JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2002, p. 1254–1258 Vol. 40, No. 4
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.4.1254–1258.2002

Comparison of DNA Hybridization and PCR Assays for Detection of Putative Pathogenic Enteroadherent *Escherichia coli*

Isabel C. A. Scaletsky,[1]* Sandra H. Fabbricotti,[1] Katia R. Aranda,[1]
Mauro B. Morais,[2] and Ulysses Fagundes-Neto[2]

Department of Microbiology, Immunology, and Parasitology[1] and Division of Pediatric Gastroenterology,[2]
Universidade Federal de São Paulo, Escola Paulista de Medicina,
São Paulo, SP, Brazil CEP 04023-062

Received 27 August 2001/Returned for modification 4 November 2001/Accepted 7 January 2002

The correlation of the different adherence patterns with DNA probes and PCR primers for the identification of *Escherichia coli* was analyzed in isolates from children, less than 2 years of age with or without diarrhea, from different regions of Brazil. A total of 1,428 isolates obtained from 338 patients and 322 control children were studied. The enteropathogenic *E. coli* (EPEC) adherence factor (EAF) probe was shown to be as good as the HEp-2 adhesion assay for the detection of typical EPEC strains. The DNA probes used to detect diffusely adhering *E. coli* and enteroaggregative *E. coli* (EAEC) showed low sensitivities (64 and 50%, respectively), and the best method of identifying these organisms in clinical research remains the HEp-2 adherence assay. The "bundle-forming pilus" (BFP) and the EAEC PCR assays could be used instead of the DNA probes as a screening method for typical EPEC and EAEC carrying the EAEC probe sequence in the clinical laboratory. In our study, only typical EPEC strains that carried EAF and BFP were associated with acute diarrhea.

Three distinct patterns of adherence to epithelial cells among fecal isolates of *Escherichia coli* have been identified: localized adherence (LA) (33), diffuse adherence (DA) (33) and aggregative adherence (AA) (31). Recently, Scaletsky et al. (34) have described a pattern of adherence similar to LA, called LA-like (LAL), characterized by the presence of bacterial microcolonies and few bacteria dispersed on the cells which is observed only upon prolonged incubation periods.

The LA exhibited by typical enteropathogenic *E. coli* (typical EPEC) is mediated by an inducible bundle-forming pilus (BFP), whose expression correlates with the presence of a plasmid designated the EPEC adherence factor (EAF) plasmid (1, 17). EPEC strains also cause attaching and effacing lesions on eukaryotic cells that involve a 94-kDa protein encoded by the chromosomal *eae* gene (25, 28). The pathogenicity of EPEC strains has been demonstrated in human volunteers, and their role in childhood diarrhea was confirmed in epidemiological studies (9, 10, 11, 15, 18, 26). Atypical EPEC strains do not carry the EAF plasmid, were found to exhibit LAL, and have been isolated from acute infantile diarrhea in São Paulo (35).

The adherence of many enteroaggregative *E. coli* strains requires the presence of a plasmid that contains genes encoding the AA (38). Epidemiological studies have implicated EAEC as a cause of diarrhea in children in developing countries, and the pathogenic potential of EAEC in human infections was substantiated by challenge studies (5, 10, 23, 27, 39).

Two factors, F1845 and AIDA-I were found to encode DA in diffusely adhering *E. coli* (DAEC) (4, 6). Several recent studies have implicated DAEC strains as agents of diarrhea, while other studies have not recovered DAEC strains more frequently from diarrheal patients than from asymptomatic controls (2, 15, 16, 21, 24).

DNA probes derived from the adherence-related sequences have been constructed (3, 4, 6, 17, 25, 30) and used in hybridization assays for the detection of the different putative categories of diarrheagenic *E. coli* in many epidemiologial studies. PCR primers have been also developed for several of the categories of diarrheagenic *E. coli* (8, 14, 20, 36).

In order to optimize screening methods for putative pathogenic enteroadherent *E. coli* in the clinical laboratory we analyzed the correlation of the different HEp-2 adherence patterns with DNA probes and PCR primers in *E. coli* isolates from different urban centers of Brazil.

