ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2009, p. 2450–2454 Vol. 53, No. 6
0066-4804/09/$08.00+0 doi:10.1128/AAC.01282-08

Antimicrobial Susceptibility and Mechanisms of Resistance in *Shigella* and *Salmonella* Isolates from Children under Five Years of Age with Diarrhea in Rural Mozambique[▽]

Inácio Mandomando,[1,2,3]* Dinis Jaintilal,[1] Maria J. Pons,[3,4] Xavier Vallès,[1] Mateu Espasa,[1] Laura Mensa,[3] Betuel Sigaúque,[1,2,3] Sergi Sanz,[3] Jahit Sacarlal,[1,3,5] Eusébio Macete,[1,3,6] Fátima Abacassamo,[1,5] Pedro L. Alonso,[1,3] and Joaquim Ruiz[3,4]

Centro de Investigação em Saúde da Manhiça (CISM), Maputo, Mozambique[1]; Instituto Nacional de Saúde, Ministério de Saúde, Maputo, Mozambique[2]; Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Hospital Clínic/IDIBAPS, Universitat de Barcelona, Barcelona, Spain[3]; CIBER, Epidemiologia y Salud Publica (CIBERESP), Barcelona, Spain[4]; Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique[5]; and Direcção Nacional de Saúde, Ministério de Saúde, Maputo, Mozambique[6]

Received 24 September 2008/Returned for modification 22 January 2009/Accepted 22 March 2009

The antimicrobial susceptibility and mechanisms of resistance of 109 *Shigella* and 40 *Salmonella* isolates from children with diarrhea in southern Mozambique were assessed. The susceptibility to seven antimicrobial agents was tested by disk diffusion, and mechanisms of resistance were searched by PCR or colorimetric method. A high proportion of *Shigella* isolates were resistant to chloramphenicol (Chl) (52%), ampicillin (Amp) (56%), tetracycline (Tet) (66%), and trimethoprim-sulfamethoxazole (Sxt) (84%). Sixty-five percent of the isolates were multidrug resistant. *Shigella flexneri* isolates were more resistant than those of *Shigella sonnei* to Amp (66% versus 0.0%, $P < 0.001$) and Chl (61% versus 0.0%, $P < 0.001$), whereas *S. sonnei* isolates presented higher resistance to Tet than *S. flexneri* isolates (93% versus 64%, $P = 0.02$). Resistance among *Salmonella* isolates was as follows: Tet and Chl, 15% each; Sxt, 18%; and Amp, 25%. Only 3% of *Salmonella* isolates were resistant to nalidixic acid (Nal), and none to ciprofloxacin or ceftriaxone (Cro). Among *Salmonella* isolates, multiresistance was found in 23%. Among *Shigella* isolates, antibiotic resistance was related mainly to the presence of *oxa-1*-like β-lactamases for Amp, *dfrA1* genes for Sxt, *tetB* genes for Tet, and Chl acetyltransferase (CAT) activity for Chl. Among *Salmonella* isolates, resistance was conferred by *tem*-like β-lactamases for Amp, *floR* genes and CAT activity for Chl, *tetA* genes for Tet, and *dfrA1* genes for Sxt. Our data show that *Shigella* isolates are resistant mostly to the most available, inexpensive antibiotics by various molecular mechanisms but remain susceptible to ciprofloxacin, Cro, and Nal, which is the first line for empirical treatment of shigellosis in the country.

Diarrhea disease is one of the main causes of childhood mortality worldwide. It is estimated that 17% of the 10.8 million deaths of children under 5 years of age worldwide is due to diarrhea, with developing countries being the most affected (6). Diarrhea can be caused by different agents, such as bacteria, parasites, and virus (1, 24, 35). The severity of the illness is mediated by different factors related to both the patient (nutritional status, presence of concomitant illness, and human immunodeficiency virus status) and the etiological agent (specific bacterial virulence and antimicrobial resistance). *Salmonella* spp. and *Shigella* spp. remain among the bacteria most frequently isolated from stool samples obtained from diarrhea patients, especially in rural areas from developing countries (24, 35). *Salmonella* spp. usually produce a self-limited illness, whereas infections due to *Shigella* are likely to be more severe.

The management of acute diarrhea is based on replacement of fluids (8). However, antibiotics might be required for management of the most severe cases or cases involving malnourished children. With shigellosis, appropriate antimicrobial therapy can reduce the duration of fever and the period of shedding of the pathogens (26), which is relevant to transmission of the pathogen to susceptible contacts. At the Manhiça District Hospital (MDH), the combination of ampicillin (Amp) plus gentamicin used with children younger than 2 months of age and chloramphenicol (Chl) used alone with older children are the most available therapeutic options for treating bacterial infections (32), while nalidixic acid (Nal) is recommended for cases of dysentery.

