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Virulence Markers of Enteroaggregative *Escherichia coli* Isolated from Children and Adults with Diarrhea in Brasília, Brazil

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Escherichia coli **strains isolated from sporadic cases of acute diarrhea in children and adults and from children without diarrhea were investigated for the presence of the pAA plasmid. Strains harboring the pAA plasmid were isolated at similar frequencies from children with (19.6%) and without (10.8%) diarrhea and from adults with diarrhea (11.8%). The genotypic and phenotypic virulence markers of these strains were further analyzed. Most of the strains were positive for EAST1 (73%), and this toxin was detected significantly more frequently in strains from children with diarrhea than in strains from adults with diarrhea ($P < 0.05$). Likewise, *pic* sequences were detected significantly more frequently in strains from children with diarrhea than in strains from adults with diarrhea ($P < 0.005$) and controls ($P < 0.025$). Furthermore, the association of pAA positivity (pAA$^+$) and *pic* positivity (*pic*$^+$) was more frequently found for strains from children with diarrhea than for strains from controls, indicating that pAA$^+$ *pic*$^+$ strains may represent a subset of pAA$^+$ strains associated with disease in children. Most of the strains (82.5%) adhered to cells presenting the typical aggregative pattern. The frequency of occurrence of enteropathogenic *E. coli* (EPEC) serogroups in the strains from children with diarrhea was very high (56%), while none of the strains from adults with diarrhea belonged to EPEC serogroups. Extraintestinal virulence markers were very commonly found in strains from adults with diarrhea. The frequencies of occurrence of the adhesins AFA and SFA were significantly higher in strains from adults with diarrhea than in strains from children with diarrhea. More than one extraintestinal virulence marker was found in 58% of the strains from adults with diarrhea but in only 7.7% of the strains from children with diarrhea. Our results show that pAA$^+$ strains isolated from children and adults with diarrhea present very different profiles when enteroaggregative *E. coli* virulence markers, serotypes, and extraintestinal virulence markers are considered.**

Several studies have reported on the association of enteroaggregative *Escherichia coli* (EAEC) with diarrheal disease in children (3, 4, 13, 17, 21, 23, 33). The EAEC pathotype has been defined by its aggregative pattern of adherence to tissue culture cells, but this definition seems to cover heterogeneous groups of strains, as indicated in volunteer studies (26) and investigations of the natural occurrence of the disease (3, 13, 14, 17). Nataro et al. (26) carried out a volunteer study with four different EAEC strains and showed that three of them failed to elicit diarrhea. Several epidemiological studies reported on an association of EAEC strains with sporadic diarrhea, either acute or persistent, and outbreaks in different geographic locations (3, 13, 17, 21, 23, 33). Furthermore, Steiner et al. (44) reported on an association of EAEC with growth impairment even in the absence of diarrheal symptoms. However, in some case-control investigations, EAEC strains were isolated at similar frequencies from patients with acute diarrhea and controls (15, 16, 35).

The mechanisms by which these organisms cause diarrhea are not well understood; however, several virulence-related genes have been described. Most EAEC strains harbor a 60- to 65-MDa plasmid called pAA which has been shown to encode the aggregative adherence fimbriae AAF/I (28, 29, 40) and AAF/II (8); the enterotoxin EAST1, a toxin homologous to the heat-stable toxin (ST) of enterotoxigenic *E. coli* (38, 39); and Pet, a serine protease which has been described as causing enterotoxic and cytotoxic effects (30, 31). Another serine protease denominated Pic, encoded by a chromosomal gene displaying mucinolytic activity, serum resistance, and hemagglutination, has also been associated with EAEC strains (19).

This study examined *E. coli* strains from children and adults with sporadic cases of acute diarrhea and children without diarrhea for the presence of the pAA plasmid. The strains possessing the pAA plasmid (pAA$^+$ strains) were further analyzed for their adherence patterns, serotypes, and the presence of sequences homologous to the virulence markers associated with EAEC pathotype and extraintestinal *E. coli* virulence factors.