MATERIALS AND METHODS

Patients. From August 1997 to July 1999, 338 infants younger than 2 years of age with diarrhea (286 acute and 52 persistent cases) were recruited into the study. These children took part in a study on the etiology of acute and persistent diarrhea in different regions of Brazil. The children were admitted to public hospitals for treatment in the following cities: São Paulo, Joinville, Natal, and São Luiz. Diarrhea was defined as the excretion of three or more liquid stools during the 24 h before admission. Acute diarrhea was defined as diarrhea lasting less than 14 days at the time of admission. Persistent diarrhea was defined as diarrhea of a presumably infectious etiology lasting more than 14 days. A control group containing 322 asymptomatic children matched for age was randomly selected from the well-child outpatient clinic of the same hospitals and was examined during the same study time period. Control infants had no gastrointestinal symptoms for at least 30 days prior to the inclusion into the study. A consent form was signed by the parents of the patients to approve their inclusion into the study.

Microbiological studies. *E. coli* strains were isolated on MacConkey agar. Four separate lactose-fermenting colonies, presumed to be *E. coli* by colony morphology, and two non-lactose-fermenting colonies of each distinct morphological type were cultivated in commercial test systems (PROBAC do Brasil, São Paulo, Brazil) for biochemical confirmation of species or genus. All *E. coli* isolates were tested with specific DNA probes designed to detect enterotoxigenic *E. coli* (LT and ST probes), enteroinvasive *E. coli* (Inv probe), and Shiga-toxin-producing *E.*

* Corresponding author. Mailing address: Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, Rua Botucatu, 862, 04023-062 São Paulo, SP, Brazil. Phone: 55764537. Fax: 55716504. E-mail: scaletsky@ecb.epm.br.

