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Ordinary and Opportunistic Enteropathogens Associated with Diarrhea in Senegalese Adults in Relation to Human Immunodeficiency Virus Serostatus

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ABSTRACT

Objectives: A survey was conducted in Dakar, Senegal, to identify major types and prevalences of bacteria, parasites, fungi, and Rotaviruses associated with diarrhea in relation to human immunodeficiency virus (HIV) serostatus with the goal to provide guidance to physicians for case management.

Methods: Etiologic agents were identified in a case-control study: cases were HIV-infected patients with diarrhea (HIV+ D+) and HIV seronegative patients with diarrhea (HIV- D+); controls were HIV-infected patients without diarrhea (HIV+ D-) and seronegative controls without diarrhea (HIV- D-). Ordinary enteric pathogens were identified by conventional methods. Different Escherichia coli pathotypes were characterized by polymerase chain reaction (PCR), identification of HEp-2 cell adherence pattern, Sereny test, GM1-ELISA, and the suckling mouse assay. Opportunistic parasites, such as Cryptosporidium and Microsporidium, were identified by the Kinyoun method and trichromic stain of Weber, respectively. Rotaviruses were identified with a commercial latex agglutination kit. Antimicrobial susceptibility testing was carried out by the disk diffusion method.

Results: Among the 594 patients examined, 158 were HIV+ D+, 121 were HIV- D+, 160 were HIV+ D-, and 155 were HIV- D-. The main etiologies of diarrhea were different according to HIV serostatus of patients. In immunocompetent adults the main causes of diarrhea were Shigella sp (12.4%), Entamoeba histolytica (10.7%), Salmonella enterica (6.6%), and Giardia (4.9%). In the immunocompromised host the more frequent pathogens were entericaggregative E. coli (19.6%), Microsporidium (8.4%), Cryptosporidium sp (8.2%), Rotavirus (8.2%), Shigella sp (7.6%), Candida albicans (7.6%), E. histolytica (5.1%), S. enterica (4.4%), and Isospora belli (4.4%). Also, Blastocystis hominis has to be considered as an opportunistic parasite, because it was identified only in HIV-infected patients, with higher prevalence in adults with diarrhea (2.5% in HIV+ D+ patients; 0.6% in HIV+ D- patients). High level of asymptomatic carriage of Ascaris lumbricoides and Trichuris trichiura and some cases of multiple infections were observed. Fungi, Cryptosporidium sp and Microsporidium sp, were often identified in patients with low CD4 counts (range, 70-250 cells/mL). Independently from HIV-serostatus, CD4 count was lower in diarrheic persons, suggesting that diarrhea is a debilitating illness and that effective management of diarrhea can prevent immunosuppression. Isolated enteropathogenic strains displayed high resistance to most antibiotics used in Senegal for treating diarrhea (ampicillin, tetracycline, cotrimoxazole); they were susceptible to amikacin, gentamicin, and norfloxacin.

Conclusion: These epidemiologic data suggest that guidelines for the management of diarrhea during HIV infection in Dakar should be updated.

Key Words: Dakar, diarrhea, etiologies, HIV, Senegal

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According to new estimates from the joint United Nations Programme on human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS) (UNAIDS) and the World Health Organization (WHO), about 33 million adults are living with HIV and close to 70% of the total of HIV-positive people are from sub-Saharan Africa. Diarrheal illnesses remain a major clinical problem for patients infected with HIV, particularly those with the life-threatening AIDS. In Africa, diarrhea occurs in 60 to 90% of HIV infection3 and "slim disease," prolonged diarrhea, and wasting usually, attributable to coccidian
parasites, is pathognomonic of AIDS in central Africa.\textsuperscript{1,2} Seroprevalence of HIV in Senegal is about 1.6%, and nearly 80% of adults with AIDS present initially with diarrhea and weight loss. A wide variety of bacterial, viral, and parasitic pathogens can cause diarrhea in patients with advanced HIV infection. Although diarrhea occurs frequently, its optimal management remains controversial.\textsuperscript{3} Bacterial pathogens in AIDS patients may manifest differently from infections in immunocompetent hosts. Other pathogens, including Cryptosporidium and Microsporidium, are difficult to diagnose and have no effective therapy. Moreover, enteric viruses and HIV itself may contribute to the diarrhea.\textsuperscript{3} Only limited information is available regarding the etiology, clinical consequences, and immunologic effects of infection with diarrheal agents in the immunocompromised host. Consequently, the authors conducted a survey in Senegal among HIV-seropositive and HIV-seronegative subjects to identify major types and prevalences of bacteria, parasites, fungi, and Rotaviruses associated with diarrhea in adult patients, with the goal to provide guidance to physicians for case management.

