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Rate of Occurrence and Pathogenic Effect of Enteroaggregative *Escherichia coli* Virulence Factors in International Travelers

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One or more putative enteroaggregative *Escherichia coli* (EAEC) virulence factors (*aggA, aggR, aspU,* or *aafA*) were identified in 60 (70%) of 86 EAEC isolates from travelers with diarrhea compared with a rate of 7 (8%) of 90 in patients with diarrhea who were infected with nonadherent *E. coli* (odds ratio, 27.36; 95% confidence interval, 11.30 to 65.91). The presence of *aggR* or one or more virulence factors in EAEC from patients with diarrhea was associated with a statistically increased concentration of interleukin-8 (IL-8) in feces compared with that in EAEC negative for these factors: for *aggR* positive (9 of 12 [75%]; median, 800 pg/ml) versus *aggR* negative (5 of 18 [28%]; median, 0), $P < 0.05$; and for isolates positive for ≥1 virulence factor (13 of 21 [62%]; median, 360 pg/ml) versus those negative for ≥1 virulence factor (1 of 9 [11%]; median, 0), $P < 0.05$. Other fecal cytokines (IL-1β and IL-1ra) were found in increased concentrations ($P < 0.05$ when at least one EAEC virulence factor was present compared with the concentrations when EAEC negative for multiple virulence factors was found in patients with diarrhea. Putative virulence factors were commonly found in EAEC from patients with diarrhea, and the pathogenicity of many strains was suggested by showing an association between the presence of plasmid-borne virulence factors and the presence of fecal cytokines. The different patterns of virulence factors of EAEC revealed several clusters demonstrating diversity among the isolates from the various regions.

Enteroaggregative *Escherichia coli* (EAEC) strains have been identified as a possible important cause of persistent diarrhea in children (17) and adult travelers (1) in developing countries. Strains of EAEC differ in their pathogenicity (16), but the pathophysiology of EAEC diarrhea and the virulence traits that enable the organism to cause diarrhea are not well understood. It has been shown that most EAEC strains possess a 60- to 65-MDa plasmid (designated pAA) which encodes several putative virulence factors, including the AA fimbria characterized as AAF/I or AAF/II (8). AAF/II mediates adherence to the intestinal mucosa (7). AAF/I-related genes include *aggA,* which encodes the major fimbrial subunit; the corresponding AAF/II subunit has been designated *aafA.* The biogenesis of AAF/I and that of AAF/II both require the action of the transcriptional activator *aggR.* It is notable, however, that many strains carrying the *aggR* gene express neither AAF/I nor AAF/II (8). In addition, the pAA plasmid in many EAEC strains has a cryptic gene called *aspU,* which encodes a secreted protein and which is apparently recognized by patient serum (8). Although none of these factors has been directly linked to virulence in vivo, each has at least a plausible role in EAEC pathogenesis.

Recent studies have documented in vitro production of interleukin-8 (IL-8) by EAEC-infected epithelial cells (21). These findings are reminiscent of those for inflammatory bowel disease in which elevated levels of the fecal cytokines IL-1β (6), tumor necrosis factor alpha (TNF-α) (22), and IL-8 (13) have been demonstrated. Elevated TNF-α and IL-6 levels in the stools and sera of children with shigellosis have been associated with disease complications (12).

There is little information about the presence of EAEC virulence factors in EAEC strains compared to their presence in HEp-2-nonadherent *E. coli* strains from patients with diarrhea. The aims of our study were (i) to examine the relationship between the possession of EAEC plasmid-borne genes *aggA*, *aggR*, *aafA*, and *aspU* and the ability of *E. coli* strains isolated from patients with traveler's diarrhea to adhere to HEp-2 cells in an aggregative pattern; (ii) to determine the relationship between enteric infection with plasmid factor-positive EAEC and the release of fecal cytokines; and (iii) to determine the genetic relationships of EAEC isolates by virulence factor content in patients with acute diarrhea studied in different geographic locations.

MATERIALS AND METHODS

Study population. Our study population included 176 U.S. and European travelers with acute diarrhea acquired during short-term stays in Goa, India; Montego Bay, Jamaica; or Guadalajara, Mexico (14, 23). Diarrhea was defined in our studies as passage of three or more unformed stools in a 24-h period plus one or more signs or symptoms of enteric illness (nausea, vomiting, abdominal pain or cramps, fecal urgency, or dysentery). Stool samples were collected and submitted to the field laboratories located in each of the three locations, where they were examined for form (formed, soft, or watery) and other characteristics: positive for gross blood or mucus, fecal leukocytes, or occult blood.