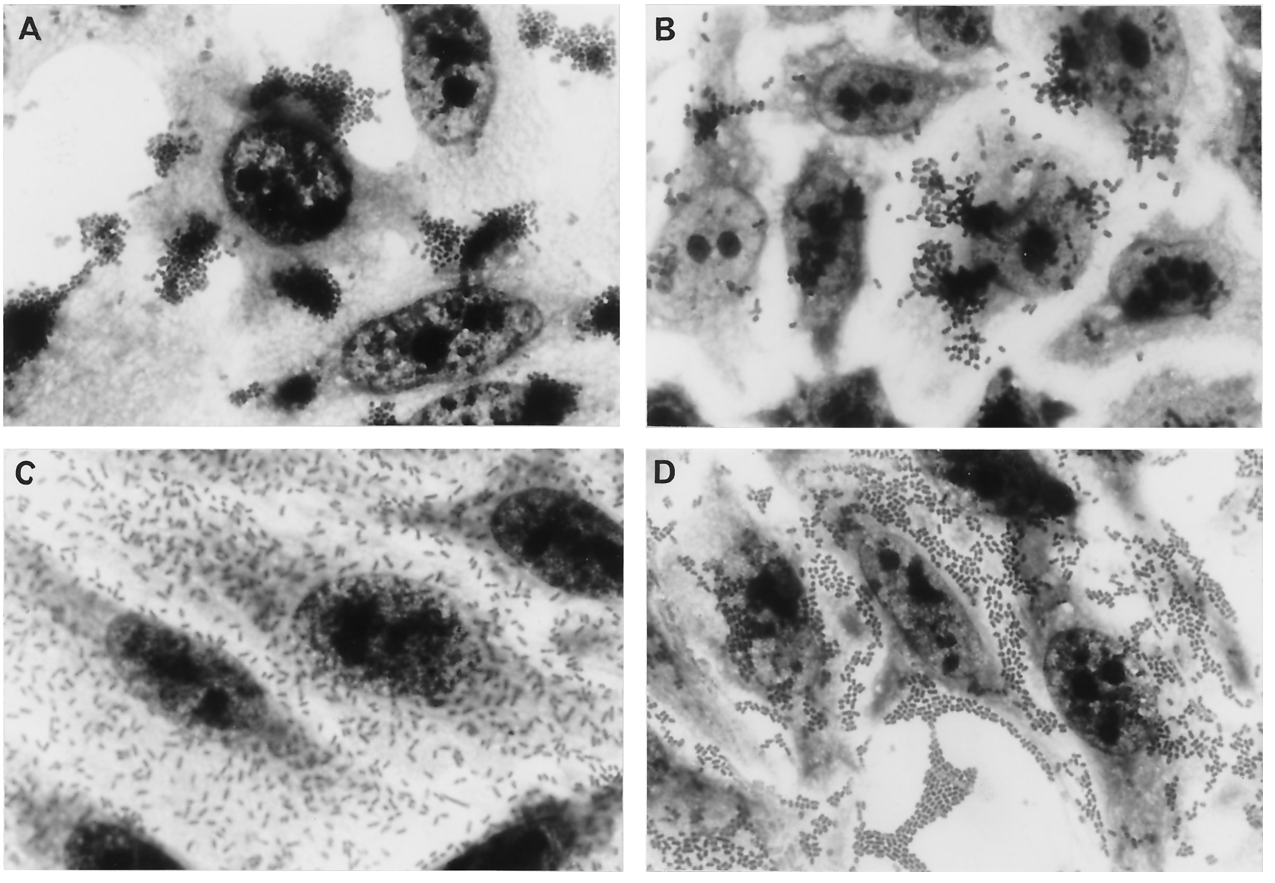

FIG. 1. HEp-2 adherence patterns of *E. coli*. (A) LA pattern; (B) LAL pattern; (C) DA pattern (DA and DA6h); (D) AA pattern (AA and AA6h).

coli (Stx1 and Stx2 probes), as described previously (29, 32, 37). The presence of *Shigella* spp., *Salmonella* spp., *Giardia lamblia*, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium* spp., or rotavirus was determined by standard methods (12, 13, 22). All strains were maintained on nutrient agar slants at room temperature.

HEp-2 adherence test. All *E. coli* isolates were characterized by the pattern of adherence to HEp-2 cells in the presence of D-mannose according to the method described by Scaletsky et al. (33). Briefly, monolayers of 10^5 HEp-2 cells were grown in Dulbecco modified Eagle medium (Gibco-BRL, Gaithersburg, Md.) containing 10% fetal bovine serum in 24-well tissue culture plates (Falcon Becton Dickinson, Franklin Lakes, N.J.). Bacterial strains were grown statically in 3 ml of tryptic soy broth (Difco, Detroit, Mich.) for 16 to 18 h at 37°C. The monolayers were infected with ca. 3×10^7 bacteria (40 μl of bacterial cultures added to 1 ml of Dulbecco modified Eagle medium) and incubated at 37°C for 3 h. The infected monolayers were washed with sterile phosphate-buffered saline, fixed with methanol, stained with May-Grünwald–Giemsa stain, and examined under a light microscope. When the adherence pattern was weak or negative, a new preparation was made and the monolayers were examined after a 6-h incubation period.

DNA hybridization. All *E. coli* isolates were tested by colony DNA hybridization with the following specific gene probes: EAF (EPEC adherence plasmid), a 1-kb *Bam*HI-*Sal*I fragment derived from plasmid pMAR2 (30); *bfpA* (encoding the major subunit of the bundle-forming pilus of EPEC strains), an 852-bp *Eco*RI fragment of pMSD207 (17); *eae* (encoding intimin, an outer membrane protein involved in the attaching and effacing lesions promoted by EPEC), a 1-kb *Sal*I-*Kpn*I fragment from plasmid from pCVD434 (25); *daaC* (associated with the biogenesis of F1845, a fimbrial adhesin involved in DA), a 350-bp *Pst*I fragment of pSLM852 (6); AIDA-I (protein associated with the DA phenotype), a 6.2-kb *Sph*I-*Cla*I fragment of pIB264 (4); and EAEC (EAEC adherence plasmid), a 1-kb *Eco*RI-*Pst*I fragment of pCVD432 (3). Colony blots were prepared with Whatman 541 filter papers. The fragment probes were prepared by extracting plasmids by the method of Birnboim and Doly (7), digesting them with appropriate restriction endonucleases, and purifying fragments by gel extraction. The fragments were then labeled by random priming with [α-^{32}P]dCTP by using a commercially available labeling kit (Amersham Pharmacia Biotech) and removing unincorporated nucleotides by passage through Sephadex G-50 microcolumns (Amersham Pharmacia Biotech). Hybridization was carried out under high-stringency conditions employing a hybridization buffer of the following composition: 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate, 10 mM EDTA, 1× Denhardt's solution, and 100 μg of sonicated salmon sperm DNA/ml. Colony blots were hybridized at 65°C overnight, washed with 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C, and exposed to X-ray film overnight at −80°C.

