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Prevalence of Antibiotic Resistant *Salmonella* Species and Selected Intestinal Protozoan Parasites in Harar Hiwot Fana Hospital, Ethiopia

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ABSTRACT

The objective of this study was to investigate the prevalence and to track associated risk factors of antibiotic resistant *Salmonella*, *E. histolytica* and *G. lamblia* in Harar, Eastren Ethiopia. A total of 384 stool samples were collected from Harar Hiwot-Fana Hospital and analyzed in Harari Regional Laboratory. The results of the study revealed that 96(25%), 80(20.6%) and 56(14.6%) of the samples were positive for *Salmonella*, *E. histolytica* and *G. lamblia*, respectively. The antimicrobial sensitivity test showed that all (100%) of the *Salmonella* isolates were sensitive to ciprofloxacin while 85% were sensitive to nalidixic acid. Of the 56(14.6%) *Salmonella* isolates, 100, 100, 85 and 71.2% were resistant to ampicillin, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol, respectively. This study indicated that *Salmonella*, *E. histolytica* and *G. lamblia* were prevalent in Harar and this enterophathogens should be considered routinely in the diagnosis of patients with diarrhoea. Moreover, physicians should also prescribe appropriate drugs either after sensitivity testing or in areas where there are no facilities for culturing and they have to refer updated information on local sensitivity patterns.

Key words: Prevalence, *Salmonella*, *E. histolytica*, *G. lamblia*, antibiotic resistance, diarrhoea, entropathogens

INTRODUCTION

Infectious gastrointestinal illnesses cause significant morbidity, mortality and socioeconomic burden worldwide (Guerrant *et al*., 2002). *Salmonella*, *G. lamblia* and *E. histolytica* are the most common aetiological agents of human diarrhoeal diseases worldwide and account for a significant proportion of morbidity and mortality in developing countries (Heyworth, 1992). It is estimated that up to 200 million people are chronically infected with *G. lamblia* globally and 500,000 new cases are reported annually (WHO, 1998). The prevalence of the disease varies from 2-5% in developed and from 20-30% in developing countries (Flanagan, 1992). Amebiasis is also one of the world's most prevalent and fatal infectious diseases. Next to malaria and schistosomiasis, amebiasis ranks

third on the list of parasitic causes of death worldwide (Walsh, 1986). Around 500 million peoples are infected worldwide while, 75,000 die of the disease annually (Walsh, 1986). Salmonellosis causes more disease burden than any other food borne pathogen worldwide. An estimated 93.8 million cases of gastroenteritis caused by *Salmonella* species occur globally each year (Majowicz *et al*., 2010). The other major epidemiological development in Salmonellosis is the emergence of multiple-antibiotic resistant *Salmonella* in the developing countries (Okeke *et al*., 2005).

In Ethiopia, various studies invariantly concluded that diarrhoeal disease is the cause of morbidity and mortality among infants, childrens, adults and elder (WHO, 2005). About 39 million episodes of diarrhoea per year were estimated to occur in Ethiopia out of which 230,000 would be children below 5 years of age and these would result to death (WHO, 2004). Harar is one of ancient and historical towns in eastern Harerge, Ethiopia. In eastern Hararge, particularly in Harar town and the surroundings, risk factors which lead to gastrointestinal infections are common. According to HMIS (2010), annual records of Harar Hiwot Fana Hospital revealed that the incidence of diarrhoeal disease was very high. There is, however, information gaps on major aetiological agents of diarrhoeal diseases in the study area. Therefore, the objective of this study was to determine the prevalence of three entropathogens (*Salmonella*, *E. histolytica* and *G. lamblia*) and evaluate the drug resistance patterns of *Salmonella* isolates from diarrhoeal patients in Hiwot Fana Hospital.

MATERIALS AND METHODS

Description of the study area: The study was conducted in the Harar Hiwot Fana Hospital in Harar town, eastern Ethiopia which is located 525 km to the East from Addis Ababa. It has an altitude of 1850 m above sea level with 596 mm mean annual rainfall and 24.02°C average annual temperature. Hiwot Fana Hospital was selected purposefully for this study due to its high patient influx.

