

Characterization of Pathogenic *Escherichia coli* in Human Immunodeficiency Virus–Related Diarrhea in Senegal

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Stool samples obtained from 594 Senegalese patients were examined for characterization of pathogenic *Escherichia coli* in human immunodeficiency virus (HIV)–related diarrhea. Multiple virulence genes were observed in stool samples obtained from HIV-infected patients with diarrhea. Enteroaggregative *E. coli* and enteroinvasive *E. coli* were present in stool samples obtained from patients with diarrhea significantly more often than in stool samples obtained from patients without diarrhea ($P = .000001$). Quinolones may be an effective alternative treatment for *E. coli*–related diarrhea in HIV-infected adults in Senegal.

It has been proven that persistent diarrhea is responsible for a substantial proportion of diarrhea-associated morbidity and mortality in human immunodeficiency virus (HIV)–infected (HIV⁺) patients, particularly in those with AIDS. In developing countries, diarrhea is common, occurring in 60%–90% of HIV⁺ patients [1]. HIV itself has been associated with enteropathy in patients with AIDS [2]. Alteration of the lamina propria predisposes these patients to enteric infections; as a result, they are susceptible to low doses of some pathogens, which would produce only asymptomatic or mild infection in immunocompetent hosts.

Among enteric pathogens, *Escherichia coli* has been recognized as being strongly associated with persistent diarrhea; this

versatile organism has different virulence factors (e.g., adhesins, invasins, and toxins) that are responsible for a wide range of types of diarrheal disease [3, 4]. Six categories of diarrhegenic *E. coli* are defined by the presence of specific genes that encode for their virulence factors: *LT* (heat-labile enterotoxin) and *Sta* (heat-stable enterotoxin), which encode enterotoxigenic *E. coli*; *eaeA* (*E. coli* attaching and effacing) and *bfpA* (bundle-forming pilus), which encode enteropathogenic *E. coli* (EPEC); *ipaH* (invasion plasmid antigen H), which encodes enteroinvasive *E. coli* (EIEC); SLTI (Shiga-like toxin I) and SLTII (Shiga-like toxin II), which encode enterohemorrhagic *E. coli* (EHEC); *Eagg*, which encodes enteroaggregative *E. coli* (EaggEC); and *afa* (afimbrial adhesin), which encodes diffusely adherent *E. coli* (DAEC) [5]. To our knowledge, this is the first study conducted in Senegal to identify pathogenic *E. coli* in HIV-related diarrhea.

We examined groups of HIV⁺ patients with and without diarrhea (D⁺ and D⁻, respectively); we also examined D⁺ and D⁻ patients who were not infected with HIV (HIV⁻). The aim of the present study was to characterize virulence genes in pathogenic *E. coli*, to determine the adherence phenotypes, and to evaluate the current antimicrobial-susceptibility patterns of virulent strains. We hoped that our findings would help identify an appropriate treatment that could significantly alleviate morbidity associated with diarrhea in HIV⁺ patients.

Patients and methods. From June 1997 through December 1999, stool and blood samples were obtained from patients >18 years old who were hospitalized in a university teaching hospital-based infectious diseases clinic (Centre Hospitalier Universitaire Fann) and in an urban hospital (Hôpital Principal) in Dakar. Written, informed consent was obtained from all participants, and all aspects of the study were approved by the ad hoc Ethical Committee. These subjects were randomly selected to be part of a case-control study of HIV-associated diarrhea (D⁺ HIV⁺ and D⁺ HIV⁻ patients vs. D⁻ HIV⁺ and D⁻ HIV⁻ patients). We defined case patients as those with diarrhea (D⁺) with frequent watery stools (>3 unformed stools/day). Control patients were those who had no history of diarrheal illness during the preceding month (D⁻) and who were hospitalized during the study period. Control patients were not matched to case patients. Patients' data were recorded by a physician. The data collected included age, sex, marital status, living conditions, contact with animals, medications, medical history, and access to potable water.

