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Enteroadherent *Escherichia coli* and Diarrhea in Children: a Prospective Case-Control Study

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The relative contribution of diarrheagenic *Escherichia coli* was examined during a 1-year prospective study of hospitalized children in Clermont-Ferrand, France, including 220 case patients (with diarrhea) and 211 matched controls. Fecal isolates were characterized by means of their pattern of adherence to HEp-2 cells and by colony hybridization with DNA probes specific for the six categories of diarrheagenic *E. coli*. No enteroinvasive or enterotoxigenic *E. coli* isolates were isolated. Twenty-eight (6.5%) *eae*-positive isolates and 39 (9%) enteroaggregative *E. coli* isolates characterized with the aggregative adherence probe and/or by their adherence pattern were detected; they were equally distributed among the patients and the controls. Diffusely adhering *E. coli* was the predominant pathotype: 30.7% were detected by their adherence pattern and 13.7% were detected with the *daaC* probe. They were isolated with similar frequencies from the patients and the controls, thereby showing no association with diarrhea. However, *daaC*-positive strains were significantly associated with a past record of urinary tract infections. These results suggest that the diffusely adhering *E. coli* organisms isolated in the present study are not true intestinal pathogens but may be regarded as resident colonic strains.

Escherichia coli strains associated with diarrheal disease have been divided into categories on the basis of pathogenic mechanisms. By using an assay measuring mannose-resistant bacterial adherence to the HEp-2 cell line, three pathotypes of *E. coli* have been distinguished so far (39). The localized adherence (LA) pattern refers to the formation of distinct microcolonies or bacterial clusters on the HEp-2 cell surface. This phenotype is characteristic of enteropathogenic *E. coli* (EPEC) responsible for infantile diarrhea in developing countries (9). The second pattern, called aggregative adherence (AA) refers to a pattern whereby bacteria adhere to each other and form a stacked brick-like lattice on the epithelial cells and on glass coverslips (39). Several epidemiological studies with infants and children in developing countries have noted an association between this adherence pattern and persistent diarrhea (6, 10, 52). The last group is referred to as diffusely adhering *E. coli* (DAEC). Many epidemiological studies concerning the involvement of this last group in infantile diarrhea have been conducted, but they led to contradictory results (2, 8, 11, 20, 21, 23, 32, 34, 36).

These discrepancies in the results could be attributed either to epidemiological factors or to the qualitative nature of the adherence test, whose interpretation is subjective, as well as to technical differences in the cell adherence procedure, as noted by Vial et al. (51). For these reasons, a method of detection of diarrheagenic *E. coli* by use of specific DNA probes has been developed. This method also allows for the detection of two other groups of diarrheagenic *E. coli*: enterotoxinogenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC). The DNA fragments used as probes detect either adhesins or toxin-encoding genes (7, 14, 27, 37, 42, 48) or nonrelated but closely linked DNA sequences (3, 38).

This study was designed to define the prevalence of the different *E. coli* pathotypes in children from an industrialized country and to determine their relationship to clinical signs. We carried out a 1-year (January to December 1994) prospective study including diarrheal (cases) and nondiarrheal (controls) stool specimens from randomly selected children hospitalized in Clermont-Ferrand, France. For each isolate, we determined the adhesion phenotype with HEp-2 cells and we looked for virulence factor-encoding genes by colony hybridization assays using a panel of specific DNA probes and by PCR assays. The main aim of this study was to determine if the presence of adherent *E. coli* strains in children's stools was associated with clinical manifestations.

MATERIALS AND METHODS

Patients. Stool specimens were collected from 220 children with diarrhea and 211 children without diarrhea hospitalized in the pediatric wards of the Clermont-Ferrand hospitals. Diarrhea was defined as three or more stools per day for at least 3 days with a decrease in the consistency to an unformed state. An episode was considered persistent if it continued for ≥14 days. All the children were 0 to 168 months old and were randomly selected. Stool samples were collected during the first 2 days of hospitalization in order to avoid the collection of nosocomial strains. A standardized questionnaire was given to the parents to obtain additional demographic, clinical, and exposure information. Symptoms, including indicators of gastroenteritis, were recorded on charts. Fever was defined as an oral temperature of >37.8°C (>100°F). Dehydration was defined by clinical characteristics, e.g., tacky mucous membranes, sunken eyes or fontanelles, and poor skin tugor. Additional demographic, clinical, and exposure information was obtained. Dietary habits during the 2 weeks before hospitalization, such as lactose-free, cow's milk protein-free, gluten-free, and/or low-residue diet, were recorded. Moreover, information regarding clinical history and treatment with antimicrobial agents during the previous weeks, as well as bowel habits for 5 days after collection of the stool specimen, was collected. The interviewing and specimen collection team consisted of trained medical personnel.

