ETIOLOGY OF DIARRHEA IN CHILDREN YOUNGER THAN 5 YEARS OF AGE ADMITTED IN A RURAL HOSPITAL OF SOUTHERN MOZAMBIQUE

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Abstract. Diarrhea is one of the main causes of morbidity and mortality among children in sub-Saharan Africa and one of the main causes of hospital admissions in rural areas. Stool samples were collected from 529 children admitted with diarrhea to the Manhiça District Hospital (September 2000 to September 2001) and processed to detect bacterial enteropathogens, parasites, and virus. Diarrheagenic Escherichia coli, isolated from 120 samples (22.6%; enteropathogenic [9.6%], enterotoxigenic [6.8%], enteroaggregative [4.3%], and verotoxigenic [1.9%]) was the most frequently isolated pathogen, followed by Ascaris lumbricoides (9.3%). Others detected included Salmonella spp. and Giardia lamblia (2.5% each) and Campylobacter spp. (1.7%). A. lumbricoides (92% versus 8%; P < 0.001) and Strongyloides stercoralis (100% versus 0%; P = 0.008) were most frequently isolated in children older than 12 months of age. Resistance to trimethoprim-sulphamethoxazole and ampicillin was high. Etiologic data on diarrheal diseases and susceptibility patterns of diarrheal pathogens are important tools for clinical management and control strategic planning.

INTRODUCTION

Diarrhea is one of the main causes of morbidity and mortality in children younger than 5 years of age, in developing countries, where the average number of episodes of diarrhea per child per year within this age group is 3.2-12 Twenty-one percent of childhood mortality in children younger than 5 years old in these countries is associated with diarrhea, resulting in 2.5 million deaths per year.2 In sub-Saharan Africa, mortality caused by acute diarrhea varies from 1.9% of all deaths in The Gambia to 37% in Nigeria, with most of the deaths occurring during the first year of life.3 Even though morbidity caused by diarrhea is still high, mortality has been decreasing worldwide, mainly because of improved management.4-6

In Mozambique, diarrhea has been recently reported to be the third cause of death (10%) among children from 0 to 14 years old in Maputo City.7 In the Manhiça district, diarrhea is the fourth cause of death among children between 12 and 59 months of age (J. Sacarlal, personal communication, 2004).

There are many different pathogens, including bacteria, viruses, and parasites, that can cause diarrhea in both developed and developing countries. The most common isolated diarrheagenic pathogens include Escherichia coli, Rotavirus, Salmonella spp., Shigella spp., Campylobacter jejuni, Entamoeba histolytica, and Giardia lamblia.8-11

Appropriate antimicrobial therapy can shorten both the bacterial excretion and clinical periods. However, the incidence of multidrug-resistant isolates is increasing.12,13 Thus, adequate fluid and electrolyte replacement and maintenance remains the central key to managing diarrheal illness.14,15 In diarrheal diseases, antibiotics are recommended for severe cases or chronic diarrhea.

In the Manhiça District, ampicillin—either alone or in combination with other antibiotics—continues to be the first line of treatment of infectious diarrhea in children younger than 2 years of age, whereas chloramphenicol is given to older children. Trimethoprim-sulphamethoxazole is used when oral administration is tolerated, and nalidixic acid is specifically used in cases of Shigellosis. Data on the etiology of diarrhea are important information to use while planning and implementing control strategies to reduce diarrhea-caused childhood morbidity and mortality in a country. Despite this fact, in most developing countries and especially in rural areas, no data are available on diarrhea-causing pathogens. Mozambique is not an exception, with no data currently available on the etiology of infectious diarrhea in its rural areas. The aim of this study was to describe the main etiologic infectious agents of diarrhea and the bacterial antimicrobial susceptibility in admitted children from a rural area of Southern Mozambique.

