Antibiogram of E. coli serotypes isolated from children aged under five with acute diarrhea in Bahir Dar town

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Abstract

Background: Diarrheal disease and its complications remain a major cause of morbidity and mortality in children. The prevalence and antibiogram of *E. coli* as causative agents of diarrhea vary from region to region, and even within countries in the same geographical area.

Objectives: To determine the serotype and antimicrobial susceptibility of *E. coli* in children under-five years of age.

Methods: A cross-sectional study was conducted among 422 children with diarrhea from December 2011 to February 2012. Identification of *E. coli* and antimicrobial susceptibility testing were done following standard procedures.

Results: The overall isolation rate of *E. coli* was 48.3%. Poly 2 sero-groups, poly 3 sero-groups, poly 4 sero-groups and E. coli O157:H7 accounted for 80 (39.2%), 40 (19.6%), 25 (12.3%), and 59 (28.9%) of the isolates, respectively. Poly 2 sero-groups, constituting isolates belonging to enteropathogenic *E. coli* were the most commonly isolated serotypes. *E. coli* exhibited high levels of antimicrobial resistance to ampicillin (86.8%), tetracycline (76%) and cotrimoxazole (76%). Low levels of resistance to ciprofloxacin (6.9%) and norfloxacin (9.3%) were documented.

Conclusion: High prevalence of diarrheagenic *E. coli* compounded by alarming antimicrobial resistances is a serious public health problem. Regular determination of antibiogram and public education are recommended.

Keywords: *E. coli*, antimicrobial susceptibility, diarrhea, Ethiopia

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Introduction

Diarrheal diseases are the cause of almost 3 million deaths annually, mainly among children younger than 5 years of age. Globally 1.3 billion cases of acute diarrhea occur in children below 5 years annually, of which more than 3 million are fatal; 80% of these deaths are in children below 2 years of age[1]. Diarrhea remains the second leading cause of death among children under five globally. One in five child deaths – about 1.5 million each year, is due to diarrhea. Diarrhea kills more young children than do AIDS, malaria and measles combined[2]. In developing countries, diarrhea is one of the main causes of morbidity and mortality in children younger than 5 years of age, with the average number of episodes of diarrhea per child per year within this age group being 3.2. Twenty-one percent of childhood mortality in children younger than 5 years of age in these countries is associated with diarrhea, resulting in 2.5 million deaths per year[3]. The prevalence of diarrhea in children under five years of age is 13% and it accounts for about 20% of deaths[4,5].

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A broad range of microorganisms such as viruses, parasites and bacteria is associated with diarrhea. The bacterial pathogens associated with diarrhea include species of *Shigella*, *Campylobacter*, *Salmonella*, *Escherichia coli* and *Yersinia enterocolitica*. Escherichia coli is one of the leading causes of acute diarrhea in developing countries in children under 5 years old, with significant morbidity and mortality[6].

The prevalence, antibiogram and epidemiological features of *E. coli* as the causative agent of diarrhea vary from region to region around the world, and even between and within countries in the same geographical area[7]. However, the lack of rapid diagnostic methods for the detection of *E. coli* hampers the implementation of empirical treatment regimens based on the epidemiological knowledge of the prevalent agents and the corresponding antimicrobial susceptibility. Data on the prevalence and antimicrobial susceptibility are important information to use while planning and implementing control strategies to reduce diarrhea-based childhood morbidity and mortality in a country. However, in Ethiopia and especially in Bahir Dar, data on the prevalence and antimicrobial susceptibility of *E. coli* in children is scarce. Therefore, this study was conducted to study the prevalence and antimicrobial susceptibility of *E. coli* in under-five children, taking Bahir Dar Town as an indicator.