Stool examination. After a qualified patient had signed a written consent form, conventional bacterial enteric pathogens were sought by published methods (14); these pathogens included *Shigella* spp., *Salmonella* spp., *Vibrio* spp., *Campy-*

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lobacter jejuni, Yersinia enterocolitica, Aeromonas spp., and *Plesiomonas shigelloides. Entamoeba histolytica, Cryptosporidium* spp., and *Giardia lamblia* were identified by enzyme immunoassays (14). The presence of rotavirus and other viral enteric pathogens was not tested in this study. Five lactose-positive colonies were retrieved from MacConkey agar plates from each stool sample and were inoculated into individual peptone stabs. Oligonucleotides probes for heat-labile and heat-stable enterotoxins of enterotoxigenic *E. coli* (ETEC) were hybridized with the five lactose-positive colonies for detection of ETEC (14). *E. coli* O157:H7 and Shiga toxin-producing *E. coli* strains were not sought in this study.

EAEC and non-EAEC identification. At least three of the five lactose-positive colonies with *E. coli* morphology per diarrheal stool sample were tested for the presence of EAEC on the basis of a characteristic pattern of adherence to cultured HEp-2 cells (9). Briefly, a chamber slide (Dynatek, Naperville, Ill.) was seeded with HEp-2 cells (American Type Culture Collection) that had been grown at 37°C in 5% CO_2 in minimal essential medium (Gibco BRL, Gaithersburg, Md.) supplemented with 10% fetal calf serum. *E. coli* isolates to be tested were grown overnight at 37°C without shaking in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 1% D-mannose. Then 25 μl of bacterial culture was added to each chamber. The cell culture medium in the chamber slide was then replaced with minimal essential medium containing 1% D-mannose without antibiotics, *E coli* was added, and the culture was incubated at 37°C for 3 h. The slide was washed three times with phosphate-buffered saline, fixed with 100% methanol, and stained with crystal violet (Difco, Detroit, Mich.). A positive control EAEC strain, JM221, isolated in Mexico from a U.S. traveler with diarrhea and a nonadherent, normal *E. coli* strain, HS, from a healthy subject were included in each assay. Each *E. coli* strain was tested twice in blinded fashion. A sample was interpreted as positive for EAEC if it showed the characteristic "stacked-brick" aggregative appearance as described by Donnenberg and Nataro (9). EAEC diarrhea was defined as diarrhea in a subject who had one or more HEp-2 cell-adherent *E. coli* colonies among the three to five colonies isolated from a stool sample. For comparison with EAEC, non-HEp-2 cell-adherent *E. coli* strains from patients with diarrhea were included in the study. Patients who were selected for study were negative for all other enteric pathogens tested, including ETEC.

Virulence factor identification. The presence of EAEC plasmid-borne genes was assessed among *E. coli* isolates from 176 patients with traveler's diarrhea without conventional bacterial and parasitic enteric pathogens, including *Shigella* spp., *Salmonella* spp., *Vibrio* spp., *C. jejuni, Y. enterocolitica, Aeromonas* spp., *P. shigelloides,* ETEC, *E. histolytica, Cryptosporidium* spp., and *G. lamblia.* In addition, 10 asymptomatic U.S. travelers to Mexico with EAEC infection and *E. coli* isolates from 10 healthy pathogen-negative U.S. travelers to Mexico were examined for the plasmid-borne factors. DNA from *E. coli* isolates was purified using the ReadyAM DNA purification system (Promega, Madison, Wis.). Oligonucleotide primers for EAEC genes *aggA, aggR, aafA,* and *aspU* were selected for study based on previous investigations (8, 17). Positive controls for *aggA, aggR, aspU,* and *aafA* were 17-2, JM221, 60A, and 042, respectively. They were used in each PCR assay. The final amplification mix contained 98 μl of PCR mix (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2 mM $MgCl_2$, 100 μM each dATP, dCTP, dGTP, and dTTP, 2.5 U of Ampli *Taq* polymerase [Perkin-Elmer, Norwalk, Conn.]), 25 pmol of each primer, and 2 μl of DNA template. The reaction mixtures were heated to 94°C for 5 min and then were subjected to 35 cycles (94°C for 30 s, 50°C for 1 min, and 72°C for 45 s) and then to a final extension at 72°C for 7 min in a DNA thermal cycler (Perkin-Elmer). Then 10 μl of each of the amplified PCR products was added to a 1% agarose electrophoresis gel with a 1-kb DNA molecular weight marker (Gibco BRL). A positive reaction was defined as the presence of the PCR product of the expected size.

