Enteroaggregative *Escherichia coli* as a Potential Cause of Diarrheal Disease in Adults Infected with Human Immunodeficiency Virus

Christine A. Wanke, Howard Mayer,* Rainer Weber, Reinhard Zbinden, D. Ashley Watson,* and David Acheson

Department of Medicine, Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard Medical School, and Division of Geographic Medicine, Tufts University School of Medicine, Boston, Massachusetts; Department of Medicine, Division of Infectious Disease and Hospital Epidemiology, University Hospital, and Department of Medical Microbiology, University of Zurich, Zurich, Switzerland

Stools of 68 human immunodeficiency virus (HIV)–infected adults with diarrhea and 60 without diarrhea were examined for enteroaggregative *Escherichia coli* (EAggEc) by HeLa cell adherence assay. EAggEc were present in stools of 30 patients with and 18 without diarrhea ($P = .05$). CD4 cell counts of patients with EAggEc and diarrhea were significantly lower than those of patients with EAggEc without diarrhea ($P = .02$). There was no difference in the mean duration of diarrheal symptoms or in the number of stools per day between patients with EAggEc and those without. None of the EAggEc strains were positive by polymerase chain reaction for adherence fimbria, but 11 strains were positive for EAggEc heat-stable toxin EAST/1. Of the EAggEc strains, 51% were resistant to trimethoprim-sulfamethoxazole and 65% were resistant to ampicillin. EAggEc may be a pathogen in HIV-infected patients with diarrhea; HIV-infected patients with EAggEc appear to be more symptomatic when HIV disease is more advanced.

Enteroaggregative *Escherichia coli* (EAggEc) have been associated with persistent diarrheal disease in children in the developing world [1–6]. The mechanisms by which these organisms cause diarrheal disease are not well understood. Various strains of EAggEc from diverse populations of children with diarrhea from around the world have demonstrated different potential virulence traits, which have not been found consistently among strains from all geographic regions [7–9]. Strains all appear to aggregate in the HeLa or HEp-2 tissue culture assay, and this phenotype serves, to date, as the most reliable means to identify EAggEc. Organisms have been described to have a pilus adhesin, AAF1, composed of a subunit AggA, and AggR, a regulator, which has been shown to be responsible for the aggregative adherence of the organisms [10–13]. EAggEc organisms have also been shown to produce a heat-stable toxin, EAggEC heat-stable toxin (EAST/1), which appears to mediate secretion through cGMP [14, 15]. Additionally, some strains have been shown to produce a larger protein toxin that activates interleukin-8 [16]. Additional strains have demonstrated the ability to invade HeLa or intestinal cell cultures and subvert cellular tyrosine phosphorylation or produce a novel cryohemagglutinin [17, 18]. None of the potential virulence traits has been identified in all or even a majority of EAggEc strains from diverse geographic areas. The precise clinical relevance of any of these virulence traits remains unclear.

Equally disturbing is the fact that healthy adult volunteers who ingest EAggEc strains and are colonized by them do not consistently develop diarrheal disease [19]. This raises the possibility that EAggEc may function as a more opportunistic pathogen in young or immunologically compromised patients. That is, EAggEc may cause diarrhea in some immunologically normal hosts when the numbers of organisms or some host factor favors the organism but more easily can routinely cause diarrhea in persons who are somewhat compromised in terms of intestinal function or nutritional status or who manifest some other immunologic defect. As these organisms cannot be identified on routine stool culture and must be identified by the time- and labor-intensive tissue culture adherence assay, their presence and prevalence in the stools of adults throughout the world and in more developed countries are not known. EAggEc are known to be associated with sporadic outbreaks of diarrheal disease in adults and children in the developed world, but the frequency with which these outbreaks occur is not known [20–22]. EAggEc have been reported to be associated with diarrheal disease in patients infected with human immunodeficiency virus (HIV) [9, 23–25].

Patients who are infected with HIV frequently develop persistent diarrheal disease for which the etiology remains enigmatic [26, 27]. We elected to examine a group of HIV-infected

Received 26 September 1997; revised 28 January 1998.

Presented in part: 31st US-Japan Cholera and Related Diarrheal Diseases Meeting, Kiawah Island, South Carolina, 1–3 December 1995.

Approval for the study was obtained from the Institutional Review Board of the West Campus of the Beth Israel Deaconess Medical Center. Verbal informed consent was obtained.