Until recently, *Salmonella* spp. were highly susceptible to the most commonly used antibiotics (34). However, in the last decade, the emergence of multidrug-resistant nontyphoidal *Salmonella* strains, including isolates resistant to quinolones, has been described worldwide (14, 33, 36). Also, the increase in the number of *Shigella* isolates resistant to most of the antibiotics available in countries where the choice of treatment is limited (14, 16, 21, 25, 36) represents an important health problem.

The mechanisms of antimicrobial resistance are associated with intrinsic resistance, point mutations, and acquired or extrachromosomal resistance (31). A wide range of molecular mechanisms, such as the presence of β-lactamases, dihydrofolate reductase, Chl acetyltransferase (CAT) enzymes and many

* Corresponding author. Mailing address: Centro de Investigação em Saúde da Manhiça (CISM), Vila da Manhiça, Rua-12, P. O. Box. 1929, Maputo, Mozambique. Phone: 258 21 810 002. Fax: 258 21 810 181. E-mail: inacio.mandomando@manhica.net.

▽ Published ahead of print on 30 March 2009.

others (7, 10, 11, 22, 27), have been described. However, few studies have investigated molecular mechanisms of antimicrobial resistance among isolates from sub-Saharan Africa, due mainly to the limited number of laboratories and research facilities with an adequate infrastructure available on the continent (10, 21).

Currently, data about antimicrobial resistance among diarrheagenic bacteria in Mozambique are scarce. This study describes the antimicrobial susceptibility and the molecular mechanisms of resistance in *Salmonella* and *Shigella* isolates from children with diarrhea in a rural hospital in Mozambique.

MATERIALS AND METHODS

Study site and population. The study was conducted by the Centro de Investigação em Saúde da Manhiça (CISM), Maputo, Mozambique, at the Manhiça District Hospital (MDH), a 110-bed referral health facility for Manhiça District, a rural area located 80 km north of Maputo in the Maputo province in southern Mozambique. The area has a subtropical climate with two distinct seasons, a warm and rainy season between November and April and a cool and dry season during the rest of the year. The district has an estimated population of 140,000 inhabitants. A full description of the geographical and socio-demographic characteristics of the study community can be found elsewhere (2). The CISM has been running a continuous demographic surveillance system since 1996 in an area that currently extends 500 km^2 and includes 82,000 inhabitants. The CISM is adjacent to the MDH, and since 1997 they have jointly operated round-the-clock surveillance of all pediatric visits to the outpatient department and admissions to the wards.

Patients and bacterial isolation. Patients were children <5 years of age who presented to the outpatient department with diarrhea. Antibiotic therapy with Nal was started among children presenting bloody diarrhea, according to national guidelines. When shigellosis was confirmed, a field worker visited the household to provide medication.

A total of 109 *Shigella* isolates and 40 *Salmonella* isolates, which were recovered between July 2001 and July 2003 from children under the age of five who were attended to at the MDH, were investigated. Briefly, after a consent form granting permission for the use of the sample for research purposes was signed by the parent/legal guardian, rectal swab specimens were collected and cultured onto solid media (MacConkey's agar, *Salmonella-Shigella* agar, xylose lysin deoxycholate agar) for bacteria isolation. Presumptive bacterial identification was done on the basis of colony color and morphology. Putative *Shigella* or *Salmonella* organisms were selected and inoculated into Kliger's iron agar slants and urea agar. *Shigella* spp. and *Salmonella* spp. were characterized biochemically and confirmed by an API-20E (bioMerieux, Marcy l'Etoile, France).

Shigella serogroups were determined by slide agglutination using specific antisera according to the manufacturer's instructions (Difco Laboratories, Detroit, MI). *Salmonella enterica* serotyping was performed at Instituto de Salud Carlos III, Madrid, Spain, using somatic and flagellum antiserum, as previously described (19). Isolates were stored in the Microbank system (Pro-Lab Diagnostics, Cheshire, United Kingdom) at −70°C and recovered at a later stage for molecular characterization.

Susceptibility testing. Antimicrobial susceptibility to Amp, ceftriaxone (Cro), Chl, Nal, tetracycline (Tet), and trimethoprim-sulfamethoxazole (Sxt) was performed by disk diffusion in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (9). Additionally, Nal-resistant strains were tested for ciprofloxacin resistance.

In this study, multidrug resistance (MDR) is defined as the presence of resistance to at least two nonrelated antimicrobial agents.