E. coli was considered to be the sole etiologic agent of diarrhea when it was obtained as a pure culture in a nonselective, solid, bromocresol-purple medium and when a significant vir-

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ulence gene was identified on at least 3 of 5 tested colonies. Five colonies of *E. coli* obtained as a pure culture were selected by standard biochemical tests (API 20E; Bio Mérieux). DNA was extracted by boiling and was stored at -20°C . Virulence factors were screened by use of polymerase chain reaction (PCR) using the following primers: *eaeA* (5'-GCA AAT TTA GGT GCG GGT CAG CGT T-3' and 5'-GGC TCA ATT TGC TGA GAC CAC GGT T-3'), *bfpA* (5'-CAA TGG TGC TTG CGC TTG CT-3' and 5'-GCC GCT TTA TCC AAC CTG GT-3'), *SLTI* (5'-GAA GAG TCC GTG GGA TTA CG-3' and 5'-AGC GAT GCA GCT ATT AAT AA-3'), *SLTII* (5'-TTA ACC ACA CCC ACG GCA GT-3' and 5'-GCT CTG CAT GCA TCT CTG GT-3'), *LT* (5'-GCG ACA AAT TAT ACC GTG CT-3' and 5'-CCG AAT TCT GTT ATA TAT GT-3'), *Sta* (5'-CTG TAT TGT CTT TTT CAC CT-3' and 5'-GCA CCC GGT ACA AGC AGG AT-3'), *afa* (5'-GCT GGG CAG CAA ACT GAT AAC TCT C-3' and 5'-CAT CAA GCT GTT TGT TCG TCC GCC G-3'), *Eagg* (5'-CTG GCG AAA GAC TGT ATC AT-3' and 5'-CAA TGT ATA GAA ATC CGC TGT T-3'), and *ipaH* (5'-GCT GGA AAA ACT CAG TGC CT-3' and 5'-CCA GTC CGT AAA TTC ATT CT-3') [5].

Amplified DNA products were resolved by conventional electrophoresis. All PCR amplifications were performed in duplicate. *E. coli* adherence pattern was assayed in Hep-2 cells, as described by Cravioto et al. [6]. Positive and negative controls were included in each assay. All assays were blind-read by 2 investigators. Antimicrobial-susceptibility testing was done by the disk-diffusion method on Mueller Hinton agar (Becton Dickinson) with commercial antimicrobial disks (Sanofi Diagnostics Pasteur), in accordance with NCCLS guidelines [7].

HIV serostatus was determined by 2 EIAs (GENELAVIA MIXT [Sanofi-Diagnostics Pasteur] and VIRONOSTIKA HIV UNI-FORM II [Organon Teknika]) and was confirmed by Western blotting (NEW LAV BLOT; Sanofi Diagnostics Pasteur). CD4 lymphocyte counts were determined by use of a laser-based FACSCount system (Becton Dickinson Immunocytometry Systems) for directly determining absolute CD4 lymphocyte count.

Epi Info (version 6.01; CDC) and SAS (version 6.12; SAS) software were used for statistical analysis. For normally distributed continuous variables, means were compared by use of Student's *t* test. Categorical variables were compared by use of the χ^2 test and Fisher's exact test, where appropriate.

Potential risk factors of diarrhea among HIV⁺ patients were analyzed by univariate model. All variables associated with diarrhea for which $P < .3$ were included in the analysis. Logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

Results. A total of 594 stool samples were examined: 279 (47%) were from case patients (158 D⁺ HIV⁺ and 121 D⁺ HIV⁻ patients), and 315 (53%) were from control patients (160 D⁻

HIV⁺ and 155 D⁻ HIV⁻ patients). CD4 geometric mean lymphocyte counts were lower in HIV⁺ patients than in HIV⁻ patients, among both case patients (D⁺ HIV⁺, 109.9 ± 205.3 cells/mm³; D⁺ HIV⁻, 674.9 ± 197.9 cells/mm³) and control patients (D⁻ HIV⁺, 187.6 ± 197.9 cells/mm³; D⁻ HIV⁻, 839 ± 71.9 cells/mm³) ($P < .000001$). CD4 lymphocyte counts were also significantly lower in case patients than in control patients, among both HIV⁺ ($P = .0007$) and HIV⁻ ($P < .0001$) patients. Decreased CD4 lymphocyte counts (<200 cells) were associated with an increased risk of diarrhea (95% CI, 1.18–19.01; $P = .03$). Other risk factors included poor housing (95% CI, 1.04–4.90; $P = .04$) and age (patients <40 years old were a high-risk group [95% CI, 0.76–2.55; $P = .28$]). There was no significant difference with regard to age or sex between case and control patients. Of the 318 patients, 289 (90.9%) were infected with HIV-1, 17 (5.3%) were infected with HIV-2, and 12 (3.8%) were coinfecting with both HIV-1 and HIV-2.

