Magnitude of Pathogenic Enteric Bacteria, Associated Factors, and Antimicrobial Susceptibility Pattern among Pediatric Patient with diarrhea in Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia

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Abstract

Background: Diarrheal disease remains the most predominant public health problem in developing countries, including Ethiopia. However, there is limited information on the extent of pathogenic enteric bacteria among pediatric patients with diarrhea in the eastern part of Ethiopia. Therefore, this study aimed to determine the prevalence of enteric bacterial pathogens, associated factors, and antimicrobial susceptibility patterns among diarrheic pediatric patients at Hiwot Fana Specialized University Hospital, Harar, eastern Ethiopia March 15 to May 15, 2021.

Methods: A hospital-based cross-sectional study was conducted among 210 diarrheic pediatric patients. Stool samples were collected and enteropathogenic bacteria were identified by the standard microbiological techniques and antimicrobial susceptibility testing was done using the disk diffusion method. Data were analyzed using Statistical Package for Social Science version 20. A multivariable logistic regression model was constructed and, a P-value <0.05 was considered a statistically significant association.

Results: The overall prevalence of enteric bacterial pathogens was 15.7% (95% CI (10.8, 20.6)). The most frequently identified isolates were *Shigella flexneri/boydii* and *EHEC O157:H7* with the same rate of 30.30%. All isolated bacteria were 90.9% susceptible to piperacillin, 87.9% to amoxicillin-clavulanate, and 78.8% to ciprofloxacin, besides, 81.8% and 57.6% of isolated enteropathogenic bacteria were resistant to ampicillin and trimethoprim/sulfamethoxazole, respectively. The overall prevalence of multi-drug resistance was 42.4% (95% CI; 24.2, 60.6.). Parents/guardians with an untrimmed fingernail (AOR=3.8, 95% CI; 1.4, 10.4), parents/guardians who hadn't a habit of washing hands before feeding (AOR=4.8, 95% CI; 1.51, 15.4) were factors associated with isolated bacteria.

Conclusions: The prevalence of pathogenic enteric bacteria was high. Parent/guardians with untrimmed fingernails, and who hadn't a habit of washing hands before feeding were factors associated with isolated bacteria. The overall prevalence of multi-drug resistance was high. Piperacillin, amoxicillin-clavulanate, and ciprofloxacin can be used as part of the empirical list of drugs for enteric bacteria. Therefore, clinicians should also consider stool culture and antimicrobial susceptibility for better management and control of enteric bacterial diarrhea.

Keywords: *Enteric bacteria; Diarrhea; Pediatric; Antimicrobial susceptibility test; Eastern Ethiopia*

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Introduction

Diarrheal diseases are a major cause of morbidity and mortality worldwide (Mokomane *et al*., 2018) .It is considered to be responsible for 15% of all childhood deaths worldwide, with estimates ranging from 25% in Africa to 31% in Southeast Asia (Kosek *et al*., 2003). In sub-Saharan Africa, it is also the second leading cause of illness and mortality among children (Bartram *et al*., 2014).

Global estimates for the prevalence of specific types of bacterial diarrhea among all diarrheal causes include *E.coli* 10% to 25%, S*higella* 10%, and *Salmonella 3*%, and *Campylobacter* 3 to 6% (Gutmans, 2023). Non-typhoidal *Salmonella sp*. yearly causes 93,800,000 cases of gastroenteritis and 155,000 deaths internationally (Bialvaei *et al*., 2017). However, bacterial enteritis is very common in low- and middle-income countries (LMICs), (Kotloff *et al*., 2013; Mokomane *et al.,* 2018). The prevalence of enteric bacterial pathogens varied from country to country 40.75%, 42.3%, and 31.6% Zanzibar (Omar, 2015), Jordan (Youssef *et al.,* 2000), and Sub-Saharan Africa respectively (Oppong *et al*., 2020).

Antibiotic resistance patterns among enteric bacteria such as *Shigella, Vibrio cholerae, Enteropathogenic E. coli (EPEC), Salmonella* Typhi*, and S. enteriditis* are becoming a major concern worldwide. Moreover,

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in Ethiopia isolates of *Shigella* and *Salmonella* showed inconsistent prevalence rates and a high rate of drug resistance to the frequently used antibiotic agents in different regions (Mengistu *et al.,* 2014).

