were removed by centrifugation for 20 min at 2,000 × *g*. The supernatant was centrifuged for 60 min at 45,000 × *g*. The pellet that was obtained, which contained the cell envelopes, was suspended in 2 mM Tris hydrochloride (pH 7.8). The concentration of protein in the cell envelopes was determined by the method of Lowry et al. (12).

SDS-PAGE. Cell envelopes of *Aeromonas* strains were suspended to a final concentration of 1 mg of protein per ml in a buffer containing 62.5 mM Tris hydrochloride (pH 6.8), 2% (wt/vol) SDS, 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 0.001% (wt/vol) bromophenol blue and boiled for 5 min. SDS-PAGE was performed by the procedure of Lugtenberg et al. (13) with a concentration of 12% acrylamide in the running gel and a concentration of 5% in the stacking gel. Boiled samples (20 μl) containing approximately 20 μg of protein were applied to the gel and electrophoresed at a constant current of 30 mA for 4 h in a buffer containing 0.1 M Tris hydrochloride (pH 8.7), 0.77 M glycine, and 0.4% SDS. Gels were stained with 0.1% (wt/vol) Coomassie brilliant blue in 10% (vol/vol) acetic acid–25% (vol/vol) methanol–65% distilled water and destained with the same solution without Coomassie brilliant blue. We used the following molecular weight standards (Pharmacia, Uppsala, Sweden): phosphorylase *b* (94,000), albumin (67,000) ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,000), and α-lactalbumin (14,000).

Separation of inner and outer membranes. Separation of inner and outer membranes was performed as described previously (14, 24). Cell envelopes were suspended in a buffer containing 2 mM Tris hydrochloride (pH 7.8). $MgCl_2$ was added to a final concentration of 10 mM and the mixture was incubated for 1 h at 0°C. After the addition of sodium lauryl sarcosinate (final concentration, 0.5%; Sarkosyl; CIBA-GEIGY Corp., Summit, N.J.) and incubation for 1 h at 23°C, the outer membrane fraction was obtained as the pellet after ultracentrifugation for 1 h at 260,000 × *g*. This pellet was suspended in 2 mM Tris hydrochloride (pH 7.8). The supernatant was precipitated with 96% ethanol for 18 h at −20°C, and the cytoplasmic membrane fraction was obtained after centrifugation for 20 min at 20,000 × *g* and suspended in 2 mM Tris hydrochloride (pH 7.8).

Peptidoglycan-associated proteins. Peptidoglycan-associated proteins were determined as described previously (14, 23). Cell envelopes were suspended in a buffer containing 10 mM Tris hydrochloride (pH 7.8), 2% SDS, 10% (vol/vol) glycerol, and 0.7% β-mercapthoethanol and incubated for 30 min at 60°C. The pellet with peptidoglycan-associated proteins was obtained after ultracentrifugation for 30 min at 225,000 × *g*, washed once with distilled water, and suspended in 2 mM Tris hydrochloride (pH 7.8).

DNA restriction endonuclease profiles. Total cellular DNA was extracted and subjected to REA by the method described by Langenberg et al. (11).

rRNA gene restriction patterns. rRNA gene restriction patterns were examined as described previously (4, 28). After horizontal agarose electrophoresis of restricted DNA samples, denaturation of DNA was performed by incubating the gel in 0.25 M HCl for 5 min and then in 1.5 M NaCl–0.5 M NaOH for 30 min. The gel was neutralized in 0.5 M Tris hydrochloride–1.5 M NaCl (pH 7.4) for 30 min, and DNA restriction fragments were transferred to a nitrocellulose filter (pore size; 0.45 μm; BA 85; Schleicher & Schuell, Dassel, Federal Republic of Germany) by using the method of Southern as described by Maniatis et al. (15). The transfer buffer was 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate; pH 7.0). After completion of the transfer, the filter was soaked in 1× SSC for 1 min, dried, and baked at 80°C for 2 h. The filters were stored before use in the dark at 4°C in a sealed plastic bag.

The 16S and 23S RNAs from *E. coli* (catalog number 206938; Boerhinger GmbH, Mannheim, Federal Republic of Germany) were further purified by high-pressure liquid chromatography and stored with an equal volume of ethanol 96% at −20°C. Before use, 1 μg of rRNA was dried by vacuum, suspended in 100 mM Tris hydrochloride (pH 9.5), and hydrolyzed for 5 min at 90°C. After labeling of RNA with [γ-^{32}P]ATP (Dupont, NEN Research Products, Boston, Mass.) by using T_4 polynucleotide kinase (Boehringer) as described by Maniatis et al. (15), the probe was purified by column chromatography (P-60 column; Bio-Rad Laboratories, Richmond, Calif.) and assayed for radioactivity by Cerenkov counting. The specific activity of the probes was most frequently 10^7 to 10^8 cpm/μg of rRNA.

