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Occurrence and Antimicrobial Drug Susceptibility Patterns of Commensal and Diarrheagenic *Escherichia coli* in Fecal Microbiota from Children with and without Acute Diarrhea

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Acute diarrhea is a public health problem and an important cause of morbidity and mortality, especially in developing countries. The etiology is varied, and the diarrheagenic *Escherichia coli* pathotypes are among the most important. Our objectives were to determine the occurrence of commensal and diarrheagenic *E. coli* strains in fecal samples from children under five years old and their drug susceptibility patterns. *E. coli* were isolated from 141 fresh fecal samples; 84 were obtained from clinically injured donors with acute diarrhea (AD) and 57 from clinically healthy donors without diarrhea (WD). Presumptive phenotypic species identification was carried out and confirmed by amplification of specific 16S ribosomal RNA encoding DNA. Multiplex PCR was performed to characterize the diarrheagenic *E. coli* strains. Drug susceptibility patterns were determined by the disc-diffusion method. In total, 220 strains were recovered from the fecal specimens (61.8% from AD and 38.2% from WD). Diarrheagenic *E. coli* was identified at a rate of 36.8% (n=50) in diarrheic feces and 29.8% (n=25) in non-diarrheic feces. Enteroaggregative *E. coli* was the most frequently identified pathotype in the AD group (16.2%) and the only pathotype identified in the WD group (30.9%). Enteropathogenic *E. coli* was the second most isolated pathotype (10.3%), followed by Shiga toxin-producing *E. coli* (7.4%) and enterotoxigenic *E. coli* (2.9%). No enteroinvasive *E. coli* strains were recovered. The isolates showed high resistance rates against ampicillin, tetracycline, and sulfamethoxazole-trimethoprim. The most effective drugs were ceftazidime, ceftriaxone, imipenem and piperacillin-tazobactam, for which no resistance was observed. Differentiation between the diarrheagenic *E. coli* pathotypes is of great importance since they are involved in acute diarrheal diseases and may require specific antimicrobial chemotherapy. The high antimicrobial resistance observed in our study raises a broad discussion on the indiscriminate or improper use of antimicrobials, besides the risks of self-medication.

***Keywords*:** acute diarrhea, *E. coli*, drug resistance

Diarrhea is one of the major causes of mortality in children under five years old in developing countries (Bryce *et al*., 2005; Mendez-Aranciba *et al*., 2008). Many cases are not diagnosed, either because they are mild and self-limiting, in which the patient does not seek medical attention, or because, especially in developing countries, the medical and laboratory resources are not available (Quadri *et al*., 2005). Despite the various available microbiological techniques, about half of the cases of diarrhea have no defined etiology, which complicates the implementation of policies and strategies for mapping and monitoring endemic areas of the occurrence of such pathogens (Durmaz *et al*., 2005).

Escherichia coli is one of the leading causes of acute diarrhea in Brazil and other developing countries in children under 5 years old, with significant morbidity and mortality (Podewils *et al*., 2004). Five different pathotypes of diarrheagenic *E. coli* are well recognized based on their patterns of gastrointestinal disease: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC) (Aranda *et al*., 2004; Persson *et al*., 2007).

In Brazil, as in other developing countries, the differentiation of diarrheagenic *E. coli* from non-pathogenic commensal microbiota is achieved by means of physiological characterization of the microorganisms and identification of the surface O-antigen (Durmaz *et al*., 2005; Shelton *et al*., 2006). However, serological methods may be inconclusive due to variations in cellular antigenicity, and the O-serotype does not correlate with the presence of virulence genes (Vidal *et al*., 2005). *In vitro* assays, such as cell culture and cytotoxicity for the identification of virulent strains, are expensive and time-consuming.

Due to the importance of epidemiological scientific research into the prevalence and occurrence of outbreaks, epidemics and the mapping of microbial reservoirs which can act as potential sources for the dissemination of new infections in susceptible populations, the use of molecular methods is often associated with conventional culture-based methods of bacterial isolation and identification for confirmation and validation of results (Song *et al*., 2005; Shelton *et al*., 2006).

