ORIGINAL ARTICLE

Isolation and Antimicrobial Susceptibility Profile of Shigella and Salmonella Species from Children with Acute Diarrhoea in Mekelle Hospital and Semen Health Center, Ethiopia

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ABSTRACT

BACKGROUND: *Salmonella and Shigella remain the major contributors to acute enteric infections and diarrhoea. Hence, the objective of this study was to isolate and determine the antimicrobial susceptibility pattern of Shigella and Salmonella species from children with acute diarrhoea in Mekelle Hospital and Semen Health Center.*

METHODS: *A cross sectional study was conducted among 260 children with acute diarrhoea from November 2011 to March 2012 in Mekelle, Ethiopia. Stool specimen was collected from all study participants who presented with acute diarrhoea. Microscopy, culture and confirmatory identification were done by the pattern of biochemical reactions using a standard bacterial identification system (API 20E, BioMerieux, Marcy-l'Etoile, France) and polyvalent (Poly O and H) antiseras for Salmonella species and Vi for S.typhi. Isolated colonies were assessed for antimicrobial susceptibility profile using disk diffusion method. Data was entered and analyzed using SPSS version 16.0 software.*

RESULTS: *Out of the 260 study participants, 145(55.8%) were males while 115(44.2%) were females. The majority of the patients (44.2%) were of children under five years old. A total of 120 enteropathogens were isolated. The frequency of isolation was 19(7.3%), 18(6.9%) and 83(31.9%) for Salmonella species, Shigella species and intestinal parasites respectively. Most of the Shigella isolates were resistant to ampicillin (88.9%), Tetracycline (77.8), cotrimoxazole (55.6%) and chloramphenicol (55.6%). Among the Salmonella isolates, the highest resistance was observed to ampicillin (89.5%), Tetracycline (89.5%), chloramphenicol (78.9%) and cotrimoxazole (57.9%). Multi-drug resistance was noted in 19(100%) and 16(88.9%) of Salmonella and Shigella species respectively.*

CONCLUSIONS: *Shigella and Salmonella are still challenging pathogens in children < 5 years of age. High antibiotic resistance was observed among both isolates to ampicillin, tetracycline, chloramphenicol and cotrimoxazole.*

KEYWORDS: *Diarrhoea, Salmonella, Shigella, Antimicrobial susceptibility, Ethiopia*

INTRODUCTION

Diarrhoea and acute gastroenteritis are among the leading causes of illness and deaths in infants and children throughout the world, especially in developing countries in situations where water supplies are contaminated and sanitation is poor (1). This is so in Asia, Africa and Latin America where an estimated 2.5 million deaths occur each year in children, resulting in over a quarter of all childhood deaths. Diarrhoea is a major cause of childhood morbidity and mortality especially in socio-economically developing countries, which remains the second leading cause of death among under-five children globally *(*2).

World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhoea occur every year in developing countries, resulting in 3 million deaths (3). It accounts for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America (3). Despite the wide range of treatment and prevention modalities that are available, diarrhoea still remains a major contributor to infant mortality worldwide. This is an obstacle to the achievement of Millennium Development Goal(MDG) number 4 (2). According to the Federal Democratic Republic of Ethiopia Ministry of Health (FDRE MOH)'s facility-based surveillance system reports, in 2002-03, the proportions of diarrhoea attributable causes of under-five mortality in the country have been estimated as 20%. Diarrhoeal diseases kill more children than malaria, HIV/AIDS and measles combined (4).

Diarrhoea can be caused by different agents such as bacteria, parasites and virus *(5*). The main etiology of the diarrhoea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella species*, *Shigella species*, *Vibrio cholera*, *Yersinia enterocolitica*, and *Aeromonas species*), enteroparasites (*Giardia lamblia*, *Cryptosporidium* species and *Entamoeba histolytica*), and viruses (adenovirus, Norwalk virus, and rotavirus) (5).

Among the bacterial causative agents, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks. The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella* and *Shigella* species (6).

