Prevalence and antibiogram of *Shigella* and *Salmonella* spp. from under five children with acute diarrhea in Bahir Dar Town

Maritu Admassu[1], Gebremariam Yemane[1], Mulugeta Kibret[1*], Bayeh Abera[2], Endalkachew Nibret[1] and Melaku Adal[1]

Department of Biology, Science College*, Bahir Dar University, P. O. Box 79, Bahir Dar, Ethiopia

[2]Department of Microbiology, Parasitology and Immunology, College of Medicine and Health Sciences Bahir Dar University, P. O. Box 79, Bahir Dar, Ethiopia

ABSTRACT

Diarrheal diseases remain the major cause of morbidity and mortality in children under five years of age. *Salmonella* and *Shigella* species are among the leading causes of diarrhea in children. The aim of this study was to determine the prevalence and antimicrobial profiles of *Salmonella* and *Shigella* spp. in children less than five years of age with acute diarrhea. A cross sectional study was conducted among 422 children with diarrhea from December 2011 to February 2012. A structured questionnaire was used to collect socio-demographic and clinical data. *I*dentification of *Salmonella* and *Shigella* species and antimicrobial susceptibility tests was done following standard microbiology procedures. The overall prevalence of *Salmonella* and *Shigella* was 7.8% and 9.5 %, respectively. The isolation rates of *S. flexineri*, *S.dysenteri* and *S. boydii* were 18 (45 %), 12 (30 %) and10 (25 %), respectively. The prevalence of *Salmonella* and *Shigella* were not statistically significant along age groups and gender of the children. Most of *Salmonella* and *Shigella* were resistant to ampicillin (> 88.7%) and cotrimoxazole (50%).In contrast, 83.3-89.9% of isolates showed susceptible to norfloxacin, ciprofloxacin and gentamicin. More than 90% of the *Salmonella* and 80% *Shigella* spp. were multiple drug resistant. High prevalence of *Shigella* and *Salmonella* linked with high levels of antimicrobial resistance is a major public health concern in the study area. Continuous surveillance of antimicrobial susceptibility should be done. Ciprofloxacin, norfloxacin and gentamicin appeared to be drugs of choice for empirical treatment of these infections.

Keywords: *Shigella, Salmonella* antimicrobial susceptibility, diarrhea, Ethiopia

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INTRODUCTION

Diarrhea is the leading causes of death among children under the age of five, particularly in the developing world (WHO, 2013), Elias, 2008). Diarrhea kills more young children than AIDS, malaria and measles combined (UNICEF/WHO, 2009). In developing countries, acute bacterial diarrhea is frequently disabling, recurrent, and significantly contributes to malnutrition and death (Rodríguez *et al*., 2011). In Ethiopia, diarrhea accounts to about 20% of death in 0-5 years age (FMoH, 2011). Diarrheal disease affects rich and poor, old and young, and those in developed and developing countries alike, yet a strong relationship exists between poverty, an unhygienic environment, and the number and severity of diarrheal episodes especially for under five children (Keusch *et al*., 2006) . Globally, it is estimated that shigellosis causes about 1, 1 million deaths per year, two-thirds of the patients being children under 5 years of age (Ranjbar *et al*., 2008). Despite scientific advances over the past century, the epidemiological characteristics, virulence and ability to develop drug resistance has led to renewed and increased incidence of *Shigella* (Ranjibar *et al*., 2008). Salmonellosis represents a major communicable worldwide disease problem (Laurent *et al*., 2005). Salmonellae may be present in all kinds of food grown in faecally polluted

*Corresponding author:mulugetanig@gmail.com

environments, and are commonly isolated from poultry and livestock and foods prepared from them. The threat to human health posed by antibiotic resistance is of growing concern.