Molecular mechanisms of antimicrobial resistance. For those *Shigella* and *Salmonella* isolates presenting full resistance to antibiotics, genes encoding β-lactamases (*tem*-like, *carb*-like, *shv*-like, *oxa-1*-like, *oxa-2*-like, and *oxa-5*-like) associated with Amp resistance were investigated by PCR, as described previously (22, 30). Determination of the presence of the *cmlA* and *floR* genes associated with Chl resistance was carried out as described by Cabrera et al. (7). The colorimetric method was used to detect the presence of CAT activity (4). Detection of the *tetA*, *tetB*, and *tetG* genes associated with Tet resistance was performed as previously described (7, 13), and detection of genes associated with Sxt resistance (*dfrA1*, *dfrA5*, *dfrA6*, *dfrA7*, *dfrA8*, *dfrA12*, *dfrA13*, *dfrA14*, *dfrA15*, *dfrA15b*, *dfrA16*, *dfrA16b*, and *dfrA17*) was carried out as described previously (23). Briefly, one colony of the strain was boiled in 25 μl sterile distilled water for 10 min, and 25 μl of a mixture containing 1.0 mM of each primer, 0.4 mM dNTPs, 2× PCR buffer with Mg, and 2.5 units of *Taq* polymerase was added. The mixture was overlaid with mineral oil, and the reaction was carried out in an Eppendorf thermocycler. The program consisted of 30 cycles as follows: 1-min denaturation (94°C), 1-min annealing, and 1-min elongation (72°C), plus a final extension of 10 min at 72°C. Annealing temperatures varied for the different primers. The reaction products were run in 1.5% agarose gels with 0.5 μg/ml of ethidium bromide.

Statistical analysis. Data were double entered into a FoxPro database using visual FoxPro version 2.6 (Microsoft Corporation, Redmond, WA). The two entries were compared, and discrepancies were resolved by referring to the original forms. The statistical analysis was performed using STATA software (version 9.0). Proportions were compared using the chi-square test or Fisher's exact test, as appropriate.

RESULTS

Antimicrobial resistance. A total of 109 *Shigella* isolates (94 were *S. flexneri* and 15 *S. sonnei*) and 40 *Salmonella enterica* isolates (10 were *S. enterica* serovar Infantis, 9 *S. enterica* serovar Typhimurium, 5 *S. enterica* serovar Virchow, 3 *S. enterica* serovar Isangi, 3 *S. enterica* serovar Heidelberg, 2 *S. enterica* serovar Enteritidis, and 8 other serotypes) were assessed for their antimicrobial susceptibility patterns.

Shigella isolates showed high levels of resistance to Sxt (84%), Tet (66%), Amp (56%), and Chl (52%). No *Shigella* isolates were resistant to Nal or Cro. When the resistance was analyzed by species, it was observed that *S. flexneri* isolates were more frequently resistant to Amp (66% versus 0.0%, $P < 0.001$) and Chl (61% versus 0%, $P < 0.001$), whereas *S. sonnei* isolates were more frequently resistant to Tet (93% versus 64%, $P = 0.02$) (Table 1).

The resistance rates among *Salmonella* isolates were lower than those reported for *Shigella* isolates. Resistance to Amp and Sxt was detected in 25% and 18% of isolates, respectively. For Tet and Chl, resistance rates were 15% each. One isolate (3%) was found to be resistant to Nal, while for this same isolate, no resistance to Cro or ciprofloxacin was detected. By serotypes, 67% of *Salmonella* serovar Typhimurium isolates were resistant to Amp. Additionally, a higher proportion of *S.* Typhimurium isolates were resistant to Sxt and Chl compared to other serotypes (Table 1). All *Salmonella* serovar Infantis isolates were susceptible to all antibiotics tested, and one isolate of *Salmonella* serovar Virchow was resistant to Tet. Three isolates of *S.* serovar Isangi were found to be MDR (Table 2).

MDR was detected with 65% of *Shigella* isolates and 23% of *Salmonella* isolates. Resistance to two antibiotics was observed in 1% of *S. flexneri* isolates, 80% of *S. sonnei* isolates, and 5% of *Salmonella* isolates. Ten percent of *S. flexneri* isolates and 5% of *Salmonella* isolates presented resistance to three agents, while 49% of *S. flexneri* and 8% of *Salmonella* isolates were resistant to four antimicrobial agents (Table 2).