Concerning the prevalence of virulence genes identified by PCR, at least 1 target gene was found in 252 patients (42.4%), and target genes were found more often in case patients (160/279 [57.3%]) than in control patients (92/315 [29.2%]; $P = .000001$) (table 1). Multiple virulence genes were observed more often in D⁺ HIV⁺ patients than in the other patients (21/158 D⁺ HIV⁺ vs. 4/121 D⁺ HIV⁻ patients [$P = .01$] and 21/158 D⁺

Table 1. Virulence genes of *Escherichia coli* isolates from 594 patients.

Virulence genes	Case patients		Control patients	
	D ⁺ HIV ⁺ (n = 158)	D ⁺ HIV ⁻ (n = 121)	D ⁻ HIV ⁺ (n = 160)	D ⁻ HIV ⁻ (n = 155)
Single virulence genes ^a				
<i>bfpA</i>	9 (5.7)	2 (1.6)	8 (5.0)	...
<i>eaeA</i>	9 (5.7)	2 (1.6)	4 (2.5)	1 (0.6)
<i>LT/Sta</i>	2 (1.3)
<i>ipaH</i>	10 (6.3)	6 (4.9)	2 (1.2)	...
<i>Eagg</i>	31 (19.6)	3 (2.4)	3 (1.8)	4 (2.5)
<i>SLTI</i>
<i>SLTII</i>
<i>afa</i>	52 (32.9)	34 (28.0)	39 (24.3)	31 (20.0)
Multiple virulence genes				
<i>afa + bfpA</i>	3 (1.8)	...
<i>afa + Eagg</i>	11 (6.9)	1 (0.8)	1 (0.6)	1 (0.6)
<i>afa + ipaH</i>	5 (3.1)	2 (1.6)	2 (1.2)	...
<i>eaeA + bfpA</i>	4 (2.5)	1 (0.6)	2 (1.2)	...
<i>afa + eaeA + bfpA</i>	1 (0.6)

NOTE. Data are no. (%) of genes. *afa*, afimbrial adhesin; *bfpA*, bundle-forming pilus; D⁺, with diarrhea; D⁻, without diarrhea; *eaeA*, *E. coli* attaching and effacing; *Eagg*, enteroaggregative *E. coli*; HIV⁺, infected with human immunodeficiency virus; HIV⁻, not infected with human immunodeficiency virus; *ipaH*, invasion plasmid antigen H; *LT/Sta*, heat-labile enterotoxin/heat-stable enterotoxin; *SLTI*, Shiga-like toxin I; *SLTII*, Shiga-like toxin II.

^a Gene categories are not mutually exclusive.

HIV⁺ vs. 8/160 D⁻ HIV⁺ patients [$P = .02$]). Only 1 strain of *E. coli* carrying 3 virulence genes (*afa*, *eaeA*, and *bfpA*) was identified in an HIV⁺ patient with chronic diarrhea. The *Eagg* gene and the *ipaH* gene were present significantly more often in stool samples obtained from D⁺ patients than in stool samples obtained from D⁻ patients ($P = .000001$). In the present study, 12.1% (34/279) of D⁺ patients had evidence of EaggEC, and the majority of EaggEC strains were found in D⁺ HIV⁺ patients (31/158 [19.6%]). The *ipaH* gene was detected in 5.7% (16/279) of D⁺ patients. Isolation of EIEC was not dependent on HIV serostatus. In contrast, the *Eagg* gene was isolated more frequently in HIV⁺ patients (34/318 HIV⁺ vs. 7/276 HIV⁻ patients; table 1). Our findings showed a high prevalence of the *afa* gene in both D⁺ and D⁻ patients; the *afa* gene was present either alone (66/279 vs. 63/315) or in association with other virulence genes (86/279 vs. 70/315). In D⁺ patients, the prevalence of the *eaeA* and *bfpA* genes was 3.9% (11/279), and that of the *LT/Sta* gene was 0.7% (2/279). No EHEC isolates were found.

