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Prevalence and Antimicrobial Susceptibility Patterns of *Salmonella serovars* and *Shigella* species

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Abstract

Background: Salmonellosis and shigellosis are global human health problems, especially in developing countries such as Ethiopia, where substandard hygiene and unsafe water supplies prevail which is aggravated by multidrug resistance. We determined the prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* isolates among diarrheic patients. Which helps in disease management by showing the disease burden and allowing for selection of appropriate antibiotics for empiric treatment in rural communities of resource limited countries such as Ethiopia.

Result: Forty (10.5%) *Salmonella* and 17 (4.5%) *Shigella* strains were isolated from 382 patients. The *Salmonella* strains isolated were 6 (15%) group A (Somatic antigen O, O:2), 5 (12.5%) each of group B (O:4), D1 (O:9) and D2 (O:9,46) and 3 (7.5%) group C (O:7/O8) isolates while 16 (40%) could not be typed with the available antisera. Among 17 *Shigella* species *Shigella sonnei* founded as 6 (35.3%) followed by *Shigella flexneri* 5 (29.5%), *Shigella dysenteriae* 3 (17.6%) and *Shigella boydii* 3 (17.6%). High frequency of resistance for both *Shigella* and *Salmonella* isolates was observed to tetracycline (82.4%, 52.5%), co-trimoxazole (76.5%, 37.5%) and ampicillin (47.1%, 60%), respectively. All isolates were sensitive to ceftriaxone except 6 intermediate level *Salmonella* isolates. Fifty three percent of *Shigella* isolates were Multi-Drug Resistant (MDR) (≥ 3 drugs) as compared to 27.5% of *Salmonella* isolates.

Conclusion: *Salmonella* and *Shigella* species cause a significant amount of morbidity in rural communities. It is essential for rural hospitals to establish antimicrobial resistance monitoring policies and enforce them to prevent exacerbation of resistance.

Keywords: *Salmonella*; *Shigella*; Diarrhea; Antibiotic Resistance; Antimicrobial Susceptibility; Ethiopia

Abbreviation: API: Analytical Profile Index; ATCC: American Type Culture Collection; MDR: Multi-Drug Resistant; SOPs: Standard Operating Procedures

Background

Acute gastroenteritis is one of the leading causes of illness and death in infants, children, immuno-compromised and aged individuals throughout the world, especially in developing countries. Asia, Africa and Latin America, had an estimated 2.5 million deaths each year in children under 5 years of age [1-4]. Among the enteric pathogens *Salmonella* and *Shigella* species are of particular concern as causes of enteric fevers, food poisoning and gastroenteritis [5]. Although more prevalent in developing countries, shigellosis is a worldwide problem [6,7] with *Shigella sonnei* in predominating in Europe and US and *Shigella flexneri* more prevalent in Asian and African countries [8]. *Salmonella*, with its more than 2500 different serotypes, is a leading cause of foodborne infections worldwide [9]. *Salmonella* can be divided into two major groups of clinical importance: typhoidal salmonellosis (*Salmonella Typhi* and *Salmonella Paratyphi*) and non typhoidal salmonellosis (all *Salmonella serovars*) [10].

Antibiotic therapy for *Salmonella* gastroenteritis has long been a debated matter because of the idea that antibiotic administration prolonged *Salmonella* excretion [11] unlike that of shigellosis which needs antibiotic therapy [12]. In recent years, an increase in the occurrence of antimicrobial resistance, among *Salmonella* has been observed in many countries, such as Asia, Africa [13] and China [9] that includes resistance to quinolones and third generation cephalosporin's. The progressive increase in antibiotic resistance because of overuse and misuse of antibiotics in the treatment of diarrhea in developing countries is becoming a critical area of concern [2,14,15].

Although most of Ethiopian studies conducted retrospectively, the prevalence of *Salmonella* (5.3% -15.4%) [15-17] and *Shigella* (5% - 7.5%) [15,16,18,19] was high with antibiotic resistance pattern ranged from 0% in case of ciprofloxacin and nalidixic acid to 100% in case of ampicillin [15,16,18]. This study fills the knowledge gap on the prevalence of salmonellosis and shigellosis and antimicrobial susceptibility patterns in the study area based on controlled prospective study. Periodic epidemiological surveillance in the area among humans is of vital importance to detect outbreaks and control the diseases caused by these pathogens.

Methods and Materials

Specimen and data collection

A cross-sectional study was conducted in Butajira, central Ethiopia.

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Patients who visited the outpatient departments of Butajira health center for diarrhea (at least three loose stools or one watery diarrhea in a day) from October 2011 to June 2012 were consecutively enrolled into the study upon informed consent. Three hundred eighty two stool specimens were collected in clean, sterile, wide-mouthed containers, without disinfectant or detergent residue and tight-fitting leak-proof lids. Participants already on antibiotic treatment were excluded. Socio-demographic data using questionnaire based interview, clinical data based on signs and symptoms of patients suspected by handling clinicians were collected.

