Bacterial pathogens associated with bloody diarrhea in Uruguayan children

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ABSTRACT

Diarrheal disease continues to be a serious health problem, especially in developing countries. Bloody diarrhea represents approximately 20-30% of all cases and has higher morbidity and mortality. Treatment with antibiotics is beneficial in cases of Shigella, Campylobacter, Yersinia and Salmonella infection, principally in those children with a higher risk of invasive disease. The aims of this study were to detect the bacterial agents associated with bloody diarrhea in children and to determine their antimicrobial susceptibility patterns. Between June 2001 and January 2008, 249 children with bloody diarrhea were studied. Shigella and Shiga toxin-producing Escherichia coli (STEC) were recovered from 48 (19.3%) and 3 (1.2%) of the total of cases, respectively. In 49 out of 249 children, in whom other enteropathogens were investigated, we recovered Campylobacter jejuni from 7 children (14.3%), Salmonella spp. from 2 (4.1%) and Aeromonas spp. from 1 (2%) in addition to Shigella from 7 children (14.3%). Thirty-four (70%) Shigella isolates showed resistance to ampicillin and 13 (27%) to trimethoprim-sulfamethoxazole. All Shigella isolates were susceptible to nalidixic acid, ciprofloxacin and ceftriaxone. Salmonella and STEC isolates were susceptible to all antibiotics assayed. Thus, the use of trimethoprim-sulfamethoxazole or ampicillin would not be appropriate for the empirical treatment of Shigella – associated diarrhea.

Key words: bloody diarrhea, Shigella, antibiotics

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Diarrheal disease continues to be a health problem worldwide, especially in developing countries. In these regions, it accounts for approximately 2.5 million deaths per year in children under 5 years of age. Furthermore, acute diarrhea considerably contributes to morbidity and increases health care costs in children from industrialized countries (6). Bloody diarrhea (BD) represents approximately 20-30% of all cases, causing important inflammatory intestinal illness and, under some circumstances, producing severe complications, like sepsis, hemorrhagic colitis and hemolytic uremic syndrome (HUS) (1, 6).

The bacterial pathogens associated with BD include species of Shigella, Campylobacter, Salmonella, Escherichia coli pathotypes, especially Shiga toxin-producing Escherichia coli (STEC) and enteroinvasive E. coli (EIEC), as well as Yersinia enterocolitica. The prevalence
of these agents and their antimicrobial susceptibility patterns vary among different regions (4, 12, 13).

Treatment with antibiotics is indicated in cases of Shigella infections and in special situations of Campylobacter, Yersinia and Salmonella infections, principally in those children at higher risk of invasive disease (5, 15).

However, the lack of rapid diagnostic methods for the detection of all these agents supports the implementation of empirical treatment regimens based on the epidemiological knowledge of the prevalent agents and the corresponding antimicrobial susceptibility.

The aims of this study were to detect the bacterial pathogens associated with bloody diarrhea in children and to determine their antimicrobial susceptibility patterns.

Within the framework of an institutional program aimed at the regional surveillance of STEC infections, a prospective, multicenter study was done involving health care centers in Montevideo and other cities of Uruguay. The analysis period extended from June 2001 to January 2008, 249 children with bloody diarrhea who required medical attention in these centers were studied. Stool samples were consecutively obtained and only one sample per child was processed. Each stool specimen was sent to the Department of Bacteriology and Virology in a Cary-Blair transport medium, whereas another part of the sample without transport medium was sent in a sterile plastic vial. Both were submitted in insulated, refrigerated boxes and were examined less than 12 hours after extraction. All samples were examined for the presence of fecal leukocytes, STEC and Shigella spp. Fecal leukocytes were semiquantified by microscopic examination of smears stained with methylene blue as follows: < 1 cell (+), 1-10 cells (++) and > 10 cells (+++) by high-power field. STEC detection was performed by culture and PCR using specific primers to stx1 and stx2 genes as previously described (14). Shigella species were investigated by classical procedures (3). Furthermore, between January 2005 and June 2006, at the request of the pediatrician in charge, we also studied forty-nine (20%) of these 249 children for the presence of Salmonella, Yersinia, EIEC and Campylobacter species, as described by Torres et al. (12).

STEC, Salmonella and Shigella isolates were serotyped by standard procedures using commercial antisera (Difco®) and others available in our institute’s collection (3). Antimicrobial disk susceptibility tests for Shigella, Salmonella and STEC isolates were done according to the Clinical and Laboratory Standards Institute guidelines (2). The following agents were tested: ampicillin (AMP), trimethoprim-sulfamethoxyazole (TMP-SMX), chloramphenicol (CMP), tetracycline (TET), ciprofloxacin (CIP), nalidixic acid (NAL), gentamicin (GEM), ceftriaxone (CRO), cefazidime (CAZ), and cefoxitin (FOX). The Fisher’s test was used to assess the association between qualitative variables.