Materials and methods

Study design and sampling

A cross-sectional study was conducted in two pediatric clinics in Bahir Dar between December 2011 and February 2012. Children under five years of age attending pediatric clinics were considered in the study. Diarrhea was defined as the passage of 3 or more liquid stools in a 24 hour period. A total of 422 stool samples were collected with sterile plastic containers by experienced laboratory technicians. The specimens were transported in ice-box to the microbiology laboratory of Bahir Dar University and analyzed for detection of *E. coli.*

Study population, sample size calculation and sampling

All children under five years of age who visited Arsema and Universal pediatric clinics with acute diarrhea and whose caretakers were willing to participate in the study were included in the study. However, all children who were on antibiotic therapy for two weeks, children having acute diarrhea of more than 14 days and those whose caretakers did not agree to allow samples taken were excluded from this study. A minimum sample size of 384 calculated using single population proportion formula. Assuming 95% confidence interval, 50% prevalence and margical error of 5%8, a 10% contingency (38) was added and the sample size was arrived at as 422. The study subjects were selected using systematic random sampling method. Considering an average monthly diarrheal cases of 230 in each clinic, the estimated total number of diarrheal cases, N, for the study period was N = 4*460 = 1840. To obtain a sample size of 422, the selection interval, K, was calculated using the following formula: K = N/n = 1840/422 = 4.4. Hence, it was decided to include every fourth diarrheal case in the sample.

Inclusion criteria

Children aged less than five years with acute diarrhea or dysentery who visited Universal and Arsema pediatric clinics, whose caretakers were willing to participate in the study, were included as the population of this study.

Exclusion criteria

Children aged above or equal to 5 years, who were on antibiotic therapy for two weeks, children having of more than 14 days and those whose caretakers did not agree to allow samples taken were excluded from this study.

Isolation, identification and serotyping of E. coli

For isolation of *E. coli*, a loop-full of stool sample was mixed with 5 ml of sterile peptone water and streaked on MacConkey Agar and Xylose Lysine Deoxycholate Agar (Oxoid, England). After 24 hours of incubation under aerobic atmosphere at 37°C, nonmucoid rose red colonies on MacConkey Agar and yellow colonies on Xylose Lysine Deoxycholate Agar were identified as E. coli based on colony characteristics. Presumptive *E. coli* colonies were purified and maintained on Tryptic Soy Agar (TSA) slant for biochemical, serology and drug sensitivity tests[9,10].

Presumptive *E. coli* colonies were confirmed with biochemical tests using Sulfide indole motility medium, Kligler iron agar, Lysine deoxycholate agar, Simmons citrate agar and Christensen urea agar (Oxoid, England). Sorbitol MacConkey agar (Oxoid, England) was used to identify *E. coli* serotype O157:H7.

Strains biochemically identified as Escherichia coli were subjected to slide agglutination tests with polyvalent 2, 3 and 4 Escherichia coli agglutinating sera (Remel, Europe). The following typing sera were used: *E. coli* poly 2 (O26:K60, O55:K59, O111:K58, O119:K69, O126:K71), *E. coli* poly 3 (O86:K61, O114:K90, O125:K70, O127:K63, O128:K67) and *E. coli* poly 4 (O44:K74, O112:K66, O124:K72, O142:K86)[11].

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed on Mueller-Hinton (Oxoid, UK) using disc diffusion technique[12]. The antimicrobials tested were: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), tetracycline (30 µg), gentamycin (10 µg), chloramphenicol (30 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and ceftizoxime (30 µg) (Oxoid, UK). Morphologically identical 4-6 bacterial colonies from overnight culture were suspended in 5ml nutrient broth and incubated for 4 hours at 37°C. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. The surface of Mueller-Hinton agar plate was evenly inoculated with the culture using a sterile cotton swab. The antibiotic discs were applied to the surface of the inoculated agar. After 18-24 hours of incubation, the diameters of growth inhibition around the discs were measured and interpreted as sensitive, intermediate or resistant according to clinical and laboratory standards institute[13]. Reference strain of *E. coli* ATCC 25922 was used as quality control for antimicrobial susceptibility tests.

Data analysis

Association between socio-demographic characteristics of the study subjects, clinical data, sanitation and hygiene and also breast-feeding status of the mothers were analyzed using chi-square test, and a p-value of less than 0.05 was considered statistically significant.

Ethical consideration

The study was ethically approved by the institutional ethics review board of Bahir Dar University. Written consent was obtained from parents/guardians of the children before enrolment into the study.

Results

The demographic and clinical characteristics of the children enrolled in the study are shown in Table 1. The age of study subjects ranged from 0-59 months, with a mean age of 16.71 months. Males in this study constituted 239 (56.6 %) and females were 183 (43.4%), with a female to male ratio of 1:1.31.

Table 1. Demographic characteristics of the children/caretakers enrolled in the study, Bahir Dar town, 2012.

