Epidemiological and Microbiological Aspects of Acute Bacterial Diarrhea in Children from Salvador, Bahia, Brazil

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In the few cases of acute childhood diarrhea that require antimicrobial therapy, the correct choice of the drug depends on detailed previous knowledge of local strains. In order to establish such parameters in our city, we reviewed the results of all 260 positive stool cultures of children between 0 and 15 years of age during two years at a pediatric tertiary care facility in Salvador, Brazil. Bacterial strains had been presumptively identified by culturing in selective media and by biochemical testing, and their antimicrobial susceptibility patterns were automatically detected by the MicroScan Walkaway System. Data about patients' sex and age, monthly distribution of the cases, pathogens isolated and their antimicrobial resistance patterns were recorded. Males corresponded to 55.4% of our sample, and most of our patients (42.7%) were between one and four years of age. *Shigella* was the commonest pathogen, being found in 141 (54.3%) cultures, while *Salmonella* was found in 100 (38.4%) cultures and Enteropathogenic *E. coli* in 19 (7.3%). *Salmonella* was the main causal agent of diarrhea in children younger than five years old, whereas *Shigella* was the most frequent pathogen isolated from the stools of children between five and 15 years old. The peaks of incidence correspond to the periods of school vacations. *Shigella* specimens presented a very high resistance rate to trimethoprim-sulfamethoxazole (90.1%) and to ampicillin (22.0%), while *Salmonella* presented very low resistance rates to all drugs tested. These data are useful for practitioners and they reinforce the need for continuous microbiological surveillance.

Key Words: Diarrhea, antimicrobial, resistance, children, epidemiology.

Severe presentations of acute diarrhea constitute one of the commonest challenges faced by the medical team in pediatric ambulatories and emergency rooms in the developing world. Supportive anti-dehydration treatment is the cornerstone of therapy and must be promptly started, but specific antimicrobial treatment may be required, depending on the severity of the disease and on the risk of complications. As stool cultures take several days to provide adequate information about pathogens and their susceptibility patterns, empirical treatment must be immediately adopted in such cases. To guide the empirical choice of antibiotics, it is crucial to know both which pathogens are most likely to be infecting the patient in a particular geographic area and the most effective antibiotics for treating them.

All over the world, severe acute bacterial gastroenteritis is caused mainly by *Shigella*, whereas *Salmonella*, *E. coli* (chiefly enteropathogenic *E. coli*, or EPEC, but also enterohemorrhagic *E. coli* or EHEC, enteroinvasive *E. coli* or EIEC and other types), *Campylobacter* and *Vibrio* spp. have also been shown to play a role in the epidemiology of diarrhea, especially in certain areas of the globe [1-5].

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Some years ago, the World Health Organization (WHO) suggested that bloody diarrhea in children should be treated with trimethoprim-sulfamethoxazole (TMP-SMX) or nalidixic acid. However, antimicrobial susceptibility patterns are ever-changing and are highly variable from one geographic zone to another. Cases of bloody acute bacterial gastroenteritis have been successfully treated with ampicillin or TMP-SMX for a long time, but reports of growing resistance have limited the empirical use of those drugs, even though they may have a role in therapy guided by the results of stool cultures. Nalidixic acid initially replaced ampicillin and TMP-SMX satisfactorily, but as resistance emerged and spread quickly [6] it has no longer been considered a mainstay of antimicrobial therapy of bacterial diarrhea. Currently, third generation cephalosporins and the newer quinolones, chiefly ceftriaxone and ciprofloxacin, respectively, are considered the most effective drugs for the selected cases of acute infectious diarrhea in which antimicrobial treatment is indicated. In spite of the profusion of reports from different parts of the world concerning trends in antimicrobial resistance, few data addressing such patterns in Brazil are available, especially for the pediatric population.

This study was carried out to identify and to establish the antimicrobial susceptibility pattern of the most important pathogens involved in the epidemiology of acute diarrhea in children who sought medical attendance at a pediatric tertiary care facility in Salvador, Brazil. General demographic data about the patients and the disease were also recorded.

Material and Methods

Patients

We reviewed the results of all stool cultures that presented bacterial growth that were taken from children between 0 and 15 years of age from January 2002 to December 2003 at a pediatric tertiary care facility in Salvador, Brazil. Data about patients' sex and age, monthly distribution of the cases, pathogens isolated and their antimicrobial resistance patterns were recorded. During that period, 1,991 patients had their stools cultured and 260 samples presented bacterial growth.