PCR assay. All adherent *E. coli* isolates were tested by PCR with oligonucleotide primers to detect EPEC, DAEC, and EAEC, as described previously (8, 14, 20, 36). Three to six bacterial colonies from each isolate were pooled for template DNA preparation immediately prior to PCR testing, suspended in 300 μl of sterile distilled water, and boiled for 10 min. A 10-μl aliquot of this suspension was added to 90 μl of PCR mixture (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 1 mM $MgCl_2$, 0.25 mM concentrations of each deoxynucleoside triphosphate, and 2.5 U of *Taq* polymerase) and subjected to PCR. The amplified DNA products were resolved by agarose gel electrophoresis and visualized by UV translumination after ethidium bromide staining.

Statistical analysis. Data derived from children with diarrhea and from control subjects were compared by a two-tailed chi-square or Fisher's exact test.

RESULTS

***E. coli* adherence to HEp-2 cells.** A total of 1,428 *E. coli* isolates were tested for HEp-2 adhesion. The HEp-2 assay differentiated four phenotypes of adherent *E. coli.* The typical LA pattern was characterized by clusters of bacteria (Fig. 1A),

TABLE 1. Comparison of DNA probe hybridization and HEp-2 adherence results for *E. coli* strains

Adherence pattern (*n*)	No. (%) of strains that hybridized with DNA probe[b]:				
	daaC	EAEC	EAEC/*daaC*	*bfpA*	None
LA (81)	0	0	0	81 (100)	0
LAL (20)	0	0	0	3 (15)	17 (85)
DA (185)	122 (65.9)	0	2 (1,1)	0	53 (28.6)
DA6h (17)	7 (41.2)	1 (5.9)	0	0	9 (52.9)
AA (162)	10 (6.2)	79 (48.8)	9 (5.6)	0	64 (39.5)
AA6h (27)	4 (14.8)	8 (29.6)	0	0	15 (55.5)
DE (112)	12 (10.7)	11 (9.8)	1 (0.9)	0	88 (78.6)
NA (820)	17 (2.1)	18 (2.1)	0	0	785 (95.7)

[a] *n* = number of strains.
[b] The EAF and AIDA-I probes were not included.

and the LAL pattern, observed only in a 6-h assay, was characterized by the formation of microcolonies or clusters less dense and less compact than those displayed by typical LA (Fig. 1B). Two types of DA were detected: typical DA, in which the bacteria adhere over the entire surface of the cells detected in the 3-h assay, and DA that could be clearly discerned only in the 6-h assay (DA6h) (Fig. 1C). Regarding the AA pattern, two types were detected: typical AA, in which the bacteria adhere to HEp-2 cells as well as to the glass between the cells in a characteristic "stacked-brick" appearance, and AA that could be detected only in the 6-h assay (AA6h) (Fig. 1D). The adherence patterns of 112 isolates could not be determined because they promoted complete detachment (DE) of the HEp-2 cells monolayers in the 3-h assays. Among the remaining 1,316 *E. coli* isolates tested, 820 (57%) were nonadherent (NA).

Correlation of HEp-2 adherence patterns with DNA hybridization by using the EAF, *bfpA*, *daaC*, AIDA-I, and EAEC probes. The *E. coli* isolated in this study were examined by DNA hybridization with probes designed to identify strains exhibiting the LA, DA, and AA adherence patterns (Table 1). All of the isolates with LA hybridized with EAF and *bfpA* probes, whereas none of the isolates with LAL reacted with the EAF probe, and three isolates with LAL reacted with the *bfpA* probe. The *daaC* probe reacted with 124 of 185 isolates with DA, with 7 of the 17 isolates with DA6h (sensitivity, 64.3%), and with 53 isolates that exhibited adherence patterns distinct from DA or that were NA (specificity, 95.7%). The AIDA-I probe reacted with only six isolates with DA, four of which hybridized with the *daaC* probe. The EAEC probe detected 88 of the 162 with AA, 8 of the 27 isolates with AA6h (sensitivity, 50.2%), and reacted with only 33 isolates showing none of the different AA types (specificity, 97.3%). Most (78%) of the cytodetaching (DE) isolates did not hybridize with any of the probes used (Table 1).