MATERIALS AND METHODS

Study area and population. The study was conducted at the Manhiça District Hospital (MDH), the 110-bed referral health facility for the Manhiça District, a rural area in Maputo Province, Southern Mozambique. The area has a warm, rainy season between November and April and a cool, dry season during the rest of the year. The district has an estimated population of 130,000 inhabitants, mostly subsistence farmers, as well as workers in two large sugar and fruit-processing factories in Maragra and Xinavane. An increasing number of small traders have established shops and businesses along the road. There are two small towns (Manhiça and Xinavane), although most of the population lives in small, dispersed hamlets. Villages in this area typically are comprised of a loose conglomeration of compounds separated by garden plots and grazing land. Houses are simple, with walls made of cane with thatched or corrugated roofs. Within the town areas, houses are often grouped into family compounds and surrounded by grass fences.16

The Manhiça Health Research Center (Centro de Investigação em Saúde da Manhiça [CISM]) is adjacent to the MDH. A continuous demographic surveillance system (DSS) for vi-
Diarrhea has been a major public health concern in rural Mozambican children. In 1996, approximately 69,000 inhabitants in the area were being followed under the DSS. Since 1997, CISM and the MDH have jointly operated a round-the-clock surveillance system of all pediatric visits to the outpatient department (OPD) and all hospital admissions of children younger than 15 years of age.

**Subjects and sample collection.** The study was conducted between September 2000 and September 2001. Fecal samples were collected from one out of every two hospitalized children younger than 5 years of age presenting diarrhea in their diagnosis. On admission, the mother or guardian of a child that met the criteria for sample collection was given a stool container and instructions for sample collection. Samples were collected in approximately one half of the diarrhea cases. Once collected, samples were kept in a cool box until processed. All children were assessed for other clinical conditions, following the inpatient pediatric protocol at the MDH. A questionnaire including clinical and laboratory data was completed for all cases.

**Definitions.** Diarrhea was defined as three or more watery loose stools in the previous 24 hours, as noted by the mother or caretaker. *Plasmodium falciparum* infection was defined as one or more asexual parasites after observation of 200 leukocytes. An agreement was obtained for readings from two independent microscopists. Nutritional status was assessed by weight for age Z-score. Mild malnutrition was defined as a weight for age (WAZ) Z-score between –2 and –1, moderate malnutrition was between –3 and –2, and severe malnutrition was < –3. Well-nourished children were defined as < –1 Z-score. Bloody diarrhea was defined as the presence of visible blood in stools.

**Laboratory methods.** Stool samples were cultured in various solid selective media and selenite broth. Plates were incubated for 18–24 hours at 37°C, except for *Campylobacter* medium, which was incubated 48 hours at 42°C under microaerophilic conditions (gas packs; Oxoid, London, UK). After a 24-hour incubation, the selenite broth was sub-cultured onto *Salmonella-Shigella* (SS) agar. Colonies were initially identified based on morphologic characteristics. When suspected to be relevant, identification was done using the appropriate biochemical tests, followed by an API20E (BioMérieux, Marcy-l’Etoile, France). *Salmonella, Shigella,* and *Vibrio* strains were agglutinated using specific antisera (Difco Laboratories, Detroit, MI). Identification of *Escherichia coli* was restricted to lactose fermenting strains, and confirmation was done with biochemical tests. Identification of the different diarrheagenic *E. coli* (enteropathogenic [EPEC], enterotoxigenic [ETEC], enterohaemagglutinin [EAEC], and verotoxigenic [VTEC]) was done by polymerase chain reaction (PCR) with previously described primers and conditions. *Campylobacter* spp. were identified based on morphologic characteristics and an oxidase test and confirmed by Gram staining.

The antimicrobial susceptibility to ampicillin, chloramphenicol, trimethoprim-sulphamethoxazole, tetracycline, nalidixic acid, and ciprofloxacin was determined by disc diffusion. Susceptibility testing for amoxicillin plus clavulanic acid was done for *E. coli* isolates resistant to amoxicillin.

The presence of parasites in feces was determined by direct stool observation. Presence of leukocytes and erythrocytes was determined microscopically. The presence of *Cryptosporidium* and *Cyclospora* was established using the modified Kinyoun carbolufchsin technique. Children with fever or having an episode of fever within the previous 24 hours were tested for malaria parasites in blood.

The presence of rotavirus was determined using an agglutination kit (Pastorex latex agglutination kit; Sanofi Diagnostic Pasteur, Paris, France) following the manufacturer's instructions.

**Statistical methods.** The values of variables were counted and summarized in tables of frequency. The χ² test was used for the analysis of categorical variables. When the expected frequency in a cell was lower than five, Fisher exact test was used instead of χ².