Bacteriology

All bacteriological examinations were performed at the Hospital Aliança microbiology laboratory. Briefly, fresh feces were collected from diarrhoeal patients and sent to the laboratory for immediate culture. The specimens were inoculated into selenite broth and on MacConkey, Salmonella-Shigella (SS) and Karmali agars.

MacConkey and SS agar plates were incubated at 35° C for 18 to 24 hours, and selenite broth at 35° C for 12-18 hours. After incubation, selenite broth was inoculated in SS agar at 35° C for 18 to 24 hours. Suspected colonies were inoculated in EPM-MILI-Citrate medium at 35° C for 18 to 24 hours for biochemical testing and presumptive identification. After biochemical testing, colonies were tested for serum agglutination with polyvalent sera (Probac) against *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* and *Shigella dysenteriae*, classic enteropathogenic *E. coli* A, B and C, entereoinvasive *E. coli* A and B, and flagellar and somatic *Salmonella* antigens.

Karmali agar plates were incubated under microaerophilia at 42° C. After 48 hours, any colonies found were assessed for *Campylobacter* spp. by Gram staining.

Shigella, *Salmonella* and *E. coli* colonies identified on serum agglutination had their identification confirmed and their antimicrobial susceptibility patterns established by microdilution in a WalkAway-96 machine (Dade Behring, West Sacramento, California, USA). Standardization and quality control tests were performed every week using *E. coli* ATCC25922, as currently recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [7]. We used the Neg BP Combo 12 panel (B1017-131, Dade Behring, West Sacramento, California, USA). The antibiotics tested for susceptibility and their respective concentrations were as follows: ampicillin (8 µg/mL and

16 μg/mL), ampicillin + sulbactam (8/4 μg/mL and 16/8 μg/mL), aztreonan (8 μg/mL and 16 μg/mL), ceftazidime (8 μg/mL and 16 μg/mL), ceftriaxone (8 μg/mL and 32 μg/mL), ciprofloxacin (1 μg/mL and 2 μg/mL), imipenem (4 μg/mL and 8 μg/mL), piperacillin (16 μg/mL and 64 μg/mL), and trimethoprim + sulfamethoxazole (2/38 μg/mL). Strains were evaluated for extended spectrum beta-lactamase (ESBL)-production by the MicroScan System and those described as possible ESBL-producers were further tested by the double disk synergy test in Mueller-Hinton agar, as recommended by NCCLS [7].

Results

During the two-year period, the stools of 1,991 diarrhoeal patients aged 0 to 15 years were cultured and 260 (13.1%) samples presented bacterial growth and were included in the study. Of these patients, 144 (55.4%) were male, while the other 116 (44.6%) were females. Only nine (3.5%) of them were younger than one year, while 111 (42.7%) were between one and four years of age, 81 (31.1%) were between five and nine years and 59 (22.7%) between 10 and 15 years (Figure 1).

Shigella spp. was the most frequent pathogen, being found in 141 (54.3%) cultures (113, or 80.1%, were *S. sonnei* and 28, or 19.9%, were *S. flexneri*), while *Salmonella* spp. was found in 100 (38.4%) cultures and *E. coli* was found in 19 (7.3%). No typhoidal *Salmonella* spp. specimens were isolated. The age distribution data revealed that *Salmonella* was the main causal agent of diarrhea, both in children younger than one year and in those between one and four years-old, whereas *Shigella* was the most frequent pathogen isolated from the stools of children between five and nine years-old and between 10 and 15 years old (Table 1). *E. coli* was an uncommon cause of diarrhea in our sample, occurring mostly in children between one and four years old.

We observed a peak of incidence in the summer: 20 cases (7.7%) in January, 33 (12.7%) in February and 24 (9.2%) in March and another in the winter, in June (36 cases, or 13.8%) and July (29 cases, or 11.1%). *Shigella* spp. was the pathogen with the highest impact on the epidemiology of acute bacterial diarrhea in these children, since it was responsible for most cases during most months of the year, especially during the peak months of incidence (Table 2).

Considering all 260 pathogens isolated, 135 (51.9%) were found to be resistant to TMP-SMX and 40 (15.4%) to ampicillin, while 22 (8.5%) were resistant to ampicillin + sulbactam and 18 (6.9%) to piperacillin. Low resistance rates were found to ciprofloxacin among *Shigella* (0.7%) and *Salmonella* (2.0%) isolates. None of the 260 isolates was found to be resistant to ceftriaxone or to aztreonan, and no ESBL-producing strain was isolated.