Studies from developed and developing countries showed that multidrug resistant *Salmonella* and *Shigella* was increasing from time to time (Kariuki *et al*., 2006; Gizachew Yismaw *et al*., 2006). Pathogen occurrence and susceptibility profiles show substantial geographic variations as well as significant differences in various populations and environments (Leegaard *et al*., 2000; Nagal *et al*., 2006). However, few studies have been conducted on the prevalence of these pathogens in Ethiopia (Moges Tiruneh, 2009; Getachew Debas *et al*., 2011). Thus, updated data on *Salmonella* and *Shigella* at the local level is required for appropriate recommendations for optimal empirical therapy. Therefore, this study was conducted to determine the prevalence and antibiogram of *Salmonella* and *Shigella* spp. in under five children in Bahir Dar Town, Ethiopia.

METHODS

Study area

The study was conducted in Bahir-Dar which is the capital of Amhara National Regional State, located northwestern Ethiopia approximately 578 km from Addis Ababa. The town has latitude and longitude of 11^{o}36'N 37^{o}23'E and an elevation of 1840 meters above sea level. The town has a total population of 256,999 (CSA, 2011). This study was specifically conducted at Arsema and Universal Pediatric clinics.

Study design and period

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.

Study population, sample size 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. A minimum sample size of 384 was calculated using single population proportion formula, assuming 95% confidence interval, 50% prevalence and margical error of 5% (Daniel, 1999). A 10% contingency (38) was added and a sample size of 422 was obtained. The study participants were selected using systematic random sampling method. Considering average monthly diarrhea cases of 230 in each clinic, the estimated total number of diarrheal cases, N, for the study period was, 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. Hence, it was decided to include every fourth case in the sample.

Inclusion criteria

Children aged less than five years with acute diarrhea who visited Arsema and Universal pediatric clinics whose caretakers were willing to participate in the study were included.

Exclusion criteria

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

Sample Collection

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 *Salmonella* and *Shigella* spp. A pre-tested structured questionnaire was used to collect socio-demographic characteristics and clinical data of the children.

Culture and identification

Stool samples were enriched in Selenit F broth for 8 hours prior to inoculating into MacConkey agar and Xylose-lysine deoxycholate (XLD) agar (Oxoid, England). The plates were incubated under aerobic atmosphere at 37°C and examined after 24 hours. Typical colorless colonies on MacConkey agar and pink to red colonies on XLD agar were picked and further identified through a series of biochemical tests as per standard method (Collins and Lyne, 2004; Cheesbrough, 2006). Serological identification of *Shigella* species was performed by slide agglutination test (Cheesbrough, 2006). The antisera used for *Shigella* serotypes identification were: *S. boydii* polyvalent 1(1-6), *S boydii* polyvalent 2(7-11), *S. boydii* polyvalent 3(12-15), *S. dysenteriae* polyvalent (1-10), *S. flexineri* polyvalent (1-6, X & Y), and *S. sonnei* (phase 1 and 2) (Oxoid, England).

Antimicrobial susceptibility testing

In Vitro antimicrobial susceptibility testing was performed for 33 *Salmonella* and 40 *Shigella* isolates on Mueller-Hinton Agar (Oxoid, England) using disk diffusion technique (Bauer *et al*., 1966). The antimicrobials tested were: ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), tetracycline (30µg), gentamicin (10µg), chloramphenicol (30µg), norfloxacin (10µg), ciprofloxacin (5µg), 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 on the surface of the inoculated agar. After 18-24 hours of incubation, the diameter of growth inhibition around the discs were measured and interpreted as sensitive, intermediate or resistant according to Clinical and Laboratory Standards Institute (CLSI, 2011). Reference strain of *E. coli* ATCC 25922 was used as quality control for antimicrobial susceptibility tests.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences version 20 software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Association between socio-demographic characteristics of the study participants, some clinical data and prevalence of *Salmonella* and *Shigella* 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 child before enrolment into the study.