The filters were pretreated in 6× SSC–10× Denhardt solution with shaking at room temperature for 1 h. Prehybridization was performed in a buffer containing 2× SSC, 5× Denhardt solution, 0.02 M Tris hydrochloride (pH 7.4), 0.1% SDS, and 46% (vol/vol) formamide at 37°C for 2 h, corresponding to a temperature of 75°C. Hybridization was done under conditions of moderate stringency in the same buffer containing ^{32}P-labeled rRNA (10^6 to 10^7 cpm/ml of hybridization solution) at 37°C for 16 h. After hybridization, the filters were washed three times for 15 min in 2× SSC–0.1% SDS at 37°C. The hybridized bands were visualized by autoradiography on XAR film (Eastman Kodak Co., Rochester, N.Y.) at −70°C by using two intensifying screens.

RESULTS

Characteristics of OMPs of *Aeromonas* species. The composition of cell envelope proteins of *Aeromonas* species was characteristic of those of gram-negative bacteria: major bands were seen in the 25,000- to 45,000 molecular-weight range after SDS-PAGE. The patterns were not influenced by growth at 22 or 37°C in brain heart infusion broth in a shaker water bath, as was analyzed for one strain each of HG-1, HG-4, and HG-8. Changing from cultures supplied with air to standing cultures, however, resulted in both qualitative and quantitative differences in the OMP patterns. The patterns were not influenced by growth in different media (nutrient broth, brain heart infusion broth, tryptic soy broth, tryptic soy broth with 10% horse serum, and sheep blood agar plates) at 37°C, but differences were observed in some of the media when cultures were grown at 22°C. Cultures grown in brain heart infusion broth at 37°C with shaking were chosen for further study.

Extraction with Sarkosyl (CIBA-GEIGY Corp.) revealed that the major proteins remained associated with the membrane fraction, and therefore act most like OMPs. Peptidoglycan-associated proteins were not detected at 60°C.

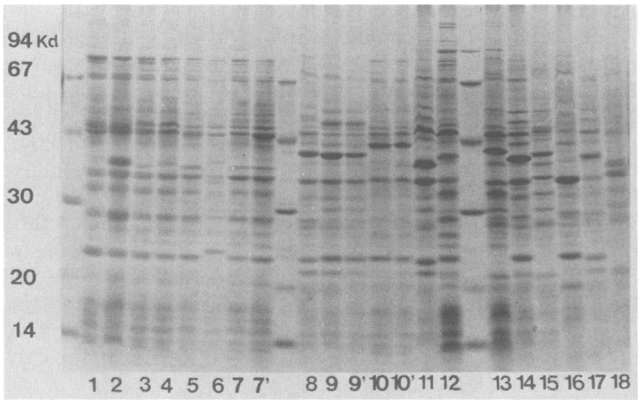

FIG. 1. SDS-PAGE profiles of 21 *Aeromonas* strains from three different hybridization groups. Lanes 13, 15, and 18, isolates of HG-1; lanes 1 to 7, isolates of HG-4; lanes 8 to 12, 14, 16, and 17, isolates of HG-8. Isolates subcultured five times were compared with the original isolates in lanes 7′ and 7, lanes 9′ and 9, and lanes 10′ and 10.

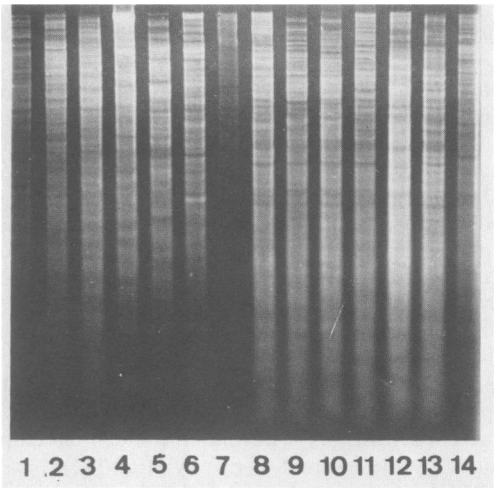

FIG. 2. DNA restriction endonuclease profiles with *Sma*I of different *Aeromonas* strains. Lanes 5, 6, and 14, isolates of HG-1; lanes 8 to 13, isolates of HG-4; lanes 1 to 4, isolates of HG-8; lane 7, DNA from *S. fonticola* cleaved by *Hin*dIII.

