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Laboratory Investigations on the Low Pathogenic Potential of *Plesiomonas shigelloides*

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The pathogenic properties of 16 *Plesiomonas shigelloides* strains recovered from humans with extraintestinal and intestinal illnesses, infected animals, and environmental sources were investigated. Most strains possessed a high cell charge and low surface hydrophobicity analogous to those of *Shigella* spp.; additionally, serogroup O:17 strains reacted with *Shigella* group D antisera. However, unlike the shigellae, *P. shigelloides* strains did not universally bind Congo red, were noninvasive in HEp-2 cell assays, and did not produce a Shiga-like toxin on Vero cells. On HEp-2, Y1, and possibly Vero cells, a low-level cytolysin was consistently produced by all 16 *P. shigelloides* strains when grown in either Evan Casamino Acids-yeast extract or Penassay broth. The median 50% lethal dose for all 16 *P. shigelloides* strains in outbred Swiss Webster mice was 3.5×10^8 CFU (range, 3.2×10^7 to $>1 \times 10^9$ CFU). Animal pathogenicity did not correlate with cytolysin expression, possession of a ≥120-MDa plasmid, protein profile, or resistance to complement-mediated lysis. No strain analyzed produced siderophores or a heat-stable enterotoxin. The results suggest that members of the genus *Plesiomonas* have an overall low pathogenic potential, irrespective of the site of isolation or phenotypic, serologic, or surface properties shared with other traditional enteropathogens.

Plesiomonas shigelloides is a facultatively anaerobic rod that has commonly been associated with sporadic episodes and rare outbreaks of diarrheal disease in humans (3). Recently, an increasing number of extraintestinal infections attributed to this bacterium have been reported and include such illnesses as bacteremia, meningitis, cholecystitis, osteomyelitis, and pseudoappendicitis (1, 3, 7, 18, 26). In the case of neonatal meningitis, fatality rates approach 80%. Although predisposing factors leading to disseminated *P. shigelloides* infections are at present poorly defined, humans with conditions leading to an impaired immune function are thought to be at increased risk of developing systemic disease.

Few studies have addressed the issue of the relative pathogenicity of individual *Plesiomonas* strains (17). Investigations on the virulence potential of plesiomonads have been further complicated by the difficulty in obtaining isolates recovered from patients with systemic infections, failure of previous studies to consistently detect potential virulence-associated factors, and the lack of relevant animal models to assess various aspects of pathogenicity. Over the past several years, a number of reputed virulence-associated factors have been described which include a cholera toxin-like activity (8), a heat-stable enterotoxin (16), and a large plasmid (>120 MDa) which may facilitate the uptake or invasion of *P. shigelloides* in the gastrointestinal tract (10). In view of these recent findings, we undertook a study of a collection of plesiomonads from both clinical (localized and systemic infections) and environmental sources to evaluate the relative virulence of individual isolates and to expand the number of investigations aimed at identifying potential pathogenic mechanisms in this group of bacteria.

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MATERIALS AND METHODS

Bacterial strains. Sixteen strains of *P. shigelloides* were investigated in this study and are listed in Table 1; all isolates were identified as *P. shigelloides* by standard morphologic and biochemical criteria (12). The following strains were received from the indicated sources; 2599-78, 1776-81, and 2447-88, F. W. Hickman-Brenner, Centers for Disease Control; PS-3 and PS-4, E. Falsen, University of Goteborg, Goteborg, Sweden (4); PS-16, K. Harrington, Louisiana State University; 801-3, R. S. Nolte, University of Rochester (18); and 14029^T, American Type Culture Collection, Rockville, Md. All other *Plesiomonas* strains originated from the Microbial Diseases Laboratory or from the personal collection of one of us (J.M.J.). Working cultures of each strain were maintained on beef extract agar slants at ambient temperatures during the course of this study.