RESULTS AND DISCUSSION

A total of 422 children (239 males and 183 females) aged 0-59 months with acute diarrhea were enrolled in the study. Of the 76 children below six months, 32 were exclusively breastfed. Two hundred and eighty one (66.6 %) children exhibited non bloody diarrhea followed by watery diarrhea in 134 (31.8%) while 7 (1.6%) had bloody diarrhea (Table 1).

Both *Salmonella* (2.6%) and *Shigella* (2.9%) were highly prevalent in children of age category 6-11 months. However, the differences were not statistically significant along age groups and gender of the children (Table 1). Non-bloody mucoid was the most able1. Socio-demographic characteristics and clinical data of the study subjects and prevalence of *Shigella,* spp. and *Salmonella* at Bahir Dar Health, Ethiopia, 2012 (n= 244)

Variables	*Salmonella*			*Shigella*		
	Positive No (%)	Negative No (%)	P value	Positive No (%)	Negative No (%)	P- value
Age (months)						
0-5	8 (1.9)	53 (12.6)	0.2	7 (1.7)	54 (12.8)	0.17
6-11	12 (2.9)	106 (25.1)		11 (2.6)	107 (25.4)	
12-23	8 (1.9)	133 (31.5)		5 (1.2)	103 (24.4)	
24-35	3 (0.7)	52 (12.3)		2 (0.5)	31 (7.3)	
36-47	0 (0.0)	28 (6.6)		4 (0.9)	30 (7.1)	
48-59	2 (0.5)	17 (4.0)		11 (2.6)	57 (13.5)	
Sex						
Male	15 (3.5)	224 (53.1)	0.2	16 (3.8)	167 (39.6)	0.65
Female	18 (4.3)	165 (39.1)		24 (5.7)	215 (50.9)	
Exclusive breast Feeding (0-6 months)						
Yes	1 (1.3)	31 (41.9)	0.02	8 (10.7)	67 (89.3)	0.72
No	9 (12.2)	35 (44.6)		0 (0.0)	1 (1.3)	
Nutritional status						
Normal	1 (0.2)	69 (16.4)	0.02	32 (7.6)	317 (75.1)	0.63
Malnourished	32 (7.6)	320 (75.8)		8 (1.9)	65 (15.4)	
Type of diarrhea						
Watery	6 (1.4)	128 (30.4)	0.27	13 (3.1)	121 (28.7)	0.01
Bloody	0 (0.0)	1 (0.2)		1 (0.2)	-	
Non bloody	27 (6.4)	254 (60.2)		25 (5.9)	256 (60.7)	
Bloody mucoid	0 (0.0)	6 (1.4)		1 (0.2)	5 (1.2)	

common form of diarrhea observed in 281 (66.6%) children, of whom 25 (5.9%) were positive for *Shigella*. Statistically significant association was found between appearance of diarrhea and prevalence of *Shigella* (p=0.01). This result is in line with the finding of Jafari *et al*. (2008).

There was statistically significant association between breast feeding practice of the mothers (p=0.02) and nutritional status of the children (p = 0.02) and the prevalence of *Salmonella*. This finding is in agreement with the findings of Rowe *et al*. 2004 and Vesta *et al*. (2010). Most mothers nowadays are advised to feed their babies with breast-milk only and not give their babies supplementary food items until the age of 6 months (Abdullahi, 2010).

As shown in Table 2, the prevalence of *Salmonella* species was 33 (7.8%). This study is in agreement with the study conducted in Ethiopia (Getnet Beyene *et al*., 2011) however it is higher than the prevalence of *Salmonella* species in Saudi Arabia (3.3%) (Johargy *et al*., 2010) but it is lower than 15.4% prevalence in Jimma, Ethiopia (Abebe Mache *et al*., 1997). Nagal *et al*. (2006) found out that the isolation rate of *Salmonella* species varies from time to time and from place to place and show seasonal variation Among a total of 40 *Shigella* spp. isolated, 18 (45%), 12 (30 %) and 10 (25%) were *S. flexineri, S. dysenteri and S. boydii*, respectively (Table 2). This result is in line with the finding reported from Central African Republic (Manirakiza *et al*., 2010). This result is lower compared to that reported from Ethiopia (Moges Tiruneh, 2009) and Botswana (Urio *et al*., 2011), however the prevalence recorded in this study is higher than the prevalence reported from Cameroon (Yongsi, 2008) and Tanzania (Moyo *et al*., 2011).