OMP typing of unrelated strains. Typing of OMPs from 16 isolates of HG-4 showed that at least five different patterns were distinguishable, based on minor differences in proteins of approximately 25, 36, and 45 kilodaltons (kDa). Isolates of HG-4 differed from isolates belonging to HG-1 and HG-8 by the absence of major proteins in the region between 40 and 45 kDa (Fig. 1). Almost all 20 isolates of HG-8 showed a unique OMP pattern with respect to variations of major proteins between 30 and 45 kDa. Minor differences were present in proteins of approximately 24 to 25 kDa. Ten isolates of HG-1 also demonstrated unique patterns of the OMP proteins between 30 and 45 kDa. All isolates of HG-1 lacked an OMP of 24 to 25 kDa, which was always present in isolates of HG-4 and HG-8.

OMP typing of disease-related isolates. The SDS-PAGE profiles of *Aeromonas* strains that were isolated from primary and repeat cultures of fecal samples from 11 patients showed that 2 patients had different *Aeromonas* isolates in their second (patient A-127) or third (patient A-196) fecal culture when compared with their first culture. The profiles of *Aeromonas* isolates from patients A-58 and A-203 were indistinguishable from one another; both isolates belonged to HG-8. Patient A-97 developed diarrhea after surfing in surface water; *Aeromonas* strains that were isolated from water samples were clearly different from those isolated from the patient. Another patient (not included in Table 1) developed diarrhea after swimming in surface water; *Aeromonas* strains isolated from water samples differed from those isolated from the patient.

Three to five different *Aeromonas* colonies were isolated from the primary culture plate of nine fecal samples and subsequently were used to prepare cell envelopes. The SDS-PAGE patterns of *Aeromonas* isolates from eight patients were identical. Two different isolates were found in the fecal sample of the ninth patient.

DNA restriction endonuclease profiles. *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Sau*3A, *Apa*I, *Bam*HI, and *Xba*I (Boehringer); *Acc*I (Pharmacia); *Bss*HII, *Cla*I, and *Sph*HI (New England BioLabs, Inc., Beverly, Mass.); and *Kpn*I, *Sst*II, *Sal*I, *Sma*I, and *Pst*I (Bethesda Research Laboratories, Gaithersburg, Md.) were tested. After digestion with *Sma*I and *Pst*I, sufficient banding was seen to distinguish 23 tested *Aeromonas* strains (5 isolates of HG-1, 8 isolates of HG-8, and 10 isolates of HG-4) from one another, since each strain showed a unique pattern. The differences were most pronounced in the high-molecular-weight fractions of the gels. The best DNA digestive patterns were obtained with *Sma*I (Fig. 2). *Pst*I was unable to digest 15% of the tested strains. The restriction patterns were reproducible; when DNA was extracted from two strains which were subcultured five times, the patterns were identical. Table 1 shows the results of REA typing compared with the results of OMP typing, phenotypic identification, and hybridization studies of isolates from 18 patients with *Aeromonas*-associated diarrhea. REA typing was not able to distinguish HGs 1, 4, and 8 from one another, in contrast to OMP typing. Biotyping did not clearly discriminate between HG-8 and HG-1.

REA of *Aeromonas* isolates that were isolated from primary and repeat fecal cultures showed that 2 of 11 patients

TABLE 1. Results of OMP, REA, and rRNA typing of clinical *Aeromonas* isolates

Patient	HG[a]	Phenotypic identification	Type by:		
			OMP	REA	rRNA
A-23	4	*A. caviae*	I_a	A	R1
A-76	4	*A. caviae*	I_b	B	NT[b]
A-133	4	*A. caviae*	I_b	C	NT
A-181	4	*A. caviae*	I_c	D	R2
A-182	4	*A. caviae*	I_d	E	R3
A-277	4	*A. caviae*	I_a	F	NT
A-278	4	*A. caviae*	I_b	G	NT
A-21	8	*A. veronii*	II_a	H	R4
A-58	8	*A. hydrophila*	II_b	I	NT
A-114	8	*A. hydrophila*	II_c	J	NT
A-122	8	*A. veronii*	II_d	K	R5
A-196	8	*A. veronii*	II_e	L	R6
A-203	8	*A. hydrophila*	II_b	M	NT
A-224	8	*A. veronii*	II_f	N	R7
A-97	1	*A. hydrophila*	III_a	O	R8
A-127	1	*A. hydrophila*	III_b	P	NT
A-176	1	*A. hydrophila*	III_c	Q	R9
A-274	1	*A. hydrophila*	III_d	R	NT

[a] The HGs of *Aeromonas* species are as follows: HG-1 is *A. hydrophila*, HG-4 is *A. caviae*, and HG-8 is *A. veronii*.
[b] NT, Not typed.