Surface characteristics. All *P. shigelloides* strains were evaluated for their reactivity against phase I (smooth) and phase II (rough) group D (*Shigella sonnei*) antisera; the specific serovar of each isolate, based on the presence of somatic (O) and flagellar (H) antigens (22), was kindly provided by T. Shimada and R. Sakazaki (National Institutes of Health, Tokyo, Japan). The relative surface hydrophobicity for each strain was determined from the results of four separate assays which included autoagglutination in brain heart infusion broth, colonial uptake of crystal violet dye, binding of intact bacteria to octyl-Sepharose CL-4B (hydrophobic interaction chromatography), and aggregation of plesiomonads in various molarities of ammonium sulfate (salt aggregation test); the procedures used for these assays have been described in detail elsewhere (13, 20). Of the 16 *P. shigelloides* isolates, 5 (PS-3, PS-11, PS-16, PS-28, and PS-33) were evaluated for cell surface adhesins in hemagglutination assays by using the rock tile test with rabbit, horse, chicken, goose, sheep, and human O erythrocytes; *Escherichia coli* LY 72 (O4:H1) and LY 91 (O6:H31) served as positive controls for these studies.

Congo red binding and siderophore production. The ability

of *Plesiomonas* strains to bind Congo red was determined on CRAMP agar as described previously (20). Siderophore production by *P. shigelloides* was evaluated by the method of Schwyn and Neilands (22) by using Chrome Azurol S (CAS) agar. Both assays were performed at 35°C, with final readings taken after 72 h of incubation.

Serum resistance. The susceptibility of individual plesiomonads to the complement-mediated lysis of 65% pooled human serum (PHS) was quantitatively evaluated as described previously (20); inoculum concentrations for each strain in this serumcidal assay ranged from 5×10^6 to 3×10^7 CFU; the endpoint for each determination was 120 min. Serum resistance was defined by the criteria of Taylor (24).

Toxin assays. The ability of plesiomonads to elaborate an extracellular toxin(s) was investigated as follows. Individual strains were inoculated into tubes of Evan Casamino Acids-yeast extract broth (CAYE) and incubated statically for 18 to 20 h at 35°C. Sterilized cell-free supernatants from each *P. shigelloides* strain were then serially diluted in the appropriate tissue culture medium before inoculation onto confluent monolayers. Mouse Y1 adrenal (courtesy of F. Knoop, Creighton University) and HEp-2 (obtained from S. Falkow, Stanford University) cells were propagated in RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.) and CHO cells (American Type Culture Collection) were propagated in nutrient Ham F-12 medium; both media were supplemented with 10% fetal bovine serum, 1% L-glutamine, and antibiotics. For toxin assays, cells were seeded into 96-well flat-bottom tissue culture plates, and monolayers were visually observed at 24 h postinoculation for evidence of toxin-like activity; the cell-free supernatant from a hemolytic (enterotoxigenic) strain of *Aeromonas hydrophila* (AH-25) served as a positive control for these experiments. In addition to the cell lines described above, *P. shigelloides* supernatants were additionally screened for Shiga-like toxin activity on Vero cells propagated in medium 199 (Sigma) supplemented in 10% fetal bovine serum–1% L-glutamine–antibiotics.

To characterize toxin-like effects on epithelial cell monolayers further, HEp-2 cells were seeded into 24-well Falcon plates and isotopically labeled with 5 μCi of [5-^{3}H]uridine (ICN Biomedicals, Costa Mesa, Calif.) per well. After overnight incubation, the resulting semiconfluent monolayers were washed three times in Hanks balanced salt solution and then subsequently overlayed with various dilutions of the cell-free toxigenic supernatant diluted in RPMI 1640 medium without antibiotics. At various intervals, an aliquot of this supernatant was removed and mixed with Econolume (ICN Biomedicals) and then counted in a Beckman model LS 3801 liquid scintillation counter. Results are expressed as the percentage of the total incorporated isotope released minus that released by the control (no toxin).