Isolation and identification of bacteria

For detection of *Salmonella* and *Shigella* isolates, specimens were plated directly on Oxoid primary media (Oxoid, England): MacConkey agar, Xylose Lysine Deoxycholate (XLD) and *Salmonella-Shigella* (SS) agar and Selenite F enrichment broth with in two hours of collection. For those negative specimens on primary sold media, sub-culturing from enrichment broth to primary media was performed to improve recovery of the isolates. All of the inoculated media were incubated at 37oC for 18-24 hours.

Using Analytical Profile Index (API) 20E (BioMerieux, France) a total of 20 biochemical tests were performed and the isolates were identified with the help of Bergey's manual of Systematic Bacteriology (2007) [20], API Web identification software version 4 (BioMerieux, France) and the manual/ leaflet of the kit supplied by the company [21]. The biochemical tests included in the kit were Ortho-Nitrophenyl-β-galactoside (ONPG), Arginin-dehydrolase (ADH), Lysine decarboxylase (LDC), Ornithine decarboxylase (ODC), Sodium Citrate utilization (CIT), H2S production (H2S), Urease (URE), Tryptophane deaminase (TDA), Indole production (IND), Acetoin production (VP), Gelatinase (GEL), Glucose (GLU), Manitol (MAN), Inositol (INO), Sorbitol (SOR), Rhamnose (RHA), Sacharose (SAC), Melibiose (MEL), Amygdaline (AMY) and Arabinose (ARA) [21]. Isolates were typed by serological agglutination with specific antisera (Denka-Seiken, Japan) against somatic (O) antigen of *Salmonella serovars* and *Shigella* species according to manufacturer's instructions [22].

Antimicrobial susceptibility testing

Disk diffusion assay was performed to assess the antibiotic resistance/ susceptibility pattern of *Salmonella* and *Shigella* isolates. The antimicrobial susceptibility testing of all strains were carried out on Muller-Hinton agar (Oxoid, England) with antibiotic discs (Oxoid, England) using the single disc diffusion [23] technique against ampicillin (10 µg), trimethoprim/sulphamethoxazole (co-trimoxazole, 1.25/23.75 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), gentamicin (10 µg) and tetracycline (30 µg) based on the Standard Operating Procedure (SOP) adapted from Clinical and Laboratory Standards Institute (CLSI) and results were reported as sensitive, intermediate and resistance. To standardize the inoculum density for a susceptibility test, a BaSO4 turbidity standard, equivalent to a 0.5 McFarland standard was used by strictly following the SOP for the preparation and standardization [23]. An isolate was defined as being multidrug resistant if it is resistant to three or more of the antimicrobial agents tested [24].

Quality control

A standard bacteriological procedure was followed to keep the quality of all laboratory tests [25]. American Type Culture Collection (ATCC) strains (*E. coli* ATCC 25922, *S. Typhi* ATCC 13311, *S. Entritidis* ATCC 13076, *S. sonni* ATCC 25331, *P. aeruginosa* ATCC 27853 and *P. mirabilis* ATCC 35659) were used as controls for culture and sensitivity testing.

Data analysis

The data were entered and analyzed using the SPSS statistical package version 20 (IBM SPSS Statistics, USA) and Microsoft excel 2010 (Microsoft corporation, USA) statistical software.

Ethical clearance

The study was reviewed and approved by Departmental research and ethical review committee of school of medical laboratory sciences, College of Health Sciences, Addis Ababa University and Armauer Hansen Research Institute / All Africa Leprosy, Tuberculosis and Rehabilitation Training center (AHRI/ALERT) ethical review committee. All results were sent to the handling physician timely to address ethical considerations. Before the study was begun, a detailed discussion was made with participants and/or guardians/parents about objective of the study. Patients were enrolled upon informed consent or assent.

Result

The patients included were from rural and urban residents and clients of diverse socioeconomic and ethnic backgrounds. Stool specimens from 382 patients were examined by culture and among them 40 (10.5%) were positive for *Salmonella* and 17 (4.5%) had *Shigella* spp confirmed by biochemical and serotyping tests. The *Salmonella* strains isolated were 6 (15%) group A (O:2), 5 (12.5%) each of group B (O:4), D1 (O:9) and D2 (O:9,46) and 3 (7.5%) group C (O:7/O8) isolates while 16 (40%) could not be typed with the available antisera. Sergroup D (*S. sonnei*) was the most frequently isolated *Shigella* species 6 (35.3%) followed by serogroup B (*S. flexneri*) 5 (29.4%), serogroup A (*S. dysenteriae*) 3 (17.6%), and serogroup C (*S. boydii*) 3 (17.6%).

