JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2005, p. 1968–1972 Vol. 43, No. 4
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.4.1968–1972.2005
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Heterogeneity among Strains of Diffusely Adherent *Escherichia coli* Isolated in Brazil

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Received 11 May 2004/Returned for modification 6 August 2004/Accepted 16 December 2004

One hundred twelve diffusely adherent *Escherichia coli* strains isolated from children in a case control study were evaluated for virulence-associated characteristics, serotyping, antibiotic resistance, and plasmid profiles. Half of the strains hybridized with the probes for *icuA* (aerobactin) and *fimH* (type 1 pili); *daaE* (F1845 fimbriae), *afa* (afimbrial Dr adhesin), *agg-3A* (aggregative adhesion fimbria type III fimbriae), *pap* (P fimbriae), *astA* (EAST1 toxin), and *shET1* (*Shigella* enterotoxin 1) sequences were present in <20% of the strains. The *shET1* gene was noted most frequently in strains isolated from patients. A minority (7%) of the strains produced hemolysin or colicin or showed cytotoxic effects on Vero cells. Forty-five different serotypes were found. The majority (70%) of the strains presented multiple antibiotic resistance. Antibiotic resistance and diffuse adherence were located on the same conjugative plasmids. These results suggest that the transfer of these potential virulence markers could be important in the epidemiology of diffusely adherent *E. coli*.

Diffusely adherent *Escherichia coli* strains that are identified by their diffuse adherence (DA) pattern on cultured epithelial cells in vitro (24) have been recognized as the sixth class of diarrheagenic *E. coli* (17).

Two putative adherence factors have been described for diffusely adherent *E. coli* strains. Bilge et al. (6) have characterized surface fimbriae (designated F1845) that are responsible for the DA phenotype in a prototype strain, C1845. The gene cluster that encodes this fimbriae can be found on either the bacterial chromosome or a plasmid. F1845 fimbriae are homologous at the nucleotide and amino acid levels with members of the Afa/Dr family of adhesins (Afa-I, Afa-III, Dr, Dr-II, and F1845) (18). Afa/Dr diffusely adherent *E. coli* strains are identified in epidemiological studies by hybridization to a specific probe, *daaC*, which is common to operons encoding Afa/Dr adhesins (6). A second putative adhesin associated with the DA phenotype, an outer membrane protein, designated AIDA-I, has been described by Benz and Schmidt (4).

In a number of epidemiological studies, diffusely adherent *E. coli* strains have been associated with diarrheal disease in different geographic areas (2, 11, 12, 13). However, the diffusely adherent *E. coli* virulence markers associated with diarrhea are yet to be clarified. In this paper, 112 diffusely adherent *E. coli* strains isolated in a case control study (61 from patients and 51 from healthy control subjects) (25) were tested for a number of phenotypic and genotypic characteristics associated with potential *E. coli* virulence factors. We further characterized these strains by determining their serotypes, antibiotic resistance patterns, and the role of plasmids in mediating the diffuse adherence.

Strains were screened for hemolytic activity on blood agar base plates containing 5% sheep erythrocytes washed with phosphate-buffered saline. The plates were observed for lysis of erythrocytes after 24 h of growth at 37°C. Strains were tested for enterotoxin production by the Y1 adrenal cell culture method of Donta et al. (10) and by the infant mouse assay of Dean et al. (9). Cytotoxin production was detected by the Vero cell culture assay (15). Colicin production was detected by using the agar overlay method of Azevedo and Costa (1).

DNA probes for virulence factors associated with other pathogenic *E. coli* strains were employed in hybridization experiments as described previously (25) (Table 1).

Serotyping was performed by the method of Ørskov and Ørskov (21) with antisera against *E. coli* O antigens 1 to 181 and H antigens 1 to 53.

Antibiotic susceptibility testing was performed by the standard disk diffusion method of Bauer and Kirby (3). The antibiotic disks used were ampicillin (10 μg), cephalothin (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), cotrimoxazole (10 μg), lomefloxacin (10 μg), ceftazidime (30 μg), ofloxacin (5 μg), streptomycin (10 μg), gentamicin (10 μg), nalidixic acid (30 μg), sulfonamide (300 μg), and tetracycline (30 μg).