**RESULTS**

Between September 2000 and September 2001, 4,590 children younger than 5 years of age were admitted to the MDH. Among these, diarrhea was one of the diagnoses in 1,029 (22%). Samples were collected from 529 (51%) of those with diarrhea. The mean age of children with diarrhea was 13.1 ± 7.8 (SD) months. Fifty-four percent (285/529) were younger than 12 months of age, and 37% (198/529) were between 12 and 24 months of age. Two hundred ninety-four children (56%) were boys.

Of the 529 samples examined, 223 (42.2%) were positives for at least one pathogen: pathogenic bacterial were isolated from 144 (27.2%) samples, parasites from 76 (14.4%), and viruses from 3 (0.6%). The presence of *Ascaris lumbricoides* (92% versus 8%; *P < 0.001*) or *Strongyloides stercoralis* (100% versus 0%; *P = 0.008*) was statistically related to age (Fisher exact test), being more frequent among children younger than 12 months of age, whereas EPEC was more frequent in children younger than 1 year of age (78% versus 22%; *P = 0.021*, Fisher exact test). Rotaviruses were only isolated in three children, all younger than 24 months of age.

 Seventy-five percent (398) of the children with diarrhea were diagnosed with malaria, and among these, an enteropathogen was isolated in only 143 of the cases (36%).

The incidence of cases of diarrhea was higher during the rainy season than during the dry season. When taking into account all enteropathogens, no seasonal difference in pathogen isolation was observed. However, when *E. coli* strains were excluded from the analysis, bacterial pathogens seem to be more frequently isolated during the rainy season (7% versus 2%; *P = 0.015*). The incidence of diarrhea and pathogens isolation over time is shown in Figure 1.

The average number of days of admission was 6.5 ± 7.3 days, and the duration of diarrhea was of 2.62 ± 1.9 days. Fever and cough were the most frequent clinical finding in all children, independent of having had an enteropathogen isolated, whereas vomit was more frequently found among children presenting *E. coli, A. lumbricoides, G. lamblia,* or rotavirus. Watery diarrhea was the most common consistency found in all stools.

Twenty-three percent (121/528) of the children had mild malnutrition (−2 to −1), 122 (23%) had moderate malnutrition (−3 to −2), 142 (27%) had severe malnutrition (−3), and 143 (27%) were well nourished (< −1). The case fatality rate was 2.8% (15/529 patients). All deaths were in children younger than 24 months of age; the percentage was slightly
higher in children between 12 and 24 months of age than in younger ones (60% versus 40%), although no statistical significance was found ($P = 0.147, \chi^2$ test). A comparison of the mortality rate between the moderate plus severe malnutrition groups and the mild malnutrition plus nourished groups shows that the rate was higher in the first group, although not statistically significant ($P = 0.056, \chi^2$ test).

*Escherichia coli* (22.6%), *A. lumbricoides* (9.3%), *Salmonella* spp. (2.5%), *G. lamblia* (2.5%), *Campylobacter* spp. (1.7%), and *S. stercoralis* (1.1%) were the most frequently found pathogens, with others accounting for < 1% (Table 1). Lactose-fermenting bacterial strains were detected in 404 (76%) samples. Among these, the presence of *E. coli* was confirmed in 335 (83%). One hundred twenty of the isolated *E. coli* (35.8%) were identified as being pathogenic, mainly EAE (15.2%), but also ETEC (10.7%), EPEC (6.9%), and VTEC (3.0%). Co-infections were found in 24 patients, accounting for 10.6% of the total number of positive cultures. Of these, 17 were bacteria/parasite co-infections, 6 were bacteria/bacteria, and only 1 was bacteria/virus. ETEC (11 cases) and *A. lumbricoides* (8 cases) were the enteropathogens most frequently found to be involved in co-infections.

The antimicrobial resistance to the antibiotics tested ranged between 38% and 89% for trimethoprim sulphamethoxazole, 62% and 72% for ampicillin, and 8% and 45% for chloramphenicol. Resistance to quinolones was mainly observed in *Campylobacter* strains (Table 2). The only *Shigella flexneri* isolated was resistant to all antimicrobials, with the exception of quinolones, whereas *Vibrio* spp. were susceptible to all antibiotics tested.