Distribution of *Salmonella* and *Shigella* isolates by age and gender is shown in Figure 1. The mean age of the patients from whom either *Salmonella* or *Shigella* microbes were isolated was 17.8 years (SD ± 15.5) and median age of 18 with (IQR= (5-23)) years with children less than 15 years of age comprising of 45.6% and a male proportion of 57.9%. There was one infant (6 months old) and one elder woman (80 years old) with *Salmonella* serogroup A and *S. boydii,* respectively. The age ≤

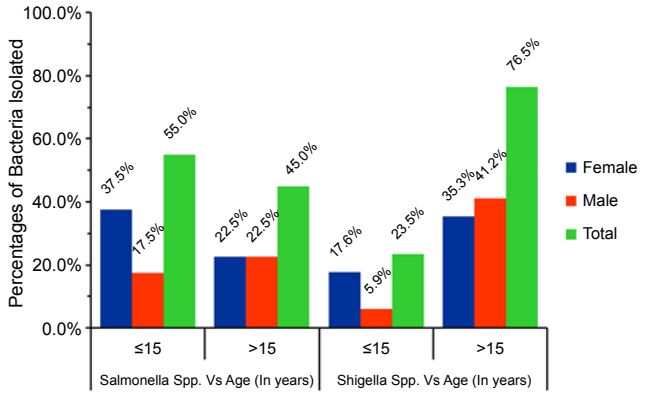

Figure 1: Age and gender distribution of patients who were positive for Salmonella (n=40) and *Shigella* (n=17) species, Butajira, Ethiopia from October 2011 to June 2012.

15 years had less risk of getting shigellosis (OD=0.274, 95% CI=0.088-0.857, P=0.026) compared to age greater than 15 years old whereas no significant association is observed to salmonellosis (P=0.647).

Most patients infected by *Salmonella* or *Shigella* reported to health center within 1 to 5 days duration of diarrhoea onset. Mucoid diarrhoea was more common among *Salmonella* 20 (50.0%) and *Shigella* 6 (35.5%) positive cases as compared to negatives (Table 1).Among the 40 patients who were positive for *Salmonella*, 30 (75%) had abdominal pain which was the commonest clinical findings followed by fever 23 (57.5%), tenesmus 20 (50%), vomiting 17 (42.5%) and in 14 (35%) of them frequent feeling of thirsty was observed. In case of the 17 *Shigella* infected individuals abdominal pain was the most frequent complains of patients which was 14 (82.4%) followed by fever 12 (70.6%), tenesmus 11 (64.7%), vomiting 5 (29.4%) and frequent thirst 3 (17.6%).

The antimicrobial susceptibility testing results of all 40 *Salmonella* and 17 *Shigella* isolates are shown in Table 2. The highest level of resistance was detected for tetracycline 14 (82.4%) and co-trimoxazole 13 (76.5%) by *Shigella* spp. and ampicillin 24 (60%) and tetracycline 21 (52.5%) by *Salmonella* spp. In other hand, the highest level of susceptibility was detected for Ceftriaxone and Ciprofloxacin, were, respectively, 100% (17) and 88.2% (15) of the *Shigella*; and 85% (34) and 92.5% (37) of the *Salmonella* isolates (Figure 2).

A total of thirteen distinct antibiograms (resistance patterns) were found among all isolates of *Salmonella serovars* (Table 3). Only 1 (2.5%) *Salmonella* isolate was resistant to six antimicrobial agents, 11 isolates (27.5%) to 3 and 2 isolates (5.0%) to 4 drugs were resistant with different antibiogram patterns. A total of ten distinct antibiograms were found among all isolates of *Shigella* species (Table 3). Resistance to one or more antimicrobial agents was detected in 16 (94.1%) of the *Shigella* strains, of which 9 (56.25%) showed multi-resistance patterns.

Discussion

The isolation rate of *Shigella* species (4.5%) in our study was comparable to the study done in Jimma and Addis Ababa (5%) [16] and another African country, Ghana (4.04%) [26]. Our result is lower when compared to the study conducted in Eastern Ethiopia (2011) (6.7%) [15], Jimma (2002) (20.1%) [17], Gonder (2009) (7.5%) [18], North West Ethiopia (2006) (8.7%) [27], and other countries such as, South Africa (2009) (8.5%) [28] and South America (2008) (8%) [29]. There is no research conducted around Butajira on identification and characterization of *Shigella* isolates to see prevalence variation over time. But, the low isolation rate of *Shigella* in our study relative to the previous studies in elsewhere might be due to improved awareness of the community about personal and environmental hygiene from the continuous interventions made by the health extension workers being implemented by the government.

Consistency of the Stool	Types of Isolate		Total (n. %)
	Salmonella (n, %)	*Shigella* (n, %)	
Mucoid	20 (50.0)	6 (35.3)	26 (45.6)
Unformed	9 (22.5)	4 (23.5)	13 (22.8)
Muciod &Watery	5 (12.5)	3 (17.6)	8 (14.0)
Watery	4 (10.0)	3 (17.6)	7 (12.3)
Bloody & Mucoid	1 (2.5)	1 (5.9)	2 (3.5)
Bloody	1 (2.5)	0 (0.0)	1 (1.75)
Total	40 (100.0)	17 (100.0)	57 (100.0)

Table 1: Consistency of diarrhea in patients with Salmonella and *Shigella* infection in Butajira health center, Ethiopia from October 2011 to June 2012.