MATERIALS AND METHODS

Specimens and strains. *E. coli* strains were isolated from fecal specimens from 143 diarrheic children (ages, 0 to 24 months) and from 145 diarrheic adults who attended an outpatient clinic or who were admitted to three hospitals in Brasília, Brazil. An average of three *E. coli* isolates were obtained from each patient by standard procedures (11). *E. coli* isolates from 37 fecal specimens from children without diarrhea (controls) who attended the same outpatient clinic and who

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TABLE 1. Fragment probes used for DNA hybridization experiments

Pathotype	Target gene or plasmid	Fragment probe used	Reference
EAEC	Aggregative adherence plasmid (pAA)	pCVD432	Baudry et. al. (2)
EPEC	*eaeA*	pCVD434	Jerse et al. (22)
	bfp A	pMSD	Donnenberg et al. (9)
	EPEC adherence factor plasmid	pJPN16	Nataro et al. (27)
EHEC	EHEC plasmid	pCVD419	Levine et al. (25)
	stx_1	pJN37-19	Newland et al. (32)
	stx_2	pNN110-18	Newland et al. (32)
ETEC	*esth* (LT)	pCVD427	Echeverria et al. (10)
	estp (STp)	pCVD423	Echeverria et al. (10)
	elt (STh)	pCVD403	Echeverria et al. (10)

belonged to the same age group as the children without diarrhea were also examined. All the specimens were collected over a period of 18 months. Diarrhea was defined as the occurrence of two or more watery stools in a 24-h period. Neither patients nor controls had been treated with antibiotics in the 10 days preceding sampling.

DNA probes and colony hybridization. Table 1 shows the DNA probes used to detect the plasmid of EAEC strains (pAA), the *eaeA* and *bfpA* genes and plasmid EAF of enteropathogenic *E. coli* (EPEC) strains, plasmid EHEC and genes *stx1* and *stx2* of enterohemorrhagic *E. coli* (EHEC) strains, and genes coding for the heat-labile toxin (LT) and heat-stable toxins (STs) STp and STh of enterotoxigenic *E. coli* (ETEC). Probes were kindly provided by J. P. Nataro (Center for Vaccine Development, School of Medicine, University of Maryland, Baltimore). DNA fragments were labeled by random priming with [α-^{32}P]dATP and a random primer DNA labeling kit (New England Biolabs, Beverley, Mass.). Colony blots were prepared, processed, and hybridized under high-stringency conditions as described previously (36). The following *E. coli* strains were used as positive or negative controls: *E. coli* 17-2 (EAEC), *E. coli* 2348/69 (EPEC), *E. coli* 933 (EHEC plasmid), *E. coli* C600W (Shiga-like toxin [Stx] Stx2), *E. coli* C600J (Stx1), *E. coli* H10407 (ETEC), and *E. coli* HS (negative control).

Serotyping. Determination of O and H antigens was carried out as described previously (18, 34) with all available O-antigen (O1 to O181) and H-antigen (H1 to H56) antisera.

PCR. All strains that hybridized with the pCVD432 fragment probe (pAA$^+$ strains) were analyzed by PCR with the primers described in Table 2 to amplify fragments of genes encoding the fimbrial adhesins AAF/I, AAF/II, PAP, and SFA; afimbrial adhesin of the AFA/Dr family; the siderophore aerobactin; heat-stable enterotoxin EAST1; the cytotoxins cytotoxic necrotizing factor (CNF) and α-hemolysin; and the serine proteases Pet and Pic.

TABLE 2. Primers used for detection of virulence markers

Virulence marker	Gene	Primer sequence	Reference
AAF I	*aggA*	GCTAACGCTGCGTTAGAAAGACC GGAGTATCATTCTATATTCGCC	This work
AAF II	*aafA*	GACAACCGCAACGCTGCGCTG GATAGCCGGTGTAATTGAGCC	This work
EAST1	*astA*	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	Yamamoto and Echererria (48)
Pet	*pet*	CCGCAAATGGAGCTGCAAC CGAGTTTTCCGCCGTTTTC	Henderson et al. (20)
Pic	*pic*	TTCAGCGGAAAGACGAA TCTGCGCATTCATACCA	This work
PAP	*papC*	GAC GGC TGTACTGCAGGGTGTGGCG ATATCCTTTCTCTGCAGGGATGCAATA	Le Bouguence et al. (24)
SFA	*sfaD-sfaE*	CGGAGGAGTAATTACAAACCTGGCA CTCCGGAGAATCGGGTGCATCTTAC	Le Bouguenec et al. (24)
AFA	*afaB-afaC*	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	Le Bouguenec et al. (24)
Aerobactin	*aerA*	TACCGGATTGTCATATGCAGACCGT AATATCTTCCTCCAGTCCGGAGAAG	Yamamoto et al. (47)
CNF1 and CNF2	*cnf*	CTGGACTCGAGGTGGTGG CTCCTGTCAACCACAGCC	Blanco et al. (5)
Hly	*hly*	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	Yamamoto et al. (47)

TABLE 3. Adherence patterns and EAEC virulence markers for the 63 pAA$^+$ strains[a]