### Table 1

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Prevalence</th>
<th>0–1 year</th>
<th>1–2 years</th>
<th>2–5 years</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>120 (22.6)</td>
<td>73 (61)</td>
<td>37 (31)</td>
<td>10 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>EAE</td>
<td>51 (9.6)</td>
<td>30 (59)</td>
<td>17 (35)</td>
<td>4 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>ETEC</td>
<td>36 (6.8)</td>
<td>19 (53)</td>
<td>13 (36)</td>
<td>4 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>EPEC</td>
<td>23 (4.3)</td>
<td>18 (78)</td>
<td>5 (22)</td>
<td></td>
<td>0.021*</td>
</tr>
<tr>
<td>VTEC (VT1)</td>
<td>10 (1.9)</td>
<td>6 (60)</td>
<td>3 (30)</td>
<td>1 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>13 (2.5)</td>
<td>5 (39)</td>
<td>6 (46)</td>
<td>2 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>9 (1.7)</td>
<td>5 (67)</td>
<td>3 (33)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>49 (9.4)</td>
<td>4 (8)</td>
<td>32 (65)</td>
<td>13 (27)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>13 (2.5)</td>
<td>7 (54)</td>
<td>6 (46)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><em>S. stercoralis</em></td>
<td>6 (1.1)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>3 (0.6)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>2 (0.4)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>2 (0.4)</td>
<td>2 (100)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Anclylostoma spp.</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>3 (0.6)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

*Fischer exact test.*

NS, not significant.
On the contrary, Vibrio/H11505 E. coli Percent/H11505 Data from our isolated strain was resistant to Amp, Chl, SXT and Tet, whereas the Campylobacter spp. was susceptible to all tested antibiotics.

The role of diarrheagenic E. coli was the most frequently isolated enteropathogen, supporting the well-documented role of E. coli in diarrheal disease.\textsuperscript{9,19,21} Moreover, despite restricting the determination of E. coli to lactose-fermenting isolates, a frequency of 22.6% from the total number of samples is comparable with that found in a previous study, where a prevalence of E. coli of 35.7% was found among Tanzanian children.\textsuperscript{9} The main E. coli pathotype found was EAEC (9.6%); a similar percentage (9.1%) of this pathotype was also reported in cases of diarrhea in an urban area of the country.\textsuperscript{22} The role that EAEC plays in diarrheal disease has been previously described in several studies,\textsuperscript{11,22,23} although in others, it was not found to be associated with diarrhea.\textsuperscript{8,24,25} Data from our study are not conclusive for the role that EAEC plays, because there was not a control group.

The low frequency of verotoxin-producing E. coli (VTEC-VT1) reported is consistent with what has been previously found in others studies, referring to both epidemic and sporadic isolates, carried out in sub-Saharan Africa and including Mozambican neighboring countries such as South Africa, Swaziland, and Tanzania.\textsuperscript{12,26–28} Prevalence of VTEC in childhood diarrhea in developing countries has been shown to be lower than that of ETEC or EAEC.\textsuperscript{26,29} On the contrary, VTEC is highly prevalent in developed countries.\textsuperscript{30}

### DISCUSSION

In this study, at least one pathogen was isolated from 42.2% (223/529) of the stools of patients with diarrhea. This percentage is lower compared with previous studies carried out in developing countries.\textsuperscript{8,24,25} Various factors may account for such a difference. Antimicrobial therapy previous to sample collection was given in some cases where it was recommended, and it is known that this can reduce the percentage of bacterial enteropathogens isolation.\textsuperscript{20} The fact the samples collected during the night were only processed on the following morning or an underestimation of the prevalence of certain pathogens caused by methodological problems could also account for these results. Finally, the direct effect of other pathologies, such as malaria, cannot be discarded. It has been previously described that between 5% and 38% of malaria cases present diarrhea as one of the main symptoms. Thus, even taking into consideration the aforementioned study limitations, including the possible presence in the area of other enteropathogens that we did not test for, such as some diarrheagenic viruses, we must not underestimate the impact of malaria, which may account for an important percentage of the diarrhea among children.

Diarrheagenic E. coli was the most frequently isolated enteropathogen, supporting the well-documented role of E. coli in diarrheal disease.\textsuperscript{9,19,21} Moreover, despite restricting the determination of E. coli to lactose-fermenting isolates, a frequency of 22.6% from the total number of samples is comparable with that found in a previous study, where a prevalence of E. coli of 35.7% was found among Tanzanian children.\textsuperscript{9} The main E. coli pathotype found was EAEC (9.6%); a similar percentage (9.1%) of this pathotype was also reported in cases of diarrhea in an urban area of the country.\textsuperscript{22} The role that EAEC plays in diarrheal disease has been previously described in several studies,\textsuperscript{11,22,23} although in others, it was not found to be associated with diarrhea.\textsuperscript{8,24,25} Data from our study are not conclusive for the role that EAEC plays, because there was not a control group.