Antimicrobial resistance has complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole (7). Since most diarrhoeal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens. In Ethiopia, there is a great need to establish the identity and antibiotic susceptibility patterns of different bacterial agents which cause enteric infections in order to introduce effective treatment for diarrhoeal illness (8).

According to Tigray Regional Health Bureau's annual report from Hospitals in 2010, diarrhoea was one of the top ten in under-five children OPD visits, admissions and deaths (9). In Mekelle, there is lack of adequate information on bacterial enteric pathogens and their antimicrobial resistance trend. Hence, this study aimed to isolate and determine the antimicrobial susceptibility profiles of *Shigella* and *Salmonella* species from children with diarrhoea in Mekelle.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted from November 2011 to March 2012 in Mekelle Hospital and Semen Health Center, Mekelle, Northern Ethiopia. These sites have higer patient load, catchment population and nearest to regional laboratory.

Inclusion and exclusion criteria: Participants who meet the following inclusion criteria were included in the study: having acute diarrhoea (the passage of loose stool by an individual, at least three times a day for <14 days in duration) (1), age of 6 month to 14 years and no anti-infective therapy for all of the antibiotics. We used in this

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study 48 hours prior to recruitment and informed written consent from the children's parents or guardians. Children with antibiotic treatment within 48 hours before recruitment, whose parents did not agree to participate, were less than 6 month old and those who were greater than 14 years old were excluded.

Sample collection, handling and transport: Two hundred and sixty stool specimens were collected from all study participants who presented with acute diarrhoea using dry, clean, leak proof and wide mouth stool containers after informed consent was obtained through their legal and competent guardians or parents and parasitological examination was done directly after collection by direct microscopy in the study site.

Then, after transported into the Microbiology laboratory of Mekelle Regional Health Research Laboratory using Cary Blair transport media within 2-4 hours of collection for bacteriological examination and antimicrobial susceptibility testing. The sample size was determined based on the prevalence rate of *shigella* spicies done by Mache on children in Jimma(16).

Processing of stool specimens: Direct microscopy of the smears in saline (0.85% Nacl solution) was performed for the detection of ova, larvae, trophozoites and cysts of intestinal parasites. Stool samples were directly inoculated using sterile cotton swabs onto MacConky agar (Oxoid; UK) and Xylose Lysine Deoxycholate (XLD) agar (Oxoid; UK), and the plates were incubated aerobically at 37°C for 24 hours. The same stool samples were also inoculated onto Selenite F broth enrichment media (Mast Diagnostics, UK) and incubated at 37°C for 24 hours, which is intended for the best recovery of *Salmonella* species. Following the incubation of Selenite F broth, sub-cultures were done onto both MAC and XLD plates and incubated at 37°C for 24 hours. The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on MAC (NLF, smooth, colorless colonies, sometimes with black centered) and XLD agar (small red colonies and black-centered colonies). Confirmatory identification was done by the pattern of biochemical reactions using a standard bacterial identification system (API 20E, BioMerieux, Marcy-l'Etoile, France) and polyvalent (Poly O and H) antiseras for *Salmonella* species and Vi for *S.typhi* (10). We used polyvalent antisera to identify salmonella typhi from others and supported also using the API 20 E to both salmonella and shigella species differentiation

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was performed using the disk diffusion method. In brief, a McFarland 0.5 standardized suspension of the bacteria in 0.85% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar (Oxoid, UK) with a sterile cotton swab (10). The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 9 antibiotic discs (Mast Diagnostics, UK) was then delivered onto the inoculated surface of Muller-Hinton plate with the following concentrations of the discs; ampicillin (AP) (10 µg), chloramphenicol (C) (30 µg), gentamycin (CN) (10 µg), norfloxacin (NOR) (10 µg), cotrimoxazole (TS) (25 µg), nalidixic acid (NA) (30 µg), ceftriaxone (CRO) (30 µg), ciprofloxacin (CIP) (5 µg) and tetracycline (T) (30 µg) (Mast Diagnostics, UK).