**MATERIALS AND METHODS**

**Study Population**

Four groups of patients hospitalized in two Senegalese hospitals (Hôpital Principal and Fann Teaching Hospital) were examined: group 1 comprised HIV-infected patients with diarrhea (HIV+ D+); group 2, HIV seronegative patients with diarrhea (HIV− D+); group 3, HIV-infected patients without diarrhea (HIV+ D−); group 4, HIV seronegative controls without diarrhea (HIV− D−). Control stool specimens (group 3 and group 4) were obtained from HIV-seropositive and HIV-seronegative adults selected from individuals who had no history of diarrheal illness during the preceding month. Cases (group 1 and group 2) were HIV-seropositive and HIV-seronegative hospitalized adults with diarrhea who had not received antibiotics during the previous 2 weeks. All subjects underwent complete physical examination and patient environment and history were recorded on a standardized questionnaire filled out by a physician. All aspects of the study were approved by the ad hoc Ethical Committee, and prior informed consent was obtained from included subjects.

**HIV Serology and CD4 Counts**

Human immunodeficiency virus serology was tested by two enzyme immunoassays (Genelavia Mixt, Sanofi Diagnostics Pasteur, Marnes la Coquette, France, and Vironostika HIV Uni-Form II, Organon Teknika, Boxtel, The Netherlands), and confirmed by Western blotting (New Law Blot, Sanofi Diagnostics Pasteur). CD4 lymphocyte counts were undertaken using a laser-based FACSCount system (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) for directly determining absolute CD4 count.

**Microbiologic Analysis**

All cases and controls had stool specimens collected and processed in the same way. No transport media were used; specimens were quickly examined in the laboratory after collection. Freshly collected stool samples were examined immediately after collection, by dark-field microscopy, for motile bacteria, trophozoites, red blood cells, and leukocytes. Stool specimens were treated with saline and iodine preparations and examined for intestinal parasites; furthermore, formalin-ether concentrates and smears stained with Merthiolate-iodine-formaldehyde solution were prepared from each of the specimens and examined microscopically. Coccidia and Microsporidia were detected respectively by Kinyoun stain and modified trichrome stain with a high concentration of chromotrope 2 R.\textsuperscript{3} The modified Kinyoun acid-fast stain was used for detection of mycobacteria. Rotaviruses were identified with a commercial latex agglutination test (Sanofi Diagnostics Pasteur). Bacteria such as Escherichia coli, Salmonella spp, Shigella spp, Yersinia spp, Aeromonas spp, Plesiomonas spp, Vibrio spp, Campylobacter spp, and fungi were identified by standard methods.\textsuperscript{6} Identification of Clostridium spp was undertaken only if gram-positive rods were seen after Gram staining. Genotypic differentiation of pathogenic E. coli strains was performed by polymerase chain reaction (PCR), according to Tornieporth et al.\textsuperscript{7} Oligonucleotide primers to detect enterotoxigenic E. coli (ETEC),\textsuperscript{8−10} enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC), and diffuse adherent E. coli (DAFC) by PCR tests are shown in Table 1. Phenotypic characterization of pathogenic E. coli was performed by a microtiter ganglioside enzyme linked immunosorbent assay (GM1-ELISA) for the thermostable enterotoxin,\textsuperscript{11} the suckling mouse assay for the thermostable enterotoxin,\textsuperscript{12} and the guinea pig keratoconjunctivitis assay for enteroinvasive E. coli.\textsuperscript{13} All E. coli isolates were examined for HEP-2 cell adherence in eight-well chamber slides (LAB-TEK, Nunc Inc., Naperville, IL, USA), according to Vial et al.\textsuperscript{14} HEP-2 cell adherence was characterized as localized (LA), aggregate (AA), or diffuse (DA) adherence. HEP-2 adherence pattern and virulence gene were performed on five colonies of E. coli. Escherichia coli was considered to be the etiologic agent of the diarrheal illness when a significant virulence gene was identified on at least three of five tested colonies. Serotypes of Salmonella spp and Shigella spp were determined using slide agglutination. The antimicrobial susceptibility of bacterial enteropathogens was determined using the disk diffusion test.\textsuperscript{15}
Table 1. Oligonucleotide Primers Used to Differentiate Pathogenic Enteric Escherichia coli

| Target Gene (Enteropathogen) | Primer Sequences | Location (base pairs) From 5' end (base pairs) | Amplicon Size (base pairs) |
|------------------------------|------------------|-----------------------------------------------|--------------------------|
| bfp gene (EPEC)              | 5'CAATGGTGCGCCTGCTGCT3' | 119-138                          | 325                     |
|                              | 5'GGGCTTCATTGTGCGCCATTGCTGCT3' | 2412-2436                  | 494                     |
| eaeA (EPEC)                  | 5'GCAATTTAGCCGCTGGCCTGCTGCT3' | 59-76                           | 707                     |
| LT gene (ETEC)               | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| Sta gene (ETEC)              | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| ipaH (EIEC)                  | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| EAggEC                       | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| LT 1                         | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| LT 2                         | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |
| afa (DAEC)                   | 5'GCCGGCTAATCGTCTGAGGACGGGAT3' | 708-746                         | 182                     |