Parameters	Frequency (%)	Negative No (%)	Positive No (%)	P value
Age (in months)				
0-5	61 (14.5)	35 (8.3)	26 (6.2)	0.14
6-11	118 (28)	60 (14.2)	58 (13.7)	
12-23	141 (33.4)	80 (19)	61 (14.5)	
24-35	55 (13)	25 (5.9)	30 (7.1)	
36-47	28 (6.6)	13 (3.1)	15 (3.6)	
48-59	19 (4.5)	5 (1.2)	14 (3.3)	
Sex				
Female	183 (43.4)	95 (22.5)	88 (20.9)	0.93
Male	239 (56.6)	123 (29.1)	116 (27.5)	
Residence of the caretaker				
Rural	19 (4.5)	9 (2.1)	10 (2.4)	0.70
Urban	403 (95.5)	209 (49.5)	194 (46)	
Educational status of caretaker				
No-formal education	68 (16.1)	36 (8.5)	32 (7.6)	0.79
Primary completed	47 (11.1)	21 (5)	26 (6.2)	
Secondary completed	169 (40.0)	88 (20.9)	81 (19.2)	
Tertiary completed	138 (32.7)	73 (17.3)	65 (15.4)	

Out of 422 stool samples examined, 204 (48.3%) were positive for *E. coli*. Of these isolates, 80 (39.2%) were poly 2 sero-groups (O26:K60, O55:K59, O111:K58,O119:K69,O126:K71), 40 (19.6%) were poly 3 sero-groups (O86:K61,O114: K90,O125:K70,O127:K63,O128:K67), 25 (12.3%) were poly 4 sero-groups (O44:K74,O112: K66,O124:K72,O142:K86) and 59 (28.9%) were *E. coli* O157:H7 serotype. Poly 2 sero-groups, constituting isolates belonging to enteropathogenic *E. coli* were the most commonly isolated (39.2%) of the serotypes (Table 2).

Table 2. The isolation rate of *E. coli* serotypes in under-five children, Bahir Dar town, 2012

Isolated Sero-groups/serotype	Frequency	Isolation rate (%)
Poly 2 Sero-groups (O26:K60, O55:K59, O111:K58, O119:K69, O126:K71)	80	39.2
Poly 3 Sero-groups (O86:K61, O114:K90, O125:K70, O127:K63, O128:K67)	40	19.6
Poly 4 Sero-groups (O44:K74, O112:K66, O124:K72, O142:K86)	25	12.3
E. coli O157:H7 Serotype	59	28.9
Total	204	48.3

Three hundred and fifty two (83.4%) of the caretakers reported that they wash their hands with soap, and 70 (16.6%) reported that they do not wash their hands with soap after visiting toilet. Most, 378 (89.6%) reported that they have a regular habit of hand-washing with soap before feeding their children. Sanitation and hygiene practices of the caretakers of children are shown in Table 3. In this study, there was a statistically significant association between diarrhoea associated with *E. coli* and boiling of drinking water (p=0.04), washing hands with soap after visiting toilet (p=0.04) and before feeding the child (p = 0.001).

Table 3. Sanitation and hygiene conditions and prevalence of *E.coli*, Bahir Dar town, 2012

Parameters	Frequency No (%)	Positive No (%)	Negative No (%)	*P*-value
Access to a toilet				
No	9 (2.1)	3 (0.7)	6 (1.4)	0.26
Yes	413 (97.9)	215 (50.9)	198 (46.9)	
Hands-washing with soap after visiting the toilet				
No	70 (16.6)	41 (9.7)	29 (6.9)	0.21
Yes	352 (83.4)	177 (41.9)	175 (41.5)	
Hand-washing with soap before feeding your child				
No	44 (10.4)	14 (3.3)	30 (7.1)	0.005
Yes	378 (89.6)	204 (48.3)	174 (41.2)	
Clean the child's feeding utensils				
No	33 (7.8)	15 (3.4)	18 (4.3)	0.45
Yes	389 (92.2)	203 (48.1)	186 (44.1)	
Boiling drinking water				
No	380 (90.0)	192 (45.5)	188 (44.5)	0.16
Yes	42 (10.0)	26 (6.2)	16 (3.8)	
Source of drinking water				
Piped tape water	363 (86.0)	190 (45)	173 (41)	0.40
Private hand dug well	10 (2.4)	7 (1.7)	3 (0.7)	
Untreated surface water	12 (2.8)	5 (1.2)	7 (1.7)	
Bottled water	37 (8.8)	16 (3.8)	21 (5)	

The antimicrobial susceptibility profiles of *E. coli* are shown in Table 4. High rate of antibiotic resistance was documented for ampicillin (86.8%), cotrimoxazole (76%) and tetracycline (76%). However, *E. coli* were susceptible to ciprofloxacin (78.4%) and norfloxacin (80.4%).