Study design: A hospital based cross sectional study was conducted to determine the prevalence of three entropathogens. Data was collected using a pre-tested structured questionnaire and laboratory based diagnosis. A pre-tested structured questionnaire was used to collect data regarding socio-demographic factors and risk factors of the study subjects. Standard bacteriological and parasitological techniques were used at the Harer Reginal Laboratory (HRL), to detect the presence of *Salmonella* species, *E. histolytica* and *G. lamblia*.

Study population: All patients who came to the Out Patients Departments (OPD) of Hiwot Fana Hospital (HFH) with complaints of diarrhoea were enrolled. In this study, patients less than 15 years of age were considered as children and their stool samples were collected with the help of their parents/care takers.

Sample size determination: Since, there was no previous investigation conducted on the same title in the study area, a p-value of 0.5 was taken to ensure the sample that size was large enough to satisfy the precision and confidence constraints. By taking this into consideration, the sample size for the unknown population was calculated based on the 95% confidence limits and 5% sampling error using a equation described by Hassan (1991):

$$n = \frac{(Z_{\alpha/2})^2 \, P(1-p)}{d^2}$$

Where:
n = No. of sample size
P = Prevalence of *Salmonella* and selected intestinal protozoan parasites
d = Marginal error between the sample and the population
$Z_{\alpha/2}$ = Critical value at 95% certainty (1.96), considering 5% non responsive rate

Therefore, the calculated sample size for this study was 384.

Sampling method: A serial sampling method was used where all patients coming to the OPD with diarrhoeal cases were recruited as they came until the required sample size was reached.

Specimen collection, handling and transport: Before collection of stool samples, patients above 15 years of age and children's parents or care-takers were given orientation on how to take samples. The laboratory technician also provided them with materials to place the stool specimens. In addition, about 2 g of fresh stool samples were collected by the principal investigator and assistant data collectors together from each study subject on the same day of enrolment. Each sample was labeled with the code on the corresponding patients questionnaire. Finally, the collected stool specimens were delivered to the Harari Regional Laboratory which is very close to HFH, without using transport media for analysis on the same day.

Culture and isolation procedures of *Salmonella*: About 1 g of stool sample was added in a tube containing sterile saline solution to prepare a faecal suspension. A loopfuls of feacal suspension was transferred to 9 mL selenite broth and incubated aerobically at 37°C for 18 h. A loopful of faecal suspension from selenite broth was sub-cultured on Xylose Lysine Deoxycholate Citrate (XLD) agar and incubated at 37°C in aerobic incubator for 18 h. Typical colonies with black centres and a lightly transparent zone with a reddish color were considered as presumptive *Salmonella* species. Typical colonies with the above morphology were further confirmed by urease test, triple sugar iron agar, kligler iron agar test and lysine iron agar motility test, indole test and citrate utilization test. *Salmonella* species are urease negative, citrate positive, LIA positive, motile, indole negative and yields a red slope (alkaline) and yellow (acid) butt with/out gas or H_2S production (Cheesbrough, 2006).

Antibiotic susceptibility tests for *Salmonella* isolates: The standard Kirby-Bauer disk diffusion test was used to determine the antimicrobial sensitivity profiles of the *Salmonella* isolates. Broad spectrum antimicrobial agents were chosen since they are frequently prescribed by general practitioners to humans suffering from salmonellosis, after visiting the Hiwot Fana Hospital. These antibiotics include ampicillin, tetracycline, trimethoprim-sulfamethoxole, chloramphericol, nalidixic acid and ciprofloxacin.

Nutrient broth inoculated with *Salmonella* isolates were used to prepare bacterial suspensions that was compared to a turbidity of a 0.5 McFarland standard. Mueller-Hinton agar plates were spread-plated with these bacterial suspensions. Different antimicrobial agent containing disks were placed in intimate contact with the cultures on the inoculated plates. The plates were incubated at 35°C for 18 h. The diameters (mm) of the clear zones of growth inhibition around the antimicrobial disks were measured. Sensitive strains of *E. coli* (Amrical type Culture Collection 25922) were used as a negative control in this experiment. The break points used to categorize

isolates as resistant, intermediate resistant and sensitive to each antimicrobial agent were based on recommendations proposed by the Clinical and Laboratory Standard Institute (NCCLS, 2000).