bfp = bundle forming pilus; EPEC = enteropathogenic E. coli; eaeA = E. coli attaching and effacing gene A; LT = heat-labile enterotoxin; ETEC = enterotoxigenic E. coli; STa = heat-stable enterotoxin; ipaH = invasion plasmid H; EIEC = enteroinvasive E. coli; EAggEC = enterogaugergative E. coli; SLT 1 = Shiga-like toxin 1; SLT 2 = Shiga-like toxin 2; DAEC = diffuse adherent E. coli.

Statistical Analysis

Statistical analyses were performed using Epi Info, version 6.01 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SAS, version 6.12 (SAS Inc., Cary, NC, USA). For continuous variables normally distributed, means were compared using Student's t-test or the analysis of variance. Categoric variables were compared using the chi-squared test and Fisher's exact test where appropriate.

Potential risk factors of diarrhea among HIV-infected individuals were analyzed by univariate methods to determine possible inclusion in the multivariate model. All variables associated with diarrhea with a P-value of less than 0.3 were included in the multivariate analysis. Logistic regression analysis was performed to calculate odds ratio (OR) and 95% confidence interval (95% CI).

RESULTS

Among the 594 stool and blood samples analyzed, 279 were from cases and 315 were from controls. The characteristics of the studied population are described in Table 2. The more frequent serotype, HIV-1, was identified in 90.9% (289/318) of the population; HIV-2 was identified in 5.3% (17/318), and 3.8% (12/318) of the study population were co-infected by both serotypes HIV-1 and HIV-2. As expected, CD4 geometric mean cell counts were lower in HIV-infected than in HIV-seronegative patients in cases as well as in controls (P < 0.000001). CD4 count was also significantly lower in diarrheic patients in HIV+ (P = 0.0007) as well as in HIV- (P < 0.0001) patients. There were no significant differences of age or gender among the four groups.

Identified bacterial pathogens are listed in Table 3. No Yersinia spp, Vibrio spp, Plesiomonas spp, EHEC, Clostridium perfringens, or Mycobacterium spp were identified during this study. Shigella spp and Salmonella spp were the most frequently identified bacterial pathogens during acute diarrhea; 28 Shigella were identified: Shigella flexneri (n = 14), Shigella dysenteriae (n = 12), and Shigella sonnei (n = 2); no Shigella boydii were identified. All Shigella strains were from cases except one S. sonnei isolated from a HIV-seronegative patient without diarrhea. No asymptomatic carriage of Shigella was observed in HIV-infected patients. Eighteen strains of Salmonella enterica (15 in cases; 3 in controls) were identified. The various serotypes were S. enteritidis (5 among cases; 1 among controls), S. kentucky (2 among cases), S. typhi (1 among cases), S. sangera (1 among cases), S. magnera (1 among cases), S. tambacounda (2 among cases; 1 among controls), S. glostrup (1 among cases), and S. typhimurium (1 among cases).
Enteropathogens Associated with Diarrhea and HIV in Senegal / Gassama et al

Table 3. Prevalence of Bacterial Pathogens

| Pathogen                      | D+ HIV+ n = 158 (%) | D+ HIV- n = 121 (%) | D- HIV+ n = 160 (%) | D- HIV- n = 155 (%) |
|-------------------------------|---------------------|---------------------|---------------------|---------------------|
| Enteroaggregative E. coli*    | 31 (19.6)           | 8 (6.6)             | 5 (3.1)             | 4 (2.6)             |
| Enteroinvasive E. coli        | 5 (3.2)             | 4 (3.2)             | 0                   | 0                   |
| Shigella                      | 12 (7.6)            | 15 (12.4)           | 0                   | 1 (0.6)             |
| Salmonella                    | 7 (4.4)             | 8 (6.6)             | 1 (0.6)             | 2 (1.3)             |
| Campylobacter                 | 1 (0.6)             | 2 (1.6)             | 0                   | 0                   |
| Aeromonas                     | 1 (0.6)             | 2 (1.6)             | 0                   | 0                   |
| Klebsiella                    | 7 (4.4)             | 0                   | 0                   | 0                   |
| Citrobacter                   | 3 (1.9)             |                    |                     |                     |

*E. coli displaying enteroaggregative pattern on HEp-2 cells and/or with the EAgg PCR gene; 'E. coli positive in the guinea pig keratoconjunctivitis assay or with ipaH gene. D = diarrhea; HIV = human immunodeficiency virus; + = positive; - = negative.