After overnight incubation at 37°C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler. The diameter of the zone was read using an interpreting chart for zone sizes. Findings of antibiotic resistance testing were recorded as susceptible, intermediate and resistant (10).

Reference strain: *E. coli* (ATCC 25922) was used as quality control throughout the study for culture and antimicrobial susceptibility testing. This strain was obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

Statistical analysis: Data was entered and analyzed using SPSS version 16.0 software. Statistical analysis was focused on the relationships between antimicrobial resistance patterns with *Salmonella* and *Shigella* species of the patients. The distribution of *Shigella* and *Salmonella* species in relation to age, sex and consistency of the stool was also analyzed. Probability values were based on two-tailed test

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results and *P* values of <0.05 was considered statistically significant.

Ethical clearance: Ethical clearance was given by the Department Research and Ethical Review Committee (DREC) of Addis Ababa University. Official permission from the study sites was secured. Written informed consent was obtained from study participants' parents/ guardians. The results of the study participants with positive for enteropathogens were communicated to the attending physician for the management of the cases.

RESULTS

The number and percentage of detection of enteropathogens in diarrhoeal stool samples obtained from pediatrics patients by culture and microscopic examination is presented in Table 1. A total of 260 study participants were participated in the study. Of these, 37 participants were positive for one or more organisms. One hundred and twenty enteropathogens were isolated. Of the total 120 identified enteropathogens, 19 *Salmonella* species, 18 *Shigella* species and 83 intestinal parasites were identified.

***Salmonella* species**: A total of 19/260 (7.3%) *Salmonella* species were isolated from children with diarrhoea. Of these, 2(10.5%) *Salmonella typhi,* 3(15.8%) *Salmonella paratyphi A* and 14(73.7%) *Salmonella species* were isolated.

Table 1: Frequency of isolation of enteropathogens from the 260 children with acute diarrhoea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Enteropathogens	**Age group in years**			
	<5	5-9	>9	Total
	No. (%)	No. (%)	No. (%)	No. (%)
Salmonella				
Salmonella species (non typhi and paratyphi A)	11 (78.6)	3 (21.4)	0(0.0)	14 (100)
Salmonella typhi	0 (0.0)	1 (50.0)	1 (50.0)	2 (100)
Salmonella paratyphi A	3 (100)	0 (0.0)	0 (0.0)	3 (100)
Total	**14 (73.7)**	**4 (21.0)**	**1 (5.3)**	**19 (100)**
Shigella				
Shigella species	12 (75.0)	4 (25.0)	0 (0.0)	16 (100)
Shigella sonnei	0 (0.0)	0 (0.0)	2 (100)	2 (100)
Total	**12 (66.7)**	**4 (22.2)**	**2 (11.1)**	**18 (100)**
Intestinal parasites				
Entameoba histolytica	17 (43.6)	14 (35.9)	8 (20.5)	39 (100)
Giardia lamblia	17 (50.0)	14 (41.2)	3 (8.8)	34 (100)
Hymenolepis nana	0 (0.0)	4 (80.0)	1 (20.0)	5 (100)
Taenea species	0 (0.0)	1 (100)	0 (0.0)	1 (100)
Trichuris trichuria	0 (0.0)	1 (100)	0 (0.0)	1 (100)
Schistosoma mansonni	1 (50.0)	1 (50.0)	0 (0.0)	2 (100)
Entrovious vermicularis	1 (100)	0 (0.0)	0 (0.0)	1 (100)
Total	**36 (43.4)**	**35 (42.2)**	**12 (14.4)**	**83 (100)**

The age distributions of *Salmonella* were 14(73.7%), 4(21.1%) and 1(5.3%) in the age group of <5, 5-9, >9 years respectively (Figure 1). *Salmonella* infections were more common among children of < 5 years of age than the other age groups (p = 0.007) (Table 2). The sex

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distributions of the isolates were 12(63.2%) and 7(36.8%) in males and female respectivelys. Sex has no statistically significance association with *Salmonelosis* (p = 0.501). Among the 19 children who were positive for *Salmonella* species 9(47.4%), 7(36.8%), 1(5.3%) And 2(10.5%) were isolated from watery, mucoid, bloody and mixed diarrhoea respectively. Isolation of *Salmonellos is* was not statistically associated with nature of diarrhoea (p >0.05).

