Isolation rate and drug resistance patterns of *Shigella* species among diarrheal patients attending at Hiwot Fana Hospital, Harar, Ethiopia.

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ABSTRACT

Over the past decades, *Shigella* species have shown a pattern of steady increase in resistance to antibiotics; and strains of *Shigella* have progressively become resistant to most of the widely used antimicrobial agents and even new antibiotics. A cross sectional study was conducted in Hiwot Fana Hospital, Harar, from February to May 2011 to determine the prevalence of *Shigella* species and their antimicrobial resistance patterns among diarrheal patients. Structured questionnaires were used to collect socio-demographic and other variables from patients/parents or guardians. A total of 384 stool samples were collected and cultured on MacConkey agar and xylose-lysine desoxycholate agar. Of the 384 stool specimens analyzed, 56 (14.6%) proved to be positive for *Shigella* species. All *Shigella* isolates were sensitive to ciprofloxacin while most were resistant to tetracycline (96.4%). Multiple drug resistance as many as to five antibiotics was detected. Lack of education, absence of regular hand washing habit, use of water from unprotected sources and absence of latrine were the risk factors that were associated with contracting *Shigella* species. High prevalence of *Shigella* spp. with multiple antibiotic resistance isolates were observed in this study. Ciprofloxacin may be used as a drug of choice for empirical treatment for *Shigella* infections. Regular, systematic monitoring of diarrheal cases is also needed to identify changes in the prevalence and antimicrobial resistance patterns of *Shigella* species.

Key words: - Drug, Diarrhea, Prevalence, Resistance, *Shigella* spp.

INTRODUCTION

Diarrheal diseases constitute a major burden of disease in the world, especially in low and middle-income countries. Dehydration resulting from diarrhea causes approximately 1.8 million deaths every year. These illnesses are particularly dangerous for young children, who are more susceptible to dehydration and nutritional losses during the episode of acute diarrhea (Ahs *et al*., 2010).

Diarrheal diseases can be caused by many etiological agents, but mainly by Enterobacteriaceae (Paniagua *et al*., 2007). Among the different pathogens responsible for diarrheal diseases, *Shigella* species play an important role in causing inflammatory diarrhea and dysentery (Hui *et al*., 2001). Shigellosis, an acute diarrheal disease, is caused *Shigella* species. Supply of untreated water, poor sanitation, and overcrowding contribute to the spread of shigellosis both by human contacts and supplies of contaminated water (Schroeder and Hilbi, 2008).

Shigellosis is currently an important health problem around the world, occurring predominantly in children younger than five years old, mainly in developing countries (Paula *et al*., 2010). In Ethiopia, as in other developing countries, shigellosis is a common cause of morbidity and mortality, particularly in children (Kahsay Huruy *et al*., 2008). Its prevalence is high

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in tropical and sub-tropical regions of the world, where living standards are very low and access to safe and adequate drinking water and proper waste disposal systems are often very limited, even absent (Moges Tiruneh, 2009).

Over the past decades, *Shigella* species have shown a pattern of steady increase in resistance to antibiotics and strains of *Shigella* have progressively become resistant to most of the widely used antimicrobial agents and even newer antibiotics (Wilson *et al.*, 2006). Recent studies in Gondar and Addis Ababa showed that *Shigella* species have become highly resistant to commonly used antimicrobial agents including ampicillin and contrimoxazole. Besides the temporal changes in the antibiogram of *Shigella* species, it is well known that antibiotic susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively, particularly in developing countries. This warrants for frequent observation on the change in the patterns of antibiogram for this organism (Urvashi *et al.*, 2011). Since the prevalence and patterns of antimicrobial resistance of *Shigella* species in the country vary from one area to another and with time, updating information is very important for the proper selection and use of antimicrobial agents in the country. Thus the objective of this study was to determine the prevalence of *Shigella* species and their antimicrobial resistance patterns among diarrheal patients visiting Hiwot Fana Hospital (HFH), Harar, Ethiopia.