Recently, the health consequences associated with diarrheagenic *E. coli* infection have been worsened by the emergence of multidrug-resistant *E. coli*. This growing phenomen-

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Table 1. Primers used for multiplex PCR reactions, amplicon size, and concentrations

Primer	Primer sequence (5′→3′)	Target	Amplicon size (bp)	Concentration (pmol)
eae1	CTGAACGGCGATTACGCGAA	*eae*	917	5.00
eae2	CCAGACGATACGATCCAG			
BFP1	AATGGTGCTTGCGCTTGCTGC	*bfp*A	326	5.00
BFP2	GCCGCTTTATCCAACCTGGTA			
EAEC1	CTGGCGAAAGACTGTATCAT	CVD432	630	5.00
EAEC2	CAATGTATAGAAATCCGCTGTT			
LTf	GGCGACAGATTATACCGTGC	LT gene	450	5.00
LTr	CGGTCTCTATATATTCCCTGTT			
STf	ATTTTTMTTTCTGTATTRTCTT	ST gene	190	6.47
STr	CACCCGGTACARGCAGGATT			
IpaH1	GTTCCTTGACCGCCTTTCCGATACCGTC	*ipa*H	600	10.00
IpaH2	GCCGGTCAGCCACCCTCTGAGAGTAC			
Stx1f	ATAAATCGCCATTCGTTGACTAC	stx_1	180	3.88
Stx1r	AGAACGCCCACTGAGATCATC			
Stx2f	GGCACTGTCTGAAACTGCTCC	stx_2	225	2.50
Stx2r	TCGCCAGTTATCTGACATTCTG			

on, which was found in this and other microbial groups and whose restraint is considered as being one of the greatest challenges of the twenty-first century for science and for medicine, already has some established consequences regarding bacteria-host relationships (Santos *et al*., 2007). Selective antibiotic pressure, associated with their inappropriate use in antimicrobial chemotherapy, as well as their use in the food industry and agropecuary are the key factors in the evolution of resistant strain phenotypes (Hawkey and Jones, 2009).

In this study we investigated the occurrence and distribution of strains of commensal and diarrheagenic *E. coli* present in fecal microbiota from children with and without clinical signs of acute diarrhea in Brazil, as well as their patterns regarding antimicrobial susceptibility.

Materials and Methods

Isolation and identification of bacterial strains

We evaluated 141 fecal samples from children with the presence and absence of clinical manifestations of acute diarrhea (n=84 and n=57, respectively), aged 0 to 5 years old, collected in Juiz de Fora, Brazil. This study was approved by the Committee of Ethics on Research of the Federal University of Juiz de Fora. A brief epidemiological survey was also undertaken to assess the clinical status of each child, how sanitary the living conditions were, and to reject those who had undergone antimicrobial chemotherapy during the last 30 days. The feces, *in natura*, were collected in disposable sterilized bottles containing transport solution (0.25% NaCl, 10% glycerine, and 0.5% agar). Bacterial strains were isolated after selective culture in Eosin-Methylene Blue Agar (Biobras, Brazil). Up to three morphologically distinct colonies were isolated and identified. The presumptive phenotypic identification included growth on Triple Sugar Iron Agar (Isofar, Brazil), indole and urease production, citrate test, the ability of lysine decarboxylation, and motility test. Genotypic species identification was confirmed by the amplification of specific 16S ribosomal RNA encoding DNA. Extraction of bacterial DNA was carried out for all presumptive *E. coli* isolates as previously described (Wani *et al*., 2004). Briefly, 1 ml of overnight cultures of all bacterial strains grown in Brain Hearth Infusion Broth (HiMedia, India) at 37°C were pelleted by centrifugation at 1,200×g for 10 min. The pellet was suspended in 150 µl of sterile distilled water and the bacteria cells were lysed by boiling for 10 min in a water bath. The lysate was centrifuged at 13,600×g at 4°C, for 10 min and the supernatant was kept at -20°C until used as the template for polymerase chain reaction (PCR) as previously described (Chotár *et al*., 2006). The primers *E. coli*-1 (5′-GCTTGACACTGAACATTG AG-3′) and *E. coli*-2 (5′-GCACTT ATCTCTTCCGCATT-3′) were used in a 25 µl reaction containing 25 µM of each primer, 2 µl of the template DNA and 12.5 µl of a ready-to-use PCR mix containing *Taq* DNA polymerase, dNTPs, $MgCl_2$, and buffers, at an optimum concentration for efficient DNA amplification (PCR Master Mix®, Promega, USA). The following amplification conditions were used: initial denaturation at 96°C, 5 min, followed by 30 cycles at 96°C, 1 min; 55°C, 1 min; 72°C, 2 min, and a final extension at 72°C, 8 min. The PCR reactions were performed in duplicate in a thermocycler Techne TC-412 (Therma Cycler, UK). The amplicons were visualized after electrophoresis (1.0% agarose gel in TBE buffer) using an ultraviolet transilluminator (GE Healthcare, UK) after treatment with ethidium bromide (Promega). The amplicon size (660 bp) was estimated with 100 bp Ladder Standard DNA (Promega) as molecular weight marker. Two different strains of *E. coli* were used for quality control (*E. coli* ATCC 35218 and *E. coli* ATCC 11229). The negative control was performed in a reaction without the DNA template.