Microbiologic methods. Fresh stool specimens obtained from patients were macroscopically examined for blood and mucus. Direct wet mounts of samples of

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TABLE 1. DNA probes used for detection of diarrheagenic *E. coli*

Organism	Pathogenic factor detected	Recombinant plasmid containing the probe	Probe obtained with restriction enzyme	Reference
ETEC	Enterotoxin LT	pEWD299	0.85-bp *Hin*dIII	37
	Enterotoxin STaII		Synthetic 30-mer probe	48
EPEC	EAF plasmid	pJPN16	1-kb *Bam*HI-*Sal*I	38
	eae	pCDV434	1-kb *Sal*I-*Kpn*I	27
EIEC	Invasion-associated locus		Synthetic *ial* 21-mer	14
EHEC	SLT-I	pJPN37-19	1.142-kb *Bam*HI	42
	SLT-II	pNN111-19	0.842-kb *Pst*I	42
EAggEC	AA	pCDV432	1-kb *Eco*RI-*Pst*I	3
DAEC	DA (*daaC*)	pSML852	0.45-kb *Pst*I	7

feces were also examined for blood and pus cells as well as for *Entamoeba histolytica* and *Giardia intestinalis*. The bacterial pathogens *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Vibrio cholerae*, and *Campylobacter* as well as *Candida* spp. were sought by standard methods. Rotavirus was detected by an enzyme-linked immunosorbent assay. Only samples without any of these pathogens were retained in this study. Stool specimens were diluted in physiological water and were streaked onto Drigalski agar plates that were incubated overnight at 37°C. From each plate, five lactose-fermenting colonies typical of *E. coli* were pooled and were stored at −80°C in 12% glycerol.

Assay of adherence to HEp-2 cells. The patterns of adherence of the 431 *E. coli* strains were examined by a method closely related to that referred to as the Center for Vaccine Development method, which allows for the detection of the three types of adherent *E. coli*: those with LA, DA, and AA (24, 50). HEp-2 cells were grown in 24-well tissue culture plates (ATGC Biotechnologie, Choisy le Grand, France) at 37°C in 5% CO_2 in Eagle's minimal essential medium (EMEM; Seromed) supplemented with 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 0.25 μg of amphotericin B per ml, and 10% fetal calf serum until a nearly confluent monolayer (90%) had formed. The *E. coli* isolates to be tested were grown from the frozen cultures overnight at 37°C on Muller-Hinton agar and were washed four times with phosphate-buffered saline (PBS; pH 7.2). The cells were washed twice with PBS, 1 ml of a suspension of about 10^8 bacteria in EMEM containing 2% (wt/vol) D-mannose without antibiotics was added, and the mixture was incubated for 3 h, uninterrupted, at 37°C. After three washes, the cells were fixed with methanol for 10 min, stained with 20% Giemsa for 20 min, and examined microscopically under oil immersion by two examiners. Adherence assays were performed twice monthly; therefore, the strains were kept frozen for no longer than 15 days before being tested in the adherence assays, and each assay was performed twice.

DNA probe hybridization. The various DNA probes used for detection of diarrheagenic *E. coli* are listed in Table 1. About 10^4 bacteria from an overnight culture were spotted onto a Hybond-N membrane (Amersham) and were transferred successively onto Whatman 3MM papers impregnated with a denaturing solution (NaOH, 0.5 N; NaCl, 1.5 M) and a neutralizing solution (Tris, 1 M [pH 7.4]; NaCl, 1.5 M). Recombinant plasmids containing DNA probe fragments as inserts were prepared, purified (Qiagen plasmid kit), and digested with restriction endonucleases (Boehringer Mannheim). The appropriate restriction fragments were purified by electroelution and were labeled by random priming with [α-^{32}P]dATP (3,000 Ci/mmol; Amersham International plc, Little Chalfont, Buckinghamshire, United Kingdom) and a random primer labeling kit (Boehringer Mannheim). Synthetic DNA probes were 5′-end radiolabeled with [γ-^{32}P]dATP (3,000 Ci/mmol; Amersham International plc). Colony hybridization was performed under stringent conditions as described previously (25). The positive control *E. coli* strains used for hybridization reactions included EDL933 (for Shiga-like toxin I [SLT-I] and SLT-II), B170 (for EPEC adherence factor [EAF] and *E. coli* attaching and effacing factor [*eae*]), C1845 (for diffuse adherence), and EDL1284 (for invasion).