To test for the presence of DA conjugative plasmids, diffusely adherent *E. coli* isolates that were resistant to ampicillin, streptomycin, and tetracycline were mated with the *E. coli* K-12 C600 strain (*supE44 hsdR thr-1 leuB6 lacY1 tonA21* Nal^r Fu^r), as described elsewhere (23). The donor and recipient strains were grown in Luria broth for 18 h, mixed on the surface of Mueller-Hinton agar plates, and incubated for 24 h at 37°C. Transconjugants were selected on plates containing nalidixic acid (100 μg/ml) with ampicillin (100 μg/ml), tetracycline (30 μg/ml), and strepto-

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TABLE 1. DNA probes hybridizing to colony blots of diffusely adherent *E. coli* isolates

Probe	Description of probe	Properties of probe (reference)	No. (%) of DAEC strains isolated from:		
			Cases (n = 61)	Controls (n = 51)	Cases and controls (total) (n = 112)
daaE	419-bp amplified fragment	F1845 fimbrial subunit (7)	6 (9.8)	9 (17.6)	15 (13.4)
afa	750-bp amplified fragment	Afimbrial adhesin (16)	9 (14.7)	8 (15.7)	17 (15.2)
aggA	450-bp amplified fragment	AAF/I fimbrial subunit (8)	1 (1.6)	0	1 (0.9)
aafA	550-bp amplified fragment	AAF/II fimbrial subunit (8)	0	0	0
agg-3A	485-bp amplified fragment	AAF/III fimbrial subunit (5)	10 (16.4)	10 (19.6)	20 (17.8)
fimH	527-bp amplified fragment	Type 1 fimbriae (14)	26 (42.6)	28 (54.9)	54 (48.2)
sfa	410-bp amplified fragment	S fimbriae (16)	1 (1.6)	2 (3.9)	3 (2.7)
pap	328-bp amplified fragment	P fimbriae (16)	3 (4.9)	3 (5.9)	6 (5.3)
iucA	1,100-bp amplified fragment	Aerobactin (20)	31 (50.8)	25 (49)	56 (50)
hly	7,000-bp fragment of pSF4000	Alpha-hemolysin (29)	4 (6.5)	2 (3.9)	6 (5.4)
pet	1,037-bp amplified fragment	104-kDa cytotoxin (28)	0	0	0
astA	111-bp amplified fragment	EAST1 toxin (30)	11 (18)	6 (11.8)	17 (15.2)
shET1	309-bp amplified fragment	*Shigella* enterotoxin 1 (28)	7 (11.5)	1 (1.9)	8 (7.1)
shET2	799-bp amplified fragment	*Shigella* enterotoxin 2 (28)	0	0	0
pic	5,800-bp amplified fragment	Mucinase (28)	0	0	0
cnf	498-bp amplified fragment	Cytotoxic necrotizing factor (22)	1 (1.6)	1 (1.9)	2 (1.8)
cdt	175-bp fragment of pCVD448	Cytolethal distending toxin (26)	0	0	0
espC	2,000-bp fragment of pMS1	Enterotoxin (27)	0	0	0

mycin (100 μg/ml) and were tested for HEp-2 cell adherence using the assay of Scaletsky et al. (24).

Colicin production was found in eight strains isolated from six patients and two healthy control subjects. Six strains isolated from four patients and two controls were hemolysin producers and hybridized with the *hly* probe. No strains produced enterotoxins as tested by the Y1 adrenal cell culture method and by the infant mouse assay. Eight strains isolated from six patients and two controls showed a cytotoxic effect on Vero cells after 3-h incubation (data not shown). A similar cytotoxic effect on HEp-2 cells was observed by Jallat et al. (13) in 16 diffusely adherent *E. coli* strains, 14 from patients and 2 from controls, in France.