MATERIALS AND METHODS

Study Design

A cross-sectional study was conducted to determine the prevalence and antimicrobial resistance profiles of *Shigella* species among diarrheal patients visiting HFH, Harar from February to May 2011. A questionnaire was administered to patients or in case of minors, to their parents or guardians to gather information in socio-demographic and risk factors including sex, age, educational status, place of residence, source of drinking water, latrine usage, personal hygiene practice and clinical features of the study subjects.

Study Population

The study included all diarrheal patients visiting the outpatient department of HFH. Diarrhea was considered as a condition of having three or more loose or liquid bowel movements per day and dysentery as the presence of blood in the stool (WHO, 2010).

A minimum sample size of 384 was determined using the following formula (Bland, 1989).

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

Where:

n= sample size

P = the estimated prevalence of *Shigella* spp. (i.e. shigellosis) (50%).

$Z_{\alpha/2}$ = the desired level of confidence, i.e.1.96.

d = the acceptable margin of error (0.05)

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

Sampling Method

All diarrheal patients attending Hiwot Fana Hospital Harar, Ethiopia were the sources of population. Diarrheal patients included in the sample were coded and assisted supervised by physicians or nurses in the outpatient department (OPD) to fill the structured questionnaires. Fresh stool specimens were also collected from diarrheal cases at HFH and transported using sterile stool cups to Harari Regional Laboratory (HRL).

Isolation and Identification of *Shigella Species*

For optimal isolation of *Shigella,* two different selective media; MacConkey agar and xylose-lysine desoxycholate (XLD) agar (Oxoid, England) were used. The plates were incubated aerobically at 35-37^{o}C for 18-24 hours. Typical colorless colonies on MacConkey agar and pink to red colonies on XLD agar were picked. The isolates were further identified by a serious of biochemical tests including glucose and lactose fermentation and H_2S production, lysine decarboxylase, motility, indole, mannitol fermentation, and citrate utilization tests. After incubation at 35-37^{o}C for 18-24 hours, the biochemical changes or reactions were determined based on the standard biochemical identification formats for *Shigella* species (WHO, 1999).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was done on *Shigella* species using Mueller-Hinton Agar following the disc diffusion technique (CLSI, 2011). Each isolate was tested for: ampicillin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), gentamicin (10 µg), chloramphenicol (10 µg), and ciprofloxacin (5 µg) (Oxoid, UK). The culture was streaked on Tryptone Soy Agar to obtain pure colonies. After incubation for 18-24 hours at 37^0 C, 4-5 pure colonies were transferred to a tube containing 5 ml sterile normal saline. Turbidity of the broth was equilibrated to match with 0.5 McFarland standards. The diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a caliper and the isolates were classified as sensitive, intermediate, and resistant as recommended by CLSI (2011). The standard reference strain *Escherichia coli* (ATCC 25922) was used as a control for the study.

Data Analysis

The data was analyzed using SPSS version 12 software. The prevalence of *Shigella* species was calculated as percentage. Chi-square was used to assess the association between selected risk factors, and *Shigella* infection. P-values less than 0.05 were considered as statistically significant.

Ethical Considerations

Ethical approval was obtained from the Ethical Review Committee of College of Health Sciences, Haramaya University. In addition, institutional consent was obtained through communications made with Harari Regional Health Bureau before conducting the study. The participation of patients in this study was purely a voluntary activity and their right not to participate was respected. Issues of confidentiality and anonymity were also maintained. Appointment date was given for all participants and appropriate treatment was given freely for those tasted positive.

RESULTS AND DISCUSSION

Prevalence of *Shigella isolates*

Three hundred eighty four stool specimens collected from diarrheal patients were examined to determine the prevalence of *Shigella* isolates. The results are shown in Table 1. Of the total specimens examined, only 56 were positive for *Shigella* isolates giving a prevalence of 14.6% in all age groups combined. This result is comparable with the previous study done at Gondar University Hospital, northwest Ethiopia, which showed that the prevalence of *Shigella* isolates was 15.8% in 2003 and 14.6% in 2005 (Gizachew Yismaw *et al*., 2006) and also the study made by Kashay Hiruy *et al.* (2008) who reported 16.9% prevalence in Gondar. The frequency of isolation of *Shigella* in this study was also higher than the reported 5.8% prevalence from Addis Ababa by Berhanu Andualem and Abera Geyid (2003) and 8.7% from Gondar by Berhanu Andualem *et al*. (2006). The variations may be attributable to the sampling time and the difference in laboratory techniques used.