Types of Antibiotic		Salmonella spp	*Shigella* spp
		Frequency (%)	Frequency (%)
TE	R	21(52.5)	14(82.4)
	I	16(40.0)	3(17.6)
	S	3(7.5%)	0(0.0)
AMP	R	24(60.0)	8(47.1)
	I	15 (37.5)	5(29.4)
	S	1(2.5)	4(23.5)
SXT	R	15(37.5)	13(76.5)
	I	2(5.0)	0(0.0)
	S	23(57.5)	4(23.5)
C	R	4(10.0)	5(29.4)
	I	7(17.5)	1(5.9)
	S	29(72.5)	11(64.7)
GM	R	2(5.0)	3(17.6)
	I	17(42.5)	4(23.5)
	S	21(52.5)	10(58.8)
NA	R	2(5.0)	1(5.9)
	I	7(17.5)	1(5.9)
	S	31(77.5)	15(88.2)
CIP	R	1(2.5)	1(5.9)
	I	2(5.0)	1(5.9)
	S	37(92.5)	15(88.2)
CRO	R	0(0.0)	0(0.0)
	I	6(15.0)	0(0.0)
	S	34(85.0)	17(100.0)

AMP=Ampicillin, C=Chloramphenicaol, CIP=Ciprofloxacin, CRO=Ceftriaxone, SXT=Cotrimoxazole, TE=Tetracycline, NA=Nalidixic Acid, GM=Gentamicin, R=Resistant, I=Intermediate, S=Sensitive

Table 2: The *in vitro* antimicrobial susceptibility patterns of Salmonella and *Shigella* isolates identified in the Butajira Health center, Ethiopia from October 2011 to June 2012.

In this study, the most frequently isolated *Shigella* species were *S. sonnei* (35.3%) followed by *S. flexneri* (29.4%); while *S. dysenteriae* and *S. boydii* have shown the same frequency (17.6%). *Shigella sonnei* (75%) and *S. flexneri* (19%) accounts the first and the second predominate *Shigella* species, respectively in the South America study conducted by Orrett [29] which is comparable to this study, even though *S.dysenteriae* (1.8%) was the least prevalent species followed by *S. boydii* (4.1%) unlike that of this study.

The distribution is different when compared to the study conducted by Asrat in Addis Ababa with a proportion of 54.0% *S. flexneri*, 22.4% *S. dysenteriae*, 15.8% *S. sonnei* and 7.8% *S. boydii* [30]; whereas Tiruneh in Gonder found 72.2% *S. flexneri*, 10% *S. dysenteriae*, 8.9% *S. boydii*, and 8.9% *S. sonnei* [18]. A study done by Beyene et al. in Jimma and Addis Ababa found 68.9% *S. flexneri*, 9.8% *S. boydii*, and 21.3% *S. sonnei* [16]; and another study from Ghana by Opintan et al. found 70.8% *S. flexneri*, 16.7% *S. dysenteriae*, 8.3% *S. sonnei* and 4.2% *S. boydii* [26].

Shigella dysenetriae was the second most prevalent *Shigella* species in the previous studies done in Addis Ababa (22.4%) [30] and Gonder (10%) [18] unlike that of this study which is the third by accounting equal percentage with *S. boydii* (17.6%). On the other hand, no *S. dysenetriae* was reported from other study conducted at Jimma and Addis Ababa in 2006 [16]. In general, *S. flexneri* was the most prevalent *Shigella* species in the previous studies from different corners of Ethiopia unlike to this study which is dominated by *S. sonnei*. The difference in the pattern of species may be due to ecological or geographical differences, study time or human host differences.

In this study, children age ≤ 15 years had less risk of getting

Number of Antimicrobial resistance†	Salmonella		*Shigella*	
	Resistance antibiogram	No. of Isolates (%)	Resistance antibiogram	No. of Isolates (%)
Zero	None *	9(22.5)	None *	1(5.9)
One	Amp	9(22.5)	AMP	2(11.8)
	SXT	1(2.5)		
	TE	2(5.0)		
Two	Amp, TE	2(5.0)	SXT, TE	5(29.4)
	SXT, TE	3(7.5)		
Three	Amp, C, TE	1(2.5)	C, SXT, TE	1(5.9)
	Amp, SXT, TE	7(17.5)	SXT, TE, GM	1(5.9)
	Amp, TE, GM	1(2.5)	Amp, TE, GM	1(5.9)
	Amp, TE, NA	1(2.5)	Amp, SXT, TE	1(5.9)
	C, SXT, TE	1(2.5)		
Four	AMP, C, SXT, TE	2(5.0)	Amp, C, SXT, TE	3(17.9)
			C, SXT, TE, GM	1(5.9)
Five			Amp, CIP, SXT, TE, NA	1(5.9)
Six	Amp, CIP, SXT, TE, NA, GM	1(2.5)		