The documented risk factors for bacterial diarrhea include younger age, low maternal education, poor water storage practices, lack of observant hand washing, poor sanitation, and lack of access to clean water supply (George *et al*., 2014). Lack of access to clean water supply, poor sanitation, and inadequate personal hygiene are responsible for 90% of bacterial diarrheal disease occurrences (Gebru *et al.,* 2014).

Number of studies have been conducted on diarrhea among young children in Ethiopia (Howe *et al.,* 2019, Tosisa *et al.,* 2020). The magnitude of enteric bacterial pathogen in Hiwot Fana Specialized University Hospital was 18.2% (Reda *et al*., 2011). However, there is no updated information about pathogenic enteric bacteria, associated factors, and antimicrobial susceptibility patterns among pediatric patients with diarrhea in Eastern Ethiopia. Therefore, this study aimed to assess the magnitude of pathogenic enteric bacteria, associated factors, and antimicrobial susceptibility patterns among pediatrics patients with diarrhea in Hiwot Fana Specialized University Hospital, Harar, eastern Ethiopia.

Materials and Methods

Study Setting, Design, and Period

A hospital-based cross-sectional study was conducted among pediatrics patients with diarrhea in Hiwot Fana Specialized University Hospital (HFSUH), Harar, eastern Ethiopia from March 15 to May 15, 2021. Harar is the capital city of the Harari regional state, which is located 525 km from Addis Ababa to the east. There are two public hospital in the region Jugal and Hiwot Fana specialized University Hospital. Hiwot Fana Specialized University Hospital was established during the occupation of Ethiopia by Italian soldiers (1928-1933). Currently, the hospital is a teaching facility for health and medical sciences students at Haramaya University, College of Health Sciences. The hospital also provides health care services to more than 3 million people around Harar and neighboring regions (Gelchu *et al*., 2019).

Population, Inclusion/ Exclusion Criteria

The study the study population included all children who experienced diarrhea. Children who had received antibiotics within previous two weeks of the data collection and were unable to give stool sample were excluded.

Sample Size and Sampling Technique

The sample size was determined using a single population proportion formula by considering the prevalence of 14.5% enteric bacterial pathogens among pediatric patients with diarrhea in selected health facilities, Addis Ababa, Ethiopia (Howe *et al*., 2019), 95% confidence interval, 5% margin of error, and 10% non-response rates. The final sample size was 210. Study participants were enrolled until the required sample size was fulfilled.

Data Collection Techniques

Data were collected by the following method;

Face-to-face interviews: were conducted by trained clinical nurses using a structured questionnaire adopted from different literature (Howe *et al*., 2019; Tosisa, 2015). The questionnaire contains about socio-demographic (age, gender, residence, educational status, marital status, occupational and income status) behavioral, and environmental factors like waste disposal system, source of drinking water and hand washing habit, and toilet usage. The trained clinical nurses also performed an appropriate physical examination and history of pediatric patients from medical registration including treatment history.

Anthropometric Assessment: The weight (W) was measured on a scale with a maximum capacity of 150 kg and an accuracy of 0.1 kg. Participants were asked to wear minimal clothing and bare feet during the procedure. Height (H) was measured in a standing position with their heads straight ahead, heels together, and shoes off using a stadiometer. The height was measured to the nearest 0.1cm (Moss *et al*., 2020). The weight, height, age, and sex of the child were entered into WHO Anthro Plus software and categorized as malnutrition when the child was under or over-nutrition (WHO, 2019).

Stool Sample Collection, Transportation, and Bacterial Identification: A stool sample was collected from the child by the parent/ guardian using a clean and sterile screw-cup container after obtaining instruction. All stool specimens were placed into Carry Blair

transport medium and selenite-F broth, which were then transported to the microbiology laboratory of Haramaya University. The stool sample was inoculated on MacConkey, Xylose lysine deoxycholate (XLD), and selenite F-broth (Cheesbrough, 2005). The culture plates were incubated for 24 hours, aerobically at 37°C. The colonies were morphologically examined for size, shape, and lactose fermentation ability. And subsequently characterized using the standard biochemical test described like, oxidase, indole test, urease production, carbohydrate fermentation, hydrogen sulfide, gas production test, citrate utilization test, motility test, lysine decarboxylase test (LDC), lysine deaminase (LDA), and mannitol fermentation were used to differentiate bacterial isolates. E.coli isolates were also sub-cultured onto Sorbitol McConkey agar which was used to differentiate *EHEC O157:H7* from other *E. coli spp*. (CLSI, 2018).