Cytokine assays. Stool cytokines from 50 subjects were studied, with selection based on stool sample availability. We included 10 subjects with asymptomatic EAEC infection, 18 subjects with EAEC diarrhea, and 10 healthy asymptomatic EAEC-negative, pathogen-negative controls from Guadalajara, Mexico. Also included were stool samples from 12 subjects with EAEC diarrhea in Goa, India. Cytokine levels were measured in stool samples by using commercial quantitative sandwich EIAs for IL-1β, IL-1ra, IL-8, gamma interferon (IFN-γ), and TNF-α (Quantakine; R&D Systems, Minneapolis, Minn.). The minimum detectable concentration of each cytokine in stool supernatant was as follows: IL-1β, 1 pg/ml; IL-1ra, 14 pg/ml; IL-8, 10 pg/ml; TNF-α, 4.4 pg/ml; and IFN-γ, 8 pg/ml.

Statistical methods. The data were processed using MS Excel (Microsoft, Redmond, Wash.) and SAS (SAS Institute, Carey, N.C.). The association of plasmid-borne factors with cytokine production was assessed using the chi-square or Kruskall-Wallis test. The phylogenetic relationships among the virulence factors in EAEC strains were inferred by distance methods (10) using the average-linkage method (19). For these genetic studies, EAEC strains differing in virulence factors were compared by the geographic region where the organism was isolated. Median values for cytokine levels were used rather than means because of the wide variation in values. Data were interpreted in a two-tailed fashion to estimate the *P* value.

TABLE 1. Presence of defined EAEC virulence factors in EAEC and HEp-2 cell-nonadherent *E. coli* strains isolated from diarrheal stools of patients with traveler's diarrhea

Virulence factor	No. (%) of patients with:		*P*
	HEp-2-adherent *E. coli* (n = 86)	HEp-2-nonadherent *E. coli* (n = 90)	
Patients with only one virulence factor	30 (35)	5 (6)	<0.001
aggA only	13	0	
aggR only	11	2	
aspU only	5	2	
aafA only	1	1	
Patients with more than one virulence factors	30 (35)	2 (2)	<0.003
aggA and *aggR*	2	0	
aggA and *aspU*	3	0	
aggA and *aafA*	2	0	
aggA and *aggR* and *aspU*	8	0	
aggR and *aspU*	9	2	
aggR and *aafA*	3	0	
aggR and *aspU* and *aafA*	3	0	
Patients with one or more virulence factors	60 (70)	7 (8)	<0.001
aggA or *aggA* with other virulence factors	28	0	
aggR or *aggR* with other virulence factors	36	2	
aspU or *aspU* with other virulence factors	28	4	
aafA or *aafA* with other virulence factors	9	1	
Patients negative for virulence factors	26 (30)	83 (92)	<0.01

RESULTS

We identified 86 patients with diarrhea and positive stools for EAEC strains but no other enteric pathogen in their stools and an additional 90 patients with diarrhea whose stools were negative for all enteric pathogens including EAEC. The 86 patients with EAEC diarrhea included 26 patients from Goa, India; 30 patients from Montego Bay, Jamaica; and 30 patients from Guadalajara, Mexico. Among the *E. coli* strains from 90 pathogen-negative subjects with diarrhea, 7 (8%) had one or more virulence factors identified (odds ratio [OR] = 27 with 95% confidence interval [CI] of 11.3 to 65.9) (Table 1). The two most prevalent virulence factors were *aggR* and *aggA,* found in 36 (41%) and 28 (32%) of EAEC strains studied, respectively. Sixty EAEC strains (70%) from 86 patients with EAEC diarrhea had one or more gene markers identified. The *aggA* gene was the most common single gene marker identified and was found in 13 EAEC strains (15%), followed by *aggR,* which was found in 11 EAEC strains (13%). When combinations were investigated, it was found that nine EAEC strains (10%) possessed *aggR* and *aspU,* followed by eight EAEC