Genetic characterization of human diarrheagenic *E. coli*

The characterization of human diarrheagenic *E. coli* strains was carried out as previously described in a set of two multiplex PCR reactions (Aranda *et al*., 2004). For the first assay we used the primers *eae*, *bfp*, and EAEC, whereas for the second assay the primers LT, ST, *IpaH*, *Stx1*, and *Stx2* were used (Table 1). Multiplex PCR was carried out in 25 µl reactions containing 2.0 µl of DNA template, the primers and the ready-to-use PCR Master Mix® (Promega). For the first assay the PCR cycling conditions were: initial denaturation 50°C, 2 min and 95°C, 5 min; 40 cycles of 95°C, 40 sec; 58°C, 1 min; 72°C, 2 min and a final extension at 72°C, 7 min. For the second assay the PCR cycling conditions were: initial denaturation 50°C, 2 min and 95°C, 5 min; 40 cycles of 95°C, 45 sec; 50°C, 1 min; 72°C, 1 min and a final extension at 72°C, 7 min. The reactions were performed

Table 2. Susceptibility patterns to antimicrobials from isolated *Escherichia coli* samples from diarrheic and non-diarrheic feces from children under 5 years old in Brazil

Antimicrobials	Acute diarrhea (n=136)			Without diarrhea (n=84)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amikacin	0.0	1.5	98.5	0.0	0.0	100.0
Ampicillin	30.9	1.5	67.6	30.2	2.3	67.5
Ampicillin-sulbactam	8.8	5.9	85.3	2.3	2.3	95.4
Cephalothin	5.9	4.4	89.7	4.6	9.3	86.1
Ceftazidime	0.0	0.0	100.0	0.0	0.0	100.0
Ceftriaxone	0.0	0.0	100.0	0.0	0.0	100.0
Levofloxacin	3.0	0.0	97.0	4.6	0.0	95.4
Gentamicin	0.0	1.5	98.5	0.0	0.0	100.0
Tetracycline	39.7	3.0	57.3	34.9	2.3	62.8
Sulfamethoxazole-trimethoprim	30.9	0.0	69.1	25.6	0.0	74.4
Imipenem	0.0	0.0	100.0	0.0	0.0	100.0
Piperacillin-tazobactam	0.0	0.0	100.0	0.0	0.0	100.0

Resistance (R), intermediate resistance (I), and susceptibility (S)

in duplicate in an automated thermal cycler TC-412 (Techne, UK). In all experiments, the pathogenic *E. coli* reference strains (EPEC, ETEC, EIEC, STEC, and EAEC) were used as positive controls, *E. coli* ATCC 11229 as the negative control, and distilled water as the internal negative control. The amplicons were visualized after electrophoresis in 1.5% or 2% agarose gels as described previously.