Cholera toxin-like activity. The ability of selected *P. shigelloides* strains to produce a cholera toxin-like toxin was evaluated by several different methods. Cell-free supernatants from Evan CAYE-grown cultures were coincubated with Y1 adrenal monolayers and monitored for the characteristic cytotonic rounding of cells typically observed when cells are exposed to the heat-labile cholera toxin. The toxin activity seen on Y1 cells was then retested and preincubated with anti-cholera toxin (Swiss Serum Institute, Bern, Switzerland) to determine whether such activity was neutralizable. CAYE supernatants were also tested for cholera toxin-like activity in the Vet-RPLA latex agglutination assay by using beads that were presensitized with rabbit immunoglobulin G anti-cholera toxin. Finally, all 16 *Plesiomonas* strains were probed for homologous sequences to the cholera toxin in colony dot hybridization assays by using the ^{32}P-labeled ctx-A11 probe of Lockman et al. (15) (courtesy of C. Kaysner, Food and Drug Administration, Bothell, Wash.). *Vibrio cholerae* 569B served as a positive control for each of the experiments described above.

HEp-2 cell invasion. HEp-2 cells were propagated in eight-chamber tissue culture slides (Lab Tek; Miles Laboratories, Naperville, Ill.) with antibiotic-free RPMI 1640 medium. A 200-μl portion containing 2×10^6 to 6×10^6 CFU of brain heart infusion broth-grown (18 to 20 h) bacteria appropriately resuspended in phosphate-buffered saline and diluted in RPMI 1640 medium was then inoculated into a single chamber of a tissue culture slide containing an almost confluent monolayer of HEp-2 cells that were previously washed three times with sterile phosphate-buffered saline. The inoculum was allowed to coincubate with HEp-2 cells for 90 min, after which the residual inoculum was aspirated, monolayers were washed 10 times in phosphate-buffered saline, and then 300 μl of RPMI 1640 medium containing 300 μg of lysozyme per ml and 50 μg of gentamicin per ml was added to each well. The HEp-2 monolayers were then reincubated in a 5% CO_2 atmosphere at 35°C for approximately 3 h, after which the maintenance medium was removed and monolayers were washed (five times in phosphate-buffered saline) and then fixed with methanol prior to Giemsa staining. Several strains of *Shigella dysenteriae* and *Yersinia enterocolitica* O3 served as positive controls for these investigations.

Suckling mice. To detect the heat-stable enterotoxins potentially produced by *P. shigelloides*, cell-free supernatants of overnight cultures of each strain were assayed in the suckling mouse model of Dean et al. (5). A minimum of three mice were used for each experiment, and a total intestinal weight/body weight ratio of 0.08 or greater was considered positive. *E. coli* 10407 and 10405 served as the heat-stable enterotoxin-positive and heat-stable enterotoxin-negative controls, respectively.

Electrophoresis. Bacterial strains were screened for the presence of plasmids on 0.75% agarose gels as described previously (20). *Plesiomonas* lysates were horizontally electrophoresed at 100 V for 2.5 h prior to ethidium bromide staining and visualization under UV light. Protein profiles of selected *P. shigelloides* strains were determined by electrophoresis of whole-cell extracts in 10% sodium dodecyl sulfate-polyacrylamide gels as described previously (20).

Studies of 50% lethal dose (LD_{50}). Each *Plesiomonas* strain was inoculated intraperitoneally into Swiss Webster mice (age, 10 to 15 weeks) as described previously (20). Infected mice were monitored for 1 week postinoculation, and deaths were recorded daily. Saline-inoculated mice served as negative controls.