TABLE 2. Defined EAEC virulence factors and fecal cytokine production of patients with EAEC diarrhea, patients with asymptomatic EAEC infection, and healthy asymptomatic pathogen-negative control subjects

Fecal cytokine identified	EAEC asymptomatic infection in Guadalajara ($n = 10$)		EAEC diarrhea in Guadalajara and Goa ($n = 30$)		Healthy controls in Guadalajara ($n = 10$)	
	No. (%) VF[a] positive ($n = 5$)	No. (%) VF negative ($n = 5$)	No. (%) VF positive ($n = 9$)	No. (%) VF negative ($n = 9$)	No. (%) VF positive ($n = 1$)	No. (%) VF negative ($n = 9$)
IL-8	0	0	13 (62)	1 (11)	0	0
IFN-γ	2 (40)	1 (20)	8 (38)	0	0	0
TNF-α	0	1 (20)	5 (24)	2 (18)	1 (100)	0
IL-1β	0	0	15 (71)	1 (11)	0	1 (11)
IL-1ra	4 (80)	3 (60)	20 (95)	8 (89)	1 (100)	9 (100)
IL-1β/IL-1ra	0	0	13 (62)	1 (11)	0	1 (11)

[a] VF, virulence factor.

isolates (9%) with the combination of *aggA, aggR,* and *aspU.* Isolates from all 90 subjects with HEp-2-nonadherent *E. coli* strains lacked the *aggA* gene marker; one strain was positive for *aafA* gene. Two nonadherent *E. coli* strains were positive for both *aggR* and *aspU.*

In Table 2, the relationship between virulence factors and the presence of fecal cytokines is presented for 10 patients with asymptomatic EAEC infection and 30 persons with EAEC diarrhea. For comparison, 10 pathogen-negative asymptomatic controls were studied. Increased levels of fecal IL-8 and IL-1β were observed for patients with EAEC diarrhea compared with those of healthy and asymptomatically infected subjects (Table 2). Other cytokines, including IFN-γ, TNF-α, IL-1ra, and IL-1β/IL-1ra, occurred with approximately equal frequency in all three groups. These findings indicated that fecal cytokine production may be related to the presence of specific EAEC virulence factors and diarrheal illness. There was no significant difference in the levels of fecal cytokines identified between virulence factor-positive and -negative isolates in asymptomatic subjects (Table 2). However, a statistically significant difference was seen in the presence or absence of virulence factors in EAEC isolates from patients with diarrhea. Patients with EAEC diarrhea wherein the infecting strain was positive for one or more virulence factors were more likely to

TABLE 3. Fecal cytokine levels of 30 patients with EAEC diarrhea in terms of the presence or absence of defined EAEC virulence factors[a]

Cytokine	Strains positive for 1 virulence factor								Strains positive for ≥1 virulence factors	
	aggA		*aggR*		*aspU*		*aafA*			
	+ ($n = 9$)	− ($n = 21$)	+ ($n = 12$)	− ($n = 18$)	+ ($n = 15$)	− ($n = 15$)	+ ($n = 7$)	− ($n = 23$)	+ (n = 21)	− ($n = 9$)
IL-8										
Median concn (pg/ml)	395.73	0	800.3	0[a]	502.29	0	544.98	0[a]	359.7	0[b]
No. (%) of positive samples	5 (56)	9 (43)	9 (75)	5 (28)	10 (67)	4 (27)	5 (71)	9 (39)	13 (62)	1 (11)
TFN-α										
Median concn (pg/ml)	0	0	3.59	0	0	0	0	0	0	0
No. (%) of positive samples	0 (0)	8 (38)	7 (58)	1 (6)	7 (47)	1 (7)	3 (43)	5 (22)	8 (38)	0 (0)
TNF-α										
Median concn (pg/ml)	0	0	0	0	0	0	0	0	0	0
No. (%) of positive samples	1 (11)	6 (28)	5 (42)	2 (11)	5 (33)	2 (13)	3 (43)	4 (17)	5 (24)	2 (22)
IL-1β										
Median concn (pg/ml)	732.1	0	385.68	0	11.83	0	740.66	0[b]	22.47	0[b]
No. (%) of positive samples	7 (78)	9 (43)	8 (67)	8 (44)	10 (67)	6 (40)	5 (71)	11 (48)	15 (71)	1 (11)
IL-1ra										
Median concn (pg/ml)	229.69	429.79	864.16	79.32	864.16	75.65[b]	429.79	229.69	429.79	79.23[b]
No. (%) of positive samples	8 (89)	21 (100)	12 (92)	18 (100)	15 (100)	14 (93)	7 (100)	22 (96)	20 (95)	9 (100)
IL-1β/IL-1ra ratio										
Median concn (pg/ml)	0.09	0	0.09	0	0.09	0	0.09	0	0.09	0
No. (%) of positive samples	5 (56)	9 (43)	8 (67)	7 (39)	10 (67)	4 (27)	5 (71)	9 (39)	13 (62)	1 (11)

