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Prevalence of antibiotic resistant *Salmonella* isolates, *Entermoeba histolytica* and *Giardia lamblia* in Harar, Eastern Ethiopia

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The objective of this study was to investigate the prevalence and to track associated risk facters of antibiotic resistant *Salmonella*, *Entamoeba histolytica* and *Giardia lamblia* in Harar, Easthen 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 show 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 indicates 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; 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 two hundred million people are chronically infected with *Giardia lamblia* globally, and 500,000 new cases are reported annually (WHO, 1998). The prevalence of the disease varies from 2 to 5% in developed and from 20 to 30% in developing countries (Flanagan, 1992).

Amebiasis is also one of the world's most prevalent and

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fatal infectious diseases. Next to malaria and schistosomiasis, amebiasis ranks third on the list of parasiticcauses 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 five 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 Health Management Information System (2010), annual records of Harar Hiwot Fana Hospital revealed that the incidenceof 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 apre-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 Hiot 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 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 formula described by Hassan (1991).

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

Where: n = number 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 sample 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)

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

Age group (years)	Male	Female	Total
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%)

and yellow (acid) butt with/out gas or H_2S production (Cheeseborough, 2006).

Antibiotic susceptibility tests for *Salmonella*isolates

The standard Kirby-Bauer disk diffusion test (Bauer et al., 1966) was used to determine the antimicrobial sensitivity profiles of the *Salmonella* isolates. Broad spectrum antimicrobial agents were chosen since they are frequently prescriped by general practitioners to humans suffering from salmonellosis, after visiting the Hiot 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 (in millimeters) of the clear zones of growth inhibition around the antimicrobial disks were measured. Sensitive strains of E*scherichia 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 10X 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 100X and 400X magnifications for the presence of cysts and trophozoites of *E. histolytica* and *G. lamblia* (Bello, 2002).

Data analysis

Quantitative data that were 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 Reginal Healthy Bureau before conducting the study. The participation of patients, however, was planned to be purely a voluntary activity and they were clearly informed that about the purpose of the research issues of confidentiality and anonymity were also maintained.

RESULTS AND DISCUSSION

Distribution of the study population

During a six month study period, stool samples of three hundred eighty four (n=384) diarrhoeal patients who attended the out patient department (OPD) of Hiwot Fana Hospital (HFH) were examined for the presence *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 groupswas based on Harari Regional Health Bureau data record system on diarrhoeal diseases prevalance, where 0.25 to 4, 4 to 14 and ≥15 age groups are reported as under five children, young children and adults, respectively. Data obtained in the study reveals that the diarrhoeal diseases in the HFH were more common among individauls in between three months to four 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

Table 2. Prevalenceof *Salmonella* species, *E. histolytica* and *G. Lamblia* among the study population (n=384) by age and sex.

Age group and sex	Number examined		*Salmonella* species		*E. histolytica*		*G. lamblia*	
	Frequency	%	No. Positive	% Positive	No. Positive	% Positive	Positivity	% Positive
0.25-4 years								
Male	96	46.2	17	17.7	24	25	27	28.1
Females	80	45.5	15	18.7	26	32.5	29	36.25
Total	176	45.8	32	18.2	50	28.4	56	31.8
5-14 years								
Males	64	30.8	9	15.6	15	23.4	8	12.5
Females	56	31.8	7	10.7	15	26.7	8	14.2
Total	120	31.3	16	13.3	30	25	16	13.3
≥15years								
Males	48	23.1	4	8.3	9	18.7	5	10.4
Females	40	22.7	4	10	7	17.5	3	7.5
Total	88	22.9	8	9.1	16	18.8	8	9
Total for all age groups	384	100	56	14.6	96	25	80	20.8

due to a lower immune status (Workmanet al., 2006; 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 these 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, to 18.5%) reported in previous studies (Getenet, 2008; Bayeh et al., 2010) conducted in North West 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 (Dawit, 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, hospital based descriptive crossectional survey was employed compared to the previous reports that involved community based logitudinal approaches (Dawit, 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 preschool children (Seyoum 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, North West Ethiopia (Bayeh 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 dwellers 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 countries 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 (U.S. EPA, 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 (Abebe, 2002). 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 ,1994), and 4.5 to 10.9% in Addis Ababa, Ethiopia (Afeworki, 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 diarrhea. About 18.2 and 13.3% positivity 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 and 25% positivity was obtained from age groups 0.25 to 4 years and 5 to 14 years, respectively. However, 18.8% positivity was obtained from age groups ≥15years.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 4).