Antimicrobial susceptibility assays

The antimicrobial susceptibility assay was performed on Mueller-Hinton agar (HiMedia, India) by the disc-diffusion method and growth inhibition zones were interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2009). The antimicrobial disks (amikacin, ampicillin, ampicillin-sulbactam, cephalothin, ceftazidime, ceftriaxone, levofloxacin, gentamicin, tetracycline, sulfamethoxazole-trimethoprim, imipenem, and piperacillin-tazobactam) were of commercial grade (Laborclin, Brazil). *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as controls.

Results

The fecal specimens were taken to the laboratory and processed immediately, during the period from May 2007 up to December 2008. The mean age of the donors was 26 months old. Approximately 52% of the fecal samples were obtained in the rainy months from December to May, out of which 59% were from patients with acute diarrhea and 41% from healthy children without diarrheic feces. Regarding basic sanitation, 100% of the families had treated water available in their residences and drainage to a septic pit or sewer system.

Considering the microbiological cultures and bacterial identification, strains of *E. coli* were recovered from 80.9% (n=68) of the diarrheic and from 73.7% (n=42) of the non-diarrheic fecal samples. All bacterial strains not identified as *E. coli* were excluded. Despite bacterial detection in fecal specimens from children in all age brackets, there was an increased frequency of recovery (40% and 46%) among children aged between 13 and 24 months in both groups sampled. A total of 220 strains (136 from diarrheic feces and 84 from non-diarrheic feces) were presumptively identified by biochemical tests, and the identity was genetically confirmed for all strains. Viable diarrheagenic *E. coli* was identified at an overall rate of 36.8% (n=50) in diarrheic feces and 29.8% (n=25) in non-diarrheic feces. Enteroaggregative *E. coli* (EAEC) harboring the aggregative adherence plasmid (CVD432) was the only pathogenic group found in 22 diarrheic (16.2%) and 26 non-diarrheic feces (30.9%). Enteropathogenic *E. coli* (EPEC) was identified according to the genotypes eae^{+} $bfpA^{+}$ and eae^{+} $bfpA^{-}$ and accounted for 14 (10.3%) of the detected diarrheagenic strains. Shiga toxin-producing *E. coli* (STEC), identified according to the genotypes $stx1^{+}$ $stx2^{+}$ and $stx1^{+}$ $stx2^{-}$, was found in 7.4% (n=10) and strains with the genotypes elt^{+} est^{+} and elt^{+} est^{-}, considered as enterotoxigenic *E. coli* (ETEC), were found in 2.9% (n=4) of the diarrheagenic strains. Enteroinvasive *E. coli* (EIEC) was not detected.

The results of the antimicrobial drug susceptibility tests are shown in Table 2, and are presented in terms of resistance, intermediate resistance and susceptibility. The drug susceptibility patterns for the quality control strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were in accordance with CLSI (2009). Overall, considering the diarrheic feces, 39.7% of the isolated bacteria were resistant to tetracycline, 30.9% to ampicillin, 8.8% to ampicillin-sulbactam, 30.9% to sulfamethoxazole-trimethoprim, 5.9% to cephalothin and 3% were resistant to levofloxacin. Intermediate resistance was observed against ampicillin-sulbactam (5.9%), cephalothin (4.4%), tetracycline (3%) and amikacin, ampicillin, and gentamicin (1.5%). Antimicrobial resistance was not observed against ceftazidime, ceftriaxone, imipenem or piperacilin-tazobactam. Considering the bacterial strains isolated from the non-diarrheic feces, antimicrobial resistance was observed against tetracycline (34.9%), ampicillin (30.2%), sulfamethoxazole-trimethoprim (25.6%), cephalothin and levofloxacin (4.6%), and ampicillin-sulbactam (2.3%). Intermediate resistance was also detected against cephalothin (9.3%), ampicillin, ampicillin-sulbactam and tetracycline (2.3%). The most efficient antimicrobial drugs were amikacin, ceftazidime, ceftriaxone, gentamicin, imipenem and piperacillin-tazobactam, for which no microbial resistance was detected.