Test and virulence marker	No. (%) of strains from:			
	Children with diarrhea	Children without diarrhea	Adults with diarrhea	Total
PCR				
AAF/I	8 (20.5)	0 (0)	4 (21)	12 (19.0)
AAF/II	4 (10.3)	0 (0)	4 (21)	8 (12.7)
EAST1	32 (82.0)[b]	3 (60)	11 (57.9)[b]	46 (73.0)
Pic	22 (56.4)[c,d]	0 (0)[d]	3 (15.8)[c]	25 (39.7)
Pet	5 (12.8)	0 (0)	1 (5.3)	6 (9.5)
Adherence pattern				
Aggregative	33 (84.6)	5 (100)	14 (73.7)	52 (82.5)
Diffuse	2 (5.1)	0 (0)	1 (5.3)	3 (4.8)
Nonadherent	2 (5.1)	0 (0)	2 (10.5)	4 (6.3)
Detaching	2 (5.1)	0 (0)	2 (10.5)	4 (6.3)

[a] Thirty-nine pAA$^+$ strains were isolated from children with diarrhea, 5 pAA$^+$ strains were isolated from controls, and 19 pAA$^+$ strains were isolated from adults with diarrhea.
[b] $P < 0.05$ for difference between strains from children and adults with diarrhea.
[c] $P < 0.005$ for difference between strains from children and adults with diarrhea.
[d] $P < 0.025$ for difference between strains from children with and without diarrhea.

Adhesion assay. Adhesion tests were carried out as described previously (7), with some modifications. HeLa cells (1.6×10^5 cells ml^{-1}, 600 μl) were grown for 48 h at 37°C in medium 199 with 10% fetal bovine serum in 24-well tissue culture plates containing a coverslip. The medium was replaced with 300 μl of medium 199 supplemented with 2% fetal bovine serum and 1% D-mannose, followed by the addition of 75 μl of an exponential-phase bacterial culture (10^8 cells ml^{-1}) grown in Trypticase soy broth. After 3 h at 37°C, the wells were washed with phosphate-buffered saline six times, fixed with methanol, and stained with May-Grünwald and Giemsa stains as described previously (41).

Statistical analysis. *P* values were calculated by using a two-tailed chi-square distribution and one-sided Fisher's test when appropriate.

RESULTS

Prevalence of EAEC. Initially, 586 *E. coli* isolates from 143 children with diarrhea, 173 *E. coli* isolates from 37 children without diarrhea, and 435 *E. coli* isolates from 145 adults with diarrhea were investigated by colony hybridization to detect the pAA plasmid of EAEC. Positive strains were found in 19.6% (28 of 143), 11.7% (17 of 145), and 10.8% (4 of 37) of the children with diarrhea, the adults with diarrhea, and the controls, respectively. The incidence of pAA$^+$ strains was not significantly different among the strains from the groups studied. No virulence markers for EPEC, ETEC, or EHEC strains were detected in the pAA$^+$ strains by use of the probes described in Table 1.

Virulence marker and adherence pattern. The 63 pAA$^+$ strains (39 from children with diarrhea, 5 from controls, and 19 from adults with diarrhea) were assayed by PCR to detect the genes for AAF/I, AAF/II, EAST1, Pic, and Pet. Table 3 shows that most of the pAA$^+$ strains were positive for EAST1 (73%), and this toxin occurred significantly more frequently in strains from children with diarrhea than in strains from adults with diarrhea ($P < 0.05$). Likewise, *pic* sequences were detected significantly more frequently in strains from children with diarrhea than in strains from adults with diarrhea ($P < 0.005$) and controls ($P < 0.025$). Since the *pic* sequence was the only virulence marker more frequently found in pAA$^+$ strains from children with diarrhea than in strains from children without diarrhea, we also analyzed the frequency of pAA$^+$ *pic*-positive (*pic*$^+$) strains in cases and controls. pAA$^+$ and *pic*$^+$ strains were detected in 57% (16 of 28) of the pAA$^+$ strains from children with diarrhea but in none of the 4 pAA$^+$ strains from controls. The one-sided Fisher's test showed a *P* value of 0.0506, which is so close to statistical significance that it is acceptable to show an association between pAA$^+$ *pic*$^+$ strains and diarrhea. Sequences for the *pet* enterotoxin were found at a low frequency in the strains from all groups studied.