[a] Listed virulence factors were any positive factors, either single or combined.
[b] *P* values for comparisons between quartiles were calculated by the Kruskall-Wallis test; $P < 0.05$.

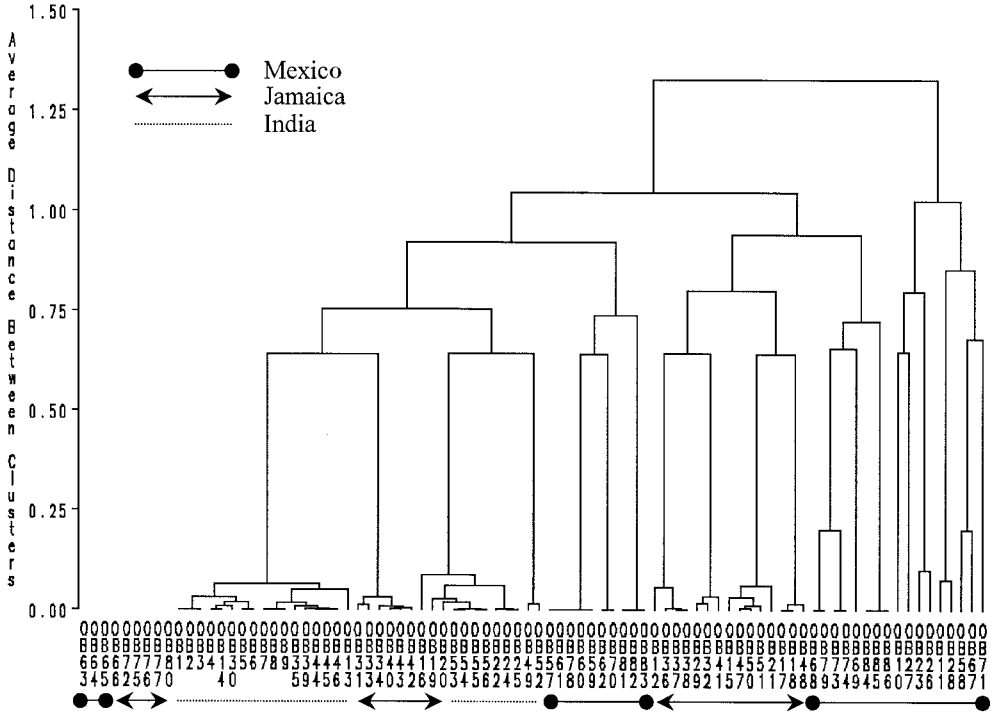

FIG. 1. Virulence factor genotype relationship of enteroaggregative *E. coli* from 86 patients with travelers' diarrhea in Goa, India; Montego Bay, Jamaica; and Guadalajara, Mexico. The dendrogram is based on the virulence factor profiles. The average distance between clusters was determined by clustering analysis based on the comparison of the similarity matrices of strains from 86 patients with traveler's diarrhea.

produce the studied fecal cytokines than were patients without identified virulence factors.