Results of the HEp-2 cell adherence assay show 3 morphologic patterns. DAEC was the most frequent pattern and was not isolated from case and control patients at different frequencies. The enteroaggregative and localized HEp-2 adherent patterns were displayed more often by *E. coli* strains isolated from D⁺ HIV⁺ patients.

Virulent strains were found to be resistant to at least 4 antibiotics, including trimethoprim-sulfamethoxazole, aminopenicillin, carboxypenicillin, and tetracycline. Of the EIEC strains, 67% were resistant to aminopenicillin, carboxypenicillin, and trimethoprim-sulfamethoxazole; 22% were resistant to amoxicillin-clavulanic acid; and 89% were resistant to tetracycline. Of the EaggEC strains, 85% were resistant to aminopenicillin, 56% were resistant to carboxypenicillin, 30% were resistant to amoxicillin-clavulanic acid, 78% were resistant to trimethoprim-sulfamethoxazole, and 96% were resistant to tetracycline. No strain was resistant to quinolone.

Discussion. In Africa, chronic diarrhea is common among HIV⁺ patients and is associated with high rates of morbidity and mortality. The lower CD4 lymphocyte counts in D⁺ HIV⁺ patients show that diarrhea itself is a debilitating illness and suggest that effective management of the diarrhea can prevent immunosuppression.

EaggEC is recognized as an emerging cause of diarrhea in HIV⁺ patients. It was often found in immunocompromised D⁺ patients [8] and can be considered as an opportunistic pathogen. The present study confirms the association between persistent diarrhea and EaggEC previously suspected in other countries [9]. EaggEC has been identified by “stacked brick” adherence to tissue culture cells. However, some of our isolates from D⁺ HIV⁺ patients displayed the “stacked brick” aggregative adherence pattern on HEp-2 cells but lacked the typical virulence factor of EaggEC strains. This phenomenon has been

observed by other investigators and highlights the necessity to look for other virulence factors [10, 11].

Isolation of EIEC was not dependent on HIV serostatus, and no asymptomatic carriage was found for this pathogen. Data are scarce with regard to an association between EIEC and diarrhea during HIV infection; a survey of fecal flora in 182 stool samples from 95 HIV⁺ patients has not detected any isolates of EIEC, but the research has shown that EIEC causes recurrent bacteremia [12].

The high frequency of the *afa* gene in our control patients suggests that pathogenic *E. coli* were those strains with at least 1 virulence factor other than the *afa* gene. This finding reveals that, in Senegal, there is no evidence for a role of enteroadherent *E. coli* in diarrhea in HIV⁺ patients, as demonstrated by another study conducted in The Netherlands [13]. Further investigations would be useful in determining whether the *afa* gene acts as a cofactor for other virulence genes. For many years, EPEC strains have been known to be important enteropathogens in children [14], and now they have emerged as being important enteropathogens in immunocompromised adults.

The high frequency of resistance to cotrimoxazole and tetracycline among EaggEC and EIEC strains could be explained by the selective pressure to which strains are exposed: in Senegal, tetracyclines and cotrimoxazole are used extensively for the treatment of diarrhea. These data exclude these 2 groups of antibiotics and make quinolones the drug of choice in treating diarrhea.

To our knowledge, this is the first study in which EaggEC strains were identified as etiologic agents of diarrhea in Senegal; it has revealed clearly the role of EaggEC in causing diarrhea in HIV⁺ adults. EaggEC strains seem to be heterogeneous; as a result, virulence factors should be evaluated and updated, with the aim of developing new diagnostic methods for this organism. Simultaneous resistance to >1 antibiotic, as shown by our data, is an alarming problem for the management of these diarrheas. A study of genetic antibiotic-resistance pattern would be very useful in determining the resistance mechanisms for these antibiotics of this widespread species (*E. coli*) and may ultimately help us establish new strategies to reduce morbidity and mortality associated with infectious diseases.

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