TABLE 4. Extraintestinal virulence markers for 63 pAA$^+$ strains[a]

Virulence marker	No. (%) of strains from:			
	Children with diarrhea	Children without diarrhea	Adults with diarrhea	Total
PAP	1 (2.5)	0	2 (10.5)	3 (4.8)
SFA	0[b]	0	2 (10.5)[b]	2 (3.1)
AFA	2 (5.1)[b,c]	1 (20.0)[c]	9 (47.4)[b]	12 (19.0)
AER	32 (82.0)	0	15 (78.9)	47 (74.6)
CNF	1 (2.5)	0	1 (5.3)	2 (3.1)
Hly	2 (5.1)	0	2 (10.5)	4 (6.3)

[a] Thirty-nine pAA$^+$ strains were isolated from children with diarrhea, 5 pAA$^+$ strains were isolated from controls, and 19 pAA$^+$ strains were isolated from adults with diarrhea.
[b] $P < 0.05$ for difference between strains from children and adults with diarrhea.
[c] $P < 0.01$ for difference between strains from children with and without diarrhea.

Only 31.7% (20 of 63) of the pAA$^+$ strains showed the fimbrial adhesin AAF/I or AAF/II. No adhesins were found in the strains from the controls. The AAF/I adhesin was detected more frequently than the AAF/II adhesin in strains from children with diarrhea, while both types of adhesins were detected at equal frequencies in strains from adults with diarrhea. It was interesting that five of the six strains positive for Pet were also positive for AAF/II (Table 3).

The 63 strains that hybridized with the probe for pAA were also assayed on HeLa cells. Table 3 shows the frequencies of the adherence patterns observed. Most of the strains (82.5%) adhered to cells presenting the aggregative pattern. Detachment from HeLa cells and nonadherence were observed for 6.3% of the strains. Three of the four detached strains exhibited a strong halo of hemolysis when grown on blood agar plates.

Extraintestinal virulence markers. The pAA$^+$ strains were also investigated for the presence of extraintestinal virulence markers (Table 4). Aerobactin was very frequently found in children with diarrhea (82%) and adults with diarrhea (78.9%), but it was not found in children without diarrhea. The frequencies of occurrence of AFA ($P < 0.01$) and SFA ($P < 0.05$) were significantly higher among the strains from adults with diarrhea than among the strains from children with diarrhea. AFA was also found significantly more frequently on strains from controls than on strains from children with diarrhea ($P < 0.05$). More than one extraintestinal virulence marker was found on 57.9% of the strains from adults with diarrhea but on only 7.7% of the strains from children with

TABLE 5. Serotypes and virulence markers for the 63 pAA$^+$ strains[a]

Serotype	No. of strains	Source (no. of isolates)	Pattern of adhesion (no. of isolates)	No. of strains with:									
				AAF	PET	PIC	EAST	PAP	SFA	AFA	HLY	AER	CNF
O1:H45	1	A	NA								1		1
O2:H2	1	A	AA			1	1		1		1	1	1
O2:H4	1	A	D				1	1		1	1	1	
O9:HNM	2	A (2)	AA (1); NA (1)	1 (I and II)[b]			2					1	
O9:H10	3	CD (2); A (1)	AA (2); D (1)		1	2	3			1		3	
O15:H18	3	A (3)	AA (3)	2 (I)		1				1		3	
O17:H33	1	CD	AA							1		1	
O21:H10	1	A	AA							1		1	
O21:H21	2	CD (1); CC (1)	AA (2)				1					1	
O36:H39	1	CD	DA				1					1	
O43:H2	1	A	AA									1	
O44:HNM	1	A	AA	1 (II)	1	1	1					1	
O44:H18	2	CD (2)	AA (2)	2 (II)	2	2	1						
O60:HNM	1	CC	AA				1					1	
O74:H11	2	A (2)	AA (1); DA (1)				2	1	1			1	
O78:H2	1	A	AA	1 (I)			1						
O86:H2	2	CD (2)	AA (2)			2	1					2	
O86:H18	3	CD (3)	AA (3)	3 (I)		3	2					2	
O110:H18	1	A	AA									1	
O125:H9	3	CD (3)	AA (3)				3				1	3	
O125:H21	1	CD	AA			1	1					3	1
O125:H33	4	CD (4)	AA (2); NA (2)			2	4					4	1
O126:HNT	2	CC (2)	AA				2			1			
O128:H10	1	CD	AA				1						
O153:HNM	1	A	AA							1		1	
O153:H2	2	CD (2)	AA (2)			2	1					2	
O174:H9	1	CD	AA				1					1	
O181:H1	1	CD	CD		1							1	
ONT:HNM	4	CD	AA (4)	3 (I)		2	4					4	
ONT:H10	1	CD	AA									1	
ONT:H21	1	CD	DA			1	1					1	
ONT:H25	3	CD (1); A (2)	AA (3)	3 (II)	1		3			1		1	
ONT:H33	3	CD (2); CC (1)	AA (3)	1 (I)		2	2					2	
ONT:HND	5	CD (5)	AA (4); D (I)	1 (I), 1 (II)	1	3	4	1				4	