Serogrouping. Boiled cells of all probe-identified potential EPEC or enterohemorrhagic *E. coli* (EHEC) (*eae*-, EAF-, SLT-I-, or SLT-II-positive) strains were serogrouped by a slide agglutination test with the following commercial antisera from Diagnostics Pasteur (Marnes-la-Coquette, France) and Difco Laboratories: O55, O26, O111, O86, O127, O119, O128, O125, O126, O124, O114, O142, and O157.

PCR assays. DNA to be amplified was released from whole organisms by boiling. Bacteria were harvested from 1 ml of an overnight broth culture, suspended in 200 μl of sterile water, and incubated at 100°C for 10 min. Following centrifugation of the lysate, 10 μl of the supernatant was used in the PCRs. Oligonucleotides specific for amplification of *afa*-specific sequences and the amplification procedure were described previously (30). The PCR cycle included denaturation for 1 min at 94°C, primer annealing for 1 min at 65°C, and extension for 1 min at 72°C (30 cycles). Each of the primers was used at 0.125 mM, with 0.2 mM (each) deoxynucleoside triphosphate (Boehringer Mannheim), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM $MgCl_2$, and 1 U of *Taq* DNA polymerase (Appligène-Oncor, Illkirch, France). Ten microliters of the reaction mixture was then analyzed by electrophoresis on 1.5% agarose gels, and the reaction products were visualized by staining with ethidium bromide. DNA from *E. coli* KS52 and a reagent blank, which contained all components except the template DNA, were included as positive and negative controls, respectively.

Statistical analysis. Isolation frequencies of organisms from case patients and controls were compared and were analyzed by the *t* test, the χ^2 test, or Fisher's exact test with Epi-Info, version 6.02, software.

RESULTS

Descriptive epidemiology. During the 1-year study, 220 patients with diarrhea and 211 matched controls were randomly selected among children hospitalized in the pediatric wards. The selected children were 0 to 168 months old (median, 8 months). The most frequent diagnoses were acute gastroenteritis (190 of 220; 86.4%) for case patients and bronchiolitis (66 of 211; 31.3%) or ear, nose, or throat infections (25 of 211; 11.8%) for controls. The length of stay was 1 to 82 days (median, 3 days). Accompanying symptoms, such as fever, vomiting, abdominal pain, dehydration (>5%), and previous antibiotic treatment were significantly more frequent in the children hospitalized for diarrhea than in the controls. From the information gathered in the questionnaires, it appeared that children admitted with diarrhea were more frequently fed cow's milk (35 versus 19%; $P < 0.008$) and were more often kept at home rather than in day-care centers (33 versus 17%; $P < 0.001$).

Detection of diarrheagenic *E. coli*. All 431 *E. coli* isolates from the feces of patients and controls were examined for adhesion to HEp-2 cells. Fewer than 5% of the isolates gave contradictory results in the two adherence assays and were thus tested once again. Of all the isolates, 152 (35.6%) adhered to HEp-2 cells with the following patterns: LA, 0.7%; AA, 4.2%; and DA, 30.7% (Table 2).

Hybridization results with the DNA probes specific for the virulence factors of diarrheagenic *E. coli* are presented in Table 3.