(2 among cases and 1 among controls). Two Campylobacter jejuni infections were identified in HIV-D+ patients, and one Campylobacter fetus was identified in a seropositive patient with persistent diarrhea. Three strains of Aeromonas spp were identified in cases: two strains of A. hydrophila in HIV-seronegative patients and one A. sobria (now referred to as A. veronii by sobria) in an HIV seropositive patient. HEp-2 cell adherence patterns of E. coli strains showed three morphologic patterns: diffusely adherent E. coli was the most frequent pattern and was not differently isolated from cases and controls. The enteroaggregative HEp-2 adherence pattern was more often displayed by E. coli strains isolated from HIV-positive patients with diarrhea (Table 4). Concerning the prevalence of different E. coli pathotypes identified by PCR (see Table 4), at least one target gene was found in 205 (34.5%), and a target gene was found more often in cases (125/279, 44.8%) than in controls (80/315, 25.3%). P = 0.00001. Results show a high prevalence of afa gene in cases (66/219) as well as in controls (63/315); consequently pathogenic E. coli were E. coli strains with at least one virulence factor different from afa gene. Inversely, enteroaggregative and enteroinvasive E. coli seem to be closely related respectively to chronic and acute diarrhea. Isolation of enteroinvasive E. coli (ipaH gene) was not dependent on HIV-serostatus, and no asymptomatic carriage was found for this pathotype. In contrast, enteroaggregative E. coli (EAgg gene) was isolated more frequently in HIV-seropositive patients (either alone or in association with other virulence genes: 24/318 in HIV-positive patients vs. 6/276 in HIV-negative patients). More than one virulence gene was more often observed in HIV-seropositive diarrheic patients (HIV+ D+ vs. HIV- D+: 21/158 vs. 3/121, P = 0.01; HIV+ D+ vs. HIV+ D-: 21/158 vs. 6/160, P = 0.2). Only one strain of E. coli carrying three virulence genes (afa, eae, EAgg) was identified in an HIV-seropositive patient with chronic diarrhea. Except for the ipaH gene, other virulence genes were identified in patients with CD4 count ranging from 11 to 259 cells/mm³. There was good correlation between genotypic and phenotypic characterization of enterotoxigenic and enteroinvasive

Table 4. HEp-2 Cell Adherence Pattern and Virulence Genes of Isolated E. coli

| HEp-2 cell adherence pattern | D+ HIV+ n = 158 (%) | D+ HIV- n = 121 (%) | D- HIV+ n = 160 (%) | D- HIV- n = 155 (%) |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|
| Adherent                    | 96 (60.7)           | 45 (37.1)           | 54 (33.7)           | 43 (27.3)           |
| Diffused adherence           | 49 (31.0)           | 31 (25.6)           | 40 (25.3)           | 33 (21.3)           |
| Localized                   | 16 (10.1)           | 6 (5.0)             | 11 (6.8)            | 5 (3.2)             |
| Aggregate                   | 31 (19.8)           | 9 (6.1)             | 3 (1.9)             | 5 (3.2)             |
| Prevalence of virulence genes |                      |                     |                     |                     |
| bfp gene                    | 4 (2.5)             | 1 (0.8)             | 3 (1.9)             | -                   |
| eae gene                    | 4 (2.5)             | 1 (0.8)             | 2 (1.2)             | 1 (0.6)             |
| LT/ST gene                  | 2 (1.3)             | -                   | -                   | -                   |
| ipaH gene                   | 5 (3.2)             | 4 (3.2)             | -                   | -                   |
| EAggEC                      | 22 (12.6)           | 2 (1.6)             | 2 (1.2)             | 3 (1.9)             |
| SLT 1                       | -                   | -                   | -                   | -                   |
| SLT 2                       | -                   | -                   | -                   | -                   |
| afa gene                    | 35 (22.1)           | 31 (25.1)           | 33 (20.6)           | 30 (19.3)           |
| Association of virulence genes |                  |                     |                     |                     |
| afa + bfp gene              | -                   | 3 (1.9)             | -                   | -                   |
| afa + EAggEC                | 11 (6.9)            | 1 (0.8)             | 1 (0.8)             | 1                   |
| afa + ipaH                  | 5 (3.1)             | 2 (1.6)             | 2 (1.2)             | (0.6)               |
| eae + bfp gene              | 4 (2.5)             | 1 (0.8)             | 2 (1.2)             | -                   |
| afa + eae + bfp gene        | 1 (0.6)             | -                   | -                   | -                   |