Detection of *E. histolytica* and *G. lamblia*

Direct wet mount method: The direct wet mount with 0.85% saline solution was prepared in the laboratory and observed for motile trophozoites of *E. histolytica* and *G. lamblia* under light a microscope at 10 and 40X magnifications. Lugol's iodine staining was also used to observe cysts of *E. histolytica* and *G. lamblia*.

Concentration method: A portion of stool samples was processed using the formalin-ether concentration method (Bello, 2002). The stool sample was sieved with cotton gauze and transferred to 15 mL centrifuge tube. Then 8 mL of 10% formalin and 3 mL of diethyl ether were added and centrifuged for 2 min at 2000 rpm. The supernatant was decanted and the residues were transferred to microscope slides and observed under light microscope at 100 and 400X magnifications for the presence of cysts and trophozoites of *E. histolytica* and *G. lamblia* (Bello, 2002).

Statistical analysis: Quantitative data that was generated from questionnaire survey about associated risk factors, socio-demographic factors and clinical features and laboratory data of the three entric pathogens were entered into a computer using statistical package for social science (SPSS) (ver. 12.0) data analyzing software. The Chi-square test was done to associate *Salmonella*, *E. histolytica* and *G. lamblia* positivity with risk factors and observed clinical features. A p-value of <0.05 was considered to indicate statistically significant differences. In the mean time, descriptive statistic cross-tabulation of SPSS 12 version was used to analyze the distribution of study subjects by age and sex and the frequency of positive results under each age and sex categories. Zone of inhibition differences between antibiotics for *Salmonella* isolates was calculated in comparison with the inhibition zone produced by the positive control strain *E. coli* (ATTC25922) and this was used to interpret the antimicrobial resistance of *Salmonella*.

Ethical clearance: First, the proposal was reviewed and approved by the ethical review committee of the College of Health Science, Haramaya University. Institutional consent was obtained through communication with Harari Regional Healthy Bureau before conducting the study. The participation of patients, however, was planned to be purely a voluntary activity and they were clearly informed about the purpose of the research. Issues of confidentiality and anonymity were also maintained.

RESULTS AND DISCUSSION

Distribution of the study population: During a 6 month study period, stool samples of 384 (n = 384) diarrhoeal patients, who attended the Out Patient Department (OPD) of Hiwot Fana Hospital (HFH), were examined for the presnce *Salmonella* species, *E. histolytica* and *G. lamblia*. The distribution of the study population by sex and age is shown in Table 1. Data obtained from the present study is of epidemiological value to the study area and therefore, the criteria to determine age groups was based on Harari Regional Health Bureau data record system on diarrhoeal diseases prevalance, where 0.25-4, 4-14 and $\geq$15 age groups are reported as under five

Table 1: Distribution of the study population (n = 384) by age group and sex examined for the presence of *Salmonella* species, *E. histolytica* and *G. lamblia*

	Male		Female		Total	
Age (years)	No.	%	No.	%	No.	%
0.25-4	96	46.2	80	45.5	176	45.8
5-14	64	30.8	56	31.8	120	31.3
≥15	48	23.1	40	22.7	88	22.9
Total	208	54.2	176	45.8	384	100.0

Table 2: Prevalence of *Salmonella* species, *E. histolytica* and *G. lamblia* among the study population (n = 384) by age and sex