By using the *eae* DNA probe, which is specific for both EPEC and EHEC, 6.5% of the isolates were positive. Further characterization of these strains was achieved by hybridization with DNA probes specific for EPEC (EAF) or EHEC (SLT-I and SLT-II probes). Only two *eae*-positive isolates from the patient cohort hybridized with the EAF probe, indicating that they belong to the EPEC pathotype. Moreover, these two

TABLE 2. Adherence pattern of *E. coli* isolated from patients and controls

Type of HEp-2 cell adherence	No. (%) of subjects		
	Total (n = 431)	Children with diarrhea (n = 220)	Controls (n = 211)
LA	3 (0.7)	3 (1.4)	0
AA	18 (4.2)[a]	8 (3.7)[a]	10 (4.8)
DA	132 (30.7)[a]	76 (34.6)[a]	56 (26.6)

[a] One isolate exhibited both the DA and AA phenotypes.

strains harbored a localized adherence phenotype characteristic of EPEC strains and belong to the classical EPEC serogroup, serogroup O127. None of the isolates were positive for both SLT-I and SLT-II. Among the seven SLT-I- or SLT-II-positive isolates, only three were also positive for *eae*: two SLT-II-positive isolates and the SLT-I-positive isolate from the patient and the control groups, respectively. Of the 26 *eae*-positive EAF-negative and the 7 SLT-I- or SLT-II-positive isolates, only 14 and 1, respectively, agglutinated with commercial sera to the traditional EPEC and EHEC serogroups (Table 4). The serogroups detected were O127 (n = 3), O111 (n = 4), O26 (n = 5), O142 (n = 2), and O126 (n = 1), and they were distributed both among the patients and the control groups.

Detection of enteroaggregative *E. coli* (EAggEC) was performed by hybridization with the AA DNA probe described by Baudry et al. (3), which consisted of a 1-kb *Eco*RI-*Pst*I fragment from a region unrelated to the adhesion gene cluster of the plasmid of the prototype strain, *E. coli* 17.2. Of all isolates, 33 were positive (8%), 12 among the case patients and 21 among the controls. However, only eight of these AA-positive isolates showed an aggregative phenotype when tested with HEp-2 cells (Fig. 1); four of them were isolated from case patients and the other four were isolated from controls. The remaining strains harbored either a diffuse adherence pattern (11 of 33) or no adhesion phenotype (15 of 33). One isolate harbored both an aggregative and a diffusely adhering phenotype.

The DNA probe used to detect DAEC isolates in this study was derived from an accessory gene (*daaC*) of the chromosomally encoded fimbrial adhesion, F1845. Positive strains were predominantly detected: 34 were isolated from case patients (15.5%), and 25 were isolated from controls (12%). The majority of these positive isolates (80%) exhibited a diffuse adherence pattern with HEp-2 cells (Fig. 1). Only five isolates showed an aggregative phenotype, including one isolate that showed both an aggregative and a diffuse adherence pattern, and eight isolates did not show any adherence properties. The *daaC* probe used to identify DAEC isolates hybridizes with all the members of a family of gene clusters encoding adhesins recognizing different epitopes of the Dr blood antigen as the receptor, including the Afa afimbrial adhesins involved in urinary tract infections. The *daa* operon and the *afa* gene clusters previously ascribed to uropathogenic *E. coli* are closely related and were both found to encode DA (7, 16, 17, 30). The detection of the presence of *afa* or *daa* sequences was also performed by using a specific PCR test; 30 of the 59 strains positive by hybridization gave a positive amplification (51%).

TABLE 3. Hybridization of *E. coli* isolates with nine probes to detect diarrheagenic strains

Organism and probe[a]	No. (%) of subjects	
	Children with diarrhea (n = 220)	Controls (n = 211)
EIEC, *ial* probe	0	0
ETEC		
ST probe	0	0
LT probe	1 (0.5)	0
EPEC or EHEC		
eae probe	15 (7)	13 (6.5)
EAF probe	2 (1)[b]	0
SLT I probe	2 (1)	1 (0.5)[b]
SLT II probe	2 (1)	2 (1)
EAggEC, 1-kb AA probe	12 (5.5)	21 (10)
DAEC, *daaC* probe	34 (15.5)	25 (12)

[a] Abbreviations: *ial*, invasion-associated locus; ST and LT, heat-stable and heat-labile toxins, respectively.
[b] Isolates also hybridizing with the *eae* probe.