With regard to the multi-drug resistance phenomenon,

Table 3. Distribution of the antimicrobial resistance phenotypes among the diarrheagenic and non-pathogenic *E. coli* strains isolated from both diarrheic and non-diarrheic feces

Source	Bacteria (n)	Resistant phenotypes observed	Frequency	
			n	%
Diarrheic feces	EAEC (18)	AMP	2	11.1
		TET	2	11.1
		TET, STX	2	11.1
		AMP, AMS	2	11.1
		AMP, STX	2	11.1
		AMP, AMS, TET	2	11.1
		AMP, AMS, STX	2	11.1
		AMP, CEP, STX	2	11.1
		AMP, TET, STX	2	11.1
	EPEC (10)	AMI	2	14.3
		TET	4	28.5
		STX	2	14.3
		AMP, CEP, TET, STX	2	14.3
	STEC (4)	TET, STX	2	20.0
		TET, STX, AMP, AMS, CEP, GEN, LEV	2	20.0
	ETEC (2)	TET, STX	2	50.0
	Non-pathogenic (54)	AMP	6	11.1
		TET	22	40.7
		STX	4	7.4
		TET, STX	2	3.7
		TET, AMP	2	3.7
		AMP, TET, STX	4	7.4
		AMP, AMS, CEP	2	3.7
		AMP, AMS, STX	2	3.7
		AMP, TET, STX, CEP	2	3.7
		AMP, AMS, STX, TET	4	7.4
		AMP, AMS, STX, TET, CEP	2	3.7
		AMP, AMS, STX, TET, CEP, LEV	2	3.7
Non-diarrheic feces	EAEC (16)	AMP	2	12.5
		TET	4	25.0
		TET, STX	4	25.0
		AMP, LEV	1	12.5
		AMP, AMS, TET	2	12.5
		AMP, TET, STX, LEV	2	12.5
	Non-pathogenic (30)	AMP	6	20.0
		TET	8	26.7
		TET, STX	2	6.7
		AMP, STX	2	6.7
		AMP, CEP, STX	2	6.7
		AMP, CEP, TET, STX	8	26.7
		AMP, AMS, CEP, TET, STX	2	6.7

EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E. coli*; STEC, Shiga toxin-producing *E. coli*; ETEC, enterotoxigenic *E. coli*; NP, non-pathogenic *E. coli*; AMP, ampicillin; TET, tetracycline; STX, sulfamethoxazole-trimethoprim; AMS, ampicillin-sulbactam; CEP, cephalothin; GEN, gentamicin; LEV, levofloxacin.

among the bacterial strains isolated from diarrheic feces, 18 (81.9%) of the EAEC, 10 (71.4%) of the EPEC, 4 (60%) of the STEC, 2 (50%) of the ETEC and 54 (62.8%) of the non-pathogenic *E. coli* were resistant to at least one of the antimicrobial agents tested. The same phenomenon was observed among bacterial strains isolated from non-diarrheic samples, where 16 (61.6%) of the EAEC and 30 (50%) of the non-pathogenic *E. coli* showed antimicrobial resistance. Overall, multi-drug resistance was observed against up to six of the twelve antimicrobials tested (Table 3).

Discussion

The average low age of the population studied reflects the elevated frequency of acute diarrhea in children younger than two years of age and the gravity of the disease in this phase of life, which leads to the search for medical care (Nanda-Kumar *et al*., 2008). The fecal specimens were obtained on

rates distributed homogeneously throughout the rainy and dry months of the year. Most cases of diarrhea associated with bacteria occur in rainy and hot months, possibly because elevated temperatures favor bacterial multiplication in the environment and rain contributes to the dissemination of the etiological agent in surface waters (Podewils *et al*., 2004; Quadri *et al*., 2005). Although family incomes were not recorded, all of the families had basic sanitary conditions in their dwellings, such as treated water and a closed sewage system.

Although it is accepted that diarrheagenic strains of *E. coli* have an aggressive potential, the research into such microorganisms is not usually performed in clinical laboratories. Thus, taking into consideration the importance of diarrheal diseases in children, an awareness of the incidence of these pathogens in children under two years of age is relevant in understanding their role in acute diarrhea, particularly in endemic areas (Lopez-Saucedo *et al*., 2003; Long *et al*., 2006). Our results corroborate data in the literature showing that such microorganisms are present in the fecal microbiota of children aged under 2 years old (Estrada-Garcia *et al*., 2009). Although the detection of diarrheagenic *E. coli* was more frequent in fecal specimens from patients with clinical signs of acute diarrhea, it was also observed in the fecal microbiota of children who participated in the study but did not present clinical signs or symptoms of acute diarrhea. Enteroaggregative *E. coli* was detected in the fecal samples of these children, as well as in the fecal specimens of children with clinical signs of diarrhea.