Financial support: Bayer Pharmaceuticals, American Gastrointestinal Association Foundation (to C.A.W.).; NIH (AI-39067 to D.A.).

Reprints or correspondence: Dr. Christine Wanke, Division of Infectious Diseases, Beth Israel Deaconess Medical Center, One Deaconess Rd., Boston, MA 02215 (cwanke@bidmc.harvard.edu).

* Present affiliations: Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut (H.M.); Infectious Disease Unit, Woden Valley Hospital, Woden, Australia (D.A.W.).

The Journal of Infectious Diseases 1998; 178:185–90

0022–1899/98/7801–0023$02.00

patients with and without diarrheal disease to determine whether EAggEc were present in those with unexplained diarrhea. We also surveyed *E. coli* from stools of persons who were not known to be infected with HIV to estimate the baseline occurrence of EAggEc in an adult population with and without diarrhea in Boston. As there has been no single factor that has consistently characterized these organisms, we characterized the EAggEc strains we recovered from the HIV-infected patients as completely as possible.

Methods

Population. Urban, hospital-based HIV clinics in Boston and Zurich were used to recruit the diarrheal disease patients and nondiarrheal control patients for the study.

HIV-infected adults who were followed at either of these two clinics who had complained of diarrheal symptoms on at least two visits to their primary care physician were screened for EAggEc as well as routine pathogens. Randomly selected, unmatched control patients infected with HIV, followed at the same clinic, who did not have diarrheal disease or any change in bowel habits, were recruited as HIV-infected controls. Patient data were obtained and/or verified by interview and by chart review. The data collected included a description of and the duration of diarrheal symptoms, medications, gastrointestinal evaluation, other medical history, and laboratory data including CD4 cell count. Exposure data including employment, travel history, pets, and source of drinking water were also collected when possible.

In addition, stools from adult patients who were not known to be infected with HIV but who presented with diarrhea and had submitted a stool culture to one of the microbiology laboratories (Boston) were also screened for the presence of EAggEc. Patient data for this group, including the level of suspicion of potential HIV exposure, were obtained by interviewing the physician who obtained the stool for culture. Stools from healthy adult volunteers without diarrhea and either with no recognized risk factor for acquisition of HIV or known to be HIV-negative were also screened for the presence of EAggEc. EAggEc strains identified from either of these groups of patients were not characterized further in this study.

Routine stool culture and examination. Stool specimens were cultured for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species and were tested for *Clostridium difficile* toxin. Stool specimens were examined for ova and parasites, cryptosporidia, microsporidia, and acid-fast bacilli by use of a fluorescent antibody stain for cryptosporidia and the modified trichrome and chitin stain for microsporidia.

EAggEc assay. Two to 5 colonies of *E. coli* were selected for each patient and control and stocked at −70°C in Luria broth and glycerol. *E. coli* were assayed in the HeLa cell adherence assay, as previously described [5, 28]. Briefly, HeLa cells (American Type Culture Collection, Rockville, MD) that had been grown to confluence in MEM (Gibco-BRL, Gaithersburg, MD) with 10% fetal calf serum but without antibiotics were plated in 8-well Labtek Slides (Dynatek, Naperville, IL) in fresh medium and incubated overnight at 37°C with 10% CO_2. Each *E. coli* strain was also grown overnight in 5 mL of Luria broth, washed, and resuspended in sterile PBS on the second day of the assay. The tissue culture cells were washed and refed with 0.5 mL of fresh medium, without fetal calf serum or antibiotics. Twenty microliters of the overnight culture of bacterial suspension was added to each well of cells, incubated for 2 h, and then washed five times with sterile PBS, fixed with 100% methanol, and stained with Giemsa. Positive and negative controls were included in each assay. All assays were read blinded by two investigators.

AAF1	5' TGGGATTGCACTCTCAGG 3'
	5' TACCAGATATAAATATAGGG 3'
	(450 bp product)
AggR	5' CGATGTATACACAAAAGAAGGA 3'
	5' GCCTAATGAAATATGATGGTACT 3'
	(640 bp product)
AggA	5' GCGTTAGAAAGACCTCCAATA 3'
	5' GCCGGATCCTTAAAAATTAATTCCGGC 3'
	(435 bp product)
EAST/1	5' CCATCAACACAGTATATCCGA 3'
	5' GGTCGCGAGTGACGGCTTTGT 3'
	(111 bp product)

Figure 1. Primers used for detection of recognized adherence fimbria and EAggEc heat-stable toxin (EAST/1).