[a] Abbreviations: A, strains from adults; CD, strains from children with diarrhea; CC, strains from control children; AA, aggregative adherence; DA, diffuse adherence; NA, nonadherent; D, detaching; NM, nonmotile; NT, not typeable; ND, not determined.
[b] The AAF type is given in parentheses.

diarrhea, showing that extraintestinal virulence markers are much more common on strains from adults with diarrhea than on strains from children with diarrhea.

Serotypes of pAA$^+$ strains. The pAA$^+$ strains from children with diarrhea belonged to 14 distinct serotypes (Table 5), and the most frequent serotypes were O86:H18, O125:H9, and O125:H33. The pAA$^+$ strains from adults with diarrhea and controls had 13 and 3 different serotypes, respectively, and among the strains from adults with diarrhea, the predominant serotype was O15:H18. Only two serotypes were found in more than one group; that is, O9:H10 strains were detected in children and adults with diarrhea, and O21:H21 strains were detected in children with and without diarrhea.

The frequency of occurrence of EPEC serogroups among the strains from children with diarrhea was very high. Fifty-six percent of the typeable strains from children with diarrhea belonged to the classical EPEC serogroups (O86, O125, and O128), while none of strains from adults with diarrhea belonged to EPEC serogroups. A high percentage (35.9%) of the pAA$^+$ strains from children with diarrhea did not react with any of the O antisera tested. It was interesting that strains from children with diarrhea with sequences for both *pic* and *pet* were only of serotypes O9:H10, O44:HNM (NM indicates nonmotile), and O44:H18.

DISCUSSION

Even though the study showed a trend for a higher frequency of pAA$^+$ strains from children with diarrhea than from healthy controls or adults with diarrhea, the differences were not statistically significant. Considering that studies on the rate of isolation of EAEC strains from diarrheic and healthy children from different geographic areas have been controversial, an association between EAEC and diarrhea should not be discounted. In southeastern Brazil, previous studies of pediatric diarrhea also found similar frequencies of EAEC strains in cases and controls (16, 35). However, in northeastern Brazil, Fang et al. (13) found the frequency of isolation of EAEC strains from patients with acute and persistent diarrhea to be significantly different from the frequency of isolation from controls. Reports from other countries associating EAEC with persistent or acute diarrhea in children (23, 33) can be found as frequently as reports showing no significant association (1, 15).

Isolation of EAEC strains from adults with diarrhea has seldom been reported. Gascón et al. (14) and Schultz et al. (42) reported on the presence of EAEC strains in patients with traveler's diarrhea, but the ages of the patients from which the EAEC strains were isolated were not specified. In this work we have shown that the frequency of isolation of EAEC strains from adults with sporadic cases of severe acute diarrhea was not significantly different from that from children with diarrhea.

The frequencies of occurrence of the virulence markers of EAEC varied with the origin of the strain. Apart from AAF/II, all the virulence markers of EAEC were more commonly found in the strains from children. In accordance with previous reports (12), adhesins AAF/I and AAF/II were found in a minority of the pAA$^+$ strains. These results indicate the presence of more EAEC virulence markers in the pAA plasmid in strains from children with diarrhea than in strains from adults with diarrhea and the diversity of the genes encoded by the pAA plasmid. However, these differences in the pAA plasmids had no effect on the ability of these pAA$^+$ strains to determine an aggregative pattern on HeLa cells, since most of the strains showed the typical aggregative adherence pattern, irrespective of their source or the adhesin-coding genes that they contained. The *pic* sequence was the only virulence marker more frequently found in pAA$^+$ strains from children with diarrhea than from controls, and the association of pAA$^+$ *pic*$^+$ strains with diarrhea was more frequent among cases than controls. This finding indicates that pAA$^+$ *pic*$^+$ strains represent a subset of pAA$^+$ strains which can be associated with disease. Nevertheless, since our control group was very small, further studies would be necessary to confirm this finding.