D = diarrhea; HIV = human immunodeficiency virus; + = positive; - = negative; bfp = bundle-forming pilus; LT = heat-labile; ST = heat-stable; EAggEC = enteroaggregative E. coli; SLT = Shiga-like toxin.
**Table 5. Antibiotic Susceptibility Pattern of Isolated Bacterial Enteropathogens**

| Characteristics | Number Tested | AM | T/C | CF | FOX | CIX | AMC | CAZ | IPM | GM | TM | AN | UB | CS | NOR | SXT | TE |
|-----------------|---------------|----|-----|----|-----|-----|-----|-----|-----|----|----|----|----|----|-----|-----|-----|
| Salmonella spp   | 18            | 19 | 13  | 13 | 0   | 0   | 0   | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 0   | 0   |
| Shigella spp     | 28            | 100| 69  | 7  | 0   | 0   | 0   | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 0   | 0   |
| Campylobacter spp| 3             | 67 | 0   | 67 | 0   | 0   | 0   | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 0   | 0   |
| Aeromonas spp    | 3             | 100| 67  | 100| 67  | 0   | 0   | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 0   | 0   |
| Klebsiella spp   | 7             | 100| 100 | 29 | 29  | 0   | 14  | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 43  | 0   | 57  |
| Citrobacter spp  | 4             | 50 | 25  | 75 | 0   | 0   | 0   | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 25  | 50  |
| Pathogenic enteric E. coli (EIEC) | 9 | 67 | 67  | 22 | 0   | 0   | 22  | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 0   | 0   |
| Pathogenic enteric E. coli (EAggEC) | 48 | 85 | 56  | 67 | 4   | 0   | 29  | 0   | 0   | 0  | 0  | 0  | 0  | 0  | 0   | 79  | 96  |

AM = ampicillin; T/C = ticarcillin; CF = cefalotin; FOX = cefoxitin; CIX = cefotaxime; AMC = amoxicillin clavulanate; CAZ = ceftazidime; IPM = imipenem; GM = gentamicin; TM = tobramycin; AN = amikacin; UB = flumequin; CS = colistin; NOR = norfloxacin; SXT = trimethoprim-sulfamethoxazole; EIEC = enteroinvasive E. coli; EAggEC = enteroaggregative E. coli.

**E. coli.** Two strains were positive in the GM1-ELISA test for heat-labile enterotoxin and in the suckling mouse model assay for heat-stable enterotoxin; all strains carrying *pha* gene were positive for the guinea pig keratoconjunctivitis Sereny test. Unusual bacterial etiologies like Klebsiella pneumoniae (n = 7), Citrobacter freundii (n = 4) were found in immunocompromised patients with diarrhea. One of the Citrobacter spp strains was isolated from a 68-year-old HIV-seronegative woman with diabetes. The other strains were from HIV-seropositive patients with very low CD4 counts. In all these cases oral antibiotic therapy proved to be effective. These opportunistic bacterial pathogens were examined for HEP-2 cell adherence, and all showed a diffuse adherence pattern except three strains of K. pneumoniae, which displayed the “stacked-brick” aggregative adherence pattern typical of enteroaggregative E. coli strains and one strain of C. freundii (different from the one isolated from the 68-year-old woman) displaying the localized adherence attaching and effacing pattern typical of enteropathogenic E. coli. Polymerase chain reaction for E. coli virulence genes was negative for almost all strains except one K. pneumoniae with *EAgg* gene and one C. freundii gene with *eae* gene. Table 5 illustrates the antibiotic susceptibility of the 117 strains of bacterial enteropathogens recovered from cases and controls. All isolates were susceptible to ampicillin, gentamicin, broad-spectrum cephalosporin, and ciprofloxacin. Shigella spp and pathogenic E. coli were highly resistant to ampicillin, ticarcillin, cotrimoxazole, and tetracycline. Most enteropathogenic strains remained susceptible to ampicillin and clavulanic acid, third generation cephalosporins and fluoroquinolones. Interestingly, Salmonella strains remain susceptible to almost all antibiotics except tetracyclines.

