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Association of *Providencia alcalifaciens* with Diarrhea in Children

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It has been demonstrated in previous studies that *Providencia alcalifaciens* can produce diarrhea by an invasive mechanism. In the present study, *P. alcalifaciens* was isolated from the stool specimens of 17 of 814 diarrheal children younger than 5 years of age (2.1%) and from those of 4 of 814 matched controls (0.49%) ($P = 0.004$), indicating that the organism is significantly associated with diarrhea. However, 71% of *P. alcalifaciens*-positive diarrheal children had simultaneous infections with other recognized enteric pathogens.

The genus *Providencia* belongs to the family *Enterobacteriaceae*. Four species of this genus have been isolated from humans: *Providencia alcalifaciens*, *P. stuartii*, *P. rettgeri*, and *P. rustigianii* (6). Before the genus *Providencia* was classified into four species, the bacteria were collectively called the *Providencia* group of organisms (13) and were found to be causative agents of diarrhea in some studies (5, 12); the clinical spectrum suggested that they caused invasive diarrhea (5, 12). A recent study in the United Kingdom reported that the proportion of diarrheal British travelers returning from developing countries from whom *P. alcalifaciens* was isolated was significantly higher than that of diarrheal patients who had not traveled overseas and had *P. alcalifaciens* (10). Although this was not a case-control study, it suggested that *P. alcalifaciens* could cause travelers' diarrhea. Our studies in laboratory models confirmed the diarrheagenic potential of *P. alcalifaciens* (1, 2, 11). However, case-control studies showing an epidemiological association of *P. alcalifaciens* with diarrhea are lacking. Therefore, we carried out a study in which we compared the rate of isolation of *P. alcalifaciens* from the stool specimens of children with diarrhea with that in matched children without diarrhea. The results are reported in this article.

Children younger than 5 years of age with acute diarrhea (defined as three or more loose stools a day) seen at the Clinical Research and Service Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka, Bangladesh, were studied from February 1993 to July 1994. These children were the routine 4% of diarrheal surveillance patients (every 25th patient) seen at the Clinical Research and Service Centre (14). The patients were first seen in a triage area, and those requiring further care were admitted to the hospital ward. A physician performed a physical examination and assessed the patients' dehydration status as none, mild, moderate, or severe according to clinical signs (15). A health assistant administered a questionnaire regarding demographic background, medical history, and previous treatment to the patient or to an adult guardian. A stool specimen collected immediately after admission was used for etiological studies.

Controls were healthy children from the same neighborhood as the case patients. After leaving the household of a patient in the community, a health worker tossed a pencil onto the street and then walked in the direction the sharpened end of the pencil pointed. After passing the first 10 houses, the health worker located the first house with an age-matched control and collected the same information from the control that had been collected from the patients. Control children had not taken antibiotics during the previous 2 weeks. Stool samples from controls were collected within 2 weeks of samples from patients and were transported in refrigerated boxes to the laboratory within a few hours.

Stool specimens were cultured for *P. alcalifaciens* on modified proteeae isolation medium (10). Characteristic colonies causing browning of the medium were further tested for *P. alcalifaciens* as described previously (1). These colonies were confirmed to be *P. alcalifaciens* by their reaction on an API-20E strip (API System, Montalieu, Vercieu, France). The isolates were studied for invasiveness and actin condensation in the HEp-2 cell monolayer as described previously (2). Briefly, for invasiveness, 10^7 CFU of bacteria were inoculated onto the monolayer and allowed to interact with it for 2 h. After the monolayer was washed to remove nonadherent bacteria, it was incubated for another hour in the presence of gentamicin. The gentamicin-resistant bacteria, which are an indicator of invasiveness, were enumerated after the monolayer was lysed with 1% Triton X-100. To detect actin condensation in the HEp-2 cell monolayer, the monolayer was incubated with bacteria for 3 h; after the nonadherent bacteria were removed by washing, the monolayer was stained with fluorescein-labeled phalloidin and viewed under a fluorescent microscope.

Stool specimens were also examined for other enteric pathogens, including rotavirus, *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* sp., and recognized bacterial pathogens as described previously (3). The bacterial pathogens sought were *Vibrio cholerae* O1 and O139, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., *Plesiomonas* sp., *Clostridium difficile*, and diarrheagenic *Escherichia coli* (enterotoxigenic, enteropathogenic [EPEC], enteroaggregative, enteroinvasive, enterohemorrhagic, and diffuse adherent [DAEC]).