Antimicrobial susceptibility test: of the isolated bacteria was done using the Kirby-Bauer disk diffusion method. A pure colony of an isolated organism was mixed with normal saline compared with 0.5 McFarland standards and inoculated in muller Hinton agar (CLSI, 2020). The following antibacterial disks: ciprofloxacin- CIP (5µg), chloramphenicol- C (30µg), cefepime- FEP (30µg), gentamicin-GN (10µg), amoxicillin-clavulanate (30µg), and trimethoprim/sulfamethoxazole - SXT (30µg), ampicillin-AMP (10µg), azithromycin AZT (30µg) and piperacillin P (10µg) were used to screen susceptibility of isolates based on local availability, pathogens, and CLSI recommendations. The diameter of each inhibition zone was measured with calipers and recorded in mm. The results were interpreted as sensitive (S), intermediate (I), or resistance (R) according to CLSI guidelines (CLSI, 2020). Moreover, the Multi Drug Resistant (MDR) bacteria were resistant to three or more antimicrobial categories (Magiorakos *et al.,* 2012).

Data Quality Control

The questionnaire was prepared first in the English language and translated into local languages and back to the English language by different language experts and pretested on a 5 % sample size at Jugal Hospital. Data collectors were trained and the raw data was checked every day for completeness and consistency during data collection. All laboratory operations were carried out following standard operating procedures (SOPs). The sterility of the culture media was tested by incubating 5% of the batch at 37 °C. Standard control strains of E. coli ATCC 25922, *Salmonella* Typhi ATCC 13311, *Salmonella enteriditis* ATCC 13076, and *Shigella sonni ATCC* 25331 were used to test growth ability and sensitivity testing of the culture media (Weinstein *et al*., 2020).

Data Processing and Analysis

Data were entered into Epi Info version 7 and transferred to SPSS version 20 for cleaning and analysis. Descriptive statistical analysis was used to describe the socio-demographic and clinical characteristics of study participants, the prevalence of enteric bacteria, and its antimicrobial susceptibility pattern. The association between associated factors and enteric bacteria was determined by using bi-variable and multi-variable logistic regression analysis. All explanatory variables with a P-value ≤ 0.25 in the bi-variable analysis were considered for a multi-variable logistic regression analysis. Variables with $p < 0.05$ in multivariable analysis were considered as statistical factors associated with enteric bacterial pathogens.

Ethical Consideration

Ethical clearance was obtained from the College of Health and Medical Science Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University with ethical reference number Ref. No. IHRERC/023/202 and an official letter of support was written to HFSUH. The purpose, objectives, procedures, potential risks, benefits of the study, and their rights to refuse or withdraw from the study at any time were explained to the hospital head and the guardians of each pediatrics. Participants' confidentiality was ensured by excluding names and identifiers from the questionnaires. The head of the hospital and guardian provided informed voluntary written, and signed consent. Moreover, positive cases were reported to the attending physician or health professional.

Results

Socio-Demographic Characteristics of Participants

A total of 210 pediatric patients with diarrhea participated in this study. The mean age of pediatrics was 5.0 ± 3.85 (SD) years. Of those, 110 (52.4%), 64 (30.5%), and 127 (60.5%) of the children were female, at kindergarten educational level and rural residences respectively. Among parents/guardians, 52 (24.8%), 82 (39.0%),87 (41.4%), and 95 (45.2%) were able to read

and write, had average family monthly income > 1500 Ethiopian birr (ETB), were housewife, and had 4-5 family sizes, respectively.

Most of the study participants (98.1%) reported that one of the household members had diarrhea last two weeks prior to data collection (Table 1).

Table 1:- Socio-demographic characteristics of the parent /guardian or pediatric with diarrhea attending Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021 (n=210).