Table 1: Prevalence of *Shigella* isolates by age and sex of the study participants

Age Group (years)	Male			Female			Both sex		
	No of Examined	No of Positive	%	No of Examined	No of Positive	%	No Examined	No of Positive	%
0-15	101	21	20.7	80	11	13.7	181	32	17.7
16-45	75	7	9.3	98	12	12.2	173	19	10.9
>45	16	1	6.2	14	4	28.6	30	5	16.7
Total	193	29	15	191	27	14.1	384	56	14.6

The prevalence of *Shigella* infection among male and female patients was 15% and 14.1%, respectively. The difference was not statically insignificant (P=0.805) (Table 2). Regarding the age of patients, Moges Tiruneh (2009) reported that 53.3% of the patients who became positive for *Shigella* species were in the pediatric age group (up to 15 years) and the remaining 47% were adults aged 16 years and above. Similarly other studies reported that *Shigella* was isolated most frequently from children 10 years of age in southern Trinidad (Orrett, 2008). Children are most susceptible to shigellosis primarily because of poor resistance, lack of previous exposure, poor personal hygiene, and higher exposure

Table 2: Risk factors associated with *Shigella* isolates among diarrheal patient visited in Hiwot Fana Hospital.

Factors	*Shigella* species		χ^2	Df	P-value
	Positive (%)	Negative (%)			
Age (in years)					
0-15	32 (17.7)	149 (82.3)	3.298	2	0.192
16-45	19 (11.0)	154 (89)			
>45	5 (16.7)	25 (83.3)			
Sex					
Male	29 (15)	164 (85)	0.061	1	0.805
Female	27 (14.1)	164 (85.9)			
Place of residence					
Urban	25 (11.7)	188 (88.3)	3.111	1	0.078
Rural	31 (18.1)	140 (81.3)			
Educational status					
Literate	14 (9.7)	130 (90.3)	4.317	1	0.037*
Illiterate	42 (17.5)	198 (82.5)			
Source of water					
Protected	20 (10.3)	174 (89.7)	5.750	1	0.016*
Unprotected	36 (18.9)	154 (81.1)			
Latrine					
Present	23 (9.8)	212 (90.2)	11.184	1	0.001*
Absent	33 (22.1)	116 (77.9)			
Hand washing habit					
Yes	17 (6.5)	243 (93.5)	41.833	1	0.000*
No	39 (31.5)	85 (68.5)			
Source of meal					
Home	42 (14.6)	245 (85.4)	0.888	2	0.641
Hotel	6 (19.4)	25 (80.6)			
Home and hotel	8 (12.1)	58 (87.9)			

Df= Degree of freedom, *significant at p<0.05

to contaminated environment due to play-related activities (Ahmed, 2010). The fact that the majority of *Shigella* isolates were from the paediatric population as reported in the literature where 70% of all infections occur in children aged less than 15 years (Urvashi *et al*., 2011). This is likely due to delay in humoral responses, poor personal hygiene, higher exposure to contaminated environments and the development of protective immunity secondary to a high level of exposure to the organism early in life in developing countries. Usually as the age increases, the level of antibody tends to increase; this is associated with future protection to infection (Ali *et al*., 2010).

Educational status of diarrheal patients included in this study showed a significant association with increase in shigellosis at 95% confidence interval ($X^2 = 4.317$ and $P = 0.037$). Out of the total positive cases, 75% were illiterate which was significantly higher than the proportion of literate patients (25%). This clearly indicates that education has a great role to reduce the prevalence of shigellosis in the community. The result of this study is in line with those reported from different countries (Ghaemi *et al*., 2007). In contrast, a study conducted in Egypt showed that educational status of patients with diarrhea was not significantly associated with the prevalence of shigellosis (Abu-Elyazeed *et al*., 2004).