Association of diarrheagenic *E. coli* with clinical signs. We looked for any significant association between the presence of adherent *E. coli* in the children's stools with either clinical signs or behavior patterns (dietary habits, clinical antecedents, antimicrobial treatments, and living habits). Table 2 indicates the frequency of isolation of localized, aggregative, and diffusely adhering *E. coli* from diarrheic and nondiarrheic patients. No significant association of any of these adherent groups with diarrhea was observed, even when sectioning the patient population into age groups. When detected by use of specific DNA probes in hybridization assays, the rates of isolation of *eae*-positive strains characteristic of both EPEC and EHEC were identical in case patients (15 of 220; 7%) and controls (13 of 211; 6.5%). Concerning the enteroaggregative pathotype, although their distribution between patients and controls was not statistically different, an EAggEC strain (AA positive) was the only potential enteric pathogen detected in the two patients with persistent diarrhea (>14 days) in which no other etiology could be demonstrated. Overall, there was no significant difference in the rates of isolation of any adherent *E. coli* between patients and controls regarding the clinical sign diarrhea.

Further analysis of additional information revealed that one clinical antecedent, i.e., a past record of urinary tract infection, was significantly associated with the presence of DAEC *daaC*-positive strains: 21% among *daaC*-positive strains and 7.4% among *daaC*-negative strains ($P < 0.05$).

DISCUSSION

This study prospectively examined the association between the presence of diarrheagenic *E. coli* in children's stools and clinical features. Epidemiological studies and laboratory experiments examining strains' pathogenesis are necessary to determine if the virulence factors identified are linked to clinical syndromes. Several epidemiological studies examining the role of the different *E. coli* pathotypes in children's diarrhea have been performed, most of them in developing countries (2, 8, 11, 20, 21, 23, 34, 36). The frequency and gravity of diarrhea due to *E. coli* in developed countries are undoubtedly less important. However, the few investigations that have been published on this topic indicated that EAggEC and DAEC are potential pathogens in children and adults, respectively (25, 47). The present study was conducted in pediatric wards of the Clermont-Ferrand hospitals and was designed to isolate and characterize *E. coli* strains from children's stools. A total of 431 randomly selected children (median age, 8 months) were in-

TABLE 4. Distribution of serogroups among potential EPEC and EHEC strains in case and control children[a]

Serogroup	No. of isolates							
	eae positive		EAF positive		SLT-I positive		SLT-II positive	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
O127	3	2	2[b]	0	0	0	0	0
O111	2	2	0	0	0	0	0	0
O26	2	2	0	0	1	0	0	0
O142	2	0	0	0	0	0	0	0
O126	0	1	0	0	0	0	0	0
Undefined	6	6	0	0	1	1[b]	2	2

[a] No agglutination was observed with the following antisera: O55, O86, O119, O128, O125, O124, O114, and O157.
[b] Isolates also hybridizing with the *eae* probe.

cluded: 220 case patients consisting of children with diarrhea and 211 matched controls consisting of children without diarrhea. We identified potential diarrheagenic *E. coli* by assays of adhesion to HEp-2 cells and by hybridization with a panel of specific DNA probes.

Of all the isolates tested, 6.5% (28 of 431) hybridized with the *eae* DNA probe, which is specific for both EPEC and EHEC strains. Only two *eae*-positive isolates, from children with diarrhea, could be characterized as EPEC since they possessed the two other characteristics of EPEC, i.e. localized adhesion to HEp-2 cells and hybridization with the EAF probe. Moreover, these two isolates belong to the classical EPEC serogroup, serogroup O127. Gomes et al. (22) previously suggested that detection of EAF in a strain with a classical EPEC serotype might be a reliable test for detecting EPEC associated with diarrhea. However, serogrouping of other potential EPEC and EHEC isolates did not bring any additional information about their pathogenic potentials. The number of strains detected was too small to draw any significant conclusion, but the five serogroups were detected in both controls and case patients.

The remaining *eae*-positive EAF-negative isolates from this study could be either avirulent strains or EPEC strains that have lost their EAF plasmid upon passage through the child host. Indeed, previous experiments performed with human volunteers demonstrated the requirement of the EAF plasmid for full virulence of EPEC strains in adults (33), and a spontaneous loss of this plasmid occurred in 67% of the volunteers that received the wild-type strain. Moreover, despite determination of the nucleotide sequence of the 1-kb *Bam*HI-*Sal*I EAF fragment, it has not yet been clarified whether this DNA is directly involved in adhesion or in another pathogenic process, since it does not show homology with any known gene sequences (13). The inadequacy of the use of the EAF probe in the detection of EPEC strains has previously been demonstrated in several epidemiological studies (19, 26, 46, 47). In the present study, *eae*-positive, EAF-negative strains were detected at similar rates in the case patient (13 of 220) and control (13 of 211) groups. Similar observations have previously been reported in a study involving both children and adults (45). Therefore, the use of the *eae* probe in hybridization assays appears to be of little diagnostic value, and the results of this study emphasize the need for detection of multiple factors involved in pathogenesis.