Variables	**Category**	**Frequency (%)**
Pediatric age group (years)	0-4	96(45.7)
	5-9	76(36.2)
	10-14	38(18.1)
Residence	Urban	83(39.5)
	Rural	127(60.5)
Educational status of pediatrics	Under age	61(29.0
	Kindergarten	64(30.5)
	Elementary school	50(23.8)
	Not formal education	35(16.7)
Educational status of parent/guardian	Unable to read and write	43(20.5)
	Able to read and write	52(24.8)
	Elementary school	50(23.8)
	High school	28(13.3)
	College and above	37(17.6)
Family monthly income (ETB)	<500	19(9.0)
	501-1000	47(22.4)
	1001-1500	62(29.5)
	>1500	82(39.0)
Occupational status	Housewife	87(41.4)
	Merchant	46(21.9)
	Farmer	40(19.0)
	Civil servant	25(11.9)
	Daily laborer	12(5.7)
Family size	2-3	40(19.0)
	4-5	95(45.2)
	≥6	75(35.7)

Clinical Features of the Study Participants

A total of 61 (29.0%), 110 (52.4%), and 45 (45.0%) of the study participants had a clinical duration of more than a week, did not receive any antimicrobial treatment for diarrheal disease and, only received Oral Rehydration Salts (ORS), respectively. Additionally, 97(46.2%) and 112 (53.3%) of the study participants were malnourished and had a fever (Table 2).

Environmental, and Behavioral Characteristics

The majority of the study participants had a latrine (81.4%) and reported having a hand washing facility attached to the latrine (60.2%). Regarding hand washing practice, only 64 (30.5%) guardians had a daily habit of washing pediatrics' hands after defecation, and 137 (65.2%) of guardians washed their hands before feeding their children. The majority of guardians (64.3%) and children (72.9%) had trimmed fingernails. Additionally, 70 (33.3%) and 70 (33.3) of participants used pipe chlorinated water and, consumed cooked food and beverages before the illness, respectively. Moreover, 134 (63.8%) and (48.57%) of the guardian reported have a domestic animal in their house and dispose of their waste in open fields, respectively (Table 2).

Table 2: Clinical features, environmental, and behavioral characteristics of the parent /guardian or pediatric with diarrhea attending Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021(n=210).

Variables	Category	Frequency (%)
No. of household members reported diarrhea	Only one	206(98.1)
	≥2	4(1.9)
Duration of diarrhea (day)	More than -7	61(29.0)
	5-7	54(25.7)
	3-4	54(25.7)
	1-2	41(19.5)
Previous history of treatment	Yes	100(47.6)
	No	110(52.4)
Types of treatment taken	ORS only	45(45.0)
	Antibiotics + ORS	26(26.0)
	Antibiotics only	29(29)
HIV serological status	Negative	64(30.5)
	Positive	57(27.1)
	Not Tested	89(42.4)
Nutritional status	Malnourished	97(46.2)
	Non-malnourished	113(53.3)
Types of diarrhea	Watery	125(59.5
	Mucoid	40(19.0)
	Bloody	24(11.4)
	Semi-fluid	21(10.0)
Clinical feature(s) other than diarrhea	Fever	112(53.3)
	More than one symptom	51(24.3)
	Dehydration	31(14.8)
	Vomiting	16(7.6)
Latrine availability	Yes	171(81.4)
	No	39(18.6)
Hand washing facility attached to the latrine	Yes	103(60.2)
	No	68(39.8)
A habit of washing pediatrics' hands after defecation	Yes	135(64.28)
	No	75(35.72)
Frequency of washing pediatrics' hands after defecation	Daily	64(30.5)
	Sometimes	71(33.8_)
	Never	75(35.7)
Fingernail status of parent /guardians	Trimmed	135(64.3)
	Untrimmed	75(35.7)
Fingernail status of a child	Trimmed	153(72.9)
	Untrimmed	57(27.1)
Drinking water source for the child	Pipe water	171(81.4)
	Bottled water	39(18.6)
Waste disposal area	Open field	102(48.57)
	Hole	39(18.57)
	Municipal	69(32.86)
Domestic animals present in the house	Yes	134(63.8)
	No	76(36.2)
Types of food/ drink taken by the child before illness	Raw meat	31(14.8)
	Raw vegetable	63(30.0)
	Unpasteurized milk	45(21.4)
	Cooked food and drink	71(33.8)
Parent /guardian hand washing habit before feeding child	Yes	137(65.2)
	No	73(34.8)

ORS: Oral Rehydration Salt, HIV: Human Immunodeficiency Virus

Prevalence of Enteric Bacterial Pathogens

The overall prevalence of enteric bacterial pathogens among diarrheic pediatric patients at HFSUH was 33(15.7%) (95% CI: 10.8, 20.6). The most frequently identified isolates were *Shigella flexneri/boydii* and *EHEC O157*:*H7* with the same rate of 30.30%, followed by *Salmonella* Typhi at 15.16% (5/33) (Fig 1).