The low frequency of verotoxin-producing E. coli (VTEC-VT1) reported is consistent with what has been previously found in others studies, referring to both epidemic and sporadic isolates, carried out in sub-Saharan Africa and including Mozambican neighboring countries such as South Africa, Swaziland, and Tanzania.\textsuperscript{12,26–28} Prevalence of VTEC in childhood diarrhea in developing countries has been shown to be lower than that of ETEC or EAEC.\textsuperscript{26,29} On the contrary, VTEC is highly prevalent in developed countries.\textsuperscript{30}

In disagreement with a previous study carried out in Maputo city,\textsuperscript{22} in which rotavirus antigen was detected in 18.2% of all symptomatic children and in 5% of asymptomatic ones, we found a low prevalence (0.6%) in this study. Some factors such as methodology and patient selection may explain the difference in these two studies. On the other hand, rotavirus has been associated with approximately one quarter of all diarrhea-related hospitalizations in South Africa\textsuperscript{31} and has been reported in other sub-Saharan African countries.\textsuperscript{9,24,31} Group A rotavirus is the most common human strain, with a worldwide distribution. Groups B and C rotaviruses are also human pathogens, with the first group mainly found in Asia, and the latter probably causes frequently undetected endemic infections.\textsuperscript{32,33} Commercial kits are designed to identify group A rotaviruses and have a limited sensitivity for other groups.\textsuperscript{33} No data on the prevalence of the different groups are available for Mozambique. A plausible explanation for the low prevalence obtained in this study would be that, in the Manhiça area, there was a high prevalence of non–A rotavirus groups that were not detected because of experimental limitations. Another possibility would be that cases of rotavirus infections were not severe or had a short duration and thus did not require hospitalization. Therefore, to determine the real rotavirus prevalence in Manhiça, as well as in other regions of Mozambique, longer studies with adequate methodology are needed. Establishing a real picture of rotavirus infection in the country is a first step to defining the possibility of introducing vaccines in the area.

The lower frequency of Salmonella spp. and Campylobacter spp. found in our study is consistent with that from other African studies in which these microorganisms were isolated in < 3% of children younger than 5 years of age with diarrhea.\textsuperscript{9,34} The low prevalence of both Shigella spp. and Vibrio spp. in our study may be explained by the fact that it only included severe cases requiring hospitalization. Furthermore, both pathogens usually appear in outbreaks, so that in the absence of an epidemic, they are not frequently isolated.\textsuperscript{35,36}

The frequency of enteric parasites found in our study is similar to that reported in Tanzanian children,\textsuperscript{8} although our data are not conclusive of the role of these pathogens in diarrheal disease. However, that G. lamblia and other protozoa are a cause of diarrhea is a well-documented fact.\textsuperscript{9,21}

In our study, we did not find a marked seasonality in pathogen isolation, even though bacterial pathogens were more frequent during the rainy season, something that seems to be statistically significant when E. coli strains are excluded from

### Table 2

Percentage of antimicrobial resistance for bacterial strains isolated from children presenting with diarrhea and admitted to the MDH

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Amp</th>
<th>Chl</th>
<th>SXT</th>
<th>Tet</th>
<th>Nal</th>
<th>Cip</th>
<th>Amox/Clav*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 13)</td>
<td>8 (62)</td>
<td>1 (8)</td>
<td>5 (38)</td>
<td>2 (15)</td>
<td>1 (8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 9)</td>
<td>NT</td>
<td>1 (11)</td>
<td>NT</td>
<td>2 (22)</td>
<td>1 (11)</td>
<td>1 (11)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 94)</td>
<td>68 (72)</td>
<td>42 (45)</td>
<td>54 (58)</td>
<td>45 (48)</td>
<td>4 (4)</td>
<td>1 (1)</td>
<td>3 (4)</td>
</tr>
<tr>
<td><em>EAEC</em> (N = 46)</td>
<td>42 (91)</td>
<td>28 (61)</td>
<td>37 (80)</td>
<td>23 (50)</td>
<td>3 (7)</td>
<td>1 (2)</td>
<td>2 (5)</td>
</tr>
<tr>
<td><em>ETEC</em> (N = 28)</td>
<td>13 (46)</td>
<td>8 (29)</td>
<td>17 (61)</td>
<td>10 (36)</td>
<td>–</td>
<td>–</td>
<td>1 (8)</td>
</tr>
<tr>
<td><em>EPEC</em> (N = 14)</td>
<td>10 (71)</td>
<td>5 (36)</td>
<td>8 (57)</td>
<td>8 (57)</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td><em>VTEC</em> (N = 6)</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The only *Shigella* isolated strain was resistant to Amp, Chl, SXT and Tet, whereas the *Vibrio* spp. was susceptible to all tested antibiotics.