	No. examined		*Salmonella* species		*E. histolytica*		*G. lamblia*	
Age (years) and sex	Frequency	%	Positivity	%	Positivity	%	Positivity	%
0.25-4								
Male	96	46.2	17	17.7	24	25.0	27	28.10
Female	80	45.5	15	18.7	26	32.5	29	36.25
Total	176	45.8	32	18.2	50	28.4	56	31.80
5-14								
Males	64	30.8	9	15.6	15	23.4	8	12.50
Female	56	31.8	7	10.7	15	26.7	8	14.20
Total	120	31.3	16	13.3	30	25.0	16	13.30
≥15								
Males	48	23.1	4	8.3	9	18.7	5	10.40
Female	40	22.7	4	10.0	7	17.5	3	7.50
Total	88	22.9	8	9.1	16	18.8	8	9.00
Total for all age groups	384	100.0	56	14.6	96	25.0	80	20.80

children, young children and adults, respectively. Data obtained in the study revealed that the diarrhoeal diseases in the HFH were more common among individauls in between 3 months to 4 years of age. This is in line with data obtained from most studies worldwide which indicate that children suffer from diarrhoeal diseases more than adults due to a lower immune status (WHO, 2011).

Prevalence of *Salmonella* species, *E. histolytica* and *G. lamblia*: The number and percentage of stool samples that were positive for the different enteropathogens based on culture and microscopic examination are shown in Table 2. In general, a total of 232(60.41%) enteropathogens were detected and/or isolated. Among 60.41% enteric pathogens, the proportion of *E. histolytica* 96(25%) was higher than that of *G. lamblia* 80(20.6%) and *Salmonella* species 56(14.6%). As shown in Table 2, intestinal protozoan parasites (*E. histolytica* and *G. lamblia*) were more frequently isolated than enteric *Salmonella* species.

In the present study, the prevalence of *E. histolytica* reported at 25% was higher than those (8.8-18.5%) reported in previous studies (Getenet, 2008; Abera *et al*., 2010) conducted in Northwest Ethiopia, respectively. These differences imply the endemicity of *E. histolytica* in the study area because of so many associated risk factors of the pathogen. However, the data obtained in the current study is lower than prevalence reports of 38 and 34.2% obtained in a rural area in eastern Ethiopia (Ayalew, 2006) and the Vhembe district, South Africa (Samie *et al*., 2009), respectively. It is, therefore, suggested that differences in the settings and designs used in these

studies may account for the patterns observed. In the current study, a hospital based descriptive cross sectional survey was employed compared to the previous reports that involved community based logitudinal approaches (Ayalew, 2006; Samie *et al*., 2009).

Similarly, high level of giardiasis was observed in this study. Out of the 384 diarrhoeal patients, 80(20.6%) were positive for *G. lamblia*. The finding of this study is higher as compared to 9.3% reported from Addis Ababa pre school children (Taticheff *et al*., 1981), 5.8% reported from Jimma University Hospital and some selected health centers in Addis Ababa (Getenet, 2008), 7.0% reported from Bahir Dar Town, Northwest Ethiopia (Abera *et al*., 2010) and 12.8% reported from Vhembe district, South Africa (Samie *et al*., 2009). The high prevalence, in this study, might be attributed to the endemicity of amebiasis in the study area because of many risk factors which predispose the dewallers in Harar and the sourrounding. However, the finding of this study is in agreement with 21.43% reported from orphanage centers Addis Ababa, Ethiopia (Sintayehu, 2010). One would expect the rate of giardiasis to be higher in developing when compared to developed countries but unfortunately most individuals are usually asymptomatic (Gilman *et al*., 1988). Patients with asymptomatic giardia infections may go unidentified and serve as carriers who potentially transmit the pathogens to healthy individuals (USEPA, 1989).

The proportion of *Salmonella* species (14.6%, 56/384) detected from diarrhoeal individuals in the out-patient unit in this study was comparable with a previous study that was conducted at Jimma University Specialized Hospital. On the contrary, these results were higher than prevalence rates of 2.9% reported in Djibouti (Mikhail *et al*., 1990), 9.2% in Manila, Philippines (Adkins *et al*., 1987), 3.3% in Lagos, Nigeria (Ogunsanya *et al*., 1994) and 4.5-10.9% in Addis Ababa, Ethiopia (Gebre-Yohannes, 1985). This increased prevalence of *Salmonella* in HFH may indicate that poor sanitary practices are highly common among individuals in the study area.