It was interesting to verify that a high proportion of the pAA$^+$ strains (27%) did not react with any of the antisera used. Untypeable strains have been also reported by Vial et al. (46), who worked with EAEC strains from children in Chile. Furthermore, an outbreak of severe diarrhea in children associated with O-untypeable EAEC strains has been reported (21). The strains from children with diarrhea were very frequently found to be of the EPEC classical serogroups, while such strains were very rarely recovered from adults. EAEC strains from children have previously been reported to belong to EPEC serogroups (6, 17, 35, 49). Rosa et al. (35) found that most of the EAEC strains from children with diarrhea belonged to EPEC serogroups, and similar to our findings, they showed that the most frequent serogroups were O86 and O125. Moreover, Valle et al. (45), in a study of a collection of strains belonging to serogroup O125, found that serotypes O125:H21 and O125:H9 were enteroaggregative. Since Smith et al. (43) reported that isolates of *E. coli* O44:H18 of diverse origins were enteroaggregative, it was also interesting to observe that only the strains belonging to serotypes O44:H18 and O44:HNM presented an adhesin and the three toxins associated with EAEC.

Most of the extraintestinal virulence markers were very commonly found in strains from adults with diarrhea, while in strains from children with diarrhea they were quite rare except for the aerobactin sequences, which were present at equally high rates in children and adults with diarrhea. Okeke et al. (33), in a study with children with diarrhea, reported that strains presenting sequences for *pap* showed weak aggregative adherence; however, the frequency of these strains was not mentioned. Santos (37) also found sequences homologous to those associated with extraintestinal virulence markers (adhesins AFA, SFA, and PAP) in a group of strains from children with and without diarrhea.

In conclusion, our results show that pAA$^+$ strains from children and adults with diarrhea present quite different profiles when EAEC virulence markers, serogroups, and extraintestinal virulence markers are considered.

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REFERENCES

1. **Albert, M. J., S. M. Faruque, A. S. G. Faruque, P. B. K. Neogi, M. Ansaruzzaman, N. A. Bhuiyan, K. Alam, and M. S. Akabar.** 1995. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. J. Clin. Microbiol. **33:**973–977.
2. **Baudry, R. S., J. Savarino, P. Vial, J. B. Kaper, and M. M. Levine.** 1990. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J. Infect. Dis. **161:**1249–1251.
3. **Bhan, M. K., P. Raj, M. M. Levine, J. B. Kaper, N. Bhandari, R. Srivastana, and S. Sazawal.** 1989. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J. Infect. Dis. **159:**1061–1064.
4. **Bhatnagar, S., M. K. Bhan, H. Sommerfelt, S. Sazawal, R. Kumar, and S. Saini.** 1993. Enteroaggregative *Escherichia coli* may be a new pathogen causing acute and persistent diarrhea. Scand. J. Infect. Dis. **25:**579–583.
5. **Blanco, M., J. E. Blanco, J. Blanco, M. P. Alonso, C. Balsalobre, M. Mouriño, C. Madri, and A. Juárez.** 1996. Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and 2 (CNF1 and CNF2). J. Microbiol. Methods **26:**95–101.
6. **Campos, L. C., T. S. Whittam, T. A. T. Gomes, J. R. C. Andrade, and L. R. Trabulsi.** 1994. *Escherichia coli* serogroup O111 includes several clones of diarrheagenic strains with different virulence properties. Infect. Immun. **62:**3282–3288.
7. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3:**95–99.
8. **Czeczulin, J. R., S. Balepur, S. Hicks, A. Phillips, R. Hall, M. H. Kothary, F. Navarro-Garcia, and J. P. Nataro.** 1997. Aggregative adherence fimbriae II, a second fimbrial antigen mediating aggregative adherence in enteroaggregative *Escherichia coli*. Infect. Immun. **65:**4135–4145.
9. **Donnenberg, M. S., J. A. Giron, J. P. Nataro, and J. B. Kaper.** 1992. A plasmid-encoded type IV fimbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence. Mol. Microbiol. **6:**3427–3437.
10. **Echeverria, P., D. N. Taylor, J. Seriwatana, and C. Moe.** 1987. Comparative study of synthetic and cloned polynucleotide enterotoxin gene probes to identify enterotoxigenic *Escherichia coli*. J. Clin. Microbiol. **25:**106–109.
11. **Edwards, P. R., and W. R. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing, Minneapolis, Minn.
12. **Elias, W. P., S. Suzart, L. R. Trabulsi, J. P. Nataro, and T. A. Gomes.** 1999. Distribution of aggA and aafA gene sequences among *Escherichia coli* isolates with genotypic or phenotypic characteristics, or both, of enteroaggregative *E. coli*. J. Med. Microbiol. **48:**597–599.
13. **Fang, G. D., A. A. Lima, C. V. Martins, J. P. Nataro, and R. L. Guerrant.** 1995. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. J. Pediatr. Gastroenterol. Nutr. **21:**137–144.
14. **Gascón, J., M. Vargas, L. Quintó, M. Corachán, M. T. Jiménez de Anta, and J. Vila.** 1998. Enteroaggregative *Escherichia coli* strains as a cause of traveler's diarrea: a case-control study. J. Infect. Dis. **177:**1409–1412.
15. **Gascón, J., M. Vargas, D. Schellenberg, H. Urassa, C. Casals, E. Kahigwa, J. J. Aponte, H. Mshinda, and J. Vila.** 2000. Diarrhea in children under 5 years of age from Ifakara, Tanzania: a case-control study. J. Clin. Microbiol. **38:**4459–4462.
16. **Gomes, T. A. T., M. A. M. Vieira, C. M. Abe, D. Rodrigues, P. M. Griffin, and S. R. T. S. Ramos.** 1998. Adherence pattern and adherence-related DNA sequences in *Escherichia coli* isolated from children with and without diarrhea in São Paulo City, Brazil. J. Clin. Microbiol. **36:**3609–3613.
17. **Gonzalez, R., C. Diaz, M. Marino, R. Cloralt, M. Pequeneze, and I. Perez-Schael.** 1997. Age-specific prevalence of *Escherichia coli* with localized and aggregative adherence in Venezuelan infants with acute diarrhea. J. Clin. Microbiol. **35:**1103–1107.