* Amox/Clav was tested only for *E. coli* strains resistant to ampicillin.
* All *Campylobacter* strains were susceptible to erythromycin.

Amp, ampicillin; Chl, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; Tet, tetracycline; Nal, nalidixic acid; Amox/Clav, amoxicillin plus clavulanic acid; NT, Not tested.

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the analysis. During February and March, an increase in bacterial isolates—mainly E. coli and Salmonella spp.—was observed, suggesting a small outbreak of these two microorganisms. Little can be said about the seasonality of Rotavirus and S. flexneri, because of the small number of isolates. However, EAEC, Shigella spp., and rotavirus were all more frequently isolated during the dry season, whereas ETBE and G. lambia were more common during the rainy period in a previous study conducted in Tanzania.7

The epidemiologic data suggest that there is an age dependency in the isolation of parasites, especially in the case of A. lumbricioides and S. stercoralis, both being more frequently isolated in older children. This could be because of the fact that children older than 12 months of age are in permanent contact with soil and thus more prone to infection. Feeding habits can also play a role in infection and could be the reason why E. coli tends to be more frequently isolated from children younger than 12 months of age, being especially significant for EPEC strains. This finding is consistent with that from another study in which age dependency was reported for different E. coli categories that were related to diarrhea.29 One of the limitations of the study was that data on risk factors were not collected. The fact that this study was conducted among hospitalized children also needs to be taken into consideration when interpreting the results.

Vomiting was more frequent in children presenting an E. coli, G. lambia, A. lumbricioides, and rotavirus. The presence of fever in most children with diarrhea could be caused by malaria, because this disease is endemic in the study area, and 75% of the children enrolled in the study were positive for P. falciparum parasites. Nevertheless, diarrhea caused by certain bacteria can also be accompanied with fever. S. flexneri was isolated from one patient with bloody diarrhea. The capacity of Shigella spp. to penetrate and multiply within the intestinal mucosa, causing ulceration and consequent blood in the stool, is well known.37

The main enteric bacteria isolated in this study presented important levels of resistance to those antimicrobials being used in the study area such as ampicillin and trimethoprim-sulfamethoxazole. High levels of trimethoprim-sulfamethoxazole resistance are distressing, especially because this antimicrobial is increasingly being used as prophylaxis for HIV-opportunistic infections.38 E. coli strains resistant to ampicillin showed high susceptibility to amoxicillin plus clavulanic acid. On the other hand, ampicillin in combination with gentamicin has been commonly used in the area to treat non-pneumococcal pneumonias in children younger than 2 years of age.

Chloramphenicol, quinolones, and third-generation cephalosporins are still effective for most bacterial pathogens from the area. However, the use of chloramphenicol and fluoroquinolones are not recommended in younger children, and both quinolones and cephalosporins are expensive and of limited supply within the country. As a result, the treatment of choice in cases in which antibiotherapy is recommended becomes a problem.

In summary, this study shows that diarrheagenic E. coli is the predominant enteropathogen isolated in children younger than five years of age admitted to the MDH and presenting diarrhea. Isolated pathogens had important levels of resistance in front of the most commonly used antimicrobials. Thus, antimicrobial surveillance should be continuous to monitor multidrug-resistant strains. Although the etiologic causes of diarrhea in children younger than 5 years of age remain unknown for most of the country, this report opens the door to further studies addressed to analyze the specific epidemiology, resistance patterns, and virulence of diarrhea-causing pathogens in Mozambique, as a first step to improve local control strategies.

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