Additional EAggEc characterization by bacterial endocytosis. All *E. coli* strains were tested for the ability to invade eukaryotic cells in the gentamicin protection assay [17]. For this assay, HeLa cells were seeded into 24-well plates for 18 h. Each well was inoculated with 20 μL of an *E. coli* strain prepared as described above and incubated for 2 h. Cells were washed with sterile PBS and refed with media with 50 μg/mL gentamicin for an additional 2 h. HeLa cells were lysed with 0.125% sodium deoxycholate. Serial dilutions of this lysate were plated on Luria agar to determine the number of viable intracellular bacteria. Isolates were considered positive if >5% of the original inoculum was internalized, as previously defined [17]. Positive and negative controls were included in each assay.

Verotoxin production. Vero cells (ATCC) were seeded in 24-well plates and incubated for 18 h. Cells were washed and refed with MEM and 5% fetal calf serum. The *E. coli* strains were grown in Luria-Bertani broth overnight and centrifuged, and the supernatant was filtered through a 0.45-μm filter; 0.5 mL of this filtered bacterial supernatant was added to each well. Cells were incubated at 37°C and examined for visible cell rounding at 24 and 48 h and for cell death by trypan blue exclusion at 48 h. Positive and negative controls were included in each assay.

Antibiotic sensitivity. Susceptibility testing was done by the disk diffusion method in accordance with National Committee for Clinical Laboratory Standards guidelines.

Polymerase chain reaction (PCR). *E. coli* strains were assayed for the presence of the recognized aggregative adherence fimbria AAF1, as well as the genes for AggR, the regulator, and AggA, a subunit, and for the presence of EAST/1 genes by PCR. The

primers used are shown in figure 1. The AAF1 primers were generated from pCVD432. Forty cycles of PCR were done directly from bacterial colonies grown on Luria-Bertani agar plates, and strain 17-2 was used as a positive control in each PCR run [29]. In our hands, the adherence probe for aggregative adherence correlates 100% with this PCR methodology (93 strains tested using the primers as described; unpublished data).

Statistical analysis. Variables were analyzed by logistic regression modeling. CD4 cell counts were analyzed as a continuous and dichotomous variable. Odds ratios (ORs) with 95% confidence intervals (CIs) were used as measures of association. Continuous variables were analyzed by Student's *t* test.

Results

Surveillance of adults without HIV infection. The stools of 68 adult patients with diarrhea or abdominal complaints that were submitted to the microbiology laboratory of the Beth Israel Deaconess Medical Center were examined for the presence of EAggEc. These patients were not known to be HIV-positive and were not at high risk for HIV infection by interview with patient physicians and/or review of patient records. Many of these patients did have other comorbid illnesses, such as inflammatory bowel disease, diabetes mellitus, or irritable bowel syndrome, or had a history of travel to the developing world. Nineteen (28%) of these patients had phenotypic evidence of EAggEc in their stool. Sixteen of these had a comorbid illness. An additional 52 adults without diarrhea who were not known to have HIV infection or to be at high risk of HIV infection and who had no significant or chronic medical illnesses were recruited to submit stools for EAggEc assay. Seven (13%) of these adults had EAggEc in their stools. EAggEc were present significantly more often in the stools of the adults with diarrhea than of those without diarrhea ($P = .05$).

Surveillance of adults with HIV infection. Of the 134 HIV-infected patients enrolled, 117 were male and 17 female. Sixty-three patients were enrolled from Zurich (33 with diarrhea and 30 without diarrhea) and 71 were enrolled from Boston (35 with diarrhea and 36 without diarrhea). Demographic data are shown in table 1. Patient ages ranged from 22 to 66 years (mean, 39). Seventy-two percent of the male patients had sex with men as the risk factor for HIV infection. Seventy-six patients (57%) were taking antiretroviral therapy; 32% of those were taking zidovudine. Forty-four patients (33%) were taking trimethoprim-sulfamethoxazole as prophylaxis for *Pneumocystis carinii* pneumonia. CD4 lymphocyte counts for the enrolled patients ranged from 0 to 1080 cells/mm^3 (mean $\pm$ SD, 173 $\pm$ 203). The median CD4 cell count for the entire group was 100 cells/mm^3.

