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Available online at www.ijit.net**Research Article****PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPECIES IN DIARRHEAL CHILDREN UNDER FIVE-YEARS OF AGE IN BAHIR DAR TOWN, ETHIOPIA****GEBREMARIAM YEMANE*, GUESH MULAW, TSIGE GAIM***Department of Biology, College of Natural and Computational Sciences, Aksum University, Axum, Ethiopia.***Corresponding author: guesh2001@gmail.com***ABSTRACT**

Diarrheal disease and its complications remain a major cause of morbidity and mortality in children, especially in developing countries including Ethiopia. Salmonellosis is one of the most common and widely distributed food-borne diseases, and presence of antimicrobial resistant *Salmonella* is a global public health problem. This study was aimed at exploring the prevalence and antimicrobial susceptibility of *Salmonella* species in diarrheal children under five-years of age in Bahir Dar town. Cross-sectional prospective survey was conducted among diarrheal children less than five years of age. Out of the total 422 stool samples collected, 33 (7.8%) showed positive results for *Salmonella* species. From the 33 *Salmonella* isolates 29 (87.9%) were *Salmonella enterica* subspecies *arizonae* and 4 (12.1%) were *Salmonella* group-A. *Salmonella* isolates in this study were highly resistant to ampicillin (93.9%) followed by augmentin (75.8%) and trimethoprim-sulfamethoxazole (48.5%). However, the isolates showed high susceptibility to ciprofloxacin and norfloxacin (93.9% each) followed by gentamicin (87.9%). Likewise, the *Salmonella* isolates showed 90.9% of multidrug-resistance. *Salmonella enterica* subspecies *arizonae* were the dominant strains of *Salmonella* isolated from children with acute diarrhea in this study.

KEYWORDS: *Salmonella*, antimicrobial resistant, diarrhea, Ethiopia, children**INTRODUCTION**

Diarrheal disease and its complications remain a major cause of morbidity and mortality in children, especially in developing countries¹⁰. It is the second most common cause of death in children under five years of age worldwide and is responsible for 2.4 million deaths each year. There are approximately 1.5 billion episodes of diarrhea per year worldwide¹⁶. While diarrheal diseases occur worldwide, 90% of diarrheal disease deaths in children under the age of five occur in developing countries. The World Health Organization (WHO) has estimated that over 1.4 billion episodes of diarrhea occur every year in children less than five years of age in Asia (excluding China), Africa, and Latin America. In Africa, diarrheal disease is the 4th leading cause of death in children under the age of 5 years. It causes 701,000 deaths per year¹⁸.

The commonest causes of infectious diarrhea include: viruses, bacteria, protozoa and unidentified and mixed infections¹⁰. The Food-borne Diseases Active Surveillance Network (Food Net) of the Centers for

Disease Control and Prevention (CDC) collects data on the incidence of diarrhea attributable to nine enteropathogens in 13% of the U.S. population (37.4 million people) living in nine states. Of these, the pathogens responsible for the most cases of diarrhea in 2002 were *Salmonella* (16.1 cases per 100,000 population), *Campylobacter* (13.4 cases per 100,000 population), *Shigella* (10.3 cases per 100,000 population), *Escherichia coli* O157:H7 (1.7 cases per 100,000 population), and *Cryptosporidium* (1.4 cases per 100,000 population). *Vibrio*, *Yersinia*, *Listeria*, and *Cyclospora* were reported in less than 1 person per 100,000. Other enteropathogens for which diagnostic testing are readily available includes *Clostridium difficile*, *Giardia*, rotavirus, and *Entamoeba histolytica*. Additional agents of infectious diarrhea for which clinical diagnostic testing is not routinely available include enterotoxigenic, enteropathogenic, enteroaggregative and enteroinvasive strains of *E. coli*, toxin-producing *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, and rotaviruses³².

Most members of the genus *Salmonella* are potentially pathogenic to humans and vertebrates and cause salmonellosis. Salmonellosis is an infection caused by ingesting *Salmonella* in food which is contaminated by faeces of animals or humans. Infected humans and animals shed *Salmonella* into the environment via faeces, and re-infection takes place by ingestion of *Salmonella*-contaminated food and water⁹. *Salmonella* infections exhibit a complex pathogenesis in which numerous virulence genes are involved. These are clustered within *Salmonella* pathogenicity islands⁶. Bacterial diarrheal diseases cause substantial morbidity and mortality in sub-Saharan Africa, but data on the epidemiology and antimicrobial susceptibility patterns of enteric bacterial pathogens are limited³¹.