In this study, there was a significant association between source of drinking water and the prevalence of shigellosis ($X^2 = 5.75$ and $P = 0.016$). Out of the total positive cases for shigellosis, patients who used protected water sources were at lower risk of contracting *Shigella* than those who used unprotected water sources (Table 2). This can be explained by the high level of contamination of water by *Shigella* from the environment. This finding was in agreement with the studies reported from Bangladesh and Vietnam by Carre *et al*. (2011) and Kelly-Hope *et al*. (2007), respectively. In addition, other studies have shown that inadequately treated water may cause *Shigella* infection in humans and this pathogen was implicated in several waterborne outbreaks in some countries (Saha *et al*., 2009; Ranjbar *et al*., 2010).

Similarly, out of the total positive cases for shigellosis, those patients who did not have the habit of regularly washing their hands before and after meal showed more than a two-fold increased risk for shigellosis compared to patients who were in the habit of washing their hands regularly (Table 2). Hence, regular hand washing seems to reduce the risk of shigellosis significantly ($P = 0.000$). Similarly, other reports indicated that transmission occurs when basic hygiene and hand washing habits are inadequate. Transmission is particularly likely to occur among toddlers who are not fully toilet-trained (CDCP, 2010; Harper, 2011).

The clinical features and their associations with *Shigella* infections among 384 diarrheal patients are summarized in Table 3. In this study, the clinical features of shigellosis ranges from a watery, mucoid, bloody diarrhea to abdominal pain, vomiting and fever. This is in agreement with an earlier report made by Sur *et al*. (2004) and Phalipon and Sansonetti (2007) which indicated that shigellosis is manifested by the passage of loose stools mixed with blood and mucous and accompanied by fever and abdominal cramps

In the present study, from the total positive patients (56) for shigellosis, most of the positive cases have abdominal pain followed by fever and vomiting which accounted 94.6%, 78.5% and 46.4%, respectively. There was significant association between fever and *Shigella* infection ($X^2 = 4.871$ and $P = 0.027$) whereas abdominal pain and vomiting were not significantly associated with *Shigella* infection (Table 3). There was also a significant association between consistency of stools and sample positivity for *Shigella* (X^2 = 8.871 and P = 0.031) as the majority of the stool samples were watery diarrhea. The remaining 30.4%, 17.8% and 1.8% of the stool samples were mucoid, bloody and mixed (blood and mucus), respectively. Clinical symptoms usually persist for 10 to 14 days or longer (Kirnpal-Kaur, 2006). Even though statistically not significant, most of the positive cases (87.5%) were noted within 1 to 5 days duration of diarrhea whereas only 12.5% the positive cases were 6 to 10 days duration. This result is comparable with earlier report made by Remon *et al*. (2004).

Test for Antibiotic Susceptibility of *Shigella* Isolates

The in vitro antimicrobial resistance profile of 56 *Shigella* isolates are shown in Table 4. As shown in the Table 4, 96.4%, 94.6%, 73.2% and 53.6% of the isolates were resistant to tetracycline, ampicillin, cotrimoxazole, and chloramphenicol, while 10.7%, 7.1%, 5.4%, 3.6 and 1.8% of them were of intermediate resistance against cotrimoxazole, gentamicin, ampicillin, chloramphenicol and tetracycline, respectively. In addition, 100% and 71.4% of the *Shigella* isolates were found to be susceptible to ciprofloxacin and gentamicin, respectively.

In this study, the highest percentage of resistant *Shigella* isolates were observed against tetracycline (96.4%) which is in line with the studies reported from Ethiopia by Daniel Asrat (2008) and Moges Tiruneh (2009) and in agreement with the report of Gizachew Yismaw *et al*. (2006). These results are also in harmony with the studies reported from India (Saxena and Dutta, 2011). In addition the next highest proportion of *Shigella* isolates developed resistance against ampicillin (94.6%), which is in agreement with the study reported by Berhanu Andualem *et al*. (2006) who showed 100% of their *Shigella* isolates from Ethiopia had developed resistance to the same antibiotic. Similarly, in southeast Iran and India,

Table 3: Clinical features and their associations with positivity of *Shigella* isolates among diarrheal patients.