According to the hybridization studies, 48% of strains harbored the *iucA* gene that is involved in the synthesis of the aerobactin siderophore (Table 1). In a recent study, it has been reported that 50% of the diffusely adherent *E. coli* strains hybridize with the *irp2* probe, which is part of the yersiniabactin operon, encoding a siderophore-dependent iron transport system (8). Therefore, it appears that the genes for aerobactin and yersiniabactin siderophore production are widely distributed among diffusely adherent *E. coli* strains, as they are for enteroaggregative *E. coli* (20).

In addition, some strains possessed genes encoding the EAST1 toxin (*astA*) and eight strains had the *shET1* sequence, virulence factors found frequently in enteroaggregative *E. coli* strains (28). The *astA* gene was detected more frequently in strains from patients (18%) than in strains from control subjects (12%). Of the eight strains with the *shET1* profile, seven (87.5%) were isolated from children with diarrhea who carried no other well-established enteropathogen. To our knowledge, this is the first report of the prevalence of *shET1* in diffusely adherent *E. coli* strains. Further studies are necessary to determine whether other virulent factors play a role in the pathogenicity of the diffusely adherent *E. coli* strains.

The *cnf1* gene encoding cytotoxic necrotizing factor 1 was found in two strains from both patients and healthy controls. This virulence factor has been found in cell-detaching *E. coli* strains from children with and without diarrhea (19). None of the 112 strains hybridized with the *cdt*, *pet*, *pic*, *shET2*, and *espC* probes.

Besides the F1845 fimbriae and AIDA-I, little is known about possible adhesins expressed by diffusely adherent *E. coli* strains. We investigated the presence of putative adhesins in an attempt to identify the factor responsible for the DA phenotype. As shown in Table 1, 15 (13%) strains carried the *daaE* (F1845 major fimbrial subunit), and 17 (15%) other strains carried the *afa* (afimbrial adhesin) sequence, an afimbrial adhesin of the Dr family. The *fimH* gene, which encodes adhesin subunit type 1 fimbriae, was the most frequently found (54 strains [48%]). This adhesin is present in nearly all *E. coli* strains, including commensal strains (14). Our results for *afa*, *agg-3A*, *sfa*, and *pap* extended the observations of previous reports that investigated the distribution of some of these adhesins in a limited number of strains (16). Indeed, 20 strains carried the *agg-3A* sequence that encodes the aggregative adhesion fimbria type III (AAF/III) fimbrial subunit, 6 strains hybridized with *pap* (P fimbria), 3 strains hybridized with *sfa* (S fimbria), and one strain hybridized with *aggA* (AAF/I fimbria) probes. Thus, at least half of the strains must have adhered by means of an adhesin different from those described so far. Further studies are necessary to characterize the adhesin(s) of diffusely adherent *E. coli* strains.

The serotypes of the 112 diffusely adherent *E. coli* strains are indicated in Table 2. In total, 45 different serotypes were found. Thirty-five strains were O nontypeable, and three were rough.

A variety of different combinations of the specific virulence DNA sequences and serotypes was observed in diffusely adherent *E. coli* isolates from both patients with diarrhea and healthy controls (Table 2). However, an interesting combination of virulence markers was found in some of the strains studied: three strains isolated from patients of serotypes O89:HNT, O99:H33, and O126:H16, for example, carried *astA*

TABLE 2. Characteristics of 112 diffusely adherent *E. coli* strains carrying different putative virulence DNA sequences