Although there are a few of studies done on the prevalence and antimicrobial susceptibility of *Salmonella* species in the region, they are not age-specific, and the prevalence and antimicrobial susceptibility of *Salmonella* species is variable from time to time and from place to place. So, updated information on their prevalence and resistance patterns is very important for proper selection and use of antimicrobial agents in a setting. Therefore, the aim of this study was to explore the prevalence and antimicrobial susceptibility of *Salmonella* species in diarrheal children under five years of age in Bahir Dar town.

MATERIALS AND METHODS

Study subjects and area

The study was conducted at two pediatric clinics (Ariseema and Universal) in Bahir Dar. Bahir Dar is the capital of Amhara National Regional State (ANRS), which is located in Northwestern Ethiopia. Children under five years of age who were outpatients/inpatients for the treatment of acute diarrhea in the two pediatric clinics were considered in the study.

Study design

A cross-sectional prospective study was conducted in Bahir Dar from December 2011 to February 2012. The required fecal specimens were collected among the study sites across the 3 months period.

Sample size

In estimating the sample size, the minimum number of the sample size (N) was determined using the statistical formula of sample size calculation¹⁴.

$$N = z^2 p (1-p)/d^2$$

Where N, is the minimum sample size required

z, 1.96 at 95% confidence interval

d, is margin of sampling error tolerated (5% marginal error was used)

p, is an estimate of the prevalence rate for the population, since the overall prevalence of the study

area was not known p was taken to be 50% for the calculation.

$$N = (1.96)^2 0.5(1-0.5)/ (0.05) = 384$$

To minimize errors arising from the likelihood of non-compliance 10% of the sample size which were added to the normal sample 384, therefore, the total sample size become 422.

Specimen collection and sampling

A total of 422 stool samples were then taken from the two pediatric clinics by purposive sampling technique. Stool specimens were collected using a sterile stool cup by the laboratory technicians in each pediatric clinic. The specimens were transported from the site of collection to the site of processing (Bahir Dar University Microbiology Laboratory Room) using an ice-bag within two hours of collection and analyzed for *Salmonella*.

Isolation and identification of *Salmonella* species

The isolation and identification of *Salmonella* was performed at the microbiology laboratory of Bahir Dar University using techniques recommended by International Organizations for Standardization⁷. A loop-full of stool was mixed in 5 ml sterile peptone water. From the homogenized stool sample, a loop full was cultured in to pre-enrichment broth (Selenite Cystine broth) to allow the multiplication of *Salmonella*. After 24 hours of incubation at 37°C a loop full of sample from the pre-enrichment broth was sub-cultured onto *Salmonella-Shigella* agar (S-S agar) and Xylose Lysine Deoxycholate agar (XLD agar) and then incubated under aerobic atmosphere at 37°C for 24 hours. If colorless and red colonies with/without black center growth were present in S-S agar and XLD agar respectively, a colony of bacteria was streaked onto Nutrient agar for purification purpose. After 24 hours of incubation at 37°C under aerobic atmosphere, a single colony of bacteria was taken from the nutrient agar and inoculated into Tryptic Soy agar slant. The slant was incubated at 37°C under aerobic atmosphere for 24 hours. Characteristic *Salmonella* colonies was confirmed biochemically using triple sugar iron agar (TSI) (Oxoid CM0277, Basingstoke, England), Christensen's urea agar (Oxoid CM53, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England), methyl red (MR) (Micromaster Thane, India), and Indole tests (Becton Dickinson, USA)⁷.

Serological identification of *Salmonella* species

Serological identification of *Salmonella* species was performed by slide agglutination test⁷. A commercial kit (polyvalent O and H antisera) were used to agglutinate and serogroup *Salmonella* species by their O and H antigens. The slide was divided into two sections with a wax pencil and one small drop of physiological saline was placed in each test section on the slide. By using a sterile inoculating loop a portion

of growth from the surface of Tryptic Soy agar was removed and emulsified in each drop of physiological saline on the slide. A bent inoculating loop was used to pick a small drop of antiserum and transferred to each of the suspensions from each antiserum (polyvalent O or H). The suspension and antiserum were mixed very well and then the slide was rocked to observe for auto-agglutination (agglutination is more visible if the slide is observed under bright light and against a black background). If the reaction is positive, clumping will appear within 30 to 60 seconds. When positive agglutination reaction was obtained in one of the antisera, the *Salmonella* subgroup was identified, and no further testing with antisera needed to be conducted².

