Diarrhea in patients infected with the human immunodeficiency virus (HIV) continues to be a common problem in Peru and other developing countries. Antiretroviral therapy (HAART) has markedly improved the long-term survival of HIV-infected patients; however, the lifetime incidence of diarrhea among HIV-infected children with diarrhea has been estimated to be 30% to 70%. However, stool samples are not routinely evaluated for enteric pathogens in clinical laboratories outside the developed world. However, stool samples are not routinely evaluated for these pathogens in clinical laboratories outside the developed world. The diarrheagenic Escherichia coli (E. coli) are categorized into six groups, enterohemorragic (EHEC or STEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enteropathogenic (E. coli) enteroaggregative (EAggEC), and diffusely adherent (DAEC). Within these groups, EAEC has been the most frequently isolated pathogen in Peru with a prevalence of 10%, and control samples (2%) infected with diarrheagenic E. coli. The diarrheagenic E. coli were studied in hospitalized children in Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Infectology, Instituto Nacional de Salud del Niño, Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Pediatrics, Hospital National Hipolito Unanue, Lima, Peru; Center for Infectious Diseases, University of Texas School of Public Health, Houston, Texas; and Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru.

**INTRODUCTION**

Diarrhea in patients infected with the human immunodeficiency virus (HIV) continues to be a common problem in Peru and other developing countries. Antiretroviral therapy (HAART) has markedly improved the long-term survival of HIV-infected patients; however, the lifetime incidence of diarrhea among HIV-infected children with diarrhea has been estimated to be 30% to 70%. However, stool samples are not routinely evaluated for enteric pathogens in clinical laboratories outside the developed world. The diarrheagenic Escherichia coli (E. coli) are categorized into six groups, enterohemorragic (EHEC or STEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enteropathogenic (E. coli) enteroaggregative (EAggEC), and diffusely adherent (DAEC). Within these groups, EAEC has been the most frequently isolated pathogen in Peru with a prevalence of 10%, and control samples (2%) infected with diarrheagenic E. coli. The diarrheagenic E. coli were studied in hospitalized children in Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Infectology, Instituto Nacional de Salud del Niño, Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Pediatrics, Hospital National Hipolito Unanue, Lima, Peru; Center for Infectious Diseases, University of Texas School of Public Health, Houston, Texas; and Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru.

**Study design.** The aims of this study were 1) to determine the prevalence of diarrheagenic E. coli in Peruvian children infected with HIV; 2) to determine the association of diarrheagenic E. coli with viral load and immunosuppression; and 3) to determine the antimicrobial resistance patterns of diarrheagenic E. colitisolated from these patients.

**PATIENTS AND METHODS.** The study was prospective, cross-sectional, descriptive, and longitudinal study of HIV-infected children in Lima to determine the prevalence of diarrheagenic E. coli and diffusely adherent E. coli (DAEC). Within these groups, EAEC has been the most frequently isolated pathogen in Peru with a prevalence of 10%, and control samples (2%) infected with diarrheagenic E. coli. The diarrheagenic E. coli were studied in hospitalized children in Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Infectology, Instituto Nacional de Salud del Niño, Lima, Peru; Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Pediatrics, Hospital National Hipolito Unanue, Lima, Peru; Center for Infectious Diseases, University of Texas School of Public Health, Houston, Texas; and Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru.
at the microbiology laboratory of each hospital. Direct exam was used for eggs of parasites, and modified Ziehl-Nielsen stain for *Cryptosporidium*, after stool concentration. Stool samples from patients without diarrhea were cultured only on MacConkey agar plates for isolation of *E. coli* colonies. Detection of diarrheagenic *E. coli* was done for both diarrhea and control samples at the Tropical Medicine Institute “Alexander von Humboldt” in Lima. Five lactose-positive *E. coli* colonies per patient were isolated from MacConkey agar plates and analyzed by a multiplex real-time polymerase chain reaction (PCR) method using previously validated specific primers for each pathotype: EAEC (AggR), ETEC (*E. coli EaeA*), STEC (*stx1, stx2*), EIEC (*IpaH*), and DAEC (*dadD*). The PCR was performed using a FTC-200 thermal cycler and real-time fluorescence monitoring by a Chromo 4 optical detector (MJ Research/Biorad, Hercules, CA), using a five-colony pool analysis as previously described. Diarrheagenic *E. coli* colonies were analyzed for their antimicrobial susceptibility by disk diffusion according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Antibiotic susceptibilities were analyzed for ampicillin (AMP, 10 μg disk), amoxicillin-clavulanic acid (AMC, 30 μg disk), cefotaxime (CTX, 30 μg disk), ceftazidine (CAZ, 30 μg disk), gentamicin (GTM, 10 μg disk), nalidixic acid (NAL, 30 μg disk), ciprofloxacin (CIP, 5 μg disk), azithromycin (AZD, 15 μg disk), tetracycline (TET, 30 μg disk), chloramphenicol (CAF, 30 μg disk), cotrimoxazole (SXT, 23.75/1.25 μg disk), and nitrofurantoin (NIT, 300 μg disk). Multi-resistance was defined as resistance to three or more unrelated antimicrobial agents.

**Ethical aspects.** This study was reviewed and approved by the Institutional Review Board (IRB) of Universidad Peruana Cayetano Heredia and by the IRB of each participating hospital. Informed consent was obtained from the parents and assent from patients over the age of 7 years.