Table 4. Antimicrobial resistance profiles of *E. coli* serotypes

	Percentage resistance for				
Antimicrobials tested	All *E. coli* Serotypes (n=240)	*E. coli* O157:H7 (n=59)	Poly 2 Sero-groups (n=80)	Poly 3 Sero-groups (n=40)	Poly 4 Sero-groups (n=25)
Ampicillin	86.8	89.8	87.5	85.0	80.0
Tetracycline	76.0	28.2	80.0	75.0	56.0
SXT*	76.0	74.6	76.3	82.5	68.0
Amoxicillin-clavulanic acid	47.5	42.4	48.8	57.5	40.0
Gentamycin	37.2	42.4	30.0	45.0	36.0
Chloramphenicol	36.2	30.5	36.3	47.5	32.0
Ceftizoxime	24.5	23.7	22.5	27.5	28.0
Norfloxacin	9.3	10.2	8.8	10.0	8.0
Ciprofloxacin	6.9	8.5	6.3	10.0	0.0

SXT* = trimethoprim-sulfamethoxazole

About 180 (88.2%) of *E. coli* isolates were resistant to two or more antibiotic agents, and only 15 (7.4 %) of isolates were sensitive to all antimicrobial agents tested. Moreover, out of the 88.2 % multi-drug resistant *E. coli* isolates, 127 (62.2%) were found to be resistant to four and more antimicrobials. The highest multiple drug resistance in *E. coli* isolates was documented against ampicillin, cotrimoxazole and tetracycline.

Discussion

In this study, *E. coli* was isolated in all age-groups examined, but the isolation rate was high among age category of 6-23 months with the association between age and diarrhea being curvilinear as observed elsewhere[14]. This could be related to the beginning of environmental exposure and increased introduction of solid foods to children whose immune system is still developing. Older children are probably more mobile and playful than younger children. They have a higher chance than children below six months to get diarrhea from hand-contamination, especially while playing in the ground, playing with their toys or other objects, and unknowingly putting their dirty fingers into their mouth. In addition, the risk of ingesting contaminated materials is high, especially in unhygienic environments. The low isolation rate of *E. coli* in children older than 23 months may be associated with the development of immunity or loss of receptors for some specific adhesion molecules[15].

The isolation rate of *E. coli* in the present study was lower than rates reported from Brazil[16] and Costa Rica[17]. However, the prevalence rate recorded in this study was higher than reported in Tanzania[18]. On the other hand, the prevalence rate reported in under-five children with diarrhea in a study conducted in Egypt[19], and the rate reported in a study conducted in Mozambique[20] concur with the observations in the present study. The similarity in these studies might be attributed to closer socio-demographic characteristics and cultural practices of the study subjects and the time when all these studies were carried out.

The highest number of *E. coli* isolated in this study belonged to the poly 2 sero-group followed by poly 3 and poly 4 Sero-groups. The results of the present study are in line with the results of study conducted in South African children with diarrheagenic *E. coli*[21]. Results of a study conducted in southern Iran and Nigeria showed that most of the cases of acute diarrhea were due to Sero-groups of enteropathogenic *E. coli* (EPEC) including O127, O86, O126, O142, O55, O119, O128, and Sero-groups O127 and O86 were more frequently found[22-23]. The prevalence of different Sero-groups of *E. coli* in the present study is lower than the prevalence rate reported from Iran[24] and Nigeria[25]. The World Health Organization recognized 12 most commonly occurring EPEC sero-groups in children as O26, O65, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158[26].