Antimicrobial susceptibility test

The antimicrobial susceptibility testing of *Salmonella* isolates was performed according to Clinical and Laboratory Standards Institute (formerly known as National Committee for Clinical Laboratory Standards (NCCLS)⁸. method using Kibry-Bauer disk diffusion test on Mulle-Hinton agar (Oxoid CM0337 Basingstoke, England). *Eshercia coli* ATCC 25922 was used as a quality control organism for the antimicrobial susceptibility test (Hendriksen, 2002). The antimicrobials tested were ampicillin (AMP, 10µg), augmentin/ amoxicillin-clavulanic acid (AC, 10µg), cefoxitin (CEF, 30µg), tetracycline (TTC, 30µg), trimethoprim-sulfamethoxazole (TXS, 25µg), gentamycin (GEN, 10µg), chloramphenicol (CAF, 10µg), norfloxacin (NOR, 10µg), and ciprofloxacin (CIP, 5µg) (Oxoid)⁷.

Data analysis

Data were analyzed using SPSS version 16.0 software. Relations of different antimicrobial agents with variable outcomes were analyzed using chi-square test. P-value less than 0.05 were considered statistically significant.

Ethical considerations

The study was conducted after obtaining institutional ethical clearance from Bahir Dar University (BDU). After the research purpose was explained, patients' parents and/or guardians gave verbal consent prior to participation in the study.

RESULTS AND DISCUSSION:

Out of the 422 stool samples collected, 33 (7.8%) showed positive results for *Salmonella* species. The 95% confidence interval for the prevalence of *Salmonella* species among diarrheal children under five years was between 5.1% and 10.5%. This study is in agreement with the prevalence of 9.5% *Salmonella* species isolated from the study conducted in children with acute diarrhea, Addis Ababa, Ethiopia²⁹. Similarly, the present study in line with the study conducted in Tikur Anbessa and Jimma Specialized

University Hospital, Ethiopia¹⁷. On the other hand, the prevalence of *Salmonella* species in the present study is higher than the prevalence of *Salmonella* species in Saudi Arabia (3.3%)²² and lower than 15.4% prevalence in Jimma, Ethiopia¹. The reason for the variation might be due to the difference in frequencies of isolation of *Salmonella* species from different clinical sources from time to time and from place to place²⁵.

In line with this, from the total 33 *Salmonella* isolates there were two subspecies observed, *Salmonella enterica* subspecies *arizonae* (*S. arizonae*) and *Salmonella* group-A. About 87.9% (29/33) of the isolates were *Salmonella enterica* subspecies *arizonae*, and the remaining 12.1% (4/33) *Salmonella* isolates were *Salmonella* group-A

Table 1: Distribution of *Salmonella* subspecies isolates (N= 33) from diarrheal children in Bahir Dar, Ethiopia, 2012.

Subspecies	Number of isolates	% of isolates
<i>S. arizonae</i>	29	87.9
S. group-A	4	12.1
Total	33	100

However, these *Salmonella enterica* subspecies *arizonae* are uncommon human pathogens. Even though gastroenteritis and systemic infections occur in immune-compromised adults and young children, most cases are in children less than 5 years of age. However, infants are at a particular risk of this pathogen³⁰. In addition, several studies suggest that snakes and reptiles harbor it and transmit it to humans, resulting in gastroenteritis and systemic infections¹⁹. In particular, rattlesnake meat, capsules and powders have been linked to infection with *S. arizonae*, although other animals or animal products have been implicated, including reptiles, poultry, sheep, dogs, and cats³. So, occurrence of *Salmonella enterica* subspecies *arizonae* dominantly in the present study would probably due to the fact that the study subjects in the present study were children of less than 5 years of age and mostly infants, and the relatively greater exposure of these study subjects to animal contact including cat, dog and chickens. The present finding supported by a literature survey yielded 17 case reports of *S. enterica* subspecies *arizonae* infection, of whom 11 were children including 4 infants²⁵.