For a long time, the diagnosis of EPEC was based on the identification of serotypes (O:H). Enteropathogenic *E. coli* is currently classified into two sub-categories, typical EPEC and atypical EPEC. While typical EPEC is an important pathogen, the importance of atypical EPEC is still unknown (Franzolin *et al*., 2005; Araújo *et al*., 2007). The classification of samples of EPEC as being typical or atypical is related to the presence of the EAF plasmid which carries the gene *bfp*A. Positive *bfp*A samples are termed typical EPEC and are important pathogens which cause diarrhea in children under two years old in developing countries (Trabulsi *et al*., 2002; Araújo *et al*., 2007). Epidemiological studies in several countries showed that the atypical EPEC strains have become a more frequent cause of diarrhea than typical EPEC (Trabulsi *et al*., 2002; Franzolin *et al*., 2005), and this may also be related to persistent diarrhea (Nguyen *et al*., 2006).

In this study, atypical EPEC was the second most frequently isolated pathotype after the EAEC. In Brazil, it is believed that this decrease in the occurrence of the typical EPEC and the increase in the atypical EPEC as a cause of diarrhea can be related to the improvement of sanitation (Franzolin *et al*., 2005). It is also justified by the fact that typical EPEC is almost exclusively detected in humans, whereas samples of the atypical EPEC can also be detected in domestic animals, which may behave as reservoir for these bacteria (Trabulsi *et al*., 2002; Paula and Marin, 2008).

Enteroaggregative *E. coli* (EAEC) is considered to be an emerging pathogen in Brazil and in other developing countries, associated with acute diarrhea in both children and adults (Huang *et al*., 2006; Weintraub, 2007). However, according to some authors, EAEC is also found in fecal microbiota of children without clinical manifestations of the disease (Huang *et al*., 2006; Weintraub, 2007; Hien *et al*., 2008). Other studies performed in Brazil also reported EAEC as the most frequent pathotype of *E. coli* in children who were both healthy and had diarrhea (Franzolin *et al*., 2005; Bueris *et al*., 2007).

Several studies with stool samples from children under 5 years of age showed similar results to the ones found in our study, where STEC was isolated with a lower frequency than EAEC and EPEC (Bryce *et al*., 2005; Araújo *et al*., 2007; Bueris *et al*., 2007). However, when taking different geographical regions into consideration, the incidence found in our study is still high compared to other Brazilian studies whose reported occurrence values varied from 0.5% to 1.5% (Chotár *et al*., 2006; Araújo *et al*., 2007). Despite the fact that in this study only genotypes *stx1*and *stx2* were evaluated, some authors suggest genotypic characterization of these bacteria based on the detection of *ehxA*, *iha*, and *saa* genes. Strains which carry the *stx2* gene are believed to be commonly associated with more severe illnesses (Oliveira *et al*., 2008). Among the five strains identified in this study, two had genotype $stx1^{-}stx2^{+}$. However, other possible etiologies were not checked in our study, and our goal was not to determine the primary etiologic agent in the cases of acute diarrhea, but the occurrence of such microorganisms.

Enterotoxigenic *E. coli* is an important pathogen related to diarrhea in children under two years old in developing countries, causing about two to three episodes of diarrhea per year in these children and resulting in more than 700,000 estimated deaths each year (Davidson *et al*., 2002). In this study, ETEC was the least frequent group of diarrheagenic *E. coli* isolated from patients with diarrhea, and none were recovered from patients in the control group, as observed by other authors in Brazil (Araújo *et al*., 2007).