Mechanisms of resistance. A great variety of molecular mechanisms of resistance were detected, although some of them in few isolates (Table 3). Among *S. flexneri* isolates, the resistance to Amp was related mainly to the presence of *oxa-1*-like genes (55 out of 61 Amp-resistant isolates; 90%), while Tet resistance was related mainly to the presence of the *tetB* gene (57 out of 58 Tet-resistant isolates; 98%). The presence of genes *dfrA1* (40 out of 71 Sxt-resistant isolates; 56%) and *dfrA14*-like (25 out of 71 Sxt-resistant isolates; 31%) was detected mainly in the resistance to Sxt. A total of 27% (19 out of 71) of *S. flexneri* isolates, from which *dfrA14*-like was found,

TABLE 1. Antimicrobial resistance (%) of *Shigella* and *Salmonella* isolates from children under 5 years of age with diarrhea at the MDH

Organism (no. of isolates)	Amp			Chl			Sxt			Tet		
	MIC range	MIC_{50}	MIC_{90}	% Resistant	MIC range	MIC_{50}	MIC_{90}	% Resistant	MIC range	MIC_{50}	MIC_{90}	% Resistant	Range	MIC_{50}	MIC_{90}	% Resistant
Shigella spp. (109)	0.5–>128	64	128	56	0.5–128	32	32	57	1–128	>128	>128	84	1–64	32	64	66
S. flexneri (94)	0.5–>128	64	128	66[a]	0.5–128	32	32	61[a]	4–128	>128	>128	82	1–64	32	64	64
S. sonnei (15)	0.5–>128	1	32	0	1–128	2	32	0	1–>128	>128	>128	100	1–64	32	64	93[b]
Salmonella spp. (40)	<0.5–>128	≤0.5	>128	25	<0.5–>128	≤0.5	>128	15	<0.5–>128	≤0.5	>128	18	<0.5–>128	≤0.5	1	15
S. Infantis (10)	≤0.5	≤0.5	≤0.5	0	≤0.5	≤0.5	≤0.5	0	≤0.5	≤0.5	≤0.5	0	≤0.5	≤0.5	≤0.5	0
S. Typhimurium (9)	≤0.5–>128	>128	>128	67	≤0.5–>128	1	>128	22	<1	1	>128	44	<0.5–1	≤0.5	1	0
S. Virchow (5)	≤0.5–32	≤0.5	≤0.5	0	≤0.5	≤0.5	≤0.5	0	≤0.5	≤0.5	≤0.5	0	<0.5	≤0.5	≤0.5	0
Other *Salmonella* isolates (16)	<0.5–>128	≤0.5	≤0.5	25	<0.5–>128	≤0.5	>128	25	<0.5–>128	≤0.5	>128	19	<0.5–>128	≤0.5	>128	25

[a] *S. flexneri* isolates were more frequently resistant to Amp and Chl than *S. sonnei* isolates ($P < 0.001$; Fisher's exact test).
[b] *S. sonnei* isolates were more frequently resistant to Tet than *S. sonnei* isolates ($P < 0.02$; chi-squared test).

TABLE 2. Profiles of *Shigella* and *Salmonella* resistance to at least two unrelated antibiotics[a]

Antibiotic resistance phenotype	No. (%) of resistant isolates of:			
	Shigella flexneri	*Shigella sonnei*	All *Shigella* spp.	All *Salmonella* spp.
Amp^r Sxt^r	1 (1)		1 (1)	2 (5)
Amp^r Chl^r	1 (1)		1 (1)	1 (3)
Amp^r Tet^r				1 (3)
Chl^r Sxt^r	1 (1)		1 (1)	
Chl^r Tet^r	1 (1)		1 (1)	
Sxt^r Tet^r		12 (80)	12 (11)	
Tet^r Nal^r				1 (3)
Amp^r Chl^r Sxt^r				2 (5)
Amp^r Chl^r Tet^r	9 (10)		9 (8)	
Amp^r Sxt^r Chl^r Tet^r	46 (49)		46 (42)	3 (8)
Total	59 (63)	12 (80)	71 (65)	9 (23)

[a] Values represent isolates exhibiting resistance to two or more unrelated antimicrobial agents.

presented a "new" restriction fragment length polymorphism (RFLP) pattern. Resistance to Chl was associated mainly with the presence of CAT activity. Meanwhile, for *S. sonnei* isolates the resistance to Sxt and Tet was related mainly to the presence of the *tetA* and *dfrA1* genes, respectively (Table 3).