†The sum of drugs resistant by a specific isolate *sensitive and/or intermediate
AMP=Ampicillin, C=Chloramphenicaol, CIP=Ciprofloxacin, CRO=Ceftriaxone, SXT=Cotrimoxazole, TE=Tetracycline, NA=Nalidixic Acid, GM=Gentamicin

Table 3: Antibiogram of Salmonella and *Shigella* isolates identified in Butajira Health center, Ethiopia from October 2011 to June 2012.

shigellosis unlike to other studies conducted in Ethiopia [31]. Because children do not spend more time outside of the house, drank and eat foods properly prepared by their parents/guardians may prevent acquiring the disease. This idea is strengthen by the study conducted on foodborne outbreaks of shigellosis from multiple restaurants [32].

Although untypable *Salmonella* spp. account to the highest percentage (40.0%), the predominant typable serogroup was serogroup A (O:2) (15.0%) followed by B (O:4), D1 (O:9) and D2 (O:9, 46) (12.5% each), and Group C (O:7/O:8) (7.5%). In earlier study from Jimma by Mache, serogroup B comprised 28.8% followed by *S. Typhi* (22%), serogroup C (22%), D (13.6%), A (8.5%) and E (5.1%) [17]. The same result was obtained from Addis Ababa's study by Asrat; serogroup B (81.1%), D (*S. Typhi*) (10.8%) and group C (8.1%) [30]. The difference in the pattern of serogroup may be due to ecological (animal reservoirs) or geographical variation, differences in the human host or study time.

Fifty percent of *Salmonella* and 35% of *Shigella* positive cases were identified from mucoid diarrheic patients and this is comparable to the study done in Harar by Reda et al., *Salmonella* (42.8%) and *Shigella* (52.9%) [15]. Seven (17.5%) of *Salmonella* and 4 (23.5%) of *Shigella* isolates were found from watery diarrhea in contrast to a study done in Harar, by Reda et al. who reported that no *Salmonella* species and only 1 (5.9%) *Shigella* species was found from watery diarrhea [15]. But, our result was supported by study conducted in Addis Ababa which reported that 82.4% of *Salmonella* and *Shigella* species were isolated from watery diarrhea samples [30]. A study conducted in Washington State by Villar et al. showed that *S. Typhimurium* results in diarrhea (100%), abdominal cramps (93%), fever (93%), and vomiting (53%) [33], which is comparable to our study; abdominal pain (77.5%), fever (52.5%), vomiting (42.5%), tenesmus (39.8%) and frequent thirst (32.5%).

Shigella species invade and replicate in cells lining the colon and rectum, cause mucosal ulceration, characterized by lower abdominal cramps, tenesmus and fever [6,34], which is similar to our study where abdominal pain (88.2%), tenesmus (64.7%) and fever (58.8%) were the predominant symptoms of culture positive *Shigella* cases followed by vomiting (23.5%) and frequent thirst (17.6%).

In recent years, an increase in the occurrence of antimicrobial resistance, including resistance to quinolone's and third generation cephalosporin's, among *Salmonella* has been observed in many countries, such as Asia, Africa [13] and china [9]. Most of Ethiopian studies had shown emergence of drug resistant *Salmonella* and *Shigella* species which will be a challenging problem in the future [15,16].

Antimicrobial resistance pattern findings of this study are displayed against findings from other parts of the country and are shown in Tables 4,5. There is high resistance of *Shigella* to tetracycline (82.4%) in this study, which is in agreement with reports from other parts of the country (86.0% [19] and 90.0% [18]). High resistance was also observed to co-trimoxazole (76.5%), which agrees with the report from Gonder (73.4% [19] and 84.6% [18]) in contrast to study from Awassa (56.0%) [35] and Addis Ababa (45.7%) [30]. This increase of resistance from those reports indicated that aggravating problem of drug resistance by these microbes over the years. This may be due to misuse or inappropriate use of drugs [2,14].

None of the *Shigella* isolates were resistant to ceftriaxone in our study that is comparable to the study done in Gonder [18]. Gentamicin resistant *Shigella* isolates were not found in previous studies from Addis Ababa [30] and Harar [15] in contrary of our study (17.6%) which was supported by the study done in Gonder (12.2%) [18]. This indicates emerging of gentamicin drug resistance *Shigella* isolates over time. Low level of *Shigella* resistant for ciprofloxacin and nalidixic acid (5.9% each) were observed in this study like that of other studies in Ethiopia [18,19,30,35].