RESULTS

Surface characteristics. Our initial analysis of the 16 *P. shigelloides* isolates involved characterization of the surface properties of each strain (Table 1). These studies included serologic characterization of each strain, since one recent report (18) suggested that shared O antigens between *P. shigelloides* and *Shigella* spp. might represent a virulence-associated property in plesiomonads. Of the 16 strains studied, 5 (31%) reacted (3+ to 4+) with *S. sonnei* (group D) antisera; four of these strains were later identified as belonging to somatic type O17, a previously recognized serogroup sharing an antigenic relationship with shigellae (23). The only other strong serologic cross-reaction observed was

TABLE 1. Surface characteristics of *P. shigelloides* strains

Strain	Source	Serovar	Reaction with *Shigella* group D	Cell surface:	
				Hydrophobicity[a]	Charge[b]
14029	Unknown	O17:H2	+	−	+++
PS-3	Gall bladder	O3:H2	−	+++	+++
PS-4	Aquarium water	O8:H5	−	−	+++
PS-10	Feces	O17:H2	+	−	+++
PS-11	Vaginal secretion	OUK:HNM[c]	−	−	+++
PS-12	Feces	OUK:H3	−	−	+++
PS-16	Pelican	O44:HUK	−	−	+++
PS-28	Feces	O17:HNM	+	−	+++
PS-29	Feces	OUK:H2	−		+
PS-32	Feces	O24:H8	−	−	+++
PS-33	Seal	R:H2	+	++	+++
PS-36	Lake water	O23:H1a1c	−	−	+++
801-3	Blood	O17:H2	+	−	+++
1776-81	Blood	O32:H16	−	−	+++
2447-88	Blood	O27:HUK	−	+	−
2599-78	Blood	R:HNM	−	−	+++

[a] Cumulative results from autoagglutination, crystal violet dye, hydrophobic interaction chromatography, and salt aggregation. Criteria for positive reactions are as follows: autoagglutination, ≥60% reduction in turbidity; crystal violet dye, strong uptake dye complex; hydrophobic interaction chromatography, ≥50% retention on column; salt aggregation, aggregation in ≥2.0 M $(NH_4)SO_4$. Each + indicates a positive reaction in a hydrophobic assay.

[b] +++, ≥90% retention; ++, 75.90% retention, +, 50 to 75% retention on DEAE-cellulose columns (20).

[c] UK, Unknown type; NM, nonmotile.

TABLE 2. Potential virulence-associated properties of *P. shigelloides*

Strain	Serovar	Crb[a]	Serum resistance[b]	Cytotoxin[c]	Plasmid of ≥120 MDa	Mouse virulence[d]
801-3	O17:H2	++	+0.2	+	−	7.5
PS-16	O44:HUK[e]	−	−1.2	+	−	7.9
PS-32	O24:H8	−	+0.6	+	+	7.9
PS-28	O17:HNM[f]	++	+0.9	+	−	8.3
1776-81	O32:H16	++	−4.5	+	−	8.4
PS-3	O3:H2	++	−5.1	+	−	8.4
PS-12	OUK:H3	++	−0.9	+	+	8.5
14029	O17:H2	+	−0.6	+	−	8.5
PS-4	O8:H5	−	−3.5	+	+	8.6
PS-29	OUK:H2	+	+0.7	+	−	8.6
PS-33	R:H2	−	−5.0	+	+	8.7
PS-10	O17:H2	+	−2.5	+	−	8.8
PS-36	O23:H1a1c	++	+0.5	+	+	8.8
PS-11	OUK:HNM	++	−2.2	+	+	>9.0
2447-88	O27:HUK	+	−0.5	+	−	>9.0
2599-78	R:HNM	ND[g]	+0.6	+	−	>9.0

[a] Crb, Congo red binding.

[b] Gain (+) or decrease (−) in $\log_{10}$ CFU of indicated strain when challenged for 120 min with 65% PHS.

[c] As detected on Y1, HEp-2, and Vero cell lines.

[d] LD_{50} ($\log_{10}$) in outbred Swiss Webster mice.

[e] UK, Unknown.

[f] NM, Nonmotile.

[g] ND, Not determined.