The antimicrobial susceptibility pattern of *Salmonella* isolates is shown in Table 2. The highest resistance was documented for ampicillin (31, 93.9%) which is in line with the study conducted in Addis Ababa³⁵ and Harre⁵ followed by augmentin (25, 75.8%) which is

in agreement with the recently reported findings in Harrer, Ethiopia⁵. This might be due to the use of this antibiotic for long period of time in the community because it is relatively cheap and easily available, which in turn encourages the use of the antibiotic without physician's prescription. The isolated *Salmonella* species tested had also resistance against some other antimicrobial agents like trimethoprim-sulfamethoxazole (16, 48.5%), tetracycline (13, 39.4%) and chloramphenicol (11, 33.3%) which is in agreement with studies in Africa, Asia, and South America, which reported high rates of antimicrobial resistance (50% to 100%) to chloramphenicol, trimethoprim-sulphamethoxazole and ampicillin²⁸. This increased resistance to antibiotics might be due to the unwise use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia, which would have lead to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries¹³. However, the present finding contradicts the findings of Assefa *et al.* (1997)⁴, which reported that *Salmonella* isolates from Ethiopia at different times showed that *Salmonella* were susceptible to chloramphenicol, ampicillin, and tetracycline. This might be due to the difference in the study period, because the two studies have more than 15 years difference. Antibiotic resistant *Salmonella* species increases from time to time²³. On the other hand, in this study, the isolated *Salmonella* species tested showed susceptibility to fluoroquinolones (ciprofloxacin and norfloxacin, 93.9% each) followed by gentamicin (87.9%). This result is in agreement with the studies conducted at different times in Ethiopia⁴, in parts of Ethiopia like Gonder¹³, Harrer⁵ and in Sudan¹⁵ and other countries^{21,12}.

Table 2: Susceptibility patterns of *Salmonella* species (N = 33) for commonly used antibiotics

Susceptibility patterns			
Antibiotics	Resistance	Intermediate	Sensitive
Ampicillin	31 (93.9)	0 (0.0)	2 (6.1)
Augmentin	25 (75.8)	4 (12.1)	4 (12.1)
Ciprofloxacin	2 (6.1)	0 (0.0)	31 (93.9)
Chloramphenicol	11 (33.3)	0 (0.0)	22 (66.7)
TXS*	16 (48.5)	0 (0.0)	17 (51.5)
Gentamicin	4 (12.1)	0 (0.0)	29 (87.9)
Norfloxacin	2 (6.1)	0 (0.0)	31 (93.9)
Cefoxitin	9 (27.3)	1 (3.0)	23 (69.7)
Tetracycline	13 (39.4)	0 (0.0)	20 (60.6)

* = trimethoprim-sulfamethoxazole P value < 0.001

As such, fluoroquinolones are the drugs-of-choice to treat the life-threatening salmonellosis²¹. Particularly in Ethiopia these fluoroquinolones and gentamicin may be the drugs of choice for treating salmonellosis⁵.

This result agreed with the study conducted in Ethiopia¹⁷ and Morocco²⁶. Similarly, some of the isolates from children adopted from Ethiopia had decreased sensitivity to ciprofloxacin¹¹. A study carried out in different parts of Ethiopia reported that some *Salmonella* strains were resistant to gentamicin^{29, 13, 17}. The reason for the emergence of resistant *Salmonella* isolates might be due to the use of antibiotics in food animals^{34, 33}. In addition, the extensive use of fluoroquinolones has made fluoroquinolone-resistant *Salmonella enterica* isolates to emerge all over the world^{21, 12}. In the present study an interesting result was observed on the low resistance (39.4%) and high susceptibility of *Salmonella* isolates to tetracycline (60.6%). In general the present study showed a statistically significant difference between the prevalence of resistant and susceptible *Salmonella* isolates to the tested antibiotics (p < 0.001) (Table 2).

Antimicrobial drug resistant *Salmonella* isolates in paediatric clinics, Bahir Dar, Ethiopia is presented in Table 3. About 93.9% of the isolated *Salmonella* species were resistant to one or more antibiotic agents including the commonly used antimicrobial agents like ampicillin, augmentin, tetracycline, and cotrimoxazole. This result is comparable with a study conducted in Sudan, which showed that 93.1% of *Salmonella* serotypes isolated from humans were found resistant to at least one of the tested nine antibacterial agents¹⁵. Similarly this result is supported by other studies^{35, 20}.

Table 3: Antimicrobial drug resistant of *Salmonella* isolates.