Enteropathogenic *E. coli* (EPEC) is commonly transmitted via the fecal-oral route in a poor hygienic environment[27]. Results of studies carried out in areas with different health and socio-demographic characteristics showed that strains of EPEC were the major cause of diarrhea in developing countries[24]. Results of a study conducted in southern Iran showed that most of the cases of acute diarrhea were due to sero-groups of EPEC including O127, O86, O126, O142, O55, O119, O128, and sero-groups O127 and O86 were more frequently found[22]. The isolation rates of enteric pathogens reported in different studies are related to socio-economic, health, and weather conditions. In Nigeria, stool samples were examined for the presence of enteropathogenic *E. coli* in children between the ages of 0-24 months. Of the total number of specimens examined, most of the specimens were positive to EPEC, and O26, O111, O119, O127, O128, O44, O55, O125, O126, O114 and O142 serotypes were most frequently isolated[23].

In the present study the isolation rate of *E. coli* serotype O157:H7 was 28.9 %, which is lower than the isolation rate documented by Rivas et al.[28] and Rivero et al.[29]. These differences could be due to sampling of children with different types of acute diarrhea, rather than the study being oriented towards bloody diarrhea or to children in contact with hemolytic uremic syndrome (HUS) patients. On the other hand, this study is in line with the finding reported by Perez et al.[17] in Costa Rica. Water used for drinking, undercooked fruits and vegetables, milk from dairy cows and foods contaminated with *E. coli* O157:H7 by cross-contamination during food preparation and by infected caretakers who do not practice good hygiene are the probable sources of *E. coli* O157:H7 in children[30]. The habit of hand-washing with soap after visiting toilet and before feeding the child by the caretakers was significantly associated with

the prevalence of *E. coli* in children. Children whose caretakers do not wash hands with soap before feeding their children were 2.5 times likely to be infected with *E. coli* than those whose care takers wash their hands afterwards (95% CI:1.29-4.88). Similarly children whose caretakers do not have access to toilets had a 2.17 chance of infection with *E. coli* than those who have access to toilets (95% CI:0.53-8.8). Similar studies were reported from different countries[31,32].

This study indicated that *E. coli* isolates showed high resistance rate against ampicillin, tetracycline and cotrimoxzole. This finding is in agreement with the reports from Thailand[14] and Kenya[33]. Similar patterns of antimicrobial susceptibility have been reported[15,16,34] in other studies. These increases in resistance may be attributed to the widespread misuse of these drugs, since some of these drugs are cheap and easily available as compared to fluoroquinolones, so that people can purchase these drugs from the open market without physician's prescription, and some of the drugs are broad-spectrum drugs that are used for a long period of time to treat bacterial diseases. It can also be explained by the indiscriminate antimicrobial usage by the healthcare workers in the region, since culture and sensitivity-testing of clinical specimens is available only in a few setups[35].

The results of this study revealed that *E. coli* are more susceptible to norfloxacin (80.4%) and ciprofloxacin (78.4%) than to drugs commonly used to treat diarrhea caused by *E. coli*, including ampicillin, cotrimoxazole, trimethoprim-sulfamethoxazole and tetracycline. This finding is in agreement with other findings conducted in different regions of developing countries[33,35]. This could mainly be due to high consumption of antibiotics, irrational use, incomplete course of therapy, and self-medication by patients, leading to the development of resistance. In underdeveloped countries, people are used to treating themselves without obtaining prescriptions from physicians[35].

The high antimicrobial resistance among *E. coli* isolates noted in this study limits the safe and effective treatment opportunities principally for children. Under circumstances where resistance to ampicillin, cotrimoxazole and tetracycline is common, appropriate antimicrobial agents for the treatment of diarrhea caused by *E. coli* are limited to fluoroquinolone. Recommendations on the use of fluoroquinolones in children suggest that these agents must remain a therapeutic alternative in selected pediatric conditions[36].

Conclusion

The isolation rate of *E. coli* in this study was high, which is a reflection of poor hygienic practices of the caretakers in the study area. *E. coli* exhibited high rates of antibiotic resistance to ampicillin, cotrimoxazole and tetracycline. Moreover the *E. coli* had high percentage of multiple drug resistance. Norfloxacin and ciprofloxacin could be antibiotics of choice for effective treatment of *E. coli*. Determination of antibiogram before antibiotic prescription for effective treatment and public education are recommended.

Conflict of interest:
None to declare

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