Prevalence of different parasites, *C. albicans*, and Rotaviruses are shown in Table 6. A high level of asymptomatic carriage of *A. lumbricoides* and *Trichuris trichiura* was observed. In the immunocompetent patients, the two main parasitic agents involved in diarrhea were *Entamoeba histolytica* and *Giardia lamblia*. The main opportunistic parasites are *Microsporidium* (9.4%), *Cryptosporidium* (8.2%), and *Isospora belli*.
Table 7. Potential Risk Factors of Diarrhea among HIV-Infected Adults in Senegal

| Factor                  | OR     | 95% Confidence Interval | P-Value |
|-------------------------|--------|-------------------------|---------|
| Age                     |        |                         |         |
| ≥ 40 y                  | Reference |                         |         |
| < 40 y                  | 1.39   | 0.76-2.55               | 0.26    |
| CD4 cells               |        |                         |         |
| ≥ 500                   | Reference |                         |         |
| 200 ≤ CD4 < 500         | 2.60   | 0.58-11.76              | 0.21    |
| CD4 < 200               | 4.74   | 1.18-19.01              | 0.03    |
| Living conditions       |        |                         |         |
| Good (tiled floor)      | Reference |                         |         |
| Poor (mud floor)        | 2.26   | 1.04-4.90              | 0.04    |

Eight different serotypes were identified from the 18 isolated strains of \textit{S. entérica}; a similar study conducted in Bangui (Central African Republic) showed only two different serotypes from 59 strains.\textsuperscript{19} The limited diversity of serotypes in Bangui can be explained by the geographic situation of the country and the quasi absence of imported foods and crops. In contrast, spirochetes were found in Bangui and not in Dakar, where 80% of the population is Muslim and has no contact with pigs. Identification of \textit{A. sobria (A. veronii bv sobria)} as an etiologic agent of diarrhea in an HIV-infected patient emphasizes the implication of this bacteria in diarrhea of the immunocompromised host. A case of acute diarrhea produced by \textit{A. sobria (A. veronii bv sobria)} in a patient colectomized for Crohn’s disease was reported by de Sola Earle et al.\textsuperscript{19} Overall, the literature suggests that \textit{Aeromonas sp}, once mainly considered an opportunistic pathogen in immunocompromised humans, is now implicated as an etiologic agent in numerous clinical situations involving immunocompetent individuals of all age groups. The alarming rates of resistance to the most affordable antibiotics found in Dakar (tetracycline, cotrimoxazole) provide evidence of the need for more rational use of antibiotics. In fact high resistance to tetracycline emerged in Senegal after an intensive use of this drug during the cholera epidemic in 1995.\textsuperscript{20}

In the present study, about 30% of cases of diarrhea were of unknown etiology. An extensive evaluation including stool studies and endoscopic biopsies of both colon and small intestine has been widely recommended to identify all potential pathogenic organisms.\textsuperscript{5} Stool viruses, such as adenovirus and coronavirus, were described as the possible causes of diarrhea in HIV-infected patients in Berlin.\textsuperscript{21} Unusual pathogens such as spirochetes have been described as a possible cause of diarrhea in HIV-infected persons in Central African Republic.\textsuperscript{19} Moreover, enteric viruses and HIV itself may contribute to the diarrhea. In addition to microbes, other factors, such as medication, immune dysregulation, automatic dysfunction, and nutritional supplementation, play a substantial role in diarrhea in patients with AIDS.\textsuperscript{4}

The present findings suggest that coccidia should be considered to be etiologic agents for immunocompromised adults; the same results have been observed in other developing African countries.\textsuperscript{22} Conflicting evidence exists as to whether \textit{B. hominis} should also be included among the infectious agents capable of causing HIV-related diarrhea. Albrecht et al found that association of \textit{B. hominis} with clinical symptoms was not evident, and it is frequently associated with the concurrent isolation of other enteric pathogens.\textsuperscript{23} In the present study,

(4.4%). \textit{Blastocystis hominis} was isolated only in sero-positive patients, with a higher prevalence in patients with diarrhea. Fungi were considered to be potential enteric pathogens when no other known enteropathogenic agent was identified and when they represented up to 50% of microorganisms observed per field under microscope. Twelve cases of massive infestation by \textit{Candida albicans} were observed in HIV-infected patients with diarrhea. In almost all these cases patients were also undergoing oral candidiasis. \textit{Candida albicans} was once isolated as the sole pathogen in an HIV-negative patient; in all cases fungi were isolated in patients with very low CD4 counts (range, 42-78 cells per mm\textsuperscript{3}). Rotaviruses were identified in 13 HIV-infected patients and in two immunocompetent patients. Co-infection by two or more enteropathogenic agents was observed 11 times: \textit{Salmonella spp + Microsporidium (n = 1), Cryptosporidium + A. lumbricoides (n = 1), Salmonella + G. lambia + T. trichiura (n = 1), Microsporidium + E. coli + A. lumbricoides (n = 2), I. belli + T. trichiura + A. lumbricoides (n = 1), Cryptosporidium + C. albicans (n = 2), S. bifida + A. lumbricoides (n = 2).}

The risk of developing diarrhea increased progressively as CD4 counts fell below 200 cells/mm\textsuperscript{3} (Table 7). The other independent risk factor was poor housing. Also patients younger than 40 years of age tended to be at higher risk.