In a separate assessment of antimicrobial resistance patterns, *Shigella* specimens presented a very high resistance rate to TMP-SMX (90.1%). Among *S. sonnei* isolates, TMP-SMX resistance rates were as high as 94.7%, while it reached 71.4% among *S. flexneri* isolates. These *Shigella* isolates also presented significant resistance rates to ampicillin, either alone (22.0%) or in combination with sulbactam (9.9%). This was especially true for *S. flexneri* isolates, which presented resistance rates as high as 60.8% (ampicillin alone) and 14.3% (ampicillin + sulbactam) (Table 3). Otherwise, *Salmonella* isolates presented very low resistance rates, varying from 1 to 5%, to all the drugs, including TMP-SMX (2.0%). *E. coli* isolates presented high resistance rates to ampicillin (both alone and associated with sulbactam), piperacillin and TMP-SMX.

Discussion

Given the importance of a careful characterization of the local epidemiology of diarrhea to guide specific antimicrobial therapy, we reviewed the results of 260 stool cultures from children aged 0 to 15 years old in which enteropathogens were detected. We detected a discretely higher propensity of males to suffer from bacterial gastroenteritis and a low incidence during the first year of life, a peak of incidence between one and

Figure 1. Distribution of patients with diarrhea according to age range

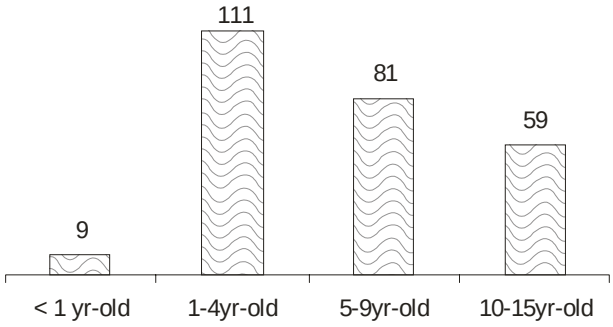

Table 1. Distribution of the pathogens in diarrheal stools according to patients' age

	Shigella spp.	*Salmonella* spp.	*E. coli*	Total
< 1 yr-old	0	7 (77.8%)	2 (22.2%)	9 (100%)
1-4 yr-old	33 (29.7%)	61 (54.6%)	17 (15.7%)	111 (100%)
5-9 yr-old	61 (75.3%)	20 (24.7%)	0	81 (100%)
10-15 yr-old	47 (79.7%)	12 (20.3%)	0	59 (100%)
Total	141	100	19	260

Table 2. Monthly distribution of acute bacterial gastroenteritis according to the pathogenic agent

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Shigella spp.	6	20	13	6	8	24	22	11	9	11	7	4
Salmonella spp.	11	13	9	9	7	9	6	3	10	3	14	7
E. coli	3	0	2	2	0	3	1	2	1	0	3	1
Total	20	33	24	17	15	36	29	16	20	14	24	12
%	7.7	12.7	9.2	6.5	5.8	13.8	11.1	6.2	7.7	5.4	9.2	4.6

four years of age (the period when children's contact with environmental pathogens increases dramatically) and decreasing incidence levels in older children (Figure 1).

More than half of the cases were caused by *Shigella* spp., reinforcing the importance of this pathogen in the epidemiology of bacterial childhood diarrhea in developing countries. Most of the *Shigella*-associated cases (80.1%) were due to *S. sonnei*, indicating that either *S. sonnei* is more common in our environment or it is associated with more severe presentations, as patients with more severe disease are more likely to seek medical help. Indeed, most patients with shigellosis require immediate hospitalization [4]. *Salmonella* was also commonly found in our patients, but no typhoidal specimens were isolated. In our sample, *E. coli* was the least frequent causative agent of acute diarrhea; however, it is more important to the epidemiology of diarrhea in poorer areas [10,11]. No cases of *Campylobacter*-associated diarrhea were

Table 3. Antimicrobial resistance of the bacterial specimens isolated from diarrheal stools

	***Shigella spp.* (141)**	***Salmonella sp.* (100)**	***E. coli* (19)**	**Total (260)**
Ampicillin + sulbactam	14 (9.9%)	5 (5.0%)	3 (15.8%)	22 (8.5%)
Ampicillin	31 (22.0%)	4 (4.0%)	5 (26.3%)	40 (15.4%)
Aztreonan	-	-	-	-
Ceftazidime	-	1 (1.0%)	-	1 (0.4%)
Ceftriaxone	-	-	-	-
Ciprofloxacin	1 (0.7%)	2 (2.0%)	-	3 (1.2%)
Imipenen	1 (0.7%)	1 (1.0%)	1 (5.3%)	3 (1.2%)
Piperacillin	12 (8.5%)	2 (2.0%)	4 (21.0%)	18 (6.9%)
Trimethoprim + sulfamethoxazole	127 (90.1%)	2 (2.0%)	6 (31.6%)	135 (51.9%)

detected. A low incidence of *Campylobacter* had already been reported in Brazil [12], while it is much more common in other countries [13-15].