Symptoms	Positive (%)	Negative (%)	χ^2	Df	P-values
Fever					
Yes	44 (17.5)	208 (82.5)	4.871	1	0.027*
No	12 (9.1)	120 (90.9)			
Vomiting					
Yes	26 (16.1)	135 (83.9)	0.546	1	0.460
No	30 (13.5)	193 (86.5)			
Abdominal pain					
Yes	53 (14.6)	309 (85.4)	0.017	1	0.897
No	3 (13.6)	19 (86.4)			
Consistency of stool					
Watery	28 (14.7)	162 (85.3)	8.871	3	0.031*
Bloody	10 (25.6)	29 (74.4)			
Mucoid	17 (15)	96 (85)			
Mixed (blood and mucus)	1 (2.4)	41 (97.6)			
Duration of diarrhea (day)					
1-5	49 (16)	258 (84)	3.568	3	0.312
6-10	7 (11.3)	55 (88.7)			
11-15	0.0	9 (100)			
≥16 days	0.0	6 (100)			

researchers have reported that 99.3% and 94% of *Shigella* isolates from the respective countries had shown resistance against ampicillin (Qureishi *et al*., 2008; Saxena and Dutta, 2011).

The high percentage of resistant isolates to ampicillin (94.6%) and tetracycline (96.4%) in this study could be either because they were frequently and unnecessarily prescribed or sold over the counter in the open market and private pharmacies without prescription. Everywhere, in hospitals, private pharmacies and in the market, people have easy access to ampicillin and tetracycline to purchase drugs without prescription. Thus, the selective pressure of these commonly used antibiotics on the bacteria circulating in the community could have resulted in high frequency of resistance among *Shigella* isolates. Similarly, other reports indicated that many factors have contributed to the development of resistance in gastrointestinal pathogens, including misuse, overuse, quality and potency of the antimicrobial agents (Bonfiglio, 2002).

In this study, the *Shigella* isolates were more susceptible to ciprofloxacin (100%) and gentamicin (71.4%). This finding is in agreement with the findings of Gizachew Yismaw *et al*. (2006), Moges Tiruneh (2009) and Abebe Mache (2001) from Ethiopia who showed 91.1%, 97.8% and 98.7%, of their respective *Shigella* isolates were susceptible to ciprofloxacin, respectively. Similarly other reports had shown that, 95% to 100% *Shigella* of isolates in Iran (Qureishi *et al*., 2008) and 96.6% in Brazil (Paula *et al*., 2010), were susceptible to the same antibiotic. Moreover, this study has also demonstrated that 71.4% of *Shigella* isolates were susceptible to gentamicin. This result was also in agreement with a recent study conducted in northwest Ethiopia that reported high sensitivity of *Shigella* isolates to gentamicin (Moges Tiruneh, 2009). Similarly, very low resistance was reported in the study conducted in north India by Urvashi *et al*. (2011)

This study revealed that high percentages of ampicillin resistant *Shigella* isolates were reported from Awassa (93%), Gondar (100%) in 2006 and Harar (94.6%). Similarly, the highest rate of tetracycline resistance was documented in Addis Ababa (Daniel Asrat, 2008), Awassa (Belay Roma *et al*., 2000), Gondar (Moges Tiruneh, 2009) and Harar (the present study) where 97%, 90%, 90% and 96.4% of the respective isolates have been shown to be resistant to this antibiotic, respectively. Though relatively lower, resistance against tetracycline was also reported from Addis Ababa (Abebe Mache *et al*., 1997) and Jimma (Abebe Mache, 2001) where 74% and 63.6% of their isolates, respectively, were found to be resistant to ampicillin. Moreover, data show during the period of 1997-2008, the prevalence of chloraphenicol, ampicillin, and tetracycline resistant *Shigella* strains from Addis Ababa increased from 50% to 74.7%, 70% to 78.7%, and 74% to 97%,

Table 4: Drug resistance pattern of *Shigella* species isolated from stool specimen at Hiwot Fana Hospital.