As indicated in Table 2, the enthropathogenic organisms investigated were isolated in all age groups. Moreover, patients below 15 years of age were positive for these enthropathogenic organisms that are associated with diarrhoea. About 18.2 and 13.3% of the *Salmonella* isolates were seen in young children between the ages of 0.25-4 years and adult children of 5-14 years old, respectively. However, 9.1% isolates were seen in patients who were ≥15 years old. The prevalence of *E. histolytica* was also shown to vary between different age groups. Hence, 28.4, 25% positivity was obtained from age groups 0.25-4 years and 5-14 years, respectively. However, 18.8% positivity obtained from age groups ≥5years. The 31.8 and 13.3% positivity of *G. lamblia* from young children and adult children, respectively is higher than the 9.1% positivity from ≥15 year of age catagories (Table 2).

The significant varations in the prevalence of *Salmonella* species, *E. histolytica* and *G. lamblia* based on age groups are similar to other previous studies (Ayalew, 2006; Samie *et al*., 2009). In these studies and the current one, the general trend is that symptomatic enteric infections frequently affect children younger than 15 years of age and incidences decline with age, particularly in developing countries (Ayalew, 2006; Samie *et al*., 2009).

The distribution of *Salmonella* species, *E. histolytica* and *G. lamblia* infection among male and female patients revealed statistically comparable frequency. In the mean time, the prevalence of all three entropathogens were shown comparable result under each age categories (Table 2). This implies that both sexes have equally at risks for acquiring and suffering from *Salmonella*, *E. histolytica* and *G. lamblia* infections in the study area.

Risk factors associated with *Salmonella*, *E. histolytica* and *G. lamblia* infections in the study area: The quantitative data that was generated from questionnaire survey and laboratory

Table 3: Association between risk factors andprevalence of *Salmonella, E. histolytica* and *G. lamblia*

	Salmonella spp.				*E. histolytica*				*G. lamblia*			
Risk factors	Isolates	%	p-value	χ^2 value	Positivity	%	p-value	χ^2 value	Positivity	%	p-value	χ^2 value
Residence												
Urban	24	15.0	0.845	0.38	38	22.8	0.735	0.326	32	20.0	0.734	24.326
Rural	32	14.3			58	26.9			48	21.4		
Educational level												
Literate	24	14.3			43	23.8			32	19.0		
Illiterate	32	14.8	0.884	0.21	53	25.9	0.635	0.226	48	22.2	0.447	0.577
Latrine												
Present	16	8.7	0.02***	9.831	32	17.4	0.001***	10.908	24	13.0	0.001***	12.98
Absent	40	20.0			64	32.0			56	28.0		
Water												
Protected	24	14.3	0.884	0.21	46	24.0	0.637	0.223	32	17.4	0.111	2.538
Unprotected	32	14.8			50	26.1			48	24.0		
Domestic animals												
Present	40	20.0	0.02***	9.831	48	24.0	0.223	0.637	72	36.0	0.000***	58.213
Absent	16	8.7			48	26.1			8	4.3		
Domestic animals house												
Separate	8	6.3	0.01***	10.704	45	19.0	0.447	0.577	8	6.3	0.00***	24.758
Cohabit	48	18.8			51	22.2			72	28.1		
Raw milk												
Used	40	17.9	0.031***	4.626	72	32.1	0.000***	14.629	56	25.0	0.017***	5.659
Unused	16	10.0			24	15.0			24	15.0		
Raw meat												
Used	48	24.0	0.000***	29.712	58	26.0	0.637	0.223	48	24.0	0.111	2.538
Unused	8	4.3			38	24.1			32	17.4		
Raw vegetables and fruits												
Used	48	20.7	0.000***	17.544	70	31.0	0.001***	11.383	64	27.6	0.000***	16.205
Unused	8	5.3			26	15.0			16	10.5		
Use of mass catering establishments for foods												
Used	40	24	0.012***	8.807	72	34.6	0.000***	22.447	56	26.9	0.000***	18.078
Unused	16	14.3			24	14.3			24	10.1		
Consumption of street vended foods												
Used	40	18.5	0.041***	6.393	68	32.3	0.000***	18.963	52	26.9	0.002***	12.409
Unused	16	11.1			28	12.1			28	18.2		

***Significant at $p<0.05$

results showed that infections caused by the three enteric pathogens is significantly associated with the absence of toilet facilities, the consumption of raw milk, consumption of raw vegetables and fruits, the common usage of mass catering foods outlets and the consumption of street vended foods products. Moreover, the possession of domestic animals and the cohabitation with animals were significantly associated with cases of *Salmonella* and *G. lamblia* infections among patients investigated. Statistically significant associations were also found between infection with *Salmonella* and consumption of raw meat (Table 3).