DISCUSSION

Diarrhea is a prominent feature of AIDS in adults, and it causes significant morbidity in HIV-infected persons. This study confirms the association between persistent diarrhea and enteroaggregative \textit{E. coli} previously suspected in other countries.\textsuperscript{16} However, some of the isolates from group 1 patients displayed the "stacked brick" aggregative adherence pattern on HEP-2 cells but lacked the typical virulence factor of enteroaggregative \textit{E. coli} strains (EAggEC gene). This phenomenon has been observed by other investigators,\textsuperscript{17} and it suggests existence of other virulence factors. The current results suggest that \textit{afa} gene is not a significant virulence gene in adults in Senegal, but it is often associated with other \textit{E. coli} virulence genes. Further investigations would be useful to determine whether \textit{afa} gene is acting as a cofactor for other virulence genes.

Eight different serotypes were identified from the 18 isolated strains of \textit{S. enterica}; a similar study conducted in Bangui (Central African Republic) showed only two different serotypes from 59 strains.\textsuperscript{19} The limited diversity of serotypes in Bangui can be explained by the geographic situation of the country and the quasi absence of imported foods and crops. In contrast, spirochetes were found in Bangui and not in Dakar, where 80% of the population is Muslim and has no contact with pigs. Identification of \textit{A. sobria (A. veronii bv sobria)} as an etiologic agent of diarrhea in an HIV-infected patient emphasizes the implication of this bacteria in diarrhea of the immunocompromised host. A case of acute diarrhea produced by \textit{A. sobria (A. veronii bv sobria)} in a patient colectomized for Crohn’s disease was reported by de Sola Earle et al.\textsuperscript{19} Overall, the literature suggests that \textit{Aeromonas sp}, once mainly considered an opportunistic pathogen in immunocompromised humans, is now implicated as an etiologic agent in numerous clinical situations involving immunocompetent individuals of all age groups. The alarming rates of resistance to the most affordable antibiotics found in Dakar (tetracycline, cotrimoxazole) provide evidence of the need for more rational use of antibiotics. In fact high resistance to tetracycline emerged in Senegal after an intensive use of this drug during the cholera epidemic in 1995.\textsuperscript{20}

In the present study, about 30% of cases of diarrhea were of unknown etiology. An extensive evaluation including stool studies and endoscopic biopsies of both colon and small intestine has been widely recommended to identify all potential pathogenic organisms.\textsuperscript{5} Stool viruses, such as adenovirus and coronavirus, were described as the possible causes of diarrhea in HIV-infected patients in Berlin.\textsuperscript{21} Unusual pathogens such as spirochetes have been described as a possible cause of diarrhea in HIV-infected persons in Central African Republic.\textsuperscript{19} Moreover, enteric viruses and HIV itself may contribute to the diarrhea. In addition to microbes, other factors, such as medication, immune dysregulation, automatic dysfunction, and nutritional supplementation, play a substantial role in diarrhea in patients with AIDS.\textsuperscript{4}

The present findings suggest that coccidia should be considered to be etiologic agents for immunocompromised adults; the same results have been observed in other developing African countries.\textsuperscript{22} Conflicting evidence exists as to whether \textit{B. hominis} should also be included among the infectious agents capable of causing HIV-related diarrhea. Albrecht et al found that association of \textit{B. hominis} with clinical symptoms was not evident, and it is frequently associated with the concurrent isolation of other enteric pathogens.\textsuperscript{23} In the present study,
B. hominis has to be considered as an opportunistic parasite, because it was identified only in HIV-infected patients, with higher prevalence in adults with diarrhea, and in these cases it was not associated with other pathogens.

As found by other investigators, results of this study suggest that declining CD4 T-lymphocyte counts are associated with increased risk of diarrhea. Furthermore, the finding of lower CD4 counts among patients with diarrhea regardless to their HIV serostatus shows that diarrhea is a debilitating illness and suggests that effective management of diarrhea can prevent immunosuppression.

CONCLUSIONS

The principles of HIV and AIDS therapy include reduction of HIV replication by antiretroviral agents, prophylaxis against the common opportunistic infections, and treatment, followed by subsequent lifelong maintenance therapy for infections when they do occur. Many developing countries cannot afford antiretroviral therapy; therefore, effective management of opportunistic infections, including diarrheal diseases, remains a realistic alternative to improve the quality of life of patients living with HIV and to delay the evolution to AIDS. Guidelines for managing bacterial diarrhea in developing countries should be updated, because in this study cotrimoxazole, one of the recommended antimicrobial agents for the treatment of diarrhea, has been shown to have little activity against Shigella spp, Salmonella spp, and enteroadherent-aggregative E. coli.