Table 2. Proportion of *Shigella* serotypes isolated from under five children with acute diarrhea, Bahir Dar, 2012.

Bacterial Isolates		Number (%)
Shigella spp.		40 (9.5%
	S. flexineri	18 (45 %)
	S.dysenteri	12 (30 %)
	S. boydii	10 (25 %)
Salmonella spp.		33 (7.8 %)

The antimicrobial susceptibility results of *Salmonella* isolates are shown in Figure 1. The highest resistance was documented for ampicillin (93.9%) followed by amoxicillin-clavulanic acid (75.8%) which is in agreement with reports from Harrar, Ethiopia (Ayalu Reda *et al*., 2011). This increased resistance to antibiotics might be due to the unwise use of antibiotics in many developing countries such as Ethiopia, which would have led to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries (Daniel Asrat, 2008). On the other hand, in this study, the isolated *Salmonella* species tested showed susceptibility to ciprofloxacin and norfloxacin, (93.9% each) followed by gentamicin (87.9%). This result is in agreement with different studies reported from Ethiopia (Ayalu Reda *et al*., 2011; Daniel Asrat, 2008; Assefa *et al*., 1997), England (Hopkins *et al*., 2006), France (Wiell *et al*., 2006) and China (Cui *et al*., 2009).

The antimicrobial resistance profiles of *Shigella* spp. is shown in Table 3. *Shigella* isolates showed high resistance rates to ampicllin, amoxycillin-clavulanic acid and tetracycline. However, *Shigella* isolates were highly sensitive to ciprofloxacin, norfloxacin, and gentamicin. The most predominant isolates of *Shigel-*

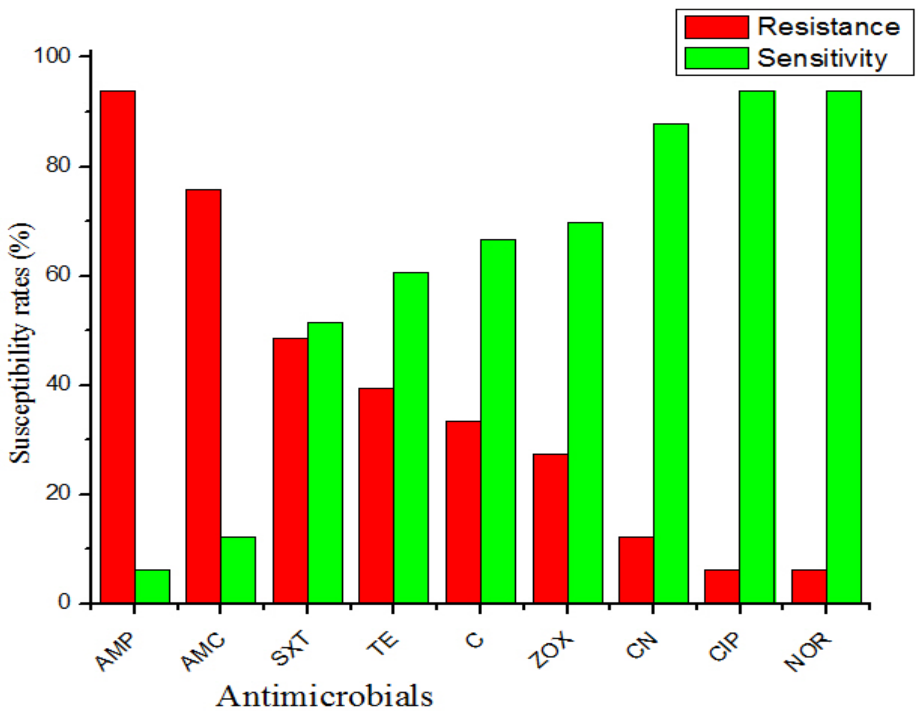

Figure 1: Antimicrobial resistance patterns of *Salmonella* spp. isolated form under-five children with acute diarrhea, Bahir Dar 2012.