Diarrhea caused by enteroinvasive *E. coli* is known to cause symptoms which are similar to those of shigellosis in adults and children. Despite being recognized as a human pathogen, little research has been conducted to identify the risk factors for infection. The lack of epidemiological attention to EIEC is related to the low incidence of this pathogen as a cause of diarrhea in relation to other strains of diarrheal *E. coli*, and, in most studies, the researchers did not report the occurrence of these microorganisms (Vieira *et al*., 2007). The low prevalence of EIEC in our study confirms the reports in the literature, which showed EIEC as having the lowest incidence of the diarrheal *E. coli.*

The observation of bacterial resistance to the tested antimicrobial drugs should be considered in empirical therapy, especially in clinical situations in which the characteristics of this disease could suggest the involvement of *E. coli*. In general, the assessment of all of the strains of *E. coli* isolated and identified in this study, whether commensal or enteropathogenic, showed that the behavior of the isolated microbial strains from diarrheal feces was similar to that observed for the strains from non-diarrheal feces. The finding of intermediate resistance to the antimicrobial drugs is significant and should enforce a regional clinical alert. These microorganisms may represent the bacterial population circulating in the community, and children younger than 2 years old should not be expected to harbor resistant bacteria since they should not have been exposed to these drugs.

Regarding the beta-lactam antibiotics, high levels of resist-

ance to ampicillin (30.9%) were observed, while low levels of resistance to the combination ampicillin-sulbactam (8.8%) were noticed. Such a difference in the susceptibility patterns to ampicillin when associated with the beta-lactamase inhibitor suggests the production of penicillinases as the predominant mechanism of resistance in those microorganisms. In general, all representatives of *Enterobacteriaceae* produce low levels of these enzymes (Qin *et al*., 2008). Moreover, a low resistance to cephalothin was previously found, a spectrum cephalosporin comparable to penicillin (Albert *et al*., 2009). The most effective beta-lactam antibiotics were ceftazidime, ceftriaxone, imipenem and piperacillin-tazobactam. Such results may indicate that the isolated strains of *E. coli* were not extended-spectrum beta-lactamase-producers, since resistance to third generation cephalosporins was not observed (Goyal *et al*., 2009). The results imply that the strains were likely to have originated from the community, which supports the observation of low levels of resistance to such drugs (Erb *et al*., 2007; Dropa *et al*., 2009).

High levels of resistance were observed for tetracycline, as well as intermediate resistance against tetracycline, amikacin, and gentamicin. Gentamicin was the most effective out of these antibiotics. Data in the literature demonstrate high rates of tetracycline resistance in strains of enteric *E. coli*, probably related to the indiscriminate use of such antibiotics (Usein *et al*., 2009). Regarding the aminoglycosides amikacin and gentamicin, low levels of intermediate resistance were found, corroborating data in the literature which suggest a good activity of these antimicrobials against enteric Gram-negative band cells. Moreover, such drugs are considered as antimicrobials used in hospitals, and resistant bacteria originating from the community are not expected (Usein *et al*., 2009).

The levels of resistance observed for trimethoprim-sulfamethoxazole reflect the results from several studies by other authors who demonstrated high rates of resistance towards enteric *E. coli* against this drug. One explanation for this could be its widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhea (Aranda *et al*., 2004; Usein *et al*., 2009).

Regarding the quinolones, low levels of resistance against levofloxacin were observed in this study. The literature has reported varying rates of resistance against both levofloxacin and ciprofloxacin, which can be explained by the high prescription of these drugs in some countries as a treatment for enteric infections caused by Gram-negative bacteria (Livermore *et al*., 2002; Yang *et al*., 2009).

Overall, our results reinforce the importance of the participation of bacterial agents in the etiology of acute diarrhea in children aged 0 to 5 years old. Among the pathotypes, there is an increasing number of EAEC which were isolated from the fecal microbiota of both clinically diseased and healthy children, and of atypical EPEC, which has also shown epidemiological importance. The data from this study draws attention to the importance of notifying diarrheal disease, as well as to the consolidation of prevention programs and health surveillance. The high level of antimicrobial resistance observed in our study raises a broader discussion about the indiscriminate use or misuse of antibiotics and the risks of empirical antibiotic therapy in children of a very young age.

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