Distribution of adherence patterns combined with DNA probes in children with or without diarrhea. The distribution of *E. coli* isolates showing different patterns of adherence and related DNA probes in cases and controls is presented in Table 2. Approximately 30% of the children studied carried more than one type of pathogenic species. The statistical analysis of the association with diarrhea was performed for those children in whose stools none of the other pathogens were identified. Only the LA pattern was associated with diarrhea (11.5 versus 2.5%, $P < 0.01$). The LAL pattern was more frequent in cases than in controls (3.6 versus 1.5%), but this difference was not significant ($P = 0.11$). DA was the most frequent pattern among isolates from both cases and controls (23.4 versus 20.2%), followed by AA (22.2 versus 15.2%). Both DA6h and AA6h were isolated with similar frequencies from both cases and controls. Isolates carrying *daaC* were the most frequent pattern among isolates from both cases and controls (15.7 versus 15.2%), followed by EAEC (14.5 versus 9.9%).

TABLE 2. Distribution of different patterns of adherence combined with DNA probes in *E. coli* isolated from 338 patients with diarrhea and 322 age-matched controls

Pattern of adherence	DNA probe	No. (%) of infants		*P*
		Patients	Controls	
LA	EAF, *bfpA*	39 (11.5)	8 (2.5)	<0.01
LAL	*bfpA*	2 (0.6)	1 (0.3)	0.59
	None	10 (2.9)	4 (1.2)	0.21
DA	*daaC*	53 (15.7)	49 (15.2)	0.95
	daaC, EAEC	2 (0.6)	0	0.17
	None	26 (7.8)	16 (5.7)	0.20
DA6h	*daaC*	1 (0.3)	4 (1.2)	NS
	EAEC	1 (0.3)	0	0.33
	None	5 (1.5)	4 (1.2)	0.79
AA	EAEC	49 (14.5)	32 (9.9)	0.09
	EAEC, *daaC*	2 (0.6)	3 (0.9)	NS
	daaC	6 (1.8)	4 (1.2)	0.58
	None	18 (5.3)	10 (3.1)	0.22
AA6h	EAEC	7 (2.1)	2 (0.6)	0.10
	daaC	2 (0.6)	2 (0.6)	0.96
	None	5 (1.5)	6 (1.9)	NS

Comparison of the DNA probes and PCR assay. A total of 496 isolates were subjected to PCR assay, and the results were compared with the DNA probes (Table 3). The results of PCR with primers of the EAF sequence demonstrated that only 17 of 81 EAF probe-positive EPEC strains with LA yielded positive EAF PCR results. None of the 20 EAF-negative strains amplified EAF, and the three *bfpA* probe-positive strains with LAL gave a positive BFP PCR. Only 53 of 131 *daaC* strains carried the *daaE* sequence as detected by PCR. Of 162 isolates which demonstrate AA to HEp-2 cells, 86 were positive with the EAEC PCR. All of these strains reacted with the EAEC probe. Six EAEC isolates gave a positive-PCR but probe-negative result, whereas two isolates gave only positive results by DNA probe. Three strains with AA6h gave a positive-PCR and probe-negative result.

DISCUSSION

Currently, in many laboratories adherent *E. coli* are detected from mixed cultures by analyzing individual colonies with the HEp-2 adherence assay. However, this technique is cumbersome and inefficient if large numbers of colonies must be analyzed. In this study we analyzed the correlation of the different adherence patterns with DNA probes and PCR primers for identification of putative pathogenic enteroadherent *E. coli* in isolates from children with or without diarrhea.