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REFERENCES

1. Bartlett JG, Belistos PC, Sears CL. AIDS enteropathy. Clin Infect Dis 1992;15:726-735.
2. O'Keefe EA, Wood R. AIDS in Africa. Stand J Gastroenterol Suppl 1996; 220:147-152.
3. Johanson JF Diagnosis and management of AIDS-related diarrhea. Can J Gastroenterol 1996; 10:461-468.
4. Framm SR, Soave R. Agents of diarrhea. Med Clin North Am 1997; 81:427-447.
5. Weber R, Bryan JR, Owen RL, Wilcox CM, Gorelikin L, Vesvesvara GS. Improved light microscopical detection of Microsporidia spores in duodenal aspirates. N Engl J Med 1992; 326:161-166.
6. Murray PR, Barron EJ, Pfaffer MA, Tenover FC, Volken RH. Manual of clinical microbiology. 6th ed. Washington, DC: American Society for Microbiology, 1995.
7. Torpieporth NG, John J, Salgado K, et al. Differentiation of pathogenic E. coli strains in Brazilian children by PCR. J Clin Microbiol 1995; 33:1371-1374.
8. Frankel G, Giron JA, Valmossio J, Schoolnick GK. Multi-genie amplification: simultaneous detection of three virulence genes in diarrheal stool. Mol Microbiol 1989; 3:1729-1734.
9. Riley TW. Infection diseases associated with Escherichia coli. In: Balows A, Hausler WJ, Ohashi M, Turano A, eds. Laboratory diagnosis of infectious diseases. Principles and practice. 1st ed. Vol 1. New York: Springer-Verlag, 1988:237-261.
10. Yamamoto T, Tamura T, Yokota T. Primary structure of heat-labile enterotoxin produced by Escherichia coli pathogenic for humans. J Biol Chem 1984; 259:5307-5344.
11. Giannella RA. Suckling mouse model for detection of heat-stable Escherichia coli enterotoxin: characteristics of the model. Infect Immun 1976; 14:95-99.
12. Dean AG, Ching YC, William RG, Harden LB. Test for Escherichia coli enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J Infect Dis 1972; 125:407-411.
13. Sereny B. Experimental keratoconjunctivitis shigellosis. Acta Microbiol Acad Sci Hung 1957; 4:367-376.
14. Vial PA, Robins-Browne R, Lior H, et al. Characterization of enteroadherent-aggregative Escherichia coli; a putative agent of diarrheal disease. J Infect Dis 1988; 158:70-79.
15. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Ann J Clin Pathol 1966; 45:493-496.
16. Mayer HR, Wanke CA. Enteroadherent-aggregative Escherichia coli as a possible cause of diarrhea in an HIV-infected patient. N Engl J Med 1995; 332:273-274.
17. Poletsky Y, Nataro JP, Kotter DJ, Barret TJ. Urenstein 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; 35:1952-1958.
18. Germani Y, Minsasart P, Vohito M, et al. Etiologies of acute, persistent, and dysenteric diarrheas in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. Am J Trop Med Hyg 1998; 59:1008-1014.
19. de Sola Earle C, Montiel Quezel-Guerraz N, Hidalgo Rojas L, Sanchez Cantos A, Garcia Alegria J. Severe acute gastroenteritis due to Aeromonas in a patient coinfected for Chohn's disease. Rev Esp Enferm Dig 1997; 89:48-50.
20. Aydemir A, Kobalav S, Boye CS, et al. Phenotypic and genotypic characterization of Vibribo cholerae isolates from a recent cholera outbreak in Senegal: comparison with isolates from Guinea Bissau. Am J Trop Med Hyg 1998; 58:163-167.
21. Schmidt W, Schneider T, Heise W, et al. Stool viruses, co-infections, and diarrhea in HIV-infected patients. Berlin Diarhea/Wasting Syndrome Study Group. J Acquir Immune Defic Syndr Hum Retrovirol 1996; 15:33-58.
22. Gomez Morales MA, Atzori C, Ludović A, Rossi P, Scaglia M, Pozio E. Opportunistic and non-opportunistic parasites in HIV-positive and negative patients with diarrhoea in Tanzania. Trop Med Parasitol 1995; 46:109-114.
23. Albrecht H, Steilbrink H, Kopfesin K, Greten H. Bistaoctysis hominis in human immunodeficiency virus-related diarrhea. Scand J Gastroenterol 1995; 30:909-914.
24. Navin TR, Weber R, Vugia DJ, et al. Declining CD4+ T-lymphocyte counts are associated with increased risk of enteric parasitosis and chronic diarrhea: results of a 3-year longitudinal study. J Acquir Immune Defic Syndr Hum Retrovirol 1999; 20:154-159.
25. Lloyd A. HIV infection and AIDS. P N G Med J 1996; 39:174-180.