In Table 3, the relationship between EAEC plasmid-borne virulence properties and the presence of cytokines in stool is presented for 30 patients with EAEC diarrhea for whom fecal cytokine assays were performed. Levels of fecal IL-8 were significantly higher ($P < 0.05$) in patients infected with *aggR*- or *aafA*-positive EAEC than in those infected with EAEC negative for these factors. If patients were infected with EAEC strains containing any of the studied plasmid-borne virulence factors, they were more likely to have increased fecal IL-8 levels than were those infected with virulence factor-negative EAEC ($P = 0.007$). IL-8 was positive in 9 (75%) of 12 fecal samples from patients infected with EAEC strains positive for *aggR* and in 5 (28%). of 18 patients infected with EAEC strains without the *aggR* gene ($P = 0.01$). IFN-γ was found in fecal samples from 7 (58%) of 12 and 1 (6%) of 18 patients with EAEC diarrhea in whom the infecting *E. coli* strains were *aggR* positive or negative, respectively ($P = 0.001$). Patients with diarrhea who were infected with EAEC strains positive for one or more virulence factors were more frequently found to have a positive result for fecal IL-8 (13 of 21 [62%], $P = 0.003$) and ratio of IL-1β to IL-1ra (13 of 21 [62%], $P = 0.003$) than were patients with diarrhea who were infected with EAEC strains without the studied virulence factors. Table 3 shows that symptomatic infections with EAEC isolates positive for *aggR* and/or *aafA* were associated with elevated levels of fecal IL-8, symptomatic infections with EAEC isolates positive for *aafA* were associated with elevated levels of fecal IL-1β, and symptomatic infection with EAEC isolates positive for *aspU* were associated with elevated levels of fecal IL-1ra.

A dendrogram of EAEC virulence factors among EAEC strains derived from a similarity matrix of the gene profiles revealed three main clusters at a distance of about 1.00. Cluster 1 consisted of all EAEC strains from India and some of the EAEC isolates from Jamaica and Mexico; most EAEC isolates in cluster 2 were from Jamaica; and most EAEC strains from Mexico were in cluster 3 (Fig. 1). Comparison of the similarity indices demonstrated a lower diversity among EAEC strains from India than among those from Jamaica and Mexico.

DISCUSSION

The importance of EAEC as a cause of pediatric diarrhea in developing regions was first identified more than 10 years ago (4, 15). This group of enteric pathogens was recently shown by our group to be the second most common cause of traveler's diarrhea in multiple areas of the world, being nearly as important as ETEC in these regions (1). EAEC strains may be found in food in areas with endemic infection (3) and asymptomatic infection occurs commonly in these areas (2). Host and environmental factors may influence the occurrence of EAEC diarrhea (e.g., diet, inoculum size, genetic makeup, stress, and relocation). The specific role of defined EAEC virulence determinants in the pathogenicity of diarrhea in humans is being investigated (21).

This study addressed the hypothesis that EAEC and nonadherent *E. coli* strains isolated from patients with diarrhea dif-

fered in terms of the presence of EAEC-associated virulence factors. In the present study, 60 (70%) of 86 EAEC strains isolated from stools from subjects with diarrhea were positive for one or more plasmid-borne factors, compared with only 7 (8%) of 90 strains of nonadherent *E. coli* from patients with diarrhea that were positive for one or more factors (OR = 27 with CI of 11.3 to 65.9). Thus, the virulence factors studied are prevalent in diarrhea-associated *E. coli* strains showing aggregative adherence to HEp-2 cells. Okeke et al. (17) found that EAEC expression of AAF/II was strongly associated with diarrhea in children in Southwest Nigeria. In that study, EAEC strains positive for the AAF/II gene probe were 3.55 times more likely to be isolated from individuals with diarrhea than from asymptomatic control subjects. These authors proposed that AAF/II is a marker for a majority of the pathogenic EAEC strains. AAF/II in these studies is closely related to *aafA,* which was being sought in the present study. The present study provides evidence that the recognized virulence factors of EAEC are prevalent in EAEC strains isolated from patients with acute diarrhea, supporting their pathogenic role. The most common virulence factors found were *aggA* (denoting AAF/I) and *aggR.* The present study did not rule out an association between the expression of other virulence factors and human infection and diarrhea. Indeed, we found a low rate of positivity for AAF/II and therefore could not assess its association with diarrhea. The variety of virulence factors of EAEC strains and the variability of their presence in infecting strains resemble the heterogeneity among colonization factor antigens in ETEC (14).