In the present study, there was a significant correlation beteween the absence of laterine and infections with all three pathogens. According to Curtis and Cairncross (2003), there was high association between the risk of contracting salmonellosis, amoebiasis and giardiasis with poor living and housing conditions.

G. lamblia and *Salmonella* infection were significantly associated with the presence and cohabitation of domestic animals. This finding agree with the fact that *G. lamblia* and *Salmonella* are important human and animal pathogens worldwide and animals are the reservoir for these enteric pathogens (Hoelzer *et al*., 2011). Unlike *G. lamblia* and *Salmonella*, *E. histolytica* did not shows association with the presence and cohabitation of domestic animals. The possible explanation for this finding is the potential sources and reservoir for this enthropathogen in human being but not domestic animals.

In the present study, there was a significant association between the consumption of raw milk and the presence of *Salmonella*, *E. histolytica* and *G. lamblia* infections in the patients. Therefore, patients who consumed raw milk were at risk of presneting with salmonellosis, giardiasis and amebiasis. This is similar to a previous finding (Jayarao *et al*., 2006) who reported that in Pennsylvania, the occurance of infections caused by enteric pathogens in humans was as a result of the consumption of raw milk. However, in some studies, the consumption of raw meat and meat products has been identified as the principal cause of increased *Salmonella* gastroenteritis worldwide (Oliveira *et al*., 2002; Haeghebaert *et al*., 2001; Fey *et al*., 2000). The finding of this study is in line with their findings. Both *E. histolytica* and *G. lamblia* revealed statistically insignificant association with consumption of raw meat.

In this study, there was a significant correlation between salmonellosis, gardiasis and amoebiasis with the consumption of raw vegetables and fruits from unhygienic sources (Table 4). Different studies also showed that *Salmonella*, *E. histolytica* and *G. lamblia* are frequently isolated from raw fruits and vegetables (Robertson and Gjerde, 2001).

Reports of food borne diseases outbreaks in various countries have resulted from unhygienic food handling and preparation practices within food establishments (CDC, 2010). Abera *et al*. (2010) reported that 41.1% out of of 384 food handlers working in different food establishments of Bahir Dar Town, Northwest Ethiopia had intestinal parasites and 6(1.6%) were found positive for *S. typhi*. In this study, infection of *Salmonella*, *E. histolytica* and *G. lamblia* were revealed high statistical significance with consumption of food from catering establishments. This may be the implication of poor sanitary condition of mass catering food establishments in the study area.

In this study, the prevalence of *Salmonella*, *E. histolytica* and *G. lamblia* was highly associated with the consumption of street vended foods which is in line with Feglo *et al*. (2004) report from Ghana, Accra. The possible explanation for this finding is street foods in Harar Town, eastern Ethiopia that are sold under unhygienic conditions with limited access to safe water, sanitary services, or garbage disposal facilities.

As indicated in Table 4, the statistical analysis of residence, educational level and water with contracting salmonellosis, giardiasis and amebiasis was revealed insignificant correlation.

Table 4: Proportion of resistant, sensitive and susceptible *Salmonella* isolates (n = 56) to six different antibiotics

Antibiotics tested against *Salmonella* isolates	No. of resistant isolates	%	No. of intermediate resistant isolates	%
Ampicillin	56	100.0	0	0.00
Tetracycline	56	100.0	0	0.00
Chloramphenicol	40	71.4	6	10.70
Co-trimoxazole	48	85.7	4	7.14
Nalidixic acid	6	10.7	2	3.50
Ciprofloxacin	0	0.0	0	0.00

Table 5: Clinical symptoms and their association with positivity of *Salmonella*, *E. histolytica* and *G. lamblia*

