In the Manhiça district, diarrhea has been recently reported to be the third cause of death (10%) among children from 0 to 14 years old in Maputo City. 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-sulfamethoxazole 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.

**Etiologic Data on Diarrheal Diseases and Susceptibility Patterns of Diarrheal Pathogens**

**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-
Since 1997, CISM and the MDH spp. were identified based on morphologic characteristics. When suspected to be relevant, identification was done using diagnostic methods. 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 vomiting 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 lower than 12 months of age.
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. lambia* (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 EAEC (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.

![Figure 1](http://www.ajtmh.org)

**Table 1**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Prevalence 0–1 year</th>
<th>Prevalence 1–2 years</th>
<th>Prevalence 2–5 years</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>120 (22.6)</td>
<td>73 (61)</td>
<td>37 (31)</td>
<td>10 (8) NS</td>
</tr>
<tr>
<td>EAEc</td>
<td>51 (9.6)</td>
<td>30 (59)</td>
<td>17 (33)</td>
<td>4 (8) NS</td>
</tr>
<tr>
<td>ETEC</td>
<td>36 (6.8)</td>
<td>19 (53)</td>
<td>13 (36)</td>
<td>4 (11) NS</td>
</tr>
<tr>
<td>EPEC</td>
<td>23 (4.3)</td>
<td>18 (78)</td>
<td>5 (22)</td>
<td>0.021* NS</td>
</tr>
<tr>
<td>VTEC (VT1)</td>
<td>10 (1.9)</td>
<td>6 (60)</td>
<td>3 (50)</td>
<td>1 (10) NS</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>13 (2.5)</td>
<td>5 (59)</td>
<td>6 (39)</td>
<td>2 (15) NS</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>9 (1.7)</td>
<td>6 (67)</td>
<td>3 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Ascaris lumbricoidea</em></td>
<td>49 (9.4)</td>
<td>4 (8)</td>
<td>32 (65)</td>
<td>13 (27) &lt;0.001*</td>
</tr>
<tr>
<td><em>Giardia lambia</em></td>
<td>13 (2.5)</td>
<td>7 (54)</td>
<td>6 (50)</td>
<td>NS</td>
</tr>
<tr>
<td><em>S. stercoralis</em></td>
<td>6 (1.1)</td>
<td>1 (33)</td>
<td>2 (50)</td>
<td>3 (50) 0.008*</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>3 (0.6)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>2 (0.4)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>2 (0.4)</td>
<td>2 (100)</td>
<td>1 (100)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Ankylostoma</em> spp.</td>
<td>1 (0.2)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>3 (0.6)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Fischer exact test.
  NS, not significant.
Various factors may account for the low prevalence of both E. coli and other protozoa as the diarrhea among children. Moreover, despite restricting the screening to defined pathogens, the absence of an epidemic, they are not frequently isolated. Both pathogens usually appear in outbreaks, so that in the absence of an epidemic, they are not frequently isolated.

Diarrheagenic E. coli was the most frequently isolated enteropathogen, supporting the well-documented role of E. coli in diarrheal disease. 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. The role that EAEC plays in diarrheal disease has been previously described in several studies, although in others, it was not found to be associated with diarrhea. 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-VTI) reported is consistent with what has been previously found in other 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. Prevalence of VTEC in childhood diarrhea in developing countries has been shown to be lower than that of ETEC or EAEC. On the contrary, VTEC is highly prevalent in developed countries. In disagreement with a previous study carried out in Maputo city, in which rotavirus antigen was detected in 12.2% 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 and has been reported in other sub-Saharan African countries.

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**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 than previous studies carried out in developing countries. Various factors may account for such a difference. Antimicrobial therapy prior 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. 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. 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. 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. The role that EAEC plays in diarrheal disease has been previously described in several studies, although in others, it was not found to be associated with diarrhea. 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-VTI) reported is consistent with what has been previously found in other 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. Prevalence of VTEC in childhood diarrhea in developing countries has been shown to be lower than that of ETEC or EAEC. On the contrary, VTEC is highly prevalent in developed countries. In disagreement with a previous study carried out in Maputo city, in which rotavirus antigen was detected in 12.2% 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 and has been reported in other sub-Saharan African countries. 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. Commercial kits are designed to identify group A rotaviruses and have a limited sensitivity for other groups. 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 Manhica 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 Manhica, 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. Although the prevalence of 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.

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

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...
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 ETEC and *G. lamblia* 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. lumbricoides* 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. lamblia*, *A. lumbricoides*, 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.

Received January 25, 2006. Accepted for publication November 28, 2006.

Acknowledgments: The authors thank the parents and guardians of study participants and the Manhiça Health District Authorities. The authors thank Mariano Sitaúbe for processing the samples. This project was supported by grants from “Agencia Española de Cooperación Internacional (AECI) and Fundació Clinic de Barcelona (Hospital Clinic de Barcelona). The Centro de Salud Internacional (CSI) receives economical support from RICET and RCESP networks. J.R. has a fellowship from RICET.

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