During the period of study, 814 children with diarrhea and the same number of matched controls were tested for enteric pathogens, including *P. alcalifaciens*. *P. alcalifaciens* was cultured from 17 patients with diarrhea and four control children without diarrhea ($P = 0.004$ by χ^2 test), indicating a significant association of the organism with diarrhea. Data for the isolation of *P. alcalifaciens* in relation to that of other enteric pathogens are presented in Table 1; in terms of rank order, *P.*

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TABLE 1. Isolation of *P. alcalifaciens* in relation to that of other enteric pathogens, in rank order, from 814 children with diarrhea and 814 children without diarrhea

Pathogen	No. (%) positive		*P* value
	With diarrhea	Without diarrhea	
Diarrheagenic *Escherichia coli*	353 (43.37)	235 (28.87)	0.0001
Rotavirus	165 (20.27)	12 (1.47)	0.0001
Campylobacter jejuni	142 (17.45)	103 (12.65)	0.007
Aeromonas spp.	99 (12.20)	39 (4.80)	0.0001
Vibrio cholerae O1 and O139	71 (8.72)	3 (0.37)	0.0001
Shigella spp.	70 (8.60)	23 (2.83)	0.001
Providencia alcalifaciens	17 (2.08)	4 (0.49)	0.004
Salmonella spp.	15 (1.84)	10 (1.23)	0.314
Clostridium difficile	13 (1.60)	4 (0.49)	0.028
Cryptosporidium sp.	11 (1.35)	3 (0.37)	0.032
Plesiomonas shigelloides	8 (1.00)	10 (1.23)	0.636
Giardia lamblia	7 (0.86)	23 (2.83)	0.003
Entamoeba histolytica	5 (0.61)	1 (0.12)	0.102

alcalifaciens was next to *Shigella* spp. However, whereas *P. alcalifaciens* was the only pathogen that was isolated from five patients, other patients had copathogens (Table 2). Two of the four control children positive for *P. alcalifaciens* had copathogens; one had *Plesiomonas shigelloides*, and the other had DAEC. Two of the *P. alcalifaciens* isolates from the control children and 9 of 17 isolates from the patients were available for studies on invasion and actin condensation in the HEp-2 cell monolayer (2). All isolates from control children and patients invaded the cell monolayer (gentamicin-resistant progeny, $>10^3$ CFU per ml), with associated actin condensation (2).

The invasive nature of the isolates is consistent with our previous findings: *P. alcalifaciens* invaded HEp-2 cells, with actin condensation, produced inflammatory changes associated with the invasion of tissues in adult rabbit ileal loops, and produced invasive diarrhea in a removable intestinal tie adult rabbit diarrhea model (1). Electron microscopy of the ileal mucosa of these animals showed two modes of entry into intestinal epithelial cells, one directly by endocytosis associated with polymerization of cytoskeletal components and the other by disruption of tight junctions, with entry into and proliferation in intercellular spaces (11). Further studies with additional isolates from diarrheal stool samples in Bangladesh confirmed the invasiveness of *P. alcalifaciens* and showed that both invasion and actin condensation were inhibited by cytochalasin D, an antimetabolite that inhibits microfilament formation (2). Invasiveness and actin condensation were also shown to be present in *P. alcalifaciens* isolates from diarrheal patients in Brazil (9). Consistent with the invasive nature of *P. alcalifaciens*, four of the five patients infected only with *P. alcalifaciens* had manifestations of invasive diarrhea (Table 3).

This first case-control study of children with diarrhea to investigate the etiologic role of *P. alcalifaciens* suggested that the organism is associated with diarrhea. However, in the present study, *P. alcalifaciens* was associated with diarrhea in only a small proportion (2.1%) of the children. Many recognized enteric pathogens are normally isolated from some apparently healthy subjects in case-control studies of diarrhea in developing countries (4, 8), as in the present study. (*P. alcalifaciens* was isolated from four apparently healthy children.) The majority of the patients from whom *P. alcalifaciens* was isolated also had copathogens. The isolation of multiple enteric pathogens from stool samples is common in many developing countries, including Bangladesh (7). This problem reflects the high degree of fecal contamination in the environments of developing countries.

TABLE 2. Isolation of *P. alcalifaciens* alone or with other enteric pathogens from the stool specimens of 17 of 814 children with diarrhea

Patient no.	Age (mo)	Gender[a]	Copathogen
S-57	11	M	None
S-73	30	F	*Vibrio cholerae* non-O1 and non-O139
S-150	5	F	None
S-171	18	M	*Aeromonas* spp.
S-222	8	M	*Campylobacter* spp.
S-469	30	M	*Vibrio cholerae* O1, *Campylobacter* spp., EPEC, *Entamoeba histolytica*
S-531	40	M	*Aeromonas* spp., *Shigella flexneri* spp.
S-556	10	F	*Aeromonas* spp.
S-612	6	F	*Shigella flexneri* spp.
S-633	48	F	*Campylobacter* spp., *Giardia lamblia*, DAEC
S-689	20	M	*Shigella flexneri* spp., EPEC
S-690	5	F	None
S-700	11	F	*Campylobacter* spp., EPEC
S-727	1	F	ETEC[b]
S-732	10	M	None
S-837	11	M	ETEC
S-849	12	F	None

[a] M, male; F, female.
[b] ETEC, enterotoxigenic *Escherichia coli.*

TABLE 3. Clinical findings for five diarrheal children whose stools contained *P. alcalifaciens* but no copathogen

Parameter	No. of children positive	Frequency in 24 h
Liquid stool	5	3–15
Erythrocytes[a] in stool	1	NA[d]
Leukocytes[a] in stool	4	NA
Abdominal pain	1	NA
Vomiting	3	4–9
Fever[b]	0	NA
Dehydration status[c]	5	NA

[a] More than 10 per high-power microscopy field.
[b] Defined as ≥37.7°C.
[c] Four children had mild dehydration, and one had moderate dehydration.
[d] NA, not applicable.

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