Low frequency of *Salmonella* resistance was observed relative to *Shigella* isolates in this study. Based on the result of our findings, high level of *Salmonella* resistance was observed to ampicillin (60%) and tetracycline (52.5%) which is comparable to the study done in Jimma (59.3% each) [17] and lower than the study from Addis Ababa (81.3% and 94.5%) [30] and Harar (100% and 71.4%) [15], respectively. Even though studies from Addis Ababa [30] and Jimma and Addis Ababa [16] reported resistance levels of 75.6%, and 74.3%, respectively *Salmonella* species in this study seem to be low resistant to gentamicin (2.5%). This is similar to reports from other parts of the country, Jimma (1.3%) [17] and Harar (3.6%) [15]. Unlike study from Jimma and Addis Ababa (78.8%) [16], *Salmonella* isolates from our study are completely susceptible to ceftriaxone.

Study area and time	No. of strains	Antibiotics							
		AMP	C	CIP	CRO	SXT	TE	NA	GM
Awassa [35]	100	93.0	63.0	-	-	56.0	90.0	10.0	2.0
Gondar [19]	214	79.9	52.8	8.9	-	73.4	86.0	-	7.9
Addis Ababa [30]	76	78.7	74.7	-	-	45.7	97.3	2.7	0.0
Gondar [18]	90	78.9	67.8	2.2	0.0	84.6	90.0	0.0	12.2
Harar [15]	17	100.0	29.4	-		-	70.6	-	0.0
Butajira (This study, 2012)	17	47.1	29.4	5.9	0.0	76.5	82.4	5.9	17.6

AMP=Ampicillin, C=Chloramphenicaol, CIP=Ciprofloxacin, CRO=Ceftriaxone, SXT=Cotrimoxazole, TE=Tetracycline, NA=Nalidixic Acid, GM=Gentamicin

Table 4: Percentages of antimicrobial resistance of *Shigella* isolates, Butajira Health Centre, 2012 compared with selected previous reports from Ethiopia.

Study area and time	No. of strains	Antibiotics							
		AMP	C	CIP	CRO	SXT	TE	NA	GM
Jimma [17]	59	59.3	35.6	-	-	40.7	59.3	8.5	1.3
Addis Ababa [30]	37	81.3	83.7	-	-	75.7	94.5	37.8	75.6
Jimma & Addia Ababa [16]	65	82.3	81.4	0.9	78.8	80.5	39.8	8.0	74.3
Harar [15]	28	100.0	62.3	-	-	-	71.4	-	3.6
Butajira, (this study, 2012)	40	60.0	10.0	2.5	0.0	37.5	52.5	5	2.5

AMP=Ampicillin, C=Chloramphenicaol, CIP=Ciprofloxacin, CRO=Ceftriaxone, SXT=Cotrimoxazole, TE=Tetracycline, NA=Nalidixic Acid, GM=Gentamicin

Table 5: Percentages of antimicrobial resistance of Salmonella isolates, Butajira Health Centre, 2012 compared with selected previous reports from Ethiopia.

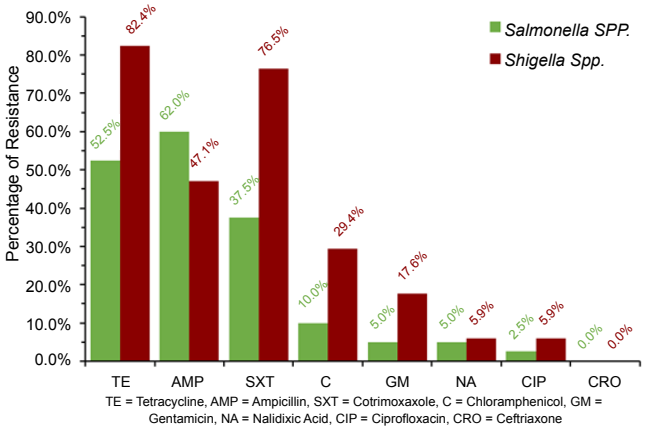

Figure 2: Antibiotic resistance patterns of 40 Salmonella and 17 *Shigella* isolates.

Comparable level of *Salmonella* resistance to ciprofloxacin (2.5%) and nalidixic acid (5.0%) were observed from other parts of the country such as Jimma and Addia Ababa (0.9% and 8.0%) [16], respectively and Jimma (nalidixic acid, 5%) [17]. In contrast to other studies from Addis Ababa [30] and Jimma and Addis Ababa [16] which reported 83.7% and 81.4%, respectively low level of resistance of *Salmonella* isolates (10%) were detected to chloramphenicol. The variation in the resistance level of *Salmonella* to different types of antibiotics in this and earlier studies can be due to difference in serovars from place to place.

Antibiotics have revolutionized the treatment of common bacterial infections and played a crucial role in reducing mortality. However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase of antibiotic resistance [2,14] including Ethiopia [15]. Poor laboratory diagnosis in developing countries enforces physicians to syndromatic diagnosis and prescription of broad spectrum antibiotics that led to emerging of drug resistant bacterial strains [36].