Amp resistance among *Salmonella* isolates was exclusively associated with *tem*-like genes, which were detected with 70% (7/10) of the isolates, of which six were determined to be *S.*

TABLE 3. Mechanisms of resistance of *Shigella* and *Salmonella* isolates from children with diarrhea in rural hospital in Mozambique

Antimicrobial or mechanism of resistance	No. of isolates with indicated mechanism of resistance/total no. of resistant isolates (%)			
	S. flexneri	*S. sonnei*	All *Shigella* isolates	All *Salmonella* isolates
Amp				
o*xa-1*-like	55/61 (90)		55/61 (90)	0/10 (0)
oxa-2-like	3/61 (5)		3/61 (5)	0/10 (0)
oxa-5-like	1/61 (2)		1/61 (2)	2/10 (20)
tem-like	3/61 (5)		3/61 (5)	7/10 (70)
shv-like	1/61 (2)		1/61 (2)	0/10 (0)
carb-like	0/61 (0)		0/61 (0)	0/10 (0)
Chl				
cmlA	1/57 (2)		1/57 (2)	2/6 (33)
floR	0/57 (0)		0/57 (0)	5/6 (83)
CAT	51/57 (89)		51/57 (89)	4/6 (67)
Tet				
tetA	1/58 (2)	10/14 (71)	11/72 (15)	2/6 (33)
tetB	57/58 (98)	0/14 (0)	52/72 (79)	0/6 (0)
tetG	13/58 (22)	5/14 (36)	18/72 (25)	0/6 (0)
Sxt				
drfA1	40/71 (56)	11/13 (85)	51/84 (61)	2/7 (29)
dfrA5	6/71 (8)	1/13 (8)	7/84 (8)	0/7 (0)
dfrA7	4/71 (6)	0/13 (0)	4/84 (5)	1/7 (14)
dfrA8	1/71 (1)	0/13 (0)	1/84 (1)	0/7 (0)
dfrA12	6/71 (8)	2/13 (15)	8/84 (10)	0/7 (0)
dfrA14	25/71 (35)	1/13 (8)	26/84 (31)	0/7 (0)
dfrA14-like[a]	19/71 (27)	3/13 (23)	22/84 (26)	0/7 (0)

[a] Isolates presenting a new RFLP pattern.

Typhimurium. CAT activity and the presence of the *floR* gene, both associated with resistance to Chl, were found with 67% and 83% of isolates, respectively. Resistance to Tet was associated with the *tetA* gene (33%; 2/6), and resistance to Sxt was associated with the presence of *dfrA1* (29%; 2/7).

With a number of isolates, the presence of more than one mechanism of resistance to the same antimicrobial agent was identified. This phenomenon was observed with 32 *S. flexneri* isolates resistant to Sxt; 18 of them involved *dfrA14*-like genes with a new RFLP pattern. With 11 isolates resistant to Tet, 5 resistant to Amp, and 1 resistant to Chl also, more than one resistance mechanism to these agents was identified. The same occurrence was observed with *S. sonnei* isolates, of which 11 presented more than one mechanism of resistance to Tet and 4 were nonsusceptible to Sxt and involved three *dfrA14*-like genes with an unrecognized RFLP pattern. Among *Salmonella* isolates, 4 of the 5 isolates resistant to Chl also presented more than one mechanism of resistance, with three showing the presence of *floR* and CAT activity, and one showing the presence of *floR* and *cmlA* (data not shown).

A number of antimicrobial-resistant isolates were without an identified mechanism of resistance. Thus, among *S. flexneri* isolates, seven Amp-resistant, three Chl-resistant, two Sxt-resistant, and two Tet-resistant isolates appeared to have mechanisms of resistance other than the ones investigated here. The same occurrence was observed with two Tet-resistant *S. sonnei* isolates. Among *Salmonella* isolates, the resistance mechanism was not identified in 3/7 (43%) isolates resistant to Sxt, in 2/6 (33%) to Tet, and 1/10 (10%) to Amp.

DISCUSSION

In this paper we have correlated the levels of antimicrobial resistance and their mechanisms among *Shigella* and *Salmonella* isolates from children with diarrhea. We report a high prevalence of antimicrobial resistance among *Shigella* isolates, with *S. flexneri* isolates being much more resistant than *S. sonnei* isolates; in contrast, the *Salmonella* isolates had a much lower incidence of resistance. Selection of resistant strains might be favored in *Shigella*, because this microorganism usually causes severe infections that are likely to require antibiotic treatment. Despite the high proportion of antimicrobial resistance observed among *Shigella* isolates, these organisms remain highly susceptible to quinolones (Nal) and Cro, supporting the current use of Nal as first-line treatment of dysentery in Mozambique. The use of quinolones in children has been controversial but has recently been demonstrated to be safely used with this population (20). The use of third-generation cephalosporines, which are also effective against other diarrhea agents, is limited by cost in resource-poor countries.

Salmonella isolates presented a lower level of resistance. Although, interestingly, a small number–all the *Salmonella* serovar Isangi isolates in this study–were more resistant to the antibiotics tested, which is consistent with other reports from the region (12, 15).