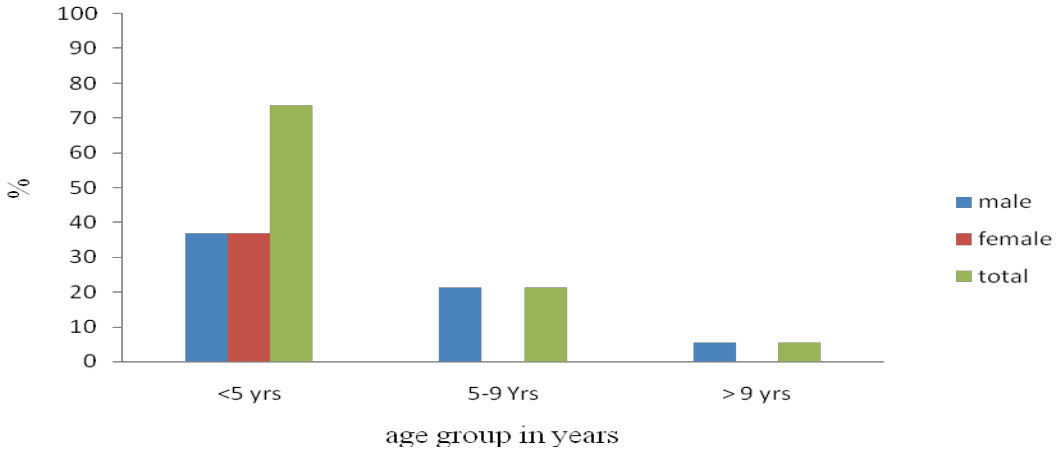

***Figure 1**: Age and sex distribution of children who were positive for Salmonella species in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.*

Table 2: Association of culture positive and negative *Salmonella* species with age group and nature of diarrhea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

	Positive (%) (*Salmonella* spp.) n=19	Negative (%) (*Salmonella* spp.) n=241	Total (%) n=260	P- value
Age group in years				
<5	Yes 14 (73.7)	101 (41.9)	115 (44.2)	0.007
	No 5 (26.3)	140 (58.1)	145 (55.8)	
5-9	Yes 4 (21.1)	104 (43.2)	108 (41.5)	0.06
	No 15 (78.9)	137 (56.8)	152 (58.5)	
>9	Yes 1 (5.3)	36 (14.9)	37 (14.2)	0.245
	No 18 (94.7)	205 (85.1)	223 (85.8)	
Nature of diarrhoea				
Watery				
	Yes 9 (47.4)	154 (63.9)	163 (62.7)	0.151
	No 10 (52.6)	87 (36.1)	97 (37.3)	
Mucoid	Yes 7 (36.8)	69 (28.6)	76 (29.2)	0.449
	No 12 (63.2)	172 (71.4)	184 (70.8)	
Bloody	Yes 1 (5.3)	11 (4.6)	12 (4.6)	0.889
	No 18 (94.7)	230 (95.4)	248 (95.4)	
Mixed	Yes 2 (10.5)	7 (2.9)	9 (3.5)	0.080
	No 17 (89.5)	234 (97.1)	251 (96.5)	

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***Shigella* species**: A total of 18/260 (6.9%) *Shigella* species were isolated from children with diarrhoea. Of these, 2(11.1) *Shigella sonnei* and 16(88.9%) *Shigella* species were isolated. The distributions of *Shigella* isolates in different age groups were 12(66.7%), 4(22.2%) and 2(11.1%) in the age group of < 5, 5-9, > 9 years respectively as shown in figure 1. *Shigella* infections were more common among children of < 5 years of age than the other age groups (p= 0.047). Among the 18 children who were positive for *Shigella* species, 6(33.3%), 8(44.4%), 3(16.7%) and 1(5.6%) were isolated from watery, mucoid, bloody and mixed diarrhoea respectively. Isolations of shigellosis were statistically associated with bloody diarrhoea (p= 0.012) (Table 3).