It has been established that inflammatory diarrheal disease caused by *Clostridium difficile* is associated with increases in fecal cytokine concentrations as a result of disease pathogenicity (18). Evidence has been provided to indicate that EAEC may cause inflammatory enteritis with secretion of IL-8 (20). We have recently shown that diarrhea caused by a variety of inflammatory bacterial enteropathogens including EAEC, *Shigella*, and *Salmonella* in adults was associated with the production of cytokines in diarrheal stools (11). In another study, we demonstrated that naturally occurring EAEC diarrhea in travelers was associated with another marker of intestinal inflammation, fecal lactoferrin (5). In the present study, IL-8 was detected at ≈200-fold-higher concentrations in fecal samples from patients with diarrhea in whom the infecting strains of EAEC were positive for *aggR*, *aafA*, or any combination of virulence factors than in fecal samples from patients without diarrhea in whom the infecting strains of EAEC were without defined virulence factors. In the present study, when the infecting EAEC isolates in the patients with diarrhea were positive for *aggR* or *aafA* factors, they were more likely to be associated with increased levels of fecal IFN-γ than were those in patients infected with EAEC without those virulence factors. Detection of high concentrations of fecal cytokines in patients with EAEC diarrhea suggests increased production and secretion from an inflamed bowel. Steiner et al. (20) demonstrated that the concentrations of IL-1β were elevated in patients with EAEC infection in either the presence or absence of symptoms. In the present study, there was a significant elevation in fecal IL-1β levels in patients infected with EAEC compared with those in healthy controls (Table 2).

One problem with comparing EAEC virulence factor profiles isolated from patients from different geographic areas is the lack of a standardized analytical method. In the current literature, unweighted pair group method with arithmetic mean (UPGMA) cluster analysis is the most commonly employed method to determine relationships between biological factors (10). We used this approach, based on similarity matrices, to compare the patterns of bacterial strains isolated from different locations. The program separated all 86 EAEC strains into three groups. Most of the EAEC isolates from Mexico and Jamaica were separated into two different subgroups. Most of the EAEC isolates from India and some of the strains from Jamaica and Mexico were in one subgroup. The different patterns of virulence factors seen in different geographic areas may be an important observation and may provide a clue to the heterogeneity of this group of pathogens. It would be worthwhile to investigate additional geographic locations for EAEC virulence factors. Information generated by such a study would help to develop specific probes for the identification of EAEC and the EAEC virulence factors.

The present study of EAEC isolates from patients with diarrhea, including characterization of their virulence factors and characterization of associated fecal cytokine profiles, furthers our knowledge of the pathogenesis of EAEC. We found an association between the possession of defined plasmid-borne factors in EAEC strains isolated from patients with diarrhea and the resultant fecal cytokine profile. The plasmid for aggregative adherence (pAA) is responsible for the aggregative adherence phenotype in some EAEC strains (7). This plasmid carries several virulence factors including *aggA*, *aggR*, *aafA* and *aspU* (7, 16). Our data are consistent with previous observations suggesting that most EAEC strains carry a partially conserved virulence pAA (8). As has been shown in this study, these plasmids vary in their genetic complement, encoding adherence factors, a transcriptional activator, and/or several secreted proteins, including toxins. While the precise plasmid-borne factors conferring disease are not apparent from our study, our data do indicate that possession of the pAA plasmid serves as a marker for a pathogen. Thus, one interpretation of our findings is that a subset of pathogenic EAEC isolates can indeed be identified and that intestinal inflammation may be their common mode of pathogenesis. It may be possible to distinguish pathogenic strains of EAEC from nonpathogenic isolates in infected patients by showing the presence of defined EAEC virulence factors in the infecting strains and by determining the level of fecal cytokines. We also note that previous studies suggest that the plasmid conferring aggregative adherence cosegregates with chromosomal genes. The presence of IL-8 in chromosomally encoded EAEC flagella (20) is one possible mechanism to explain this finding.

Our data thus suggest that *aggA* or *aggR* genes alone or in combination with other virulence factors included in the study may be used to identify pathogenic strains of EAEC. Such analyses may be useful for comparing the virulence of EAEC strains and for studying epidemiologic differences between strains isolated from various areas of the world. Common elaboration of specific fecal cytokines in EAEC strains positive for one or more virulence factors offers further support for the role of these or linked factors in the pathogenesis of EAEC diarrhea disease in humans. Further studies of the pathogen-

esis and geographic variability of EAEC strains are under way in our laboratory.

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