Genetic profile	No. (%) of strains from:		Serotype(s)[a]
	Patients (n = 61)	Controls (n = 51)	
hly shET1 cnf sfa fimH	1 (1.6)	0	O2:H6
hly cnf sfa pap fimH	0	1 (2)	O6:H27
iucA daaE agg-3A fimH	0	2 (3.9)	O21:HNT, ONT:H18
iucA hly afa pap	2 (3.3)	0	O21:HNT
iucA astA shET1 agg-3A	1 (1.6)	0	O89:HNT
iucA astA shET1 fimH	2 (3.3)	0	O99:H33, ONT:H18
iucA agg-3A fimH	1 (1.6)	3 (5.9)	O15:H16, O21:HNT, O158:H4, ONT:NM
daaE afa fimH	0	1 (2)	O1:H1
iucA afa fimH	2 (3.3)	0	O15:NM, O99:NM
agg-3A pap fimH	1 (1.6)	0	O21:HNT
iucA pap fimH	0	1 (2)	O21:H18
iucA sfa fimH	0	1 (2)	O23:HNT
astA daaE fimH	1 (1.6)	0	O86:H18
astA shET1 fimH	1 (1.6)	0	O99:H33
iucA astA shET1	1 (1.6)	0	O126:H26
hly astA fimH	0	1 (2)	O141:H19
iucA daaE agg-3A	0	2 (3.9)	O153:H2, ONT:H17
iucA daaE afa	0	1 (2)	ONT:NM
iucA daaE fimH	1 (1.6)	0	ONT:NM
aggA agg-3A fimH	1 (1.6)	0	ONT:HNT
iucA afa agg-3A	1 (1.6)	0	R
astA afa fimH	0	1 (2)	R
iucA astA	2 (3.3)	0	O5:H2, O86:H11
iucA afa	2 (3.3)	1 (2)	O11:NM, O86:H18, O101:H9
iucA shET1	1 (1.6)	0	O15:HNT
iucA fimH	6 (9.8)	5 (9.8)	O2:H4, O21:NM, O93:HNT, O153:H18, O158:NM, O164:NM, ONT:NM, ONT:H18, ONT:HNT, R:H45
shET1 fimH	0	1 (2)	O33:H32
iucA daaE	1 (1.6)	1 (2)	O86:H18, O117:H4
daaE fimH	0	1 (2)	O86:H18
astA agg-3A	1 (1.6)	0	O89:H33
afa fimH	1 (1.6)	1 (2)	O86:H18, O117:NM
iucA agg-3A	1 (1.6)	2 (3.9)	O128:NM, O153:H2
agg-3A fimH	0	1 (2)	ONT:NM
afa pap	0	1 (2)	ONT:H7
astA daaE	0	1 (2)	ONT:H18
astA fimH	1 (1.6)	1 (2)	O99:H33
hly afa	1 (1.6)	0	O102:HNT
daaE agg-3A	2 (3.3)	0	ONT:HNT
astA	1 (1.6)	2 (3.9)	O5:H10, O86:11, ONT:H34
agg-3A	1 (1.6)	0	O15:H18
daaE	1 (1.6)	0	ONT:HNT
fimH	7 (11.5)	7 (11.8)	O1:H45, O2:H17, O75:HNT, O78:HNT, O86:H18, O99:H7, O117:H7, O164:H4, ONT:H18, ONT:H23, ONT:H27, ONT:HNT
iucA	7 (11.5)	6 (11.8)	O5:H2, O5:H10, O11:H16, O83:HNT, O153:H2, O153:H18, ONT:H17, ONT:NM
afa	0	2 (3.9)	O86:H18
None	9 (13.1)	4 (9.8)	O11:H16, O86:H18, O107:NM, O153:HNT, ONT:NM, ONT:H2, ONT:H9, ONT:H10, ONT:H16, ONT:H18, ONT:HNT

[a] HNT, serotype H nontypeable; ONT, serotype O nontypeable; NM, nonmotile.

shET1 sequences, which suggests that these strains are potentially pathogenic *E. coli* strains.

Frequent antibiotic use could predispose or select for infection with antibiotic-resistant diffusely adherent *E. coli* strains. In addition, antibiotic resistance patterns could serve as markers for virulent strains whose virulence factors have not been identified. For these reasons, we sought to characterize antibiotic resistance rates in our diffusely adherent *E. coli* isolates. The frequencies of resistance to ampicillin, cephalothin, cotrimoxazole, streptomycin, sulfonamide, and tetracycline were each >50%. Resistance to chloramphenicol (20%) was less frequent. All the isolates were susceptible to ceftazidime, gentamicin, lomefloxacin, ofloxacin, and nalidixic acid, and two strains were susceptible to all antibiotics tested.