Table 3: Association of isolation of *Shigella* species with age group and nature of diarrhea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

	Positive (%) (*Shigella* spp.) n=18	Negative (%) (*Shigella* spp.) n=242	Total (%) N=260	P- value
Age group in years				
<5	Yes 12 (66.7)	103 (42.6)	115 (44.2)	0.047
	No 6 (33.3)	139 (57.4)	145 (55.8)	
5-9	Yes 4 (22.2)	104 (43)	108 (41.5)	0.085
	No 14 (77.8)	138 (57)	152 (58.5)	
>9	Yes 2 (11.1)	35 (14.5)	37 (14.2)	0.695
	No 16 (88.9)	207 (85.5)	223 (85.8)	
Nature of diarrhoea				
Watery				
	Yes 6 (33.3)	157 (64.9)	163 (62.7)	0.08
	No 12 (66.7)	85 (35.1)	97 (37.3)	
Mucoid	Yes 8 (44.4)	68 (28.1)	76 (29.2)	0.141
	No 10 (55.6)	174 (71.9)	184 (70.8)	
Bloody	Yes 3 (16.7)	9 (3.7)	12 (4.6)	0.012
	No 15 (83.3)	233 (96.3)	248 (95.4)	
Mixed	Yes 1(5.6)	8 (3.3)	9 (3.5)	0.614
	No 17 (94.4)	234 (96.7)	251 (96.5)	

Antimicrobial susceptibility pattern

***salmonella* species**: The antimicrobial susceptibility testing was done on all *Salmonella* isolates using disk diffusion method; the results are presented in Table 4. Among the 19 *Salmonella* isolates, the overall rates of resistance were ampicillin 17(89.5%), tetracycline 17(89.5), chloramphenicol 15(78.9%), cotrimoxazole 11 (57.9%), nalidixic acid 6 (31.6%), gentamicin 3(15.8%), ceftriaxone 2(10.5%) and norfloxacin 1(5.3%). All isolates were sensitive to ciprofloxacin.

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Table 4: Age and sex distribution of children positive for intestinal parasites in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Age group in year	Sex no. (%)		
	Male	**Female**	**Total**
<5	20(24.1)	16(19.3)	36(43.4)
5-9	23(27.7)	12(14.5)	35(42.2)
>9	9(10.8)	3(3.6)	12(14.4)
Total	**52(62.6)**	**31(37.4)**	**83(100)**

***Shigella* species**: The results of the antimicrobial susceptibility pattern of *Shigella* isolates are summarized in Table 5. Among the 18 *Shigella* isolates, the overall rates of resistance were ampicillin 16(88.9%), tetracycline 14(77.8%), chloramphenicol 10(55.6%), cotrimoxazole 10(55.6%), nalidixic acid 5(27.8%) and gentamicin 5(27.8%). All isolates were 94.4-100% susceptible to ciprofloxacin, ceftriaxone and norfloxacin. Resistance to one or more antimicrobial agent(s) was noted in 17(94.4%) of the isolates.

Multi-drug resistance (MDR): Multi-drug resistance (resistance to two or more drugs) was observed in 19/19(100 %) and 16/18(88.9%) of *Salmonella* and *Shigella* isolates (Table 6).