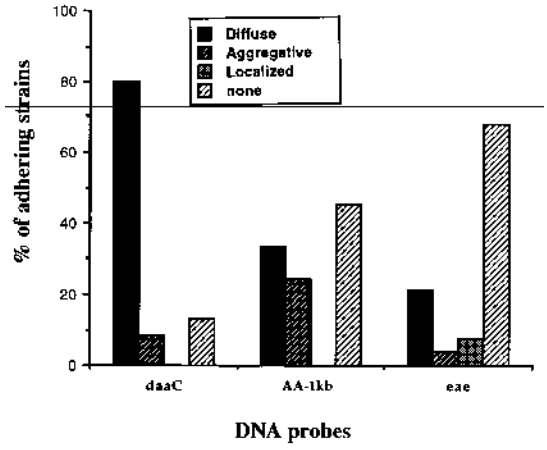

FIG. 1. Adherence pattern to HEp-2 cells of *E. coli* isolates hybridizing with the following DNA probes: DA, 0.45-kb *Pst*I fragment from *daaC* (DAEC); AA-1kb, *Eco*RI-*Pst*I fragment from pCDV432 (EAggEC); and *eae*, 1-kb *Sal*I-*Kpn*I *eae* fragment from pCDV434 (EPEC or EHEC).

Further identification by additional hybridization assays showed that three *eae*-positive isolates hybridized with DNA probes specific for EHEC toxins (SLT-I or SLT-II). However, these strains were detected in both case patient and control groups. Although they can be classified as EHEC on the basis of the hybridization results, none of these strains were associated with EHEC-specific clinical symptoms such as hemorrhagic colitis or hemolytic-uremic syndrome. Moreover, in vitro detection of the cytotoxicities of these strains with Vero cells was negative (data not shown), indicating that SLTs might not be effectively produced. The remaining two SLT-II-positive strains were *eae* negative and were isolated from the control group. Similar strains have previously been isolated from humans (15, 45, 53) and, most commonly, from animals (4, 35). One possible explanation is that these isolates have lost virulence genes, including *eae*, and lack the potential for virulence. However, these strains harbor the genetic information necessary for verotoxin production. Thus, clinical monitoring of the SLT-positive patients as well as preventive measures would avoid further development of toxin-mediated syndrome and the spread of such strains.

Forty-three *E. coli* strains characterized as enteroaggregative (EAggEC) were detected in this study. Strains belonging to this pathotype have been implicated as etiologic agents of infant gastroenteritis in both developing countries (6, 10, 52) and developed countries (46), most predominantly among patients with diarrhea that persists longer than 14 days. In this study, EAggEC strains were equally detected in patients and controls. However, they were the only potential pathogens isolated from two patients in the case group suffering from persistent diarrhea. None of the clinical and general data collected for each child was associated with the presence of EAggEC.