The strength of this study compared with previous studies on *Salmonella* and *Shigella* is in the design of the study. Our study was conducted prospectively in a manner of controlled data collection and laboratory tests, whereas the other studies were conducted retrospectively (Awassa [35], Gondar [18,19], Jimma [17], and Jimma and Addis Ababa [16]). This study may not necessarily representative of the community prevalence of the disease, because not all cases from the area were included in the study since the enrolment was based on health center visit (not community survey with representative sampling).

Conclusion

Based on this study finding the overall prevalence of salmonellosis and shigellosis was 10.5% and 4.5%, respectively. Among the five identified serogroups of *Salmonella* isolates, serogroup A had the highest percentage (15%) although, 40% of the isolates were untypable by the available antisera. In the case of 17 *Shigella* isolates, *S. sonnei* account higher percentage (35.5%) compared to other *Shigella* species.

More than 40% of *Shigella* isolates were highly resistant to

tetracycline, co-trimoxazole and ampicillin. Low resistance rate was observed for ciprofloxacin and nalidixic acid and there was no resistance detected against ceftriaxone. In *Salmonella*, more than 37% of the isolates were resistance to ampicillin, tetracycline and cotrimoxazole. Highest level of *Salmonella serovars* susceptibility was detected for ciprofloxacin, ceftriaxone and nalidixic acid. From this study relatively high level of MDR was observed in *Shigella* isolates (53%) than *Salmonella* (27.5%) isolates.

The current study suggested that, *Salmonella* and *Shigella* species are developing resistant to oral antibiotics and less resistant to intramuscular/intramuscular antibiotics, this indicated that patients may take oral antibiotic without any prescription. But, this should be verified by further studies on communities' knowledge, attitude and practice of drug use or further epidemiological study should be done on drug dispensing by different governmental and private pharmacies. Emphasis also should be given towards in prevention of further antibiotic resistance, monitoring on proper utilization of drugs and vaccine development against MDR isolates.

It is recommended that an extensive study of the prevalence, antimicrobial susceptibility pattern and drug resistance mechanisms of *Salmonella* and *Shigella* isolates be conducted. In addition, accurate diagnosis during management of infection caused by *Salmonella* and *Shigella* spp. should be employed rather than currently practiced empirical treatment. Moreover periodic epidemiological surveillance among humans is of vital importance to control the diseases caused by these pathogens.

Competing Interests

Financial competing interests: All authors have no financial relationships relevant to this article to disclose.

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Authors' Contributions

Getachew Mengistu: Mr. Getachew conceptualized and designed the study, conducted sample collection and performed the laboratory work, carried out the initial analyses and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

Tsehaynesh Lema: Mrs. Tsehaynesh designed the study, supervised the data collection and laboratory analysis, revised the manuscript, and approved the final manuscript as submitted.

Gebru Mulugeta: Mr. Gebru designed the study, revised the manuscript, and approved the final manuscript as submitted.

Abraham Aseffa: Dr. Abraham conceptualized and designed the study, supervised the data collection and laboratory analysis, revised the manuscript, and approved the final manuscript as submitted.