18. **Guinée, P. A. M., W. H. Jansen, T. Wadström, and R. Sellwood.** 1991. *Escherichia coli* associated with neonatal diarrhoea in piglets and calves, p. 126–162. *In* P. W. De Leeuw and P. A. M. Guinée (ed.), Current topics in veterinary medicine and animal science. Martinus Nijhoff, The Hague, The Netherlands.
19. **Henderson, I. R., J. Czeczulin, C. Eslava, F. Noriega, and J. P. Nataro.** 1999. Characterization of Pic, a secreted protease of *Shigella flexneri* and enteroaggregative *Escherichia coli*. Infect. Immun. **67:**5587–5596.
20. **Henderson, I. R., S. Hicks, F. Navarro-Garcia, W. P. Elias, A. D. Philips, and J. P. Nataro.** 1999. Involvement of the enteroaggregative *Escherichia coli* plasmid-encoded toxin in causing human intestinal damage. Infect. Immun. **67:**5338–5344.
21. **Itoh, Y., I. Nagano, M. Kunishima, and T. Ezaki.** 1997. Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable:H10 associated with a massive outbreak of gastrointestinal illness. J. Clin. Microbiol. **35:** 2546–2550.
22. **Jerse, A. E., J. Yu, B. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:**7839–7843.
23. **Knutton, S., R. Shaw, A. D. Phillips, H. R. Smith, G. A. Willshaw, P. Watson, and E. Price.** 2001. Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* isolated from children in the United Kingdom. J. Pediatr. Gastroenterol. Nutr. **33:**32–40.
24. **Le Bouguenec, C., M. Archambaud, and A. Labigne.** 1992. Rapid and specific detection of Pap, AFA, and SFA adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J. Clin. Microbiol. **30:**1189–1193.
25. **Levine, M. M., J. G. Xu, J. B. Kaper, H. Lior, V. Prado, B. Tall, J. P. Nataro, H. Karch, and K. Wachsmuth.** 1987. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J. Infect. Dis. **156:**175–182.
26. **Nataro, J. P., D. Yikang, S. Cookson, A. Cravioto, S. J. Savarino, L. D. Guers, M. M. Levine, and C. O. Tacket.** 1995. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. J. Infect. Dis. **171:**465–468.
27. **Nataro, J. P., M. M. Baldini, J. B. Kaper, R. E. Black, and M. M. Levine.** 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J. Infect. Dis. **152:**560–565.
28. **Nataro, J. P., Y. Deng, D. R. Maneval, A. L. German, W. C. Martin, and M. M. Levine.** 1992. Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infect. Immun. **60:**2297–2304.
29. **Nataro, J. P., D. Yikang, J. A. Giron, S. J. Savarino, M. H. Kothary, and R. Hall.** 1993. Aggregative adherence fimbriae I expression in enteroaggregative *Escherichia coli* requires two unlinked plasmid regions. Infect. Immun. **61:**1126–1131.
30. **Navarro-Garcia, F., C. Eslava, J. M. Villaseca, R. López-Revilla, J. R. Czeczulin, S. Srinivas, J. P. Nataro, and A. Cravioto.** 1998. In vitro effects of a high-molecular-weight heat-labile enterotoxin from enteroaggregative *Escherichia coli*. Infect. Immun. **66:**3149–3154.
31. **Navarro-Garcia, F., C. Sears, C. Eslava, A. Cravioto, and J. P. Nataro.** 1999. Cytoskeletal effects induced by Pet, the serine protease enterotoxin of enteroaggregative *Escherichia coli.* Infect. Immun. **67:**2184–2192.
32. **Newland, J. W., and R. J. Neill.** 1988. DNA probes for Shiga-like toxins I and II for toxin-converting bacteriophages. J. Clin. Microbiol. **26:**1292–1297.