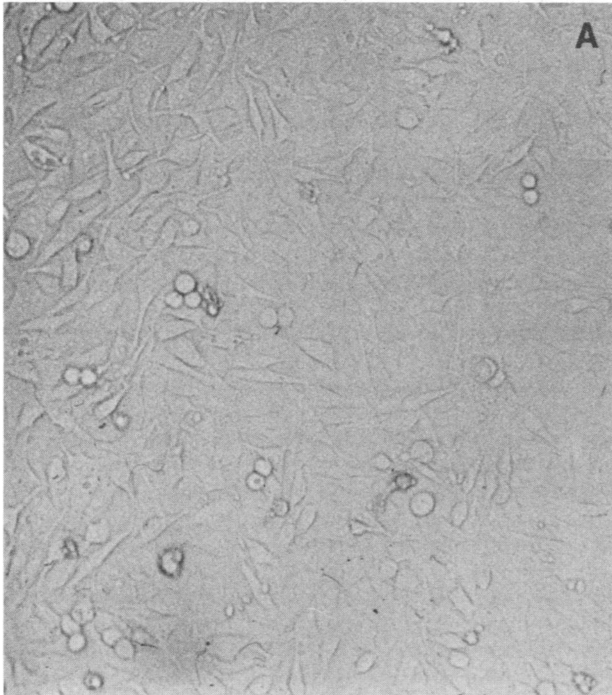
between strain 1776-81 (O32) and polyvalent group B *Shigella flexneri* sera (negative in B-type and subtype sera). Excluding O17 strains, no other somatic group was recovered on more than one occasion; three strains (16%) represented somatic groups whose O antigens have not as yet been defined (typing scheme of Shimada and Sakazaki [23]). Among flagellar antigens, H2 predominated (38%); three other isolates were found to be nonmotile.

Of the 16 *P. shigelloides* strains, 12 (75%) exhibited strong surface charges and weak hydrophobicities; two strains (PS-3, PS-33) were moderately to strongly hydrophobic with a high cell surface charge. Strains PS-29 and 2447-78 displayed no strong tendencies in either assay. There was no observable association between either of these properties and serovar, although several groups were represented by only a single isolate. Five of the *P. shigelloides* strains evaluated in this investigation, including three other isolates not described in this report, failed to exhibit adhesins that were capable of agglutinating erthrocytes in the rock tile test.

Toxin assays and virulence-associated properties. In addition to the surface characteristics, the 16 *Plesiomonas* strains were further evaluated for a number of properties potentially associated with pathogenicity and are listed in Table 2. Most strains bound Congo red, although to varying degrees; this property did not appear to relate directly to overt surface hydrophocity. Of the 16 strains screened, 6 (38%) were found to be serum resistant when challenged against 65% PHS; these strains showed quantitative increases in CFU within 2 h of incubation in PHS. Of the 10 remaining isolates, a wide variation in serum susceptibility was noted, with individual decreases in CFU ranging from a 3- to a greater than 100,000-fold reduction in viable numbers.

Interestingly, at 24 h all 16 *P. shigelloides* strains tested were found to produce a low-level, cell-free cytolysin (1:2 to 1:16) which was active on HEp-2 and Y1 cell monolayers but not on CHO cell monolayers. When susceptible cell lines were exposed to this cytolysin, toxic effects were readily observable within 24 h postinoculation. Cells became rounded with internal granulation prior to eventual lysis (Fig. 1). Although this cytolysin was always expressed, the amount produced from each static culture varied considerably. This low yield was similarly demonstrated by the slow release (20 h) of [^{3}H]uridine from the cytosol of labeled HEp-2 cells after exposure to the most concentrated form of the toxin (1:2 dilution) when compared with that released from a more dilute cell-free supernatant of *A. hydrophila* containing the beta-hemolytic cytolysin (Fig. 2). This cytolysin was not inactivated by various heat treatments (100°C for 10 min or 56°C for 30 min) or by a number of nonspecific proteases (proteinase K, pronase). Coincubation of the cytolysin in the presence of 30 μg of human glycophorin failed to inhibit its activity on susceptible cell lines; the cytolysin was further tested against erythrocytes of various animals without any observable effect.