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References

1. Abu Elamreen FH, Abed AA, Sharif FA (2007) Detection and identification of bacterial enteropathogens by polymerase chain reaction and conventional techniques in childhood acute gastroenteritis in Gaza, Palestine. Int J Infect Dis 11: 501-507.
2. Vu Nguyen T, Le Van P, Le Huy C, Nguyen Gia K, Weintraub A (2006) Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. Int J Infect Dis 10: 298-308.
3. Ren Z, Gay R, Thomas A, Pae M, Wu D, et al. (2009) Effect of age on susceptibility to Salmonella Typhimurium infection in C57BL/6 mice. J Med Microbiol 58: 1559-1567.
4. Charles AJ, Paul T, Mark W, Mark JS (2001) The Immune System in Health and Disease. In Immunology:419 New York.
5. Benson (2001) Gram-Negative Intestinal Pathogens. In Microbiological applications laboratory manual in general microbiology: 270. New York: McGraw-Hill 270.
6. Choi SY, Jeon YS, Lee JH, Choi B, Moon SH, et al. (2007) Multilocus sequence typing analysis of Shigella flexneri isolates collected in Asian countries. J Med Microbiol 56: 1460-1466.
7. Woodward DL, Clark CG, Caldeira RA, Ahmed R, Soule G, et al. (2005) Identification and characterization of Shigella boydii 20 serovar nov., a new and emerging Shigella serotype. J Med Microbiol 54: 741-748.
8. Pazhani GP, Niyogi SK, Singh AK, Sen B, Taneja N, et al. (2008) Molecular characterization of multidrug-resistant Shigella species isolated from epidemic and endemic cases of shigellosis in India. J Med Microbiol 57: 856-863.
9. Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, et al. (2010) Prevalence and antimicrobial resistance of Salmonella in retail foods in northern China. Int J Food Microbiol 143: 230-234.
10. Abdullahi M (2010) Incidence and antimicrobial susceptibility pattern of salmonella species in children attending some hospitals in kano metropolis, kano state–Nigeria. Bajopas 3: 202-206.
11. Sirinavin S, Garner P (2000) Antibiotics for treating salmonella gut infections. Cochrane Database Syst Rev: CD001167.
12. Sur D, Ramamurthy T, Deen J, Bhattacharya SK (2004) Shigellosis: challenges & management issues. Indian J Med Res 120: 454-462.
13. Xia S, Hendriksen RS, Xie Z, Huang L, Zhang J, et al. (2009) Molecular characterization and antimicrobial susceptibility of Salmonella isolates from infections in humans in Henan Province, China. J Clin Microbiol 47: 401-409.
14. Ecker L, Olarte L, Vilchez G, Ochoa TJ, Amemiya I, et al. (2011) Physicians' responsibility for antibiotic use in infants from periurban Lima, Peru. Rev Panam Salud Publica 30: 574-579.
15. Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd J-M (2011) Antibiotic susceptibility patterns of Salmonell and Shigell isolates in Harar, Eastern, Ethiopia. J Infect Dis Immun. 3: 134-139.
16. Beyene G, Nair S, Asrat D, Mengistu Y, Engers H, et al. (2011) Multidrug resistant Salmonella Concord is a major cause of salmonellosis in children in Ethiopia. J Infect Dev Ctries 5: 23-33.
17. Mache A (2002) Salmonell serogroups and their antibiotic resistance patterns isolated from diarrhoeal stools of pediatric out-patients in Jimma Hospital and Jimma Health Center, South West Ethiopia. Ethiop J Health Sci 37: 37-45.
18. Tiruneh M (2009) Serodiversity and antimicrobial resistance pattern of Shigella isolates at Gondar University teaching hospital, Northwest Ethiopia. Jpn J Infect Dis 62: 93-97.
19. Yismaw O, Negeri C, Kassu A (2009) A five-year antimicrobial resistance pattern observed in Shigella species isolated from stool samples in Gondar University Hospital, northwest Ethiopia. Ethiop J Health Dev 20.
20. Don JB, Noel RK, James TS (2007) Salmonella. In Bergey's Manual Of Systematic Bacteriology, ed. Brenner D., Krieg N., Staley J., Garrity G., 2 764. USA: Springer 764.
21. Biomerieux. Identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods.
22. Denka-seiken. Bacterial Typing antisera handbook.
23. CLSI (2006) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. M100-S17. 26: 98-104
24. Gorman R, Adley CC (2004) Characterization of Salmonella enterica serotype Typhimurium isolates from human, food, and animal sources in the Republic of Ireland. J Clin Microbiol 42: 2314-2316.
25. Cheesbrough M (2006) In District Laboratory Practice in Tropical Countries Part 2. uk: Cambridge University Press, USA.
26. Opintan J, Newman MJ (2007) Distribution of serogroups and serotypes of multiple drug resistant Shigella isolates. Ghana Med J 41: 8-29.

27. Andualem B, Kassu A, Diro E, Moges F, Gedefaw M (2007) The prevalence and antimicrobial responses of Shigella isolates in HIV-1 infected and uninfected adult diarrhoea patients in north west Ethiopia. Ethiop J Health Dev 20: 99-105.

28. Samie A, Guerrant RL, Barrett L, Bessong PO, Igumbor EO, et al. (2009) Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroeal human stools from Vhembe district, South Africa. J Health Popul Nutr 27: 739-745.

29. Orrett FA (2008) Prevalence of Shigella serogroups and their antimicrobial resistance patterns in southern Trinidad. J Health Popul Nutr 26: 456-462.

30. Asrat D (2008) Shigella and Salmonella serogroups and their antibiotic susceptibility patterns in Ethiopia. East Mediterr Health J 14: 760-767.

31. Debas G, Kibret M, Biadglegne F, Abera B (2011) Prevalence and antimicrobial susceptibility patterns of shigella species at Felege Hiwot Referral Hospital, Northwest Ethiopia. Ethiop Med J 49: 249-256.

32. Reller ME, Nelson JM, Mølbak K, Ackman DM, Schoonmaker-Bopp DJ, et al. (2006) A large, multiple-restaurant outbreak of infection with Shigella flexneri serotype 2a traced to tomatoes. Clin Infect Dis 42: 163-169.

33. Villar RG, Macek MD, Simons S, Hayes PS, Goldoft MJ, et al. (1999) Investigation of multidrug-resistant Salmonella serotype Typhimurium DT104 infections linked to raw-milk cheese in Washington State. JAMA 281:1811-1816.

34. Yang J, Wang J, Chen L, Yu J, Dong J, et al. (2003) Identification and characterization of simple sequence repeats in the genomes of Shigella species. Gene 322: 85-92.

35. Roma B, Worku S, Mariam ST, Langeland N (2000) Antimicrobial susceptibility pattern of Shigella isolates in Awassa. Ethiop J Health Dev 14:149-154

36. Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK, Opintan JA (2007) Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis 13: 1640-1646.