Since it is well-known that plasmids are responsible for the horizontal spread of antibiotic resistance between microorganisms, we wanted to know whether the DA phenotype could be cotransferred by conjugation into an *E. coli* K-12 recipient strain. It was not possible to examine all isolates for plasmids, but in conjugation experiments, the transfer of multiple drug

TABLE 3. Results of conjugation between 10 diffusely adherent *E. coli* strains and *E. coli* K-12 C600

Diffusely adherent *E. coli* strain	Source	Resistance profile[a]	Size (MDa) of plasmid(s)	Resistance profile of the transconjugant	DA phenotype	Size (MDa) of transconjugant plasmid(s)
10	Control	Ap^r Cf^r Co^r Sm^r Su^r Tc^r	44, 80, 91	Ap^r Nal^r	−	91
53	Diarrhea	Ap^r Ci^r Co^r Sm^r Su^r Tc^r	38, 75, >98	Ap^r Tc^r Nal^r	−	75
64	Control	Ap^r Cf^r Co^r Sm^r Su^r Tc^r	78, 98	Ap^r Sm^r Nal^r	−	78
66	Diarrhea	Ap^r Co^r Sm^r Su^r Tc^r	73, >98	Ap^r Sm^r Nal^r	−	73
67	Control	Ap^r Cf^r Cc^r Sm^r Su^r Tc^r	75, >98	Ap^r Sm^r Tc^r Nal^r	+	75, >98
69	Diarrhea	Ap^r Co^r Sm^r Su^r Tc^r	75, >98	Ap^r Sm^r Tc^r Nal^r	+	75
73	Control	Ap^r Co^r Sm^r Su^r Tc^r	31, 73	Ap^r Sm^r Nal^r	−	73
79	Diarrhea	Ap^r Co^r Sm^r Su^r Tc^r	68, >98	Ap^r Sm^r Tc^r Nal^r	+	68, >98
96	Diarrhea	Ap^r Co^r Sm^r Su^r Tc^r	75, >98	Ap^r Sm^r Tc^r Nal^r	+	75
97	Diarrhea	Ap^r Co^r Sm^r Su^r Tc^r	66, 95, >98	Ap^r Sm^r Nal^r	−	66

[a] Ap^r, ampicillin resistant; Cf^r, cephalothin resistant; Ci^r, ciprofloxacin resistant; Co^r, cotrimoxazole; Sm^r, streptomycin resistant; Su^r, sulfonamide resistant; Tc^r, tetracycline resistant; Nal^r, nalixidic acid resistant.

resistance was observed in 10 isolates from 6 patients and 4 controls (Table 3). In these in vitro conjugation experiments, 6 of the 10 isolates transferred only drug resistance to the recipient strain. Four isolates, however, transferred drug resistance determinants and DA phenotype to *E. coli* recipient C600. By transforming *E. coli* K-12 DH5α (*supE44 ΔlacU169 hsdR17 recA1 endA1 gyrA96 thi-1 relA1* Nal^r) (23) with plasmid DNA from two isolates (strains 67 and 79), we were able to demonstrate that genes encoding ampicillin, streptomycin, and tetracycline resistance and diffuse adherence were encoded on a 67- to 75-MDa conjugative plasmid. Curing an Ap^r Sm^r Tc^r DA transconjugant with acridine orange showed that both resistance and adherence traits were lost simultaneously.

In conclusion, our results show that diffusely adherent *E. coli* strains isolated from patients and control subjects present very different profiles when virulence markers and serotypes are considered. Among the virulence factors, *Shigella* enterotoxin 1 was noted most frequently in strains isolated from patients. The present study also demonstrated the high rate of resistance to certain antimicrobial agents in diffusely adherent *E. coli* strains in which resistance is apparently associated with conjugative plasmids. We also found plasmids encoding multiple drug resistance along with adhesion genes. The transfer of these potential virulence markers could be important in the epidemiology of diffusely adherent *E. coli* strains.

We thank Rosa M. Silva for critically reading the manuscript and helpful comments.