In contrast to these findings, we found no evidence for invasion of eucaryotic cells by any plesiomonads, as has been reported previously (2), even though we varied the length of postinfection incubation (3 to 17 h) while using both visual (cytologic) and qualitative (Triton X-100 lysis) methods for detection of gentamicin-resistant progeny; no strain was found to produce a heat-stable enterotoxin by the suckling mouse assay or siderophores in the CAS assay. Of the 16 strains studied, 10 (63%) harbored one or more low-molecular-mass plasmids ranging from 2 to 8 MDa; 6 strains were also found to possess a high-molecular-mass plasmid of approximately ≥120 MDa which has previously been reported by other investigators (10). The relative virulences of all 16 plesiomonads were determined by perform-



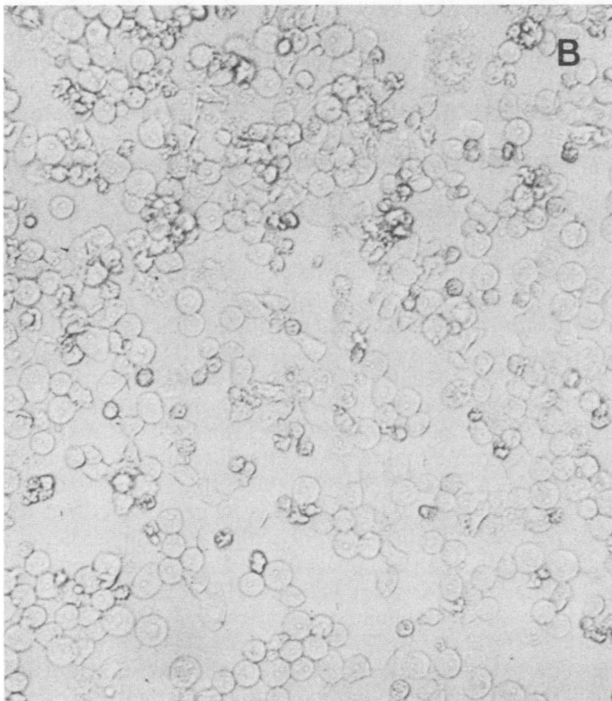


FIG. 1. Effect of *Plesiomonas* cytolysin on HEp-2 cells at 24 h. (A) Control cells (no toxin); (B) HEp-2 cells exposed to cytolysin showing rounding and granulation.

ing LD_{50} studies in outbred mice. LD_{50}s ranged from 3.2×10^7 to greater than 1×10^9 CFU per strain (mean, 3.2×10^8 CFU) (Table 2). The relative avirulence of this group was further confirmed by the slower rate that *P. shigelloides* strains killed mice compared with the rate that *Aeromonas* spp. killed mice (Fig. 3). No differences in death rates were

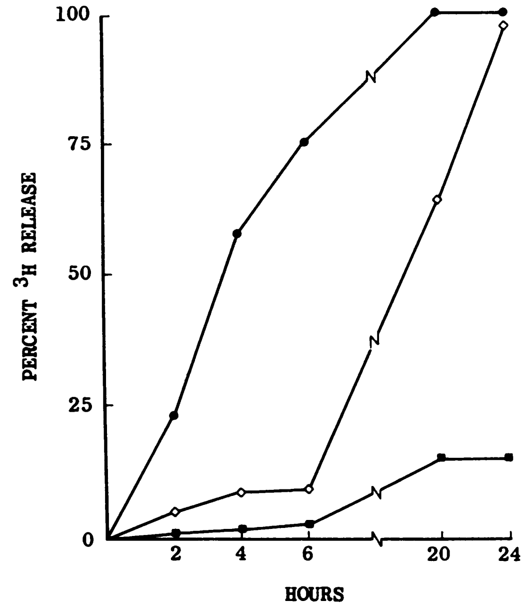


FIG. 2. Release of [^{3}H]uridine from isotopically labeled HEp-2 cells. ●, *A. hydrophila* cytolysin, 1:10 dilution; ◇, *P. shigelloides* cytolysin, 1:2 dilution; ■, *P. shigelloides* cytolysin, 1:8 dilution. Percent release = [cpm of ^{3}H released (toxin) − cpm of ^{3}H from the control (no toxin/total cellular incorporated cpm)] × 100.