Conflicting results about the role of EAggEC in persistent diarrhea have been published (5, 6, 12, 21, 32, 51). These differences may be due in part to the test procedures used to define members of this pathovar. In this study, EAggEC strains were detected by means of their HEp-2 cell adherence pattern (18 strains) and/or by hybridization with the AA probe (33 strains). The relevance of the AA probe in epidemiological studies is questionable. Indeed, many EAggEC strains contain a large 60-MDa plasmid which confers AA by means of bundle-forming fimbriae termed aggregative adherence fimbriae I (AAF/I). Genes necessary for the expression of AAF/I in the prototype EAggEC strain, *E. coli* 17.2, isolated in Chile, have been localized to two unlinked plasmid regions separated by 9 kb of DNA (41). Before identification of these loci, the AA probe was constructed of a 1-kb *Eco*RI-*Pst*I fragment from an unrelated region of the strain 17.2 plasmid (3). This DNA fragment has since been sequenced, and no significant similarity to any known bacterial gene has been detected (45). However, the AA probe has been widely used in epidemiological studies, with a sensitivity varying between 47 and 70% in field studies (44). In order to determine if the 43 EAggEC strains isolated in this study harbor the *agg* operon, PCR amplifications and hybridization assays with primers and DNA probes specific for the *agg* operon were performed. Only 14 (32%) of the EAggEC strains actually possessed an *agg* operon, having an *aggC* accessory gene similar to the one described with the reference strain, *E. coli* 17.2, but with differences in the adhesin structural gene, *aggA*, at least in the region encompassing the primers chosen for use in our PCR assays (data not shown). The 14 *aggC*-positive strains were equally distributed among stool samples collected from case and control patients, but it is noteworthy that they included the two EAggEC isolates associated with persistent diarrhea. The AA probe was highly sensitive and specific in an epidemiological study involving strains from Chile and India (3). However, in our study, we did not detect *agg* sequences in 19 AA probe-positive strains. This suggests that the use of this fragment for diagnostic purposes is questionable in geographical areas other than Chile and India. Such discrepancies in the usefulness of this DNA probe were recently reported in studies conducted in northeastern Brazil (12) and prompted us to study new EAggEC genotypes. Our results indicated that among *agg*-positive strains, heterogeneity also occurred in the adhesin-encoding gene, *aggA*. The presence of a fimbrial structure antigenically different from AAF/I and mediating AA was recently reported (40). Heterogeneity in the virulence of pathogens, especially among adhesin-encoding genes, is frequent and represents a major obstacle in the development of vaccines against adhesins.

The majority of *E. coli* strains isolated in this study belong to the DAEC pathotype: 30.7% (132 of 431) were defined phenotypically by determination of their pattern of adherence to HEp-2 cells and 13.7% (59 of 431) genotypically by hybridization with the *daaC* probe. Forty-seven isolates were detected by both methods. There is much debate over whether DAEC strains cause diarrhea. Many field reports (2, 8, 11, 20, 21, 23, 34, 36), as well as volunteer studies (49), led to contradictory results. In previous studies involving both adults and children, different groups demonstrated that DAEC strains were associated with diarrhea (18, 25). In this study, there was no significant correlation between DAEC isolates and diarrhea. However, the majority of children in the present study were under 2 years of age (82%), and they account for 85% (112 of 132) of the DAEC-positive patients. As suggested previously, DAEC may be more important as a diarrheal pathogen in older populations or in specific age groups of children (23, 32). However, heterogeneity regarding the adhesins and other virulence factors harbored by the DAEC strains could also be responsible for these discrepancies.

Analysis of general information and clinical signs other than diarrhea in children carrying DAEC revealed that a past record of urinary tract infection was associated with the presence of *daaC*-positive DAEC strains. The fimbrial adhesin F1845 encoded by the *daa* operon belongs to the same family of adhesins as the Afa afimbrial adhesin expressed by pathogenic *E. coli* strains associated with urinary tract infection or acute diarrhea (1, 31). The gene clusters have similar genetic organizations and are closely related (7, 17, 31), and their adhesins belong to the Dr family, recognizing different epitopes of the Dr blood antigen as the receptor (43). PCR assays performed with all the *daaC*-positive isolates revealed that only 51% of them harbored an Afa-encoding gene. This indicates a high degree of heterogeneity among the adhesin-encoding operons in our isolates, in opposition to the *E. coli* strains isolated in New Caledonia, in which 98% of the strains were positive by both assays (18). It is thought that most uropathogens are normal inhabitants of the bowel flora and are selected on the basis of possession of virulence factors, including adhesins that permit ascending colonization of the urinary tract (54). The F1845 adhesive factor and the Afa-I adhesin have been shown to be involved in adhesion to the human intestinal Caco-2 cell line (28, 29). Thus, isolation of DAEC strains from patients' feces may reflect the persistence of potential uropathogenic *E. coli* in the colon rather than the presence of true intestinal pathogens, at least in the patients involved in this study. Further characterization of the virulence factors of DAEC strains, including identification of the adhesins of DAEC *afa*- and *daa*-negative isolates from this study, will be necessary to confirm this hypothesis.

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