Of these 134 HIV-infected patients, 68 had diarrhea and 66 were controls, with no complaints of diarrhea. Fifty-three of the patients with diarrhea (78%) had diarrhea that had lasted $>$28 days (persistent diarrhea) and 15 (22%) had diarrheal symptoms for $<$28 days (acute diarrhea). The overall range of diarrheal symptoms was 1–52 weeks (mean, 11). The mean CD4 cell count for patients with diarrhea was 128 $\pm$ 187 cells/mm^3; the median was 50. The mean CD4 cell count for patients without diarrhea was 222 $\pm$ 193 cells/mm^3; the median was 190. The CD4 cell count of patients with diarrhea was significantly lower than that of patients without diarrhea ($P = .008$). There were no significant differences between the patients from Boston and Zurich in duration of diarrhea or CD4 cell counts.

Thirty (44%) of those with diarrhea had phenotypic evidence of EAggEc organisms in their stool cultures. Eighteen of these patients were from Zurich (55%) and 12 were from Boston (34%). These patients and the patients with diarrhea who did not have EAggEc in stool are described in table 2. The patients with EAggEc and diarrhea complained of diarrheal symptoms for a mean of 12.9 $\pm$ 12.1 weeks and had a mean of 5.1 $\pm$ 2.5 bowel movements per day. The patients with diarrhea and EAggEc in stool for whom serial weights were available had lost a mean of 7.3 kg (range, 0–22). HIV-infected patients who had diarrhea but did not have EAggEc organisms in the stool had diarrheal symptoms for a mean of 9.3 $\pm$ 9.9 weeks; this difference was not significant. These patients with diarrhea but without EAggEc in stool had a mean of 4.1 $\pm$ 2.7 bowel movements per day ($P = .08$ vs. patients with EAggEc) and had lost a mean of 3.9 kg (range, 0–13.6) ($P = .06$ vs. weight loss in HIV-infected patients with EAggEc and diarrhea). CD4 cell counts of the patients with diarrhea who had EAggEc in stool were not different from CD4 cell counts of patients with diarrhea but without EAggEc in stool. The mean CD4 cell count of patients with EAggEc and diarrhea was 94 $\pm$ 105 cells/mm^3 (median, 50), and the mean CD4 cell count of the patients without EAggEc was 157 $\pm$ 233 cells/mm^3 (median, 50). There were no significant differences between the patients from Zurich and Boston in duration of diarrhea, numbers of stools, or amount of weight loss. Stool cultures for routine enteric pathogens were negative for all of the patients enrolled in the study. Some patients had been sent for upper or lower endoscopy by their primary care physicians. Results of these investigations are shown in table 2.

Organisms phenotypically consistent with EAggEc were also present in the stools of 18 (27%) of the 66 HIV-infected patients without diarrhea; 13 of these were from Zurich and 5 were from Boston. EAggEc were present significantly more often in stools of patients with diarrhea than in stools of patients without diarrhea in the population as a whole ($P = .05$). When the population was divided by location, EAggEc were present significantly more often in patients with diarrhea than in patients without diarrhea from Boston, but the difference did not reach significance for the population from Zurich. Overall, there was a trend toward an association between the presence of EAggEc and both acute and persistent diarrhea (OR, 2.7; 95% CI, 0.73–10; vs. OR, 1.3; 95% CI, 0.6–2.7). The nondiarrheal control patients who had EAggEc identified in stool had a mean CD4 cell count that was significantly higher (188 $\pm$ 190 cells/mm^3; median, 170) than that of patients with diarrhea who had EAggEc in stool or patients with diarrhea who did not have EAggEc in

Table 1. Baseline characteristics of HIV-infected adults with and without diarrhea.

Characteristic	Boston (n = 71)	Zurich (n = 63)	Overall (n = 134)
Male	67 (94)	50 (79)	117 (87)
Female	4 (6)	13 (21)	17 (13)
Age, years, mean ± SD	39 ± 8	38.1 ± 9	39 (range, 22–66)
HIV risk factor*			
Homosexual	59	37	96 (72)
Hemophilia	1	1	2 (1)
Intravenous drug use	8	14	22 (16)
Heterosexual	4	5	9 (7)
Unknown	0	1	1
Antiretroviral therapy	44	32	76 (57)
Trimethoprim-sulfamethoxazole for PCP prophylaxis	24	20	44 (33)
Median CD4 cell count/mm^3	80	130	100
Mean CD4 cell count/mm^3 ± SD	172 ± 220	176 ± 160	173 ± 203

NOTE. Data are no. of patients or no. (%) unless indicated. PCP, *Pneumocystis carinii* pneumonia.
* More than 1 risk factor may have been reported for each person.

stool (P = .02 for each). When stratified by the presence or absence of diarrhea, the presence of EAggEc in stool was associated with a lower CD4 cell count, even when controlled for zidovudine and trimethoprim-sulfamethoxazole usage (P = .03).