observed among the *A. hydrophila*, *A. sobria*, and *A. caviae* strains tested. In these instances, only 53% of the fatally infected animals inoculated with *P. shigelloides* died within 24 h of intraperitoneal injection, in contrast to the 93% death rate observed in mice infected with *Aeromonas* spp. The slower kinetics observed in mice with *P. shigelloides* infection required twice as much time (4 days) for all fatally infected mice to succumb as opposed to that required for mice with *Aeromonas* spp. infections. The whole-cell protein profiles of both virulent and avirulent plesiomonads

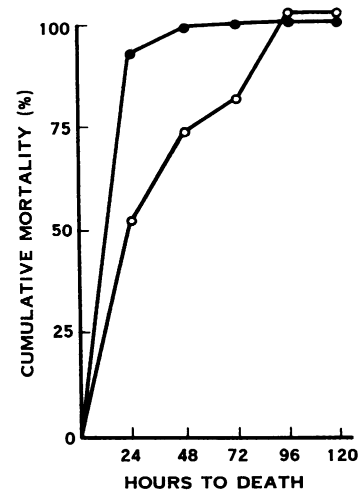


FIG. 3. Cumulative LD_{50} data for 16 strains each of *P. shigelloides* (○) and *Aeromonas* spp. (*A. hydrophila*, *n* = 6; *A. sobria*, *n* = 5; *A. caviae*, *n* = 5) (●) inoculated into Swiss Webster mice. Results are expressed as [the cumulative number of mice succumbing to infection (daily)/total number dead (day 7)] × 100.

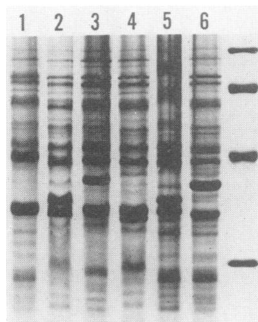


FIG. 4. Polyacrylamide gel electrophoresis (12%) of whole-cell protein profiles of the following *P. shigelloides* strains with various pathogenicities: 801-3 (lane 1), PS-16 (lane 2), PS-12 (lane 3), PS-11 (lane 4), 2447-88 (lane 5), 2599-78 (lane 6). Unmarked lane, Protein standards.

were remarkably similar (Fig. 4). Each strain contained at least two or more major proteins, as determined by polyacrylamide gel electrophoresis, in the 60- to 62-, 59-, and 44- to 46-kDa regions. No major differences in protein profiles could be detected that could potentially account for the pathogenicity. We found no direct correlation between LD_{50}s for individual strains, serum resistance, Congo red binding, presence of a high-molecular-mass plasmid, or semiquantitative expression of cell-free cytolysin.

Finally, because of a recent report of cholera toxin-like activity in a large number of *Plesiomonas* strains (8), we selected a number of our cultures for further analysis. None of the eight strains listed in Table 3 produced morphologic changes in Y1 cells consistent with cholera toxin activity, elaborated immunologically related products, or contained genetic sequences compatible with such a heat-labile enterotoxin. Cytotoxic effects previously noted on Y1 cells failed to be neutralized by preincubation of such factors with anti-cholera toxin antibodies. To rule out the possibility that this was a potentially iron-regulated toxin, we screened these same eight strains grown in syncase broth (iron depleted) on CHO cells. No biologic activity similar to that of cholera toxin-like CHO cell elongation was observed.