**Statistical analysis.** The results were analyzed using the statistical software EpInfo 3.4.3 (CDC, Atlanta, GA). The χ² (Yates) or Fisher test were used to compare pathogens or clinical data between diarrhea and control samples. Student *t* test or non-parametric tests were used in the case of continuous variables. Differences were considered statistically significant if the *P* value was less than 0.05.

**RESULTS**

One hundred thirteen HIV patients were enrolled in the study (83 from Instituto Nacional de Salud del Niño, 27 from Hospital Nacional Cayetano Heredia, and 3 from Hospital Nacional Hipolito Unanue), representing 69% of the 164 HIV-infected patients regularly followed in the three participating hospitals. The median age of the children was 6 years ranging from 2 months to 17 year of age; 50% of children were female. Overall, the median viral load (RNA copies [log10]/mL) was 4.5 (1.7–6.8); 70% had a viral load >400 RNA copies/mL; 33% had severe age-related immunosuppression; and 85% were on HAART. The most commonly used treatment schemes were zidovudine, lamivudine, and nelfinavir (66%), and lamivudine, nelfinavir, and stavudine (23%).

Ninety-four patients provided one single stool sample (diarrhea or control) and 19 patients provided more than one sample at different times during the 2-year period. Overall, 70 diarrhea samples and 70 control samples without diarrhea were analyzed. The age of children with diarrhea was 5 ± 4 years while the controls averaged 7 ± 4 years. The majority of patients with diarrhea were within the age group of 1 to 5 years, whereas the majority of patients without diarrhea were found to be within the age group of 6 to 18 years (Figure 1). Children with diarrhea had significantly worse age-related immunosuppression (based on CD4 cells by age), higher viral loads, and were less frequently on HAART (Table 1).

Diarrheagenic *E. coli* were isolated in 19% of diarrheal samples (13/70) and in 26% of control samples (18/70) (*P* = 0.416). The EAEC and EPEC were the most frequently isolated pathogens from both diarrhea and control samples (Table 2). There were no significant age-related differences in the frequency of each diarrheagenic *E. coli* group among children younger than 5 years of age and older. There were no *Shigella, Salmonella, Campylobacter, Vibrio*, or parasites isolated in the diarrheal samples. Children without diarrhea, in addition to the diarrheagenic *E. coli*, were colonized with

![Figure 1. Age distribution of human immunodeficiency virus (HIV)-infected children with (N = 70) and without diarrhea (N = 70). *P < 0.01 for the age group comparison between children with and without diarrhea.](image-url)
several parasites: Blastocystis hominis (13%), Entamoeba coli (11%), Endolimax nana (10%), Chilomastix mesnili (3%), Cryptosporidium sp. (3%), and Giardia lamblia (1%). Among the diarrheal episodes 19% were persistent, 3% dysenteric and 33% were associated with moderate or severe dehydration (Table 3). The clinical characteristics of the diarrheal episodes with an isolated diarrheagenic E. coli were similar to the characteristics of episodes without these pathogens (Table 3). The average number of positive colonies per samples was two.

Twenty-three E. coli strains were available for antibiotic susceptibility testing (74%). Resistance to ampicillin and cotrimoxazol were common (Figure 2). Of interest, there were no strains with high level resistance to nitrofurantoin (furanzolidone) and ciprofloxacin; however, 4 strains had intermediate resistance to ciprofloxacin (17%). Similarly, there were only 2 strains (9%) with high level resistance to amoxicillin-clavulanic, but 8 strains (35%) with intermediate resistance to this antibiotic. There were no differences in the resistance levels between diarrhea and control samples, except for nalidixic acid, which was significantly more resistant when isolated from diarrhea rather than control samples (63% versus 13%, \(P < 0.05\)). Multidrug resistance was present in 52% of strains. There are no break points approved for azithromycin resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; the distribution of the growth inhibitory zones by disc diffusion for this antibiotic was 4% (1 strain) less than resistance; 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for 30% to 50% of diarrheal deaths in children from diarrhea, but also with persistent diarrhea, which accounts overall for 50% of all diarrheal deaths in infants from Zaire (41%).

Persistent diarrhea is multi-factorial; it can result from multiple causes. In this study, the recruitment of patients lasted 2 years. There were few children hospitalized with diarrhea (N = 49), because the majority were on HAART. This represents a significant improvement in the quality of care of HIV-infected children in comparison to the previous decade in Lima. Because the recruitment of children with diarrhea was spread over a 2-year period, we were not able to find matched-age and season controls without diarrhea. The age difference between the diarrhea and control groups represents one of the limitations of the study. However, the age distribution in the control samples reflects the age distribution of all HIV-infected children in our setting; the fact that diarrhea was more common in children less than 5 years of age. One-third of E. coli isolated were resistant to nalidixic acid and none were highly resistant to ciprofloxacin (17% showed intermediate resistance to ciprofloxacin). However, resistance to nalidixic acid in a community is a marker of future resistance to quinolones; this is worrisome because these drugs are among the few available oral agents to treat bacterial gastroenteritis.

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In summary, HIV children with diarrhea had worse immunosuppression and viral load, and less frequently were on HAART, than HIV children without diarrhea. Diarrheagenic E. coli were the most commonly isolated pathogens in HIV-infected children and were highly resistant to ampicillin and cotrimoxazole. Because diarrhea is sometimes treatable with antibiotics, molecular studies for diarrheagenic E. coli could be part of the workup of children with HIV and prolonged diarrhea, and for surveillance studies of antimicrobial resistance.

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