Sensitivity of *Shigella* isolates (N=56)	AMP	C	CIP	SXT	CN	TE
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
R	53 (94.6)	30 (53.6)	0 (0)	41 (73.2)	12 (21.4)	54 (96.4)
I	3 (5.4)	2 (3.6)	0 (0)	6 (10.7)	4 (7.1)	54 (96.4)
S	0 (0)	24 (42.8)	56 (100)	9 (16.1)	40 (71.4)	54 (96.4)

AMP = Ampicillin C = Chloramphenicol CIP = Ciprofloxacin TE =Tetracycline
SXT = Contrimoxazole CN =Gentamicin R = Resistance I = Intermediate
S= Sensitive No = Number of isolates

Table 5: Multiple drug resistance patterns among *Shigella* species isolated in Hiwot Fana Hospital, from February to May 2011.

Resistance types	Resistant isolates No (%)
Resistance to three antibiotics	
AMP, SXT, TE	18 (32.1)
AM, C, T	7 (12.5)
AMP, CN, TE	2 (3.6)
C, SXT, TE	1 (1.8)
AMP, C, SXT	1 (1.8)
Resistance to four antibiotics	
AMP, C, SXT, TE	12 (21.4)
AM, SXT, CN,TE	1 (1.8)
Resistance to five antibiotics	
AMP, C, SXT, CN,TE	4 (7.1)

respectively. A similar trend was also observed in Gondar where the prevalence of chloramphenicol and gentamicin resistant *Shigella* isolates were shown to increase from 52.8% to 67.8% and 7.9% to 12.2%, respectively, during the years 2001-2008.

Compared to a previous report from Gondar (Gizachew Yismaw *et al*., 2006), more than a twofold increase in resistance to gentamicin was observed in the present study (from 7.9% to 21.4%). This increase might be because of the relatively increased use of gentamicin and related aminoglycosides over the past fourteen years (Teferra Abula *et al*., 2002).

Shigella species are very well noted for their ability to rapidly develop multiple drug resistance among their strains (Abera Geyid, 2004). In this study, multiple drug resistance to as many as four and five antibiotics was observed. Similar findings were observed in Ethiopia ranging from five to eight antibiotics (Kahsay Huruy *et al*., 2008; Moges Tiruneh, 2009). In this study multidrug resistance were seen against commonly used antimicrobial agents, in which resistance to ampicillin, contrimoxazole and tetracycline recorded the highest one followed by resistance to ampicillin, chloramphenicol, contrimoxazole, and tetracycline. This multiple drug resistance may be indiscriminate over use of antimicrobial agents (Moges Tiruneh, 2009).

In this study, ciprofloxacin has been shown to be the only antibiotic to which none of the *Shigella* isolates had developed resistance. Several studies show that ciprofloxacin offers advantages in the treatment of shigellosis, reaching high concentrations in serum and feces. Short courses of ciprofloxacin therapy in pediatric patients with specific enteric infections are becoming a common practice among pediatricians worldwide (Fulla *et al*., 2005). The World Health Organization recommends ciprofloxacin as the drug of choice for therapy of *Shigella* infections in both adults and children (WHO, 2005).

CONCLUSION

Highest prevalence of *Shigella* with multiple antibiotic resistance isolates were observed in this study. This highest prevalence of *Shigella* species might be the result of the consumption of water from unprotected sources, absence of regular hand-washing habit, and absence of latrine in the households. Prevention of shigellosis caused by *Shigella* relies primarily on measures that prevent spread of the organism within the community and from person to person. In all cases, health education and the cooperation of the community in implementing control measures are essential to reduce the prevalence of shigellosis. This study has also shown that ciprofloxacin may be used as the drug of choice for empirical treatment. Further studies should be conducted on the prevalence of serogroups and serotypes of *Shigella* isolates in the future. Ciprofloxacin may be used as a drug of choice for empirical treatment whereas tetracycline, ampicillin, contrimoxazole, chloramphenicol, and gentamicin should not be used as a drug of choice for the treatment of *Shigella* infection without making sensitivity tests prior to treatment. Regular systematic monitoring of diarrheal cases is also needed to identify changes in the prevalence and antimicrobial resistance patterns of *Shigella* species.

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