The significant variations in the prevalence of *Salmonella* species, *E. histolytica* and *G. lamblia* based on age groups are similar to other previous studies (Workman et al., 2006; Sorokin et al., 2007). In the previous 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 (Workman et al., 2006; Sorokin et al., 2007).

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 was shown to have comparable result under each age categories (Table 2). This implies that both sexes were equally at risks for acquiring and suffering from *Salmonella, E. histolytica* and *G. Lamblia*in fections 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 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 (Tables 3A and B)

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 co-habitation of domestic animals. This finding agrees 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 show association with the presence and cohabitation of domestic animals. The possible explanation for this finding is that the potential sources and reservoir for this enthropathogen is human being but not domestic animals.

In the present study, there was a significant association between the consumption 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 presenting with salmonellosis, giardiasis and amebiasis. This is similar to a previous finding (Jayarao et al., 2006) who reported that in Pennsylvania, the occurence 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 (WHO, 1988; Oliveira et al., 2002; Haeghebaert et al., 2001; Fey et al., 2000). The finding of this study is in line with previous 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 5). Different works 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). Bayeh et al. (2010) reported that 41.1% out of of 384 food handlers working in different food establishments of Bahir Dar Town, North West 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 to have 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 that street foods in Harar Town, Eastern Ethiopia are sold under unhygienic conditions, with limited access to safe water, sanitary services, or garbage disposal facilities.

Table 3A. The association between risk factors and prevalence of *Salmonella*, *E. histolytica* and *G. lamblia.*

Risk factor	*Salmonella* spp.			*E. histolytica*			*G. lamblia*		
	Number of isolates	P-value	X^2 value	No. positive	P-value	X^2 value	No. positivity	P-value	X^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%)		

Table 3B. The association between risk factors andprevalence of *Salmonella*, *E.histolytica* and *G. lamblia*

Risk factor	*Salmonella*			*E. histolytica*			*G. lamblia*		
	Number of isolate	P-value	X^2 value	Positivity	P-value	X^2 value	Positivity	P-value	X^2 value
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%)		

Table 3B. Cont.

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.

Table 4. Clinical symptoms and their association with positivity of *Salmonella, E. histolytica* and *G. lamblia.*

Clinical symptom	*Salmonella*			*E. histolytica*			*G. lamblia*		
	Number of isolates	P-value	X^2 value	Number of positive results	P-value	X^2 value	Number of positive results	P-value	X^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.

Table 5. The proportion of resistant, sensitive and susceptible *Salmonella* isolates (n=56) to six different antibiotics.

List of antibiotics tested against *salmonella* isolates	Antibiotics susceptibility profiles of *salmonella* isolates (n=56)			
	No. of resistant isolates	% of resistant isolates	No. of intermediate resistant isolates	% of intermediate resistant isolates
Ampicillin	56	100	0	0
Tetracycline	56	100	0	0
Chloramphenicol	40	71.4	6	10.7
Co-trimoxazole	48	85.7	4	7.14
Nalidixic acid	6	10.7	2	3.5
Ciprofloxacin	0	0	0	0

S = Sensitive, I = intermediate R = resistance.

As indicated in Table 5, the statistical analysis of residence, educational level and water with contracting salmonellosis, giardiasis and amebiasis revealed insignificant correlation.

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 our 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 to 72 h (Hohmann, 2001), which is in agreement with this study, where the duration of diarrhoea (before visiting HFH) was between one to five days in the majority of patients (Table 6).

Persons 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 wherever they go. The disease symptoms usually start after a period of 7 to 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 diarrhea (before visiting HFH) was between 6 to 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 one to two weeks (average seven days) after swallowing the cysts, which is in agreement with this study where the duration of diarrhoea (before visiting HFH) was between 6 to 10 days in majority of patients. Watery stooland abdominal cramping are the most common clinical manifestation of giardiasis (Petri and Singh, 1999), which is in line with our 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 chloramphe-nicol is much higher than previous studies reported from Addis Ababa, Ethiopia (Afeworki, 1985). However, these observations are comparable to recent reports from Addis Ababa and North West Ethiopia (Mache, 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, 70.1% out of 54 *Salmonella* isolates were resistant to this drug, respectively. In contrast, all were found to be resistant to trimethoprimsulfamethoxazole 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%) andchloramphenicol (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 agnist *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 that 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 Ngo (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. Ngo (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 *Salmonellais* is the one.

Conflict of interest

The authors declare that they have no conflict of interest.

Conclusion

The findings of this research 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 thus 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 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%).

Recommendations

Decision makers should implements awareness creation to the community regarding to the associations between risk factors and contracting *Salmonell*, *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 receive 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* at 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 a drug 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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