	Salmonella				*E. histolytica*				*G. lamblia*			
Clinical symptoms	isolates	%	p-value	χ^2 value	positivity	%	p-value	χ^2 value	positivity	%	p-value	χ^2 value
Fever												
Present	32	9.7	0.001***	13.530	40	29.4	0.139	2.186	32	23.5	0.928	0.335
Absent	24	23.5			56	22.6			48	19.4		
Vomiting												
Present	40	23.3	0.02***	12.671	46	13.3	0.640	0.219	24	20.0	0.876	0.73
Absent	16	11.2			50	30.3			56	21.2		
Abdominal pain												
Present	48	16.7	0.000***	4.014	80	27.8	0.029***	4.741	72	25.0	0.000***	12.126
Absent	8	8.3			16	16.7			8	8.3		
Duration of dairrhoea												
1-5	40	25.0	0.000***	23.892	32	28.6	0.000***	28.343	24	18.3	0.001***	13.974
6-10	8	7.1			56	35.0			40	26.0		
11-15	8	7.1			8	7.1			16	14.3		
Consistence of stool												
Watery	16	15.4			20	15.3			32	33.3		
Bloody	24	25.0	0.004***	13.558	46	48.3	0.000***	87.967	8	8.3	0.000***	18.882
Mucoid	8	8.3			8	7.7			24	23.1		
Mixed	8	9.1			22	20.3			16	18.2		
Total	56	14.6			96	25.0			80	20.6		

***Significant at $p<0.05$

Clinical features: The clinical features of *Salmonella* infections commonly present with bloody and/or watery diarrhoea, fever, head ache and abdominal cramping (Hohmann, 2001) which are similar to the findings of this study where abdominal pain and bloody and/or watery diarrhoea were the dominant symptoms of culture positive cases. The incubation period for *Salmonella* gastroenteritis is typically from 12-72 h (Hohmann, 2001) which is in agreement with this study where the duration of diarrhoea (before visiting HFH) was between 1-5 days in the majority of patients (Table 5).

Person who ingested *E. histolytica* cysts most of the time may not have any symptoms at all and may function only as carriers and spreaders, contaminating the areas where they go. The disease symptoms usually start after a period of 7-15 days of infection which is called the incubation period (Petri and Singh, 1999) which is in agreement with this study where the duration of diarrhoea (before visiting HFH) was between 6-10 days in majority of patients. Bloody diarrhoea and abdominal pain are the major symptoms of amoebiasis (Petri and Singh, 1999) which is similar to our study where abdominal pain and bloody diarrhoea were the dominant symptoms of *E. histolytica* positive patients.

Person who ingested *G. lamblia* cysts may develop acute or chronic diarrhoeal illnesses in which the symptoms occur in 1-2 weeks (average 7 days) after swallowing the cysts which is in agreement with this study where the duration of diarrhoea (before visiting HFH) was between 6-10 days in majority of patients. Watery stool and abdominal cramping are the most common clinical manifestation of giardiasis (Petri and Singh, 1999) which is in line with this study where abdominal pain and watery diarrhoea were the dominant symptoms of *G. lamblia* positive patients.

Test for antibiotic susceptibility of *Salmonella* isolates: Among 56 *Salmonella* isolates, 56(100%), 56(100%), 48(85.7%), 40(71.2%) and 6(10%) were found to have developed resistance for ampicilline, tetracycline, trimethoprim-sulphamethoxazole (Co-trimoxazole), chloramphenicol and nalidixic acid, respectively. None of the isolates were, however, resistant to ciprofloxacin. Intermediate susceptibility was found only in 6(10.7%), 4(7.14%) and 2(3.5%) of the tested isolates against chloramphenicol, trimethoprim-sulfamethoxazole and nalidixic acid, respectively. Furthermore, 56(100%), 48(85.7%), 10(17.8%) and 4(7.14%) tested isolates were susceptible to ciprofloxacin, nalidixic acid, chloramphenicol and trimethoprim-sulphamethoxazole, respectively.