There were no differences in the numbers of patients in any group for the use of trimethoprim-sulfamethoxazole, dapsone, or aerosolized pentamidine as *P. carinii* pneumonia prophylaxis nor in the presence of other epidemiologic clues for the potential acquisition of EAggEc organisms. For the patients for whom data were available, there was no association of EAggEc with employment, presence of pets, travel history, exposure to children, or source of drinking water. The Boston and Zurich groups did not differ in respect to these exposures (data not shown).

Table 2. Characteristics of HIV-infected patients with diarrhea.

Characteristic	Diarrhea with EAggEc (n = 30)*	Diarrhea without EAggEc (n = 38)†	P
Mean duration of symptoms, weeks	12.9 ± 12.1	9.3 ± 9.9	NS
Mean no. of stools/day	5.1 ± 2.5	4.1 ± 2.7	.08
Mean decrease in weight, kg	7.3 ± 6.8	3.9 ± 4.6	.06
Mean CD4 cells/mm^3	94 ± 105	157 ± 233	NS
Boston	85	145	
Zurich	104	163	
Median CD4 cells/mm^3	50	50	
Boston	45	42	
Zurich	53	80	
Other GI evaluation,‡ no. (%)	12 (40)	9 (24)	NS
Other GI pathogens identified	Microsporidia: 1 CMV colitis: 1 None: 10	Microsporidia: 1 MCV colitis: 1 None: 7	
Mean age, years	41.2 ± 10.7	37.5 ± 6.7	NS

NOTE. Means are given ± SDs. NS, not significant.
* 18 patients with diarrhea and EAggEc were from Zurich and 13 were from Boston.
† 15 patients with diarrhea without EAggEc were from Zurich and 23 were from Boston.
‡ Other gastrointestinal (GI) evaluation: these patients had been sent by their primary physician for upper or lower endoscopy as part of their evaluation for diarrheal disease.

Twenty-one patients with diarrhea had undergone further gastrointestinal evaluation; 12 of these had EAggEc in stool and 9 did not. Of the 12 with diarrhea and EAggEc, 1 patient was also found to have microsporidia, and 1 had cytomegalovirus colitis. One patient with EAggEc in stool also had lesions suggestive of bacterial enteritis in the distal ileum. For the 9 patients with diarrhea who did not have EAggEc organisms in the stool, 1 patient had microsporidia, 1 had cytomegalovirus colitis, and the remainder had unrevealing gastrointestinal evaluations.

Characterization of EAggEc organisms. EAggEc organisms in this study were identified by phenotype, so all strains considered to be EAggEc were phenotypically positive, by definition, in the adherence assay. EAggEc strains from HIV-positive patients were assayed by PCR for the presence of AAF1, AggR, AggA, and EAST/1 genes. Of 59 strains tested, 11 strains were positive for EAST/1; no strain was positive for AAF1, AggA, or AggR. None of the strains had evidence of cytotoxin production in the Vero cell assay. Four patients had EAggEc strains that were positive for invasion in the HeLa cell gentamicin protection assay. The antibiotic sensitivities of the EAggEc organisms are shown in table 3.

Discussion

EAggEc organisms are traditionally associated with persistent diarrheal disease in children in the developing world. Our

Table 3. Antibiotic sensitivities of EAggEc strains from HIV-infected adults ($n = 49$).

Antibiotic	Susceptible	Intermediate	Resistant
Trimethoprim-sulfamethoxazole	24 (49)	2 (4)	23 (47)
Ampicillin	17 (35)	4 (8)	28 (57)
Chloramphenicol	31 (63)	3 (6)	15 (31)
Gentamicin	44 (90)	1 (2)	4 (8)
Ciprofloxacin	47 (96)	1 (2)	1 (2)

NOTE. Data are no. (%).