33. **Okeke, I. N., A. Lamikanra, H. Steinrück, and J. B. Kaper.** 2000. Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. J. Clin. Microbiol. **38:**7–12.
34. **Ørskov, F., and I. Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **43:**112–142.
35. **Rosa, A. C. P., A. T. Mariano, A. M. S. Pereira, A. Tibana, T. A. T. Gomes, and J. R. C. Andrade.** 1998. Enteropathogenicity markers in *Escherichia coli* isolated from infants with acute diarrhoea and healthy controls in Rio de Janeiro, Brazil. J. Med. Microbiol. **47:**781–790.
36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
37. **Santos, S. S.** 2000. Diversidades fenotípicas e genotípicas em amostras de *Escherichia coli* enteroagregativa com variações no padrão de aderência e/ou na reatividade com a sonda EAEC. Ph.D. thesis. Universidade Federal de São Paulo, São Paulo, Brazil.
38. **Savarino, S. J., A. Fasano, D. C. Robertson, and M. M. Levine.** 1991. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J. Clin. Investig. **87:**1450–1455.
39. **Savarino, S. J., A. Fasano, J. Watson, B. M. Martin, M. M. Levine, S. Guandalini, and P. Guerry.** 1993. Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of *E. coli* heat-stable toxin. Proc. Natl. Acad. Sci. USA **90:**3093–3097.
40. **Savarino, S. J., P. Fox, D. Yinkang, and J. P. Nataro.** 1994. Identification and characterization of a gene cluster mediating enteroaggregative *Escherichia coli* aggregative adherence fimbria I biogenesis. J. Bacteriol. **176:**4949–4957.
41. **Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi.** 1984. Distinctive pattern of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. **45:**534–536.
42. **Schultz, C., J. van den Ende, F. Cobelens, T. Vervoort, A. van Gompel, J. C. F. M. Wetsteyn, and J. Dankert.** 2000. Diarrheagenic *Escherichia coli* and acute and persistent diarrhea in returned travelers. J. Clin. Microbiol. **38:**3550–3554.
43. **Smith, H. R., S. M. Scotland, G. A. Willshaw, B. Rowe, A. Cravioto, and C. Eslava.** 1994. Isolates of Escherichia coli O44:H18 of diverse origin are enteroaggregative. J. Infect. Dis. **170:**1610–1613.
44. **Steiner, T. S., A. A. M. Lima, J. P. Nataro, and R. L. Guerrant.** 1998. Enteroaggregative *Escherichia coli* produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. J. Infect. Dis. **177:**88–96.
45. **Valle, G. R. F., T. A. T. Gomes, K. Irino, and L. R. Trabulsi.** 1997. The traditional enteropathogenic *Escherichia coli* (EPEC) serogroup O125 comprises serotypes which are mainly associated with the categories of enteroaggregative *E. coli*. FEMS Microbiol. Lett. **152:**95–100.
46. **Vial, P. A., R. Robins-Browse, H. Lior, V. Prado, J. B. Kaper, J. P. Nataro, D. Maneval, A. Elsayed, and M. M. Levine.** 1988. Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease. J. Infect. Dis. **158:**70–79.
47. **Yamamoto, S., A. Terai, K. Yuri, H. Kuruzono, Y. Takeda, and O. Yoshida.** 1995. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. FEMS Immunol. Microbiol. **12:**85–90.
48. **Yamamoto, T., and P. Echeverria.** 1996. Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. Infect. Immun. **64:**1441–1445.
49. **Yatsuyanagi, J., S. Saito, H. Sato, Y. Miyajima, K. Amano, and K. Enomoto.** 2002. Characterization of enteropathogenic and enteroaggregative *Escherichia coli* from diarrheal outbreaks. J. Clin. Microbiol. **40:**294–297.