In this study, the prevalnce of resistant *Salmonella* isolates to tetracycline, ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol is much higher than previous studies reported from Addis Ababa, Ethiopia (Gebre-Yohannes, 1985). However, these observations are comparable to recent studies from Addis Ababa and Northwest Ethiopia (Mache *et al*., 1997). The percentage of isolates resistant to ampicillin, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol in the present study was also higher than those reported from Brazil, where only 88.8, 86.4, 56.8 and 55.3% of the isolates were found to be resistant, respectively (Ali *et al*., 2003). A comparable result in trimethoprim-sulfamethoxazole and chloramphenicol resistance was reported from Pakistan (Ali *et al*., 2003) where 86.8 and 70.1% out of 54 *Salmonella* isolates were resistant to this drug, respectively. In contrast, all were found to be resistant trimethoprim-sulfamethoxazole in a study at Mollorca, Spain during the period 1987-1991 (Reina *et al*., 1994).

The high resistance to ampicillin (100%), tetracycline 100%), trimethoprim-sulfame thoxazole (85.7%) and chloramphenicol (71.2%) in this study might be due to misuse of these drugs because of their easy access and affordability to the public.

In this study, however, ciprofloxacin and nalidixic acid were found still to have high potency against *Salmonella* isolate in the study area, where all *Salmonella* isolates and 85% of *Salmonella* isolates were shown susceptibility to ciprofloxacin and nalidixic acid, respectively. The possible explanation for this finding is may be ciprofloxacin and nalidixic acid are not frequently and unnecessarily prescribed or sold over the counter in the open markets and private pharmacies without prescription. Since these drugs are not easily affordable everywhere in hospitals and private pharmacies and in the market, people have no easy access to ciprofloxacin and nalidixic acid to purchase. This finding is in line with Cuong (2005) who conducted a study on the prevalence and risk factors associated with antibiotic resistance of bacteria from diarrhoeal patients in Bac Ninh Hospital Northern Vietnam. Cuong (2005) concluded that the cheapest and easily affordable drugs like ampicillin, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol are widely utilized in the community with or without prescription by health personnel and as a result, the selective pressure of these commonly used antibiotics on the bacteria circulating in the community could have resulted in high frequency of resistant pathogenic bacteria of which *Salmonella* is the one.

CONCLUSION

The findings of this study indicated that *Salmonella*, *E. histolytica* and *G. lamblia* are important enteropathogens prevalent in young children (0.25-4 years of age) and adult children (4-14 years of age) followed by adults (15 and above years of age). Therefore, these entropathogens should receive significant attention in the diagnosis and control of diarrhoeal disease caused in the study area. The associated risk factors in contracting these pathogens were found to be lack of latrine, possession and cohabitation with domestic animals, raw milk consumption, consumption of

raw meat, consumption of raw vegetables and fruits, consumption of foods from mass catering establishment and consumption of street vended foods. This study has also shown that 100 and 85% of the total *Salmonella*, isolates were sensitive to ciprofloxacin and nalidixic acid, respectively. They were found to be 100% resistant to ampicillin and tetracycline followed by trimethoprim-sulfamethoxazole (85.7%) and chloramphenicol (71.2%). Decision makers should implements awaress creation to the community regarding to the associations between risk factors and contracting *Salmonella*, *E. histolytica* and *G. lamblia*.

Regulatory body should pay due attention to strengthening compliance with good manufacturing practices by mass catering food establishments and to maintaining acceptable sanitary conditions in general and food hygiene in particular. Access to standard sanitary facilities by the general public should recive consideration. Regulatory body should also intervene with the monitoring of the health status of sick food handlers working in food establishments.

Further studies should be made to identify *Salmonella* spp. and serotype level, so that comparison with serotypes isolated from animals/food products could be possible for identifying the sources of infection. Ampicillin, tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole should not be used as adrug of choice for the treatment of enteric *Salmonella* without making sensitivity tests prior to treatment. Since considerable amount of *E. histolytica* were detected, advanced microbiological techniques such as ELISA and PCR should be conducted to differentiate invasive (*E. histolytica*) from non-invasive one (*E. dispar*).

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