The median age was 12 months (range, 5 days to 14 years of age), and 90% of the children were under five years old. More than 90% of the children were assisted at public health centers. No epidemiological link could be established among the 249 children studied. In the whole period of study, Shigella spp. was recovered from 48 (19.3%) children and STEC from 3 (1.2%) of the 249 cases. One child showed co-infection with two different STEC serogroups (O26 and O145). In those children (49) whose stool culture also included the search for Salmonella, Yersinia, EIEC and Campylobacter species, Shigella spp. was recovered from 7 (14.3%) children, Campylobacter jejuni from 7 (14.3%), Salmonella from 2 (4.1%), and Aeromonas spp. from one (2%) child. Fecal leukocytes were present in all Shigella-positive samples but only in 2 of the 10 children from whom another enteropathogen was isolated (p = 0.0018). Fecal leukocytes were present in 43 (89.6%) of the 48 fecal samples from which Shigella spp. was recovered; 32 of them showed ≥ 10 cells per field (+++). Ninety-four per cent of the cases of bloody diarrhea associated with Shigella were observed in the warmest months (between November and April). Thirty-seven isolates corresponded to Shigella flexneri and 11 to Shigella sonnei. Neither Salmonella dysenteriae nor Shigella boydii were recovered during this study. Twenty-one of the 37 S. flexneri strains corresponded to serotype 2a, 13 to serotype 3c and 3 to serotype 1.

Thirty out of 37 (81%) S. flexneri isolates showed resistance to AMP (17 isolates belonged to serotype 2a; 11 to serotype 3c and 2 to serotype 1) and 13 (35%) to TMP-SMX (7 isolates belonged to serotype 2a; five corresponded to serotype 3c and one to serotype 1). However, when we analyzed the periods 2001-2003 and 2004-2008, the results were 95% and 62% resistance to AMP; 48% and 18% resistance to TMP-SMX, respectively (p < 0.05). S. flexneri isolates showed eight antimicrobial resistance phenotypes and the TET, AMP, CMP resistance pattern was the most frequent (13 strains), followed by the TET, AMP, CMP, TMP-SMX resistance pattern (Figure 1). Two S. flexneri strains were susceptible to all antimicrobials tested. On the other hand, S. sonnei showed a unique AMP-resistance profile AMP®. Four out of 11 S. sonnei isolates had this profile and the other 7 isolates were susceptible to all antimicrobial agents tested. All Shigella isolates were susceptible to NAL, CIP, GEM, CRO, CAZ, FOX. STEC isolates belonged to serogroups O26 (2 strains), O111 (1 strain) and O145 (1 strain). All STEC strains carrying eae and ehxA genes. Isolates O26 carried stx genes, while strain O145 was positive for stx1 and strain O111 carried both stx1 and stx2 genes. The Salmonella strains corresponded to Salmonella enterica subspp. enterica serovar Enteritidis. The Salmonella and STEC cultures were susceptible to all antimicrobials tested. Neither EIEC nor Y. enterocolitica were recovered during this study.

As reported in other regions, Shigella spp. was the most frequently recovered pathogen from BD cases (11, 13). Nevertheless, when more complete stool cultures were done, C. jejuni was an important agent of BD. Therefore, in the future it would be important to perform antimi-
microbial susceptibility tests to know their resistance pattern. The absence of S. dysenteriae was not surprising. In Uruguay, S. dysenteriae strains are rarely found and they belong to serotype 2 (12). According to our results, the BD cases that occurred during the warmest months and showed fecal polymorphonuclear leukocytes were likely caused by Shigella spp. ($p = 0.017$). Serotype 2a was the most frequent among S. flexneri strains. These findings are comparable with results from Chile (9). It would be important to define antigenic determinants that should be included in a future vaccine.

As it occurs in other regions (13), we have not recovered Y. enterocolitica or EIEC in a subgroup of these 249 children. However, these results are limited due to the small sample size and probably on account of the methodology used for the detection of these agents which included only phenotypic tests. Although STEC infection represented a small proportion of all acute bloody diarrhea cases studied (1.2%), the serious associated complications, such as HUS, seem to justify the importance of searching for this agent. In this sense, 2 out of 3 children infected with STEC developed HUS during the follow-up. Salmonella isolates were susceptible to all antibiotics tested. Results obtained with Salmonella typhimurium by Macedo et al. (7) showed a marked decrease in resistance in the last years. In our country, since December 2003, the recommended drugs for empirical treatment of BD cases in children have been: oral azithromycin (5 mg/kg/day, for 5 days) for outpatients (over 6 months of age and without toxicity signs) and parenteral ceftriaxone (100 mg/kg, i/m, one dose) for inpatients (under 6 months of age or with toxicity signs). When we analyzed Shigella spp. strains isolated between 2001 and 2003 versus those isolated in 2004 and 2008, we observed that AMP resistance decreased from 95% to 62%, and TMP-SMX resistance from 48% to 18%. This trend may be explained by appropriate application of this therapeutic guideline; however, studies with larger number of samples are required to determine whether this difference is significant and sustained. In our country, like in other regions, the use of TMS or AMP continues to be inappropriate for the empirical treatment of BD cases. All Shigella strains recovered in this study were susceptible to NAL. This antibiotic has been the drug of choice for the last two decades in certain regions, such as Southern Africa and South Asia (15); nevertheless, physicians should anticipate increasing resistance to this drug as its use increases. In addition, Shigella strains resistant to NAL showed some degree of cross-resistance to CIP. Thus, the widespread use of NAL for treatment of shigellosis may reduce the efficacy of CIP (10). Fluoroquinolones are also recommended drugs for treating Campylobacter, Salmonella and Shigella-associated gastroenteritis (5, 15). According to the results of this study, ciprofloxacin could be used for the treatment of children with BD. However, we believe that this drug should be reserved for the treatment of severe cases for which there is no other treatment option available, taking into account the potential risk of joint damage.

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