study did demonstrate an association between EAggEc organisms in stool and diarrhea or abdominal complaints in a series of adults without HIV. Our study also demonstrated that EAggEc occurs in stools of patients infected with HIV with diarrhea more frequently than in stools of HIV-infected adults without diarrhea. The rates at which EAggEc were identified from diarrheal and nondiarrheal patients infected with HIV in our study are consistent with those in studies of persistent diarrheal illnesses in children in the developing world. There appears to be an association between EAggEc in patients symptomatic with diarrhea and lower CD4 cell counts as a measure of more advanced HIV illness in these patients. We believe that EAggEc may well represent an opportunistic pathogen in the HIV-infected population. As there was a tendency toward more weight loss in the HIV-infected patients with diarrhea and EAggEc in stool than in those patients with diarrhea but without EAggEc, there may also be an association of EAggEc with malnutrition in the HIV-infected patient. This finding will require additional study, as it may also reflect the tendency for these patients to have more advanced disease.

The epidemiology of EAggEc organisms has been notoriously difficult to study, because of the difficulty of identifying organisms from stool culture. EAggEc were originally identified by the adherence assay; subsequently, it was thought that the use of a DNA probe was sufficient to identify EAggEc organisms [7]. However, subsequent data have revealed that AAF1 is not universally present in organisms that clearly have the aggregative phenotype [8, 9]. Our PCR data suggest that the previously described aggregative fimbria, the subunit, and the regulator of this fimbria are not sufficient to identify aggregative organisms in HIV-positive adults in the developed world. EAST/1 has been shown to be present in a heterogeneous group of *E. coli*. Our data suggest that probing for fimbriae or EAST/1 will not assist in the identification of EAggEc organisms in the adult population or the HIV-infected population. While PCR for these genes may not be absolutely sensitive, our previous experience in comparing PCR using these primers with PCR with the AA probe (both generated from pCVD432) suggests that there is a 100% correlation in nearly 100 strains. The bioassays done in our study confirm that cytotoxin or invasion are not consistently recognizable virulence factors present in these organisms.

The antibiotic sensitivity data in our study suggest that treatment studies will need to take the resistant nature of EAggEc into account, as a high proportion of organisms are resistant to antibiotics such as ampicillin and trimethoprim-sulfamethoxazole. A previous study has reported that EAggEc organisms are fairly antibiotic-resistant and suggested that resistance may be linked to virulence [30]. In our population, such resistance may not be an intrinsic feature of the EAggEc organisms but may have occurred in the organisms in this study because of the high rate of antibiotic exposure in patients infected with HIV. Such resistance may assist in selecting for the predominance of these organisms in stools of patients infected with HIV.

Further epidemiologic data are needed to clarify the role of EAggEc in diarrheal disease and weight loss in patients with HIV throughout the world. If treatment studies could demonstrate that eradicating these organisms does alleviate the diarrheal symptoms or improve the nutritional status in HIV-infected patients who carry EAggEc, this could result in the development of a relatively simple intervention that could alleviate significant morbidity associated with HIV infection. Ultimately, the precise virulence factors in the EAggEc organisms need to be understood so that more accessible diagnostic methods for these organisms can be developed.

References

1. Wanke CA, Schorling JB, Barrett LJ, Desouza MA, Guerrant RL. Potential role of adherence traits of *Escherichia coli* in persistent diarrhea in an urban Brazilian slum. Pediatr Infect Dis J **1991**;10:746–51.
2. Bhan MK, Raj P, Levine MM, et al. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J Infect Dis **1989**;159:1061–4.
3. Bhatnagar B, Bhan MK, Sommerfelt H, Sazawal S, Kumar R, Saini S. Enteroaggregative *Escherichia coli* may be a new pathogen causing acute and persistent diarrhea. Scand J Infect Dis **1993**;25:579–83.
4. Henry FJ, Udoy AS, Wanke CA, Aziz KMA. Epidemiology of persistent diarrhea and etiologic agents in Mirzapur, Bangladesh. Acta Paediatr Suppl **1992**;381:27–31.
5. Cravioto A, Tello A, Navarro A, et al. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhoea. Lancet **1991**;337:262–4.
6. Paul M, Tsukamoto T, Ghosh AR, et al. The significance of enteroaggregative *Escherichia coli* in the etiology of hospitalized diarrhoea in Calcutta, India, and the demonstration of a new honey-combed pattern of aggregative adherence. FEMS Microbiol Lett **1994**;117:319–25.
7. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J Infect Dis **1990**;161:1249–51.
8. Fang GD, Lima AA, Martins CV, Nataro JP, Guerrant RL. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. J Pediatr Gastroenterol Nutr **1995**;21:137–44.
9. Polotsky Y, Nataro JP, Kotler D, Barrett TJ, Orenstein JM. HEp-2 cell adherence patterns, serotyping, and DNA analysis of *Escherichia coli* isolates from eight patients with AIDS and chronic diarrhea. J Clin Microbiol **1997**;35:1952–8.
10. Baldwin TJ, Knutton S, Sellers L, Hernandez HAM, Aitken A, Williams PH. Enteroaggregative *Escherichia coli* strains secrete a heat-labile toxin antigenically related to *E. coli* hemolysin. Infect Immun **1992**;60:2092–5.