TABLE 3. Comparison of results with DNA probe hybridization and PCR tests to detect the different adherence patterns in 496 isolates from patients (n = 305) and controls (n = 191)

Adherence pattern (DNA probe)	No. of isolates	Agreement (+) or disagreement (−) as determined by:	
		DNA probe	PCR
LA			
EAF	17	+	+
EAF	64	+	−
bfpA	81	+	+
LAL			
EAF	20	−	−
bfpA	3	+	+
DA (*daaC*)	53	+	+
	71	+	−
	61	−	−
DA6h (*daaC*)	7	+	−
	10	−	−
AA (EAEC)	86	+	+
	2	+	−
	6	−	+
	68	−	−
AA6h (EAEC)	8	+	+
	3	−	+
	16	−	−

The adherence-related DNA probes used showed excellent specificities (>96%), but their sensitivities varied. Compared to the LA phenotype as detected by the HEp-2 assay, the EAF probe was 100% sensitive and specific, while the *bfpA* probe was 100% sensitive and 99% specific. All isolates with LA carried *eae*, EAF, and *bfpA*, whereas three isolates with LAL carried *eae* and *bfpA*. The *bfpA* probe has been considered to be more sensitive than the EAF probe in detecting LA-producing *E. coli* (16). However, our data suggest that the *bfpA* probe detects not only isolates with LA but also some LAL-producing *E. coli*. Thus, we are inclined to use the EAF probe that is equally sensitive as the adhesion assay in detecting LA and to use the adhesion assay to detect the LAL pattern. Compared with DNA hybridization, the EAF PCR assay described by Franke et al. (14) proved to be a nonspecific and inefficient method for the detection of EPEC strains carrying the EAF plasmid. The BFP PCR was similar in sensitivity to that of the *bfpA* probe.

The different combinations of adherence patterns and DNA probes found in this study showed that the HEp-2 assay remains the "gold standard" for detection of DAEC and EAEC. The DAEC strains should be defined by the presence of the DA pattern in the HEp-2 assay. By using the *daaC* probe we found an apparent low sensitivity (64.3%), which is in agreement with the concept that 65% of the DAEC strains from around the world are positive with this F1845 gene probe (16, 19, 24). Using the PCR assay for the detection of *daaE* sequences, we found 40% DAEC-positive strains.

The presence of bacterial clusters in a stacked-brick configuration, characterizing the AA and AA6h patterns, should be used to identify the EAEC strains. By using the EAEC probe for the detection of EAEC strains, we found a correlation of 50.2% sensitivity, which is in accordance with the levels of sensitivity found in other studies (3, 19, 39). As many as 39 probe-negative EAEC strains were able to adhere to HEp-2 cells in an AA or AA6h pattern. By using the EAEC PCR, we found a sensitivity and specificity similar to those of the EAEC probe.

In our study, only EPEC strains that showed LA and that carried *eaeA*, EAF, and *bfpA* genes were associated with diarrhea in children less than 2 years of age from different regions of Brazil. Although DAEC and EAEC were very frequently found in these children, they were not significantly associated with acute diarrhea.

In conclusion, the present study confirms that the EAF probe is as good as the HEp-2 adhesion assay for the detection of EPEC strains with LA. The apparent low sensitivity of the *daaC* and EAEC probes suggests that the HEp-2 adherence test may be of greater value for detecting these categories of *E. coli*. Compared with DNA hybridization, our results showed that BFP and EAEC PCR could be used instead of the DNA probes as a screening method for typical EPEC strains and EAEC strains carrying the EAEC probe sequence, respectively, in the clinical laboratory.

Efforts to identify a DNA sequence that correlates with the DA and AA phenotypes represent a challenging avenue of investigation and could lead to a more practical assay that allows definitive epidemiological studies in the pathogenesis of diarrhea.

ACKNOWLEDGMENTS

We thank Beatriz A. Castilho and James B. Kaper for critical reading of the manuscript and helpful suggestions.