Table 5: Antimicrobial Susceptibility Profile of *Salmonella species* (n=19) isolated from children in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Antimicrobial agents	Susceptible No. (%)	Intermediate No. (%)	Resistance No. (%)
Ampicillin	2(10.5)	0(0.0)	17(89.5)
Co-trimoxazole	7(36.8)	1(5.3)	11(57.9)
Chloramphenicol	3(15.8)	1(5.3)	15(78.9)
Norfloxacin	17(89.5)	1(5.3)	1(5.3)
Tetracycline	2(10.5)	0(0.0)	17(89.5)
Gentamicin	16(84.2)	0(0.0)	3(15.8)
Ceftriaxone	17(89.5)	0(0.0)	2(10.5)
Nalidixic acid	12(63.1)	1(5.3)	6(31.6)
Ciprofloxacin	19(100)	0(0.0)	0(00.0)

Table 6. Antimicrobial Susceptibility Profile of *Shigella species* isolated from children in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Antimicrobial agents	Susceptible No. (%)	Intermediate No. (%)	Resistance No. (%)
Ampicillin	0(0)	2(11.1)	16(88.9)
Cotrimoxazole	7(38.9)	1(5.6)	10(55.6)
Chloramphenicol	8(44.4)	0(0.0)	10(55.6)
Norfloxacin	18(100)	0(0.0)	0(0.0)
Tetracycline	4(22.2)	0(0.0)	14(77.8)
Gentamicin	12(66.7)	1(5.5)	5(27.8)
Ceftriaxone	17(94.4)	1(5.6)	0(0.0)
Nalidixic acid	10(55.5)	3(16.7)	5(27.8)
Ciprofloxacin	18(100)	0(0.0)	0(0.0)

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DISCUSSION

The overall prevalence of *Salmonella* species in this study was 7.3%. This is in agreement with a study conducted in Yemen, 6.8% (11), and higher than the findings reported in Addis Ababa, 3.8% (12). But was lower than the 15.4% isolation rate reported from Jimma (13).

The variability of isolation of *Salmonella* may be attributable to the difference in study areas and period because the features of the disease vary from place to place and time to time depending on the local meteorology, geography and socio-economic elements (13).

According to this study, the isolation rate of *Salmonellosis* was higher in children of less than 5 years old, which is in accordance with previous study done in Iran (14). This might be due to age difference, because children are less likely to wash their hands after defecating than adults, more likely to put their fingers or dirty objects into their mouth and also more likely to play in soil where they may come into contact with faeces.

In this study, the majority of *Salmonella* isolates were resistant to ampicillin (89.5%), Tetracycline (89.5%), chloramphenicol (78.9%) and co-trimoxazole (57.9%). This observation is in contrast with findings from Brazil (19) where most of the isolates were sensitive to ampicillin (40.7%), chloramphenicol (64.4%), tetracycline (40.7%) and co-trimoxazole (59.3%).

Compared to a previous report from Jimma (13), greater than fourfold increase in resistance to gentamicin from 1.7% to 15.8% was observed in the present study. This increase might be because of the relatively increased irrational use of the antibiotics in Meklle. In developing countries like Ethiopia, antibiotics are carelessly used by patients and physicians. It is thus a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public contributing to the emergence and spread of antimicrobial resistance (8,15).

Resistance to two or more drugs was observed in 100% of the isolates in this study. The organisms seem to have increased their resistance to the drugs from lower levels reported earlier (20) to levels of more than 90% in reports by Asrat (8). This is similar to the pattern across the globe where the organisms have consistently increasing their resistance to these commonly used first line drugs. This is a sharp increase from earlier reports indicating the aggravating problem of drug resistance by these microbes over the years. Multi-drug resistant *Salmonella* isolates have become an issue of worldwide concern (14).

The isolation of *Shigella* species (6.9%) in this study is in agreement with those reports from Harar 6.9% (20), Gondar 7.5% (17) and Brazil 7.1% (19), but lower than findings in Addis Ababa 11.7% (12) and Jimma 20.1% (16) from similar study participants.

Higher isolation rate (66.7%) of the *Shigella* isolates was found in children of less than 5 years of old. This might indicate that *Shigellosis* was the problem of children of under 5 years of age in Mekelle, which is in agreement with studies conducted in Gondar (17).