This study has also shown that resistance to at least two antibiotics was common, with the majority of MDR in *S. flexneri* focused on Amp/Sxt/Chl/Tet, while in *S. sonnei* it was almost exclusively focused on Sxt/Tet; in contrast, the *Salmonella* isolates showed little MDR. The reason why antibiotic resistance is differently distributed among *Shigella* spp. is unknown. An explanation could be provided by differences in the genetic locations of the antimicrobial resistance genes that might possess a species-specific association (22). This hypothesis might be supported by the fact that, consistent with other studies (3, 5, 17, 21, 22), *S. sonnei* has been less resistant to Chl compared to *S. flexneri*. In addition, differences in the prevalences of genes encoding mechanisms of resistance among *S. sonnei* and *S. flexneri* have been reported previously (18, 22).

The high prevalence of the *oxa-1*-like-type β-lactamase among the Amp-resistant *S. flexneri* isolates is consistent with other findings (10, 21, 22), but the detection of other β-lactamases shows that other mechanisms of resistance could be present in *S. flexneri* and future alterations in their prevalence may occur. The genetic location of the antimicrobial resistance genes that might possess a species-specific association may help to explain the differences found with the mechanisms of resistance to Tet among *S. flexneri* (*tetB*) and *S. sonnei* (*tetA*) isolates. We found that the *dfrA1* genes were the main mechanism of Sxt resistance among *Shigella* isolates (22). Interestingly, a high proportion of *dfr14*-like genes (22/84) presented a new RFLP pattern. The DNA sequences of the new RFLP pattern are currently being determined.

Among *Salmonella* isolates, antimicrobial resistance was related to *tem*-like β-lactamase (Amp), CAT activity and the *floR* gene (Chl), and the *tetA* gene (Tet), findings which are in agreement with those published by others (7, 10, 29). The prevalence of *dfrA1* found among *Salmonella* isolates is also similar to the findings reported by Cabrera et al. (7). Differences in the prevalences of different resistance genes among *Salmonella* serotypes cannot be inferred from our data due to the small number of isolates belonging to each of the serotypes recovered.

A high number of isolates presented more than one mechanism of resistance to the same antimicrobial agent. This could facilitate adaptation to the changes of antibiotic selection pressure, as in some cases, such as with β-lactamases, the patterns of resistance conferred by each mechanism are not equal. Antibiotic resistance in a number of isolates appeared to be conferred by mechanisms other than those analyzed in this study. It should be taken into account that other mechanisms, such as overexpressed efflux pumps, may be present (28).

In summary, the present study shows that the prevalence in *Shigella* isolates of antimicrobial resistance to the most commonly used antibiotic in Mozambique is high, with *S. flexneri* isolates being much more resistant than *S. sonnei* isolates, but quinolones and the third generation of cephalosporins remain effective; resistance among *Salmonella* isolates was low. Among *Shigella* isolates, there was a difference in MDR profiles between *S. flexneri* and *S. sonnei*. *S. flexneri* Amp resistance linked with the *oxa-1*-like gene, Tet resistance with *tetB*, and Sxt resistance with *dfrA1*, *dfrA14*, and a related sequence of *dfrA14*-like; in contrast, *S. sonnei* Tet resistance was associated with *tetA*, while Sxt resistance was linked primarily to *drfA1*. Data generated in this study are also important in guiding clinicians in selection of antibiotics for empirical treatment. Continuous monitoring of antibiotic resistance in developing countries is imperative to ensure that severe diarrhea infections remain treatable.

ACKNOWLEDGMENTS

We are grateful to the parents and guardians of study participants and the Manhiça Health District Authorities. Special thanks to Ana Belen Ibarz for her dedication in editing the text. We also thank Mariano Sitaúbe, bacteriology laboratory technician of the CISM, for support in culturing and identifying *Shigella* strains. We thank Aurora Echeita and Silvia Herrera-Leon from Instituto de Salud Carlos III, Madrid, Spain, for their support in serotyping *Salmonella* isolates.

The Centre de Salut Internacional is also supported by the RICET and RCESP networks. J. Ruiz is a recipient of grants CP05/0130 and PI06/0204 from Fondo de Investigaciones Sanitarias.