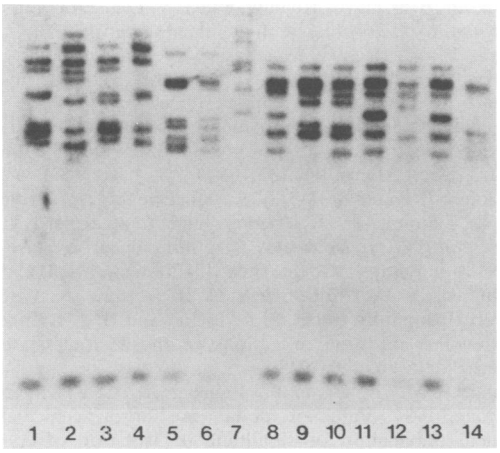

FIG. 3. rRNA gene restriction patterns (using *Sma*I) of different *Aeromonas* strains. The lanes contained the same *Aeromonas* strains as described in the legend to Fig. 2.

had obtained other *Aeromonas* isolates. These results corresponded with the results of the OMP typing. However, DNA restriction profiles of isolates from patients A-58 and A-203 were unique, whereas the SDS-PAGE patterns for these two isolates were identical.

REA of three to five different *Aeromonas* colonies from nine fecal samples showed that in one of these samples two isolates with different DNA restriction fragments were present. These findings correlated with the OMP typing data.

rRNA gene restriction patterns. DNA-rRNA hybridization for *Aeromonas* species at 75°C under conditions of moderate stringency showed 8 to 13 restriction fragments reacting with *E. coli* 16S and 23S rRNAs after cleavage with *Sma*I or *Pst*I (Fig. 3). As a control, the DNA of *Serratia fonticola* cleaved by *Hin*dIII gave seven fragments carrying rRNA gene sequences. The differences were most pronounced in the high-molecular-weight bands. Of 18 strains (5 isolates of HG-1, 6 isolates of HG-8, and 7 isolates of HG-4) that were analyzed after digestion with *Sma*I, three isolates of HG-4 (Fig. 3, lanes 8, 11, and 13) were identical to one another. However, the whole-cell DNA digest profiles (with *Sma*I) of these isolates were different (Fig. 2, lanes 8, 11, and 13). DNA-rRNA hybridization with *Pst*I as the restriction enzyme also showed remarkable differences between these isolates (data not shown). Although only strains of nine patients mentioned in Table 1 were subjected to rRNA typing, the results corresponded with the results of OMP and REA typing.

DISCUSSION

Reports of clusters of cases of *Aeromonas* hospital infections have necessitated the development of typing methods to monitor nosocomial acquisition of the organism (2, 17, 19, 20). Several methods have been developed for typing *Aeromonas* strains, including serogrouping, biotyping, esterase electrophoresis, and PAGE of radiolabeled cell proteins (2, 20, 27, 29). Biotyping could only discriminate four biotypes (2), and serotyping showed that 72% of the strains was untypable (29). Electrophoretic esterase typing revealed 44 zymotypes among 141 *Aeromonas* strains (20), and typing by SDS-PAGE of [^{35}S]methionine-labeled bacteria was able to distinguish 63 strains from one another (27). Moreover, these methods detect phenotypic variation which may not be related to genetic variation in the strains that were investigated. We therefore compared OMP profiles as a phenotypic typing method with chromosomal DNA restriction endonuclease profiles as a genetic typing method.

For SDS-PAGE studies we cultured *Aeromonas* strains in brain heart infusion broth at 37°C with shaking, since OMP profiles were influenced by differences in temperature and in the air supply of the bacterial cultures. These findings agree with the results of Statner et al. (26), who detected temperature-dependent differences in 13 of 19 *Aeromonas* strains that they tested. It was possible to identify isolates of HG-1 (*A. hydrophila*), HG-4 (*A. caviae*), and HG-8 (*A. veronii*) by their OMP profiles. The most discriminative proteins were present in the Sarkosyl extract of the cell envelopes, which probably contains all OMPs. Within the hybridization groups, almost every isolate of HG-1 and HG-8 had a unique pattern, in contrast to isolates belonging to HG-4, which were distributed in five patterns. It is not known whether OMP typing or other typing methods may help to identify all 11 DNA hybridization groups hitherto known. This may be of importance, because our ability to identify *Aeromonas* hybridization groups phenotypically is not sufficiently specific (10). The results of Stephenson et al. (27) suggest that SDS-PAGE of [^{35}S]methionine-labeled bacteria may also help to distinguish taxonomic subgroups within *Aeromonas* species. Unfortunately, their results were not compared with DNA hybridization groups of *Aeromonas* strains.