AMP;-Ampicillin; AMC- amoxicillin/clavulanic acid;SXT- Trimethoprim-sulfamethoxazole TE-tetracycline; C- chloramphenicol; ZOX- ceftizoxime; CN- gentamicin; CIP- ciprofloxacin; and NOR- norfloxacin.

Table 3: Antimicrobial resistance profiles of *Shigella* isolates from under five children with acute diarrhea for commonly used antibiotics.

Antimicrobials tested	*S.flexineri* (n=18)	*S.dysenteri* (n=12)	*S.boydii* (n=10)
Ampicillin	88.7	91.7	90.0
Amoxycillin-clavulanic acid	77.7	58.3	50.0
Chloramphenicol	61.1	16.7	10.0
Ceftizoxime	33.3	25.0	20.0
Ciprofloxacin	11.1	0.0	10.0
Trimethoprim-sulfamethoxazole	72.2	33.3	20.0
Gentamicin	16.7	8.3	0
Norfloxacin	16.7	8.3	10.0
Tetracycline	88.9	25.0	50.0

la spp. *S. flexneri*, were more resistant to amoxycillin-clavulanic acid and tetracycline than *S. dysentery* and *S. boydii* and these finding concords with results reported in Ethiopia (Belay Roma *et al*., 2000).

More than 90.9% of *Salmonella* isolates were multidrug-resistant (Table 4). The multiple antimicrobial resistances of *Salmonella* isolates in the present study are higher compared to the reports from other (Fadlalla *et al*., 2012; Okeke *et al*., 2005). This might be because multiple antibiotic resistant *Salmonella* species increase from time to time (Nagal *et al*., 2006) and differ from place to place (Kariuki *et al*., 2006). Over 80% of the isolates were resistant to two or more antimicrobials and none of the strains were sensitive to all antimicrobials tested (Table 4). High resistance rates to ampicillin, amoxycillin-clavulanic acid and tetracycline are supported by a number of studies in Ethiopia (Gizachew Yismaw

Table 4: Multiple antimicrobial resistance patterns of bacterial isolates

Antibiogram	Bacteria	
	Salmonella spp.	*Shigella* spp.
R0	6.1	0.0
R1	3.0	12.5
R2	33.3	17.5
R3	12.1	17.5
R4	18.2	7.5
R5	9.1	22.5
R6	12.1	15
R7	6.1	0.0
R9	0.0	2.5

R0 = susceptible to all; R1, R2, R3, R4, R5, R6, and R7, R9 resistant to 1, 2, 3, 4, 5, 6, 7 and 9 antimicrobials tested respectively.

et al., 2006; Moges Tiruneh, 2009) and reported in other parts of the world (Mandomando *et al*., 2009; Kavaliotis *et al*., 2002; Peridano *et al*., 2006).

CONCLUSION

The prevalence of *Shigella* and *Salmonella* were high in the study area. High prevalence and alarming rate of resistance to commonly prescribed antimicrobials is a serious public health problem in the study area. Exclusive breastfeeding and improved nutrition should be promoted. Continuous surveillance of antimicrobial susceptibility patterns should be done for choosing antimicrobials for empirical treatment. Ciprofloxacin, norfloxacin and gentamicin can be drugs of choice for empirical treatment of infections caused by these pathogens. This study has limitation because examination of stool alone does not ensure diagnosing typhoid fever caused by *Salmonella*.

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