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

REFERENCES

1. **Baldini, M. M., J. B. Kaper, M. M. Levine, D. C. A. Candy, and H. W. Moon.** 1983. Plasmid-mediated adhesion of enteropathogenic *Escherichia coli*. J. Pediatr. Gastroenterol. Nutr. **2:**534–538.
2. **Baqui, A. H., R. B. Sack, R. E. Black, K. Haider, A. Hossain, A. R. M. A. Alim, M. Yunus, H. R. Chowdhury, and A. K. Siddique.** 1992. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children <5 years of age. J. Infect. Dis. **166:**792–796.
3. **Baudry, B., S. J. Savarino, P. Vial, J. B. Kaper, and M. M. Levine.** 1990. A sensitive and specific DNA probe to identify enteroaggregative *E. coli*, a recently discovered diarrheal pathogen. J. Infect. Dis. **161:**1249–1251.
4. **Benz, I., and M. A. Schmidt.** 1989. Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. Infect. Immun. **57:**1506–1511.
5. **Bhan, M. K., P. Raj, M. M. Levine, J. B. Kaper, N. Bhandari, R. Srivastava, R. Kumar, and S. Sazawal.** 1989. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. Infect. Dis. **159:**1061–1064.
6. **Bilge, S. S., C. R. Clausen, W. Lau, and S. L. Moseley.** 1989. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J. Bacteriol. **171:**4281–4289.
7. **Birnboim, H. C., and J. Doly.** 1979. A rapid extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
8. **Campos, L. C., M. A. M. Vieira, L. R. Trabulsi, L. A. Silva, V. Monteiro-Neto, and T. A. T. Gomes.** 1999. Diffusely adhering *Escherichia coli* (DAEC) strains of fecal origin rarely express F1845 adhesin. Microbiol. Immunol. **43:**167–170.
9. **Chatkaeomorakot, A., P. Echeverria, D. N. Taylor, K. A. Bettelheim, N. R. Blacklow, O. Sethabutr, J. Seriwatana, and J. B. Kaper.** 1987. HeLa cell-adherent *Escherichia coli* in children with diarrhea in Thailand. J. Infect. Dis. **156:**669–672.