DISCUSSION

Plesiomonads share a number of features with *Shigella* spp., including biochemical similarities, serologic cross-reactivities, Congo red binding, and low hydrophobicity but high cell surface charge (9, 14, 21). The high cell surface charge, which is postulated to mediate attachment to negatively charged epithelial cell surfaces via calcium bridges in *Shigella* spp. and *Yersinia enterocolitica*, may function similarly in *P. shigelloides*, since fimbriae, cell-associated hemagglutinins, and other afimbrial attachment structures have not been identified to date. Unlike shigellae, however, the results of this study suggest that *P. shigelloides* has a relatively low pathogenic potential for humans, irrespective of the geographic source, anatomic site of isolation, or specific serotype of individual strains. Although some studies have alluded to the fact that plesiomonads possess some virulence markers associated with *Shigella* spp. (reactive lipopolysaccharide epitopes, high-molecular-mass plasmids), the evidence presented in this report does not link either characteristic predominantly with strains recovered from patients with invasive disease or documented infections or with overt pathogenicity in mice. In addition, plesiomonads were not found to elaborate invasins or to produce Shiga-like toxins, two determinants that are directly linked to the overall pathogenicity of *Shigella* spp. (9). These facts suggest that these two groups, despite some phenotypic similarities, do not possess common pathogenic mechanisms, nor do they have comparable virulence potentials for humans. Similar observations have recently been reported by Olsvik et al. (19).

The most striking findings regarding the potential virulence-associated properties of *P. shigelloides* concerned serum resistance and cytotoxin production. Approximately 40% of the *P. shigelloides* strains tested were resistant to the complement-mediated bactericidal activity of normal pooled serum; of note is the fact that 3 of the 4 (75%) most virulent strains, as judged by LD_{50} analysis, were serum resistant, whereas only 3 of the remaining 13 (23%) strains were serum resistant. However, strain PS-16 was repeatedly serum susceptible, showing between a 10- and 20-fold reduction in CFU within 2 h of incubation with PHS. This fact may indicate that such reductions in CFU (approximately 10-fold) do not represent significant serum susceptibility in vivo, as might the 10,000- to 100,000-fold reduction exhibited by other strains such as 1776-81, PS-3, and PS-33. It is also equally clear that this phenotype alone does not confer pathogenicity on a given isolate, because several avirulent strains, including 2599-78 (from blood), were also positive for this trait.

In addition to serum resistance, we universally detected low-level toxin production in all *P. shigelloides* isolates that we screened. This toxin was heat stable (100°C, 10 min) and active on cell lines such as Y1 and HEp-2, producing rounding of epithelial cells prior to death. Incubation of cell-free cytotoxin in the presence of human glycophorin (30 μg/ml) did not inhibit its activity, nor did the toxin lyse erythrocytes from a number of different animals, suggesting that it is not a typical bacterial cytolysin. Of interest is the fact that similar activity has recently been described in a strain recovered from a patient with pseudoappendicitis in which cell-free supernatants (1:16) of this isolate produced a similar morphologic effect against CHO cells (7). This cytotoxin may also be similar or identical to a Vero cytotoxin described by Hostacka and colleagues (11), since our cell-free supernatants produced morphologic alterations in Vero cells after 48 h of coincubation. Although definitive evidence

TABLE 3. Evaluation of cholera toxin-like activity among *P. shigelloides* strains

Organisms	Strain	Y1 cell morphologic effect	Toxin neutralization[a]	Latex[b]	Probe[c]
P. shigelloides	PS-3, PS-4, PS-10, PS-11, PS-32, PS-33, PS-36, 801-3	Cytotoxic	No	–	–
V. cholerae O1	569B	Cytotonic	Yes	+	+

[a] Neutralization of toxin activity on Y1 cells by preincubation with anti-cholera toxin antibodies (rabbit).
[b] Reactivity of sensitized latex beads (Vet-RPLA) with cell-free Evan CAYE supernatants from broth-grown organisms.
[c] Dot hybridization with ^{32}P-labeled ctx-A11 probe (15).

is lacking, cytotoxins have been postulated to be important virulence factors for gram-negative enteric pathogens, potentially functioning by promoting colonization or invading epithelial cells via inhibition of resident microflora or tissue destruction (6, 25). The identification of such cytotoxic activity in a large number of plesiomonads suggests that this activity could be a potential virulence factor operative in *P. shigelloides*-associated gastroenteritis and should be investigated further to characterize the molecules responsible for cellular death.

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