REFERENCES

1. **Al-Gallas, N., O. Bahri, A. Bouratbeen, A. Ben Haasen, and R. Ben Aissa.** 2007. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic Escherichia coli: prevalence, phenotyping, and molecular epidemiology. Am. J. Trop. Med. Hyg. **77:**571–582.
2. **Alonso, P. L., F. Saúte, J. J. Aponte, F. X. Gómez-Olivé, A. Nhacolo, R. Thompson, E. Macete, F. Abacassamo, P. J. Ventura, X. Bosch, C. Menédez, and M. Dgedge.** 2002. Manhiça DSS, Mozambique, p. 189–195. *In* Population and health in developing countries, vol. 1. International Development Research Centre, Ottawa, Ontario, Canada.
3. **Ashkenazi, S., I. Levy, V. Kazaronovski, and Z. Samra.** 2003. Growing antimicrobial resistance of *Shigella* isolates. J. Antimicrob. Chemother. **51:** 427–429.
4. **Azemun, P., T. Stull, M. Roberts, and A. L. Smith.** 1981. Rapid detection of chloramphenicol resistance in *Haemophilus influenzae*. Antimicrob. Agents Chemother. **20:**168–170.
5. **Bogaerts, J., J. Verhaegen, J. P. Munyabikali, B. Mukantabana, P. Lemmens, J. Vandeven, and J. Vandepitte.** 1997. Antimicrobial resistance and serotypes of *Shigella* isolates in Kigali, Rwanda (1983 to 1993): increasing frequency of multiple resistance. Diagn. Microbiol. Infect. Dis. **28:**165–171.
6. **Bryce, J., C. Boschi-Pinto, K. Shibuya, and R. E. Black.** 2005. WHO estimates of the causes of death in children. Lancet **365:**1147–1152.
7. **Cabrera, R., J. Ruiz, F. Marco, I. Oliveira, M. Arroyo, A. Aladuena, M. A. Usera, M. T. Jimenez De Anta, J. Gascon, and J. Vila.** 2004. Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveler's diarrhea. Antimicrob. Agents Chemother. **48:**3934–3939.
8. **Casburn-Jones, A. C., and M. J. G. Farthing.** 2004. Management of infectious diarrhoea. Gut **53:**296–305.
9. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial disk susceptibility tests, 9th ed. Approved standard M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Cotton, M. F., E. Wasserman, J. Smit, A. Whitelaw, and H. J. Zar.** 2008. High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing *Enterobacteriaceae* and methicillin-resistant *Staphylococcus aureus* in nasopharyngeal and blood isolates of HIV-infected children from Cape Town, South Africa. BMC Infect. Dis. **8:**40.
11. **Danes, C., M. M. Navia, J. Ruiz, F. Marco, A. Jurado, M. T. Jimenez de Anta, and J. Vila.** 2002. Distribution of beta-lactamases in *Acinetobacter baumannii* clinical isolates and the effect of Syn 2190 (AmpC inhibitor) on the MICs of different beta-lactam antibiotics. J. Antimicrob. Chemother. **50:**261–264.
12. **Govinden, U., C. Mocktar, P. Moodley, A. W. Sturm, and S. Y. Essack.** 2006. CTX-M-37 in *Salmonella enterica* serotype Isangi from Durban, South Africa. Int. J. Antimicrob. Agents **28:**288–291.
13. **Guardabassi, L., L. Dijkshoorn, J. M. Collarb, J. E. Olsen, and A. Dalsgaard.** 2000. Distribution and in-vitro transfer of tetracycline resistance determinants in clinical and aquatic *Acinetobacter* strains. J. Med. Microbiol. **49:**929–936.
14. **Kariuki, S., G. Revathi, N. Kariuki, J. Kiiru, J. Mwituria, J. Muyodi, J. W. Githinji, D. Kagendo, A. Munyalo, and C. A. Hart.** 2006. Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: zoonotic or anthroponotic transmission? J. Med. Microbiol. **55:**585–591.
15. **Kruger, T., D. Szabo, K. H. Keddy, K. Deeley, J. W. Marsh, A. M. Hujer, R. A. Bonomo, and D. L. Paterson.** 2004. Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. Antimicrob. Agents Chemother. **48:**4263–4270.
16. **Lima, A. A., N. L. Lima, M. C. Pinho, E. A. Barros Junior, M. J. Teixeira, M. C. Martins, and R. L. Guerrant.** 1995. High frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, streptomycin, chloramphenicol, and tetracycline isolated from patients with shigellosis in northeastern Brazil during the period 1988 to 1993. Antimicrob. Agents Chemother. **39:**256–259.
17. **Mates, A., D. Eyny, and S. Philo.** 2000. Antimicrobial resistance trends in *Shigella* serogroups isolated in Israel, 1990–1995. Eur. J. Clin. Microbiol. Infect. Dis. **19:**108–111.