10. **Cravioto, A., A. Tello, A. Navarro, J. Ruiz, H. Villajan, F. Uribe, and C. Eslava.** 1991. Association of *Escherichia coli* HEp-2 cells adherence patterns with type and duration of diarrhea. Lancet **337:**262–264.
11. **Donnenberg, M. S., C. O. Tacket, S. P. James, G. Losonsky, J. P. Nataro, S. S. Wasserman, J. B. Kaper, and M. M. Levine.** 1993. Role of the *eaeA* gene in experimental enteropathogenic *Escherichia coli* infection. J. Clin. Investig. **92:**1412–1417.
12. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriacea*, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
13. **Flewett, T. H., C. F. Arias, and A. Venecas.** 1989. Comparative evaluation of the W. H. O. and DAKOPATTS enzyme-linked immunoassay kits for rotavirus detection. Bull W. H. O. **67:**369–374.
14. **Franke, J., S. Franke, A. Schmidt, A. Schwarzkopf, L. H. Wieler, G. Baljer, L. Beutin, and H. Karch.** 1994. Nucleotide sequence analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. J. Clin. Microbiol. **32:**2460–2463.
15. **Germani, Y., E. Bégaud, P. Duval, and C. Le Bouguenec.** 1996. Prevalence of enteropathogenic, enteroaggregative, and diffusely adherent *Escherichia coli* among isolates from children with diarrhea in New Caledonia. J. Infect. Dis. **174:**1124–1126.
16. **Girón, J. A., T. Jones, F. Millán-Velasco, E. Castro-Munoz, L. Zarate, J. Fry, G. Frankel, S. L. Moseley, B. Baudry, J. B. Kaper, G. K. Schoolnik, and L. W. Riley.** 1991. Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J. Infect. Dis. **163:**507–513.
17. **Girón, J. A., M. S. Donnenberg, W. C. Martin, K. G. Jarvis, and J. B. Kaper.** 1993. Distribution of the bundle-forming pilus structural gene (*bfpA*) among enteropathogenic *Escherichia coli*. J. Infect. Dis. **168:**1037–1041.
18. **Gomes, T. A. T., M. A. M. Vieira, I. K. Wachsmuth, P. A. Blake, and L. R. Trabulsi.** 1989. Serotype-specific prevalence *of Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. J. Infect. Dis. **160:**755–758.
19. **Gomes, T. A. T., M. A. M. Vieira, C. M. Abe, D. Rodrigues, P. M. Griffin, and S. R. T. S. Ramos.** 1998. Adherence patterns and adherence-related DNA sequences in *Escherichia coli* isolates from children with and without diarrhea in São Paulo city, Brazil. J. Clin. Microbiol. **36:**3609–3613.
20. **Gunzburg, S. T., N. G. Tornieporth, and L. W. Riley.** 1995. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. J. Clin. Microbiol. **167:**755–758.
21. **Gunzburg, S. T., B. J., Chang, S. J. Elliot, V. Burke, and M. Gracey.** 1993. Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* from aboriginal children from the Kimberley region of Western Australia. J. Infect. Dis. **67:**755–758.
22. **Henricksens, S. A., and J. F. L. Pohlens.** 1981. Staining of *Cryptosporidia* by a modified Ziehl-Neelsen tecnique. Acta Vet. Scand. **22:**594–596.
23. **Hicks, S., D. C. A. Candy, and A. D. Phillips.** 1996. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. Infect. Immun. **64:**4751–4760.
24. **Jallat, C., V. Livrelli, A. Darfeuille-Michaud, C. Rich, and B. Joly.** 1993. *Escherichia coli* strains involved in diarrhea in France: high prevalence and heterogeneity of diffusely adhering strains. J. Clin. Microbiol. **31:**2031–2037.
25. **Jerse, A. E., Y. Jun, B. D. 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.
26. **Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, K. B. Kaper, R. E. Black, M. L. Clements, and A. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent of a plasmid encoding an enteroadhesiveness factor. J. Infect. Dis. **152:**550–559.
27. **Levine, M. M., V. Prado, R. M. Robins-Browne, H. Lior, J. B. Kaper, S. L. Moseley, K. Gicquelais, J. P. Nataro, P. Vial, and B. Tall.** 1988. Use of DNA probes and HEp-2 cell adherence assay to detect diarrheagenic *Escherichia coli*. J. Infect. Dis. **158:**224–228.
28. **Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Giannella.** 1983. Attaching and effacing of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infect. Immun. **41:**1340–1351.
29. **Mosely, S. L., I. Huq, A. R. M. A. Alim, M. So, M. Samadpour-Motalebi, and S. Falkow.** 1980. Detection of enterotoxigenic *Escherichia coli* by DNA colony hybridization. J. Infect. Dis. **142:**892–898.
30. **Nataro, J. P., M. M. Baldini, J. B. Kaper, R. E. Black, N. Bravo, and M. M. Levine.** 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J. Infect. Dis. **152:**560–565.
31. **Nataro, J. P., J. B. Kaper, R. Robins-Browne, V. Prado, P. Vial, and M. M. Levine.** 1987. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Paediatr. Infect. Dis. J. **6:**829–831.,
32. **Newland, J. W., and R. J. Neill.** 1988. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J. Clin. Microbiol. **26:**1292–1297.
33. **Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi.** 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. **45:**534–536.
34. **Scaletsky, I. C. A., J. S. Pelayo, R. Giraldi, J. Rodrigues, M. Z. Pedroso, and L. R. Trabulsi.** 1996. EPEC adherence to HEp-2 cells. Rev. Microbiol. **27**(Suppl. 1)**:**58–62.
35. **Scaletsky, I. C. A., M. Z. Pedroso, C. A. G. Oliva, R. L. B. Carvalho, M. B. Morais, and U. Fagundes-Neto.** 1999. A localized adherence-like pattern as a second pattern of adherence of classic enteropathogenic *Escherichia coli* to HEp-2 cells that is associated with infantile diarrhea. Infect. Immun. **67:** 3410–3415.
36. **Schmidt, H., C. Knop, S. Franke, S. Aleksic, J. Heeseman, and H. Karch.** 1995. Development of PCR for screening of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. **33:**701–705.
37. **Small, P. L., and S. Falkow.** 1986. Development of a DNA probe for the virulence plasmid of *Shigella* spp. and enteroinvasive *Escherichia coli*, p. 121–124. *In* L. Leive, P. F. Bonventre, J. A. Morello, S. D. Silver, and W. C. Wu (ed.), Microbiology—1986. American Society for Microbiology, Washington, D.C.
38. **Vial, P. A., R. Robins-Browne, H. Lior, V. Prado, 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.
39. **Wanke, C. A., J. B. Schorling, L. J. Barret, M. A. Desouza, and R. L. Guerrant.** 1991. Potential role of adherence traits of *Escherichia coli* in persistent diarrhea in an urban Brazilian slum. Pediatr. Infect. Dis. J. **10:** 746–751.