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

REFERENCES

1. **Azevedo, J. L., and S. O. P. Costa.** 1973. Exercícios práticos de genética, cia ed. Nacional e EDUSP, São Paulo, Brazil.
2. **Baqui, A. H., R. B. Sack, R. E. Black, K. Haider, A. Hossain, A. R. Alim, M. Yumus, H. R. Chowdhury, and A. K. Siddique.** 1992. Enteropathogens associated with acute diarrhea and persistent diarrhea in Bangladeshi children <5 years of age. J. Infect. Dis. **166:**792–796.
3. **Bauer, A. W., and W. N. N. Kirby.** 1996. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45:**493–496.
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. **Bernier, C., P. Gounon, and C. Le Bouguénec.** 2002. Identification of an aggregative adhesion fimbria (AAF) type III-encoding operon in enteroaggregative *Escherichia coli* as a sensitive probe for detecting the AAF-encoding operon family. Infect. Immun. **70:**4302–4311.
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. **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.
8. **Czeczulin, J. R., T. S. Whittam, I. R. Henderson, F. Navarro-Garcia, and J. P. Nataro.** 1999. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. Infect. Immun. **67:**2692–2699.
9. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125:**407–411.
10. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183:**334–336.
11. **Girón, J. A., T. Jones, F. Millán-Velasco, E. C. 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.
12. **Gunzburg, S. T., B. J. Chang, S. J. Elliott, V. Burke, and M. Gracey.** 1993. Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of western Australia. J. Infect. Dis. **167:**755–758.
13. **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 diffuse adhering strains. J. Clin. Microbiol. **31:**2031–2037.
14. **Johnson, J. R., and A. L. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
15. **Konowalchuk, J., J. I. Speirs, and S. Stavric.** 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. **18:**775–779.
16. **Le Bouguénec, C., M. Archambaud, and A. Labigne.** 1992. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J. Clin. Microbiol. **30:**1189–1193.
17. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
18. **Nowicki, B., A. Labigne, S. Mosely, R. Hull, S. Hull, and J. Moulds.** 1990. The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. Infect. Immun. **58:**279–281.
19. **Okeke, I. N., H. Steinruck, K. J. Kanack, S. J. Elliott, L. Sundstrom, J. B. Kaper, and A. Lamikanra.** 2002. Antibiotic-resistant cell-detaching *Escherichia coli* strains from Nigerian children. J. Clin. Microbiol. **40:**301–305.
20. **Okeke, I. N., I. C. A. Scaletsky, E. H. Soars, L. R. Macfarlane, and A. G. Torres.** 2004. Molecular epidemiology of the iron utilization genes of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. **42:**36–44.
21. **Ørskov, F., and I. Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–112.
22. **Oswald, E., P. Pohl, E. Jacquemin, P. Lintermans, K. van Muylen, A. D. O'Brien, and J. Mainil.** 1994. Specific DNA probes to detect *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 or 2. J. Med. Microbiol. **40:**428–434.
23. **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.
24. **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.
25. **Scaletsky, I. C. A., S. H. Fabbricotti, K. R. Aranda, M. B. Morais, and U. Fagundes-Neto.** 2002. Comparison of DNA hybridization and PCR assays for detection of putative pathogenic enteroadherent *Escherichia coli*. J. Clin. Microbiol. **40:**1254–1258.
26. **Scott, D. A., and J. P. Kaper.** 1994. Cloning and sequencing of genes encoding *Escherichia coli* cytolethal distending toxin. Infect. Immun. **62:** 244–251.
27. **Stein, M., B. Kenny, M. A. Stein, and B. B. Finlay.** 1996. Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic *Escherichia coli* which is homologous to members of the immunoglobulin A protease-like family of secreted proteins. J. Bacteriol. **178:**6546–6554.
28. **Vila, J., M. Vargas, I. R. Henderson, J. Gascón, and J. P. Nataro.** 2000. Enteroaggregative *Escherichia coli* virulence factors in traveler's diarrhea strains. J. Infect. Dis. **182:**1780–1783.
29. **Welch, R. A., R. Hell, and S. Falkow.** 1983. Molecular cloning and physical characterization of a chromosomal hemolysin from *Escherichia coli*. Infect. Immun. **42:**178–186.
30. **Yamamoto, T., and P. Echeverria.** 1996. Detection of enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. Infect. Immun. **64:**1441–1445.