Mucus in the stool, with or without blood, was the main characteristic of *Shigella* infection, found in 50% (9/18) of patients, although *shigellosis* was statistically associated only in patients with bloody diarrhoea, which is in agreement with reports from Harar, Ethiopia (20).

However, in contrast with a study conducted in Addis Ababa, where the majority (82.4%) of the diarrhoeal samples in which *Salmonella* and *Shigella* were isolated, had watery nature (8). This may reflect the underlying geographic variations in strain patterns from place to place. In developing countries, *Sh. dysenteriae* and *Sh. flexneri* are prevalent species causing mucoid to bloody diarrhoea, while in developed countries, *Sh. sonnei* and *Sh. boydi* species predominate. Shigellosis is commonly associated with mild watery diarrhoea. While *Sh. dysentriae* are consistently associated with dysentery, it is less common for *Sh. flexneri* to cause bloody diarrhoea (1).

Among all antibiotics tested for *Shigella* species, the highest resistance was observed with ampicillin (88.9%), tetracycline (77.8%), chloramphenicol (55.6%) and cotrimoxazole (55.6%). These findings are in agreement with the previous studies conducted in different places and

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times in Ethiopia (8,17,18). Compared to previous studies reported in Ethiopia (8,16,17,18), *Shigella* isolates had a higher level of resistance to gentamicin (27.8%). This may be due to the indiscriminate overuse of the drug in the community.

In agreement to studies conducted in Jimma, Gondar and Addis Ababa (8,16,17,18) *Shigella* isolates were highly resistant to ampicillin. However, there seems to be a lower pattern of resistance to the drug in studies reported from Brazil (19). This could be due to the fact that ampicillin has been used in Brazil for a long time and because of its easy availability and potential for misuse. The antimicrobial resistance patterns of *Shigella* species vary according to geographic region and in the same place over time, leading to a therapeutic problem. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively, particularly in developing countries.

Of the *Shigella* isolates, 17(94.4%) were found to be resistant to one or more antimicrobial agent(s), and 16(88.9%) were multi-drug resistant, which is comparable with findings from Gondar, where 90.8% and 87.8% of the isolates were resistant to one or more antimicrobial agent(s) and multi-drug resistant respectively (18).

Several factors may contribute to resistance by pathogens causing gastroenteritis in developing countries like Ethiopia. These include frequent overuse, misuse and factors related to the potency and quality of antimicrobials and the distribution of resistant strains (8). In addition, syndromic diagnosis and diagnostic imprecision usually force physicians to adopt for broad spectrum antibiotics such as amoxicillin and tetracycline, over prescribing and less antibiotic diversity which lead to the emergence and spread of antimicrobial resistance. Fortunately, there seems to be limited resistance to ciprofloxacin norfloxacine and ceftriaxon. However, given the current trends in Ethiopia, unless intensive efforts are made to stem the unrestricted use of antimicrobials in Mekelle, it will not probably be long before the microbes develop resistance to these expensive drugs and complicate effective treatment of gastroenteritis (15).

It was not possible to conduct *Shigella* and *Salmonella* serogrouping/serotyping due to lack of antisera. This study gives a brief overview of the burden and distribution of *Shigella*, *Salmonella* and intestinal parasite related diarrhoeal disease in children. The overall prevalences of *Salmonellosis*, Shigellosis and intestinal parasitosis were 7.3%, 6.9% and 31.9% respectively with greater than 2/3 of the Salmonellosis and Shigellosis cases found in children of less than 5 years old. Accordingly, *Shigella* and *Salmonella* were still challenging in children especially of less than 5 years old. High antibiotic resistance was observed among both isolates particularly to ampicillin, tetracycline, chloramphenicol and cotrimoxazole. Resistance to two or more antibiotics was observed in 100% and 88.9% of *Salmonella* and *Shigella* species respectively. Only ciprofloxacin, norfloxacin and ceftriaxone were effective for both isolates. These findings reinforce the need for continous surveillance program and strengthened infection control system to reduce the rate of infection and to apply appropriate guidelines for the use of therapeutic antibiotics.

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