Number of drug resisted	Resistant <i>Salmonella</i> isolates	
	No.	% (percent)
R0	2	6.1
R1	1	3.0
R2	11	33.3
R3	4	12.1
R4	6	18.2
R5	3	9.1
R6	4	12.1
R7	2	6.1

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

However, in a study conducted in Morocco, 42% of *Salmonella* isolates were resistant to at least one of the commonly used antimicrobial agents²⁶. This result showed a lower rate of resistant *Salmonella* isolates in Morocco than in the present study. This might be due to a difference in resistance rate of *Salmonella* isolates from place to place and from time to time. Only 2 (6.1%) *Salmonella* isolates out of the total 33

Salmonella isolates tested were susceptible to all of the nine tested antibiotics, and one *Salmonella* isolate was resistant to one tested antibiotic. Eleven (33.3%), 4 (12.1%), 6 (18.2%), 3 (9.1%), 4 (12.1%) and 2 (6.1%) of *Salmonella* isolates were resistant to two, three, four, five, six and seven antibiotics out of the nine tested antibiotics respectively. The high levels of antibiotic resistance might be due to the result of the use of antibiotics in food animals³³.

Multidrug-resistance pattern of *Salmonella* isolates is shown in Table 4. Out of the total 33 *Salmonella* isolates in this study, 90.9% (30/33) were multidrug-resistant. Relatively the rate of multidrug-resistant *Salmonella* isolates in the present study was higher than in the study conducted in Kenya²⁷ and Sudan¹⁵. This might be due to the probability that multiple antibiotic resistant *Salmonella* species increase from time to time²⁸ and differ from place to place²⁵. The highest multidrug-resistance was seen against the commonly used antimicrobial agents, in which resistance to ampicillin and augmentin recorded 75.7% followed by resistance to ampicillin, augmentin and trimethoprim-sulfamethoxazole (33.3%). Comparatively similar result was obtained by Ayalu and his colleagues in Harrer, Ethiopia⁵, by Fadlalla *et al.* (2012)¹⁵ in Sudan and by Pegues *et al.* (2005)²⁸ in Africa, Asia and South American countries. As such, due to high resistance of *Salmonella* species, these commonly used antibiotics mentioned above are occasionally used as alternatives¹². This increased resistance in these antibiotics might be due to the unwise use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia. This has led to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries¹⁵. Surprisingly two *Salmonella* isolates were resistant to seven antibiotics each from the nine tested antibiotics. One of the *Salmonella* isolates was resistant to AMP, AC, CEF, TXS, NOR, TTC, and CEP. The second *Salmonella* isolate was resistant to AMP, AC, CAF, CEP, CEF, TXS, and GEN (Table 4).

Table 4: Multidrug-resistance pattern of *Salmonella* isolates.

Resistance pattern	No (%)
Resistance to two antibiotics	
AMP, AC	25 (75.7)
Resistance to three antibiotics	
AMP, AC, TXS	11 (33.3)
AMP, AC, CEF	6 (18.2)
AMP, AC, TTC	8 (24.2)
Resistance to four antibiotics	
AMP, AC, CAF, TXS	6 (18.2)
AMP, AC, TXS, TTC	7 (21.2)
AMP, CAF, TXS, TTC	9 (27.3)

AMP, AC, CEF, TXS 4 (12.1)

Resistance to **five** antibiotics

AMP, AC, CAF, TXS, TTC 4 (12.1)

Resistance to **six** antibiotics

AMP, CAF, CEF, TXS, GEN, TTC 3 (9.1)

AMP, AC, CAF, CEF, TXS, TTC 1 (3.0)

Resistance to **seven** antibiotics

AMP, AC, CEF, CEP, TXS, NOR, TTC 1 (3.0)

AMP, AC, CAF, CEF, CEP, TXS, GEN 1 (3.0)

Note, AMP = ampicillin; AC = augmentin; CAF = chlorphenicol; CEF = cefoxitin; CEP = ciprofloxacin; TXS = trimethoprim-sulfamethoxazole; NOR = norfloxacin; GEN = gentamycin; TTC = tetracycline.

CONCLUSION

Salmonella enterica subspecies *arizonae* were the dominant strains of *Salmonella* isolated from children with acute diarrhea in this study. The current study revealed the high rate of drug resistance pattern to commonly used antibiotics among the isolated *Salmonella* species.

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