11. Knutton S, Shaw RK, Bhan MK, et al. Ability of enteroaggregative *Escherichia coli* strains to adhere in vitro to human intestinal mucosa. Infect Immun **1992**;60:2083–91.
12. Nataro JP, Yikang D, Giron JA, Savarino SJ, Kothary MH, Hall R. Aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli* requires two unlinked plasmid regions. Infect Immun **1993**;61: 1126–31.
13. Nataro JP, Yikang D, Yingkang D, Walker K. AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli.* J Bacteriol **1994**;176:4691–9.
14. Savarino SJ, Fassano A, Watson J, et al. Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of *E. coli* heat-stable toxin. Proc Natl Acad Sci USA **1993**;90:3093–7.
15. Savarino SJ, Fasano A, Robertson DC, Levine MM. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J Clin Invest **1991**;87:1450–5.
16. Eslava C, Villaseca J, Morales R, Navarro A, Cravioto A. Identification of a protein with toxigenic activity produced by enteroaggregative *Escherichia coli* [abstract B-105]. In: Proceedings of the 93rd annual meeting of the American Society for Microbiology (Atlanta). Washington, DC: ASM, **1993**.
17. Benjamin P, Federman M, Wanke CA. Characterization of an invasive phenotype associated with enteroaggregative *Escherichia coli.* Infect Immun **1995**;63:3417–21.
18. Yamamoto T, Wakisaka N, Nakae T. A novel cryohemagglutinin associated with adherence of enteroaggregative *Escherichia coli.* Infect Immun **1997**;65:3478–84.
19. Nataro JP, Yikang D, Cookson S, et al. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. J Infect Dis **1995**; 171:465–8.
20. Chan KN, Phillips AD, Knutton S, Smith HR, Walker-Smith JA. Enteroaggregative *Escherichia coli:* another cause of acute and chronic diarrhea in England? J Pediatr Gastroenterol Nutr **1994**;18:87–91.
21. Scotland SM, Willshaw GA, Smith HR, Said B, Stokes N, Rowe B. Virulence properties of *Escherichia coli* strains belonging to serogroups O26, O55, O111, and O128 isolated in the United Kingdom in 1991 from patients with diarrhoea. Epidemiol Infect **1993**;111: 429–38.
22. Scotland SM, Smith HR, Cheasty T, et al. Use of gene probes and adhesion tests to characterise *Escherichia coli* belonging to enteropathogenic serogroups isolated in the United Kingdom. J Med Microbiol **1996**;44: 438–43.
23. Mayer HB, Wanke CA. Enteroaggregative *E. coli* in the stool of an AIDS patient with previously unexplained diarrhea [letter]. N Engl J Med **1994**;332:273–4.
24. Thea DM, St. Louis ME, Atido U. A prospective study of diarrhea and HIV-1 infection among 429 Zairian infants. N Engl J Med **1993**;329: 1696–702.
25. Pichler HET, Diridl G, Stickler K, Wolf D. Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am J Med **1987**;82: 329–32.
26. Bartlett JG, Belitsos PC, Sears CL. AIDS enteropathy. Clin Infect Dis **1992**;15:726–35.
27. Lambl BB, Federman M, Pleskow D, Wanke CA. Malabsorption and wasting in AIDS patients with microsporidia and pathogen-negative diarrhea. AIDS **1996**;10:1–5.
28. Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM. Patterns of adherence diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr Infect Dis J **1987**;6:829–31.
29. Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H. Development of PCR for screening of enteroaggregative *Escherichia coli.* J Clin Microbiol **1995**;33:701–5.
30. Yamamoto T, Echeverria P, Yakota T. Drug resistance and adherence to human intestines of enteroaggregative *Escherichia coli.* J Infect Dis **1992**;165:744–9.