18. **Mensa, L., E. Pisos, S. Capilla, F. Marco, J. Vila, J. Gascón, and J. Ruiz.** 2006. Different prevalence of the main tet determinants among S. sonnei and S. flexneri. Clin. Microbiol. Infect. **12**(Suppl. 4)**:**P1603.
19. **Murray, P. R., E. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. ASM Press, Washington, DC.
20. **Murray, T. S., and R. S. Baltimore.** 2007. Pediatric uses of fluoroquinolone antibiotics. Pediatr. Ann. **36:**336–343.
21. **Navia, M. M., L. Capitano, J. Ruiz, M. Vargas, H. Urassa, D. Schellemberg, J. Gascon, and J. Vila.** 1999. Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. J. Clin. Microbiol. **37:**3113–3117.
22. **Navia, M. M., J. Gascon, and J. Vila.** 2005. Analysis of the mechanisms of resistance to several antimicrobial agents in *Shigella* spp. causing travellers' diarrhoea. Clin. Microbiol. Infect. **11:**1044–1047.
23. **Navia, M. M., J. Ruiz, J. Sanchez-Cespedes, and J. Vila.** 2003. Detection of dihydrofolate reductase genes by PCR and RFLP. Diagn. Microbiol. Infect. Dis. **46:**295–298.
24. **Okeke, I. N., O. Ojo, A. Lamikanra, and J. B. Kaper.** 2003. Etiology of acute diarrhea in adults in southwestern Nigeria. J. Clin. Microbiol. **41:**4525–4530.
25. **Oundo, J. O., S. Kariuki, J. K. Maghenda, and B. S. Lowe.** 2000. Antibiotic susceptibility and genotypes of non-typhi *Salmonella* isolates from children in Kilifi on the Kenya coast. Trans. R. Soc. Trop. Med. Hyg. **94:**212–215.
26. **Phavichitr, N., and A. Catto-Smith.** 2003. Acute gastroenteritis in children: what role for antibacterials? Paediatr. Drugs **5:**279–290.
27. **Ribera, A., F. Fernandez-Cuenca, A. Beceiro, G. Bou, L. Martinez-Martinez, A. Pascual, J. M. Cisneros, J. Rodriguez-Bano, J. Pachon, and J. Vila.** 2004. Antimicrobial susceptibility and mechanisms of resistance to quinolones and beta-lactams in *Acinetobacter* genospecies 3. Antimicrob. Agents Chemother. **48:**1430–1432.
28. **Rouveix, B.** 2007. Clinical implications of multiple drug resistance efflux pumps of pathogenic bacteria. J. Antimicrob. Chemother. **59:**1208–1209.
29. **Ruiz, J., S. Herrera-Leon, I. Mandomando, E. Macete, L. Puyol, A. Echeita, and P. L. Alonso.** 2008. Detection of *Salmonella enterica* serotype Typhimurium DT104 in Mozambique. Am. J. Trop. Med. Hyg. **79:**918–920.
30. **Salazar De Vegas, E. Z., B. Nieves, M. Ruiz, J. Ruiz, J. Vila, M. Araque, and E. Velazco.** 2007. Molecular epidemiology and characterization of resistance mechanisms to various antimicrobial agents in *Acinetobacter baumannii* isolated in Mérida, Venezuela. Med. Sci. Monit. **13:**BR89–BR94.
31. **Sanchez Garcia, J. E., R. Lopez, and J. Prieto.** 1999. Antimicrobianos en medicina. Sociedad Espanola de Quimioterapia. Prous Science, Barcelona, Spain.
32. **Sigauque, B., A. Roca, I. Mandomando, L. Morais, L. Quinto, J. Sacarlal, E. Macete, T. Nhamposa, S. Machevo, P. Aide, Q. Bassat, A. Bardaji, D. Nhalungo, M. Soriano-Gabarro, B. Flannery, C. Menendez, M. M. Levine, and P. L. Alonso.** 2009. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr. Infect. Dis. J. **28:**108–113.
33. **Soto, S. M., M. A. Gonzalez-Hevia, and M. C. Mendoza.** 2003. Antimicrobial resistance in clinical isolates of *Salmonella enterica* serotype Enteritidis: relationships between mutations conferring quinolone resistance, integrons, plasmids and genetic types. J. Antimicrob. Chemother. **51:**1287–1291.
34. **Stock, I., and B. Wiedemann.** 2000. Natural antibiotic susceptibility of *Salmonella enterica* strains. Int. J. Antimicrob. Agents **16:**211–217.
35. **Vargas, M., J. Gascon, C. Casals, D. Schellenberg, H. Urassa, E. Kahigwa, J. Ruiz, and J. Vila.** 2004. Etiology of diarrhea in children less than five years of age in Ifakara, Tanzania. Am. J. Trop. Med. Hyg. **70:**536–539.
36. **Velge, P., A. Cloeckaert, and P. Barrow.** 2005. Emergence of *Salmonella* epidemics: the problems related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other major serotypes. Vet. Res. **36:** 267–288.