Our data point to *Salmonella* as the most important causative agent of diarrhea in children younger than five years. Children younger than one year, however, are not expected to present *Shigella*-associated diarrhea, in contrast to a many children aged one to five years-old. These observations have important clinical implications, since *Salmonella* spp. were characteristically multi-susceptible, in contrast to *Shigella* spp. and *E. coli* (Table 3).

The highest levels of incidence were during the summer and the winter, when school vacations allow children to increase their exposure to environmental pathogens and to contaminated food. Consequently, we attribute the unexpected low incidence found in December to the habit of traveling out of town during this month.

In spite of the relatively small size of the sample, we believe that our data provide useful information about antimicrobial resistance. In order to help practitioners to choose an adequate antimicrobial drug to start empirical therapy in a patient with severe diarrhea without knowledge of a specific pathogen, we assessed the antimicrobial resistance patterns of all the pathogens that were isolated. We detected high resistance rates to TMP-SMX, ampicillin, ampicillin associated with sulbactam and piperacillin in *Shigella spp*. strains. Thus, we do not recommend any of these drugs for the empirical treatment of acute bloody diarrhea in children. In very severe cases, with evidence of dissemination of disease, intravenous ceftriaxone is the best choice for many, while other drugs can be adequate choices for the empirical treatment of severe acute diarrhea in children (Table 2). Obviously, as soon as the results of stool cultures are available, the therapy can be altered to a safer and/or cheaper drug based on the antimicrobial susceptibility pattern.

Separate assessment of antimicrobial resistance patterns indicates that *Shigella* is more difficult to treat and requires careful consideration at the choice of antimicrobial therapy. However, *Shigella* resistance has not reached the alarming multi-resistance rates reported in other countries, where resistance to ciprofloxacin and to ceftriaxone is a reality [16-18]. While we detected high resistance rates to TMP-SMX, ampicillin, ampicillin associated with sulbactam and piperacillin, no resistance to ceftriaxone was detected and only one out of 141 (0.7%) *Shigella* isolates was resistant to ciprofloxacin. *Shigella sonnei* isolates presented the highest resistance rates to TMP-SMX: 94.7%,

compared to 71.4% for *S. flexneri* and 51.9% for all bacteria isolated. *Shigella flexneri* presented a high rate of resistance to ampicillin compared to *S. sonnei* (60.8% and 12.4%, respectively). We found that the *Salmonella* strains were multi-susceptible, as high rates of resistance were not found against any of the drugs. Interestingly, 2.0% of those strains were found to be resistant to ciprofloxacin. While the possibility of a false result should be considered because of the automated testing method, resistance to ciprofloxacin among non-typhoidal *Salmonella* strains isolated from stool specimens has already been reported from several parts of the world [19,20]. On the other hand, a multicenter Latin American study published by Gales et al. did not isolate any strain of *Salmonella* resistant to fluoroquinolones from blood samples [21]. However, *Salmonella* spp. strains isolated from blood samples are recognized to be more susceptible than those from stools [22]. Furthermore, the flouroquinolone tested by Gales et al. was gatifloxacin, a new 8-methoxi-fluroquinolone that is supposed to be active against most ciprofloxacin-resistant bacteria. *E. coli* isolates were found to have a high rate of resistance to piperacillin, ampicillin, ampicillin + sulbactam and TMP-SMX. However, the interpretation of these data is clouded by the relatively few *E. coli*-associated cases (19, or 7.3% of the total). All in all, our susceptibility patterns are slightly more favorable than those reported by the large multicenter SENTRY Antimicrobial Surveillance Program Report for Brazil and Latin America [23].

Even though it is important to be careful with antimicrobial susceptibility patterns provided by automated methods, clinical practice has continuously endorsed their use. Furthermore, comparative studies have provided a very low testing discrepancy rate between the Walkaway system and the reference conventional agar dilution method for the identification and the antimicrobial susceptibility patterns of *Shigella*, *Salmonella* and *E. coli* [24-26]. However, no trial addressed any of those bacteria specifically nor have they involved a large number of isolates.

We did not assess the role of nalidixic acid in the treatment of acute diarrhea. In spite of the worldwide spread of resistant strains, the use of nalidixic acid is still recommended by the World Health Organization guidelines for the management of acute bloody diarrhea in children [27], and it may remain an important option for the treatment of acute infectious diarrhea, especially in services that cannot afford far more expensive drugs.

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