Whole-cell DNA restriction endonuclease profiles distinguished more *Aeromonas* strains from one another than OMP typing did, since each strain that was tested showed a unique DNA digestion pattern by *Sma*I or *Pst*I. Restriction profiles do not provide easily interpretable results because of the large number of bands, and computer-assisted laser-scanning densitometers are needed to define the more complex individual hybridization groups. The use of a broad-spectrum nucleic acid probe may reduce the number of bands obtained after restriction endonuclease digestion (4, 28). Since rRNAs are ubiquitous and extremely conserved molecules, different species contain identical rRNA operons in several copies in the bacterial chromosome (4, 28). Grimont and Grimont (4) tested 41 different species and found each species to contain a specific pattern of rRNA gene restriction fragments. Their observation that isolates of one species sometimes showed more than one pattern prompted us to use 16S and 23S RNAs from *E. coli* as probes for *Aeromonas* strains. Although we tested only 18 *Aeromonas* strains, DNA-rRNA hybridization was able to distinquish all strains when two restriction enzymes were used. The patterns were easy to interpret. Since rRNA gene restriction patterns may be useful in taxonomy and identification of bacteria, the relationship of these patterns with hybridization groups of *Aeromonas* strains needs further investigation.

OMP and REA typing done on strains isolated from patients with *Aeromonas*-associated diarrhea revealed the presence of two isolates with different OMP and REA profiles in one of nine patients. Simultaneously, recovery of various *Aeromonas* isolates from human feces is apparently rare. These findings confirm earlier observations on human samples (7), but are in contrast with the occurrence of different *Aeromonas* strains in water samples when examined by electrophoretic esterase typing (zymotyping) (20). Picard and Goullet (20) found many different zymotypes in strains coexisting in the hospital water supply. Since the zymotypes most frequently encountered in the water samples were not isolated from the patients with *Aeromonas*-

associated infections, their results suggest that zymotypes may differ in pathogenicity. *Aeromonas*-related diarrhea has been associated with the drinking of untreated water and also with swimming or wading in freshwater ponds or streams (6). We found many different *Aeromonas* strains in samples from surface water using OMP typing and DNA digestion patterns (data not shown). We were unable to establish a correlation between the bacteria from two patients who developed diarrhea after surfing and swimming in surface water and the bacteria isolated from that water source.

In 9 of 11 patients, a repeat fecal culture contained an *Aeromonas* isolate that was identical to the isolate of the primary culture. This was particularly noteworthy in one patient, since the interval between the first and last culture was 60 days. This 3-month-old female (A-76) had a moderate disturbance of liver functions and suffered from chronic diarrhea. Although the *Aeromonas* strain (HG-4) was isolated in a heavy growth from all cultures and was considered of clinical significance, the patient was not treated with antibiotics and recovered spontaneously. Of 11 patients, 2 contained a different *Aeromonas* isolate on repeat fecal culture. A 16-month-old male (A-127) developed *Aeromonas*-associated diarrhea (HG-1) while he was being treated for acute otitis media with amoxicillin, to which *Aeromonas* is usually resistant. Amoxicillin was substituted with cotrimoxazole, and diarrhea ceased within 1 week. A second sample that was cultured, taken after 10 days, was negative for *Aeromonas* strains, but a third sample that was cultured, taken after 21 days, contained a different nonenteropathogenic *Aeromonas* isolate. Another patient, a 1-year-old female (A-196), developed acute gastroenteritis associated with *Salmonella typhimurium*. The disease lasted for 1 week. An *Aeromonas* isolate (HG-8) was cultured from the same fecal specimen and remained present in the second fecal sample 4 days later. However, after 14 days a third culture contained another *Aeromonas* isolate, which also differed biochemically from the first isolate in the fermentation of raffinose. These two patients may represent patients who are colonized with different *Aeromonas* strains in a predisposed intestinal tract. We have previously shown that 66 of 169 (39%) *Aeromonas* strains were isolated from patients who had a mixed infection, from patients who had an underlying disease, and from patients who used prior medication which could predispose their intestinal tracts to colonization with *Aeromonas* strains (E. J. Kuijper, P. Bol, M. F. Peeters, Arnold G. Steigerwalt, H. C. Zanen, and D. J. Brenner, J. Clin. Microbiol., in press).

In conclusion, our data suggest that REA of whole-cell DNA is a valuable method for typing of *Aeromonas* strains. The applications of rRNA gene restriction patterns and OMP typing need further investigation for identifying more specifically all 11 *Aeromonas* hybridization groups.

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