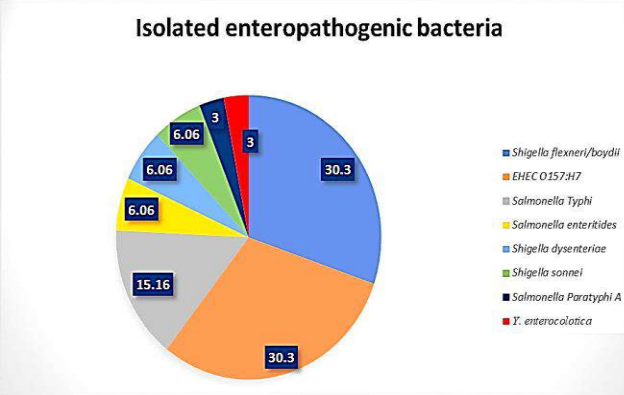

Figure 1; Isolated enter pathogenic bacteria

Factors Associated with Enteropathogenic Bacteria

Based on the bi-variable analysis; the nutritional status of pediatrics, fingernail status of guardians, a habit of washing pediatrics' hands after defecation, waste disposal area, the presence of a domestic animal in the house, and a habit of washing hands of the parent/guardians before feeding were candidates for multivariable logistic regression analysis.

On the multi-variable logistic regression analysis model; the fingernail status of parents/guardians and a habit of washing parent/guardians' hands before feeding were significantly associated with the isolation of diarrhoeagenic enteropathogenic bacteria ($P < 0.05$). Pediatrics who had guardians with untrimmed fingernails were 3.8 times (AOR=3.8, 95% CI =1.4, 10.4) more likely to be infected by diarrhoeagenic enteropathogenic bacteria compared to pediatrics who had guardians with a trimmed fingernail. Pediatrics who had guardians with hadn't a habit of washing hands before feeding were 4.8 (AOR=4.8, 95% CI =1.5,15.4) more likely to be infected by diarrhoeagenic enteropathogenic bacteria compared to pediatrics who had guardians with hand washing habits before feeding (Table 3).

Table 3: Factors associated with enteropathogenic bacteria among pediatric with diarrhea attending Hiwot Fana Specialized Hospital, Harar, Eastern Ethiopia, 2021, (n=210).

Variables	Category	Yes N (%)	No N (%)	COR (95% CI)	P-value	AOR (95%CI)
Malnourished	No	24(11.4)	89(42.4)	1		1
	Yes	9(4.3)	88(41.90)	2.6(1.16,5.99) *	0.08	2.26(0.92,5.55)
Washing pediatrics' hands after defecation	Daily	14(6.66)	50(23.8)	1		1
	Sometimes	12(5.7)	59(28.0)	1.4(0.58,3.25)	0.58	1.36(0.45,4.11)
	Never	7(3.33)	68(32.4)	2.7(1.02,7.23) *	0.12	2.4(0.79,7.38)
Fingernail status of parent/guardians	Trimmed	27(12.9)	108(51.4)	1		
	Untrimmed	6(2.9)	69(32.9)	2.9(1.12,7.32) *	0.01	3.8(1.37,10.434)*
Availability of domestic animals in-house	No	17(8.0)	59(28.0)	1		1
	Yes	16(7.6)	118(56.2)	2.1(1.00,4.50) *	0.08	2.109(0.911,4.884)
Waste disposal area	Municipal	15(7.1)	54(25.7)	1		1
	Open field	10(4.8)	92(43.8)	2.6(1.07,6.09) *	0.12	2.1(0.82,5.46)
	Hole	8(3.8)	31(14.8)	1.1(0.41,2.83)	0.21	0.427(0.11,1.60)
Parent /guardian hand washing habit before feeding child	Yes	27(12.9)	110(52.4)	1		1
	No	6(2.9)	67(31.9)	2.741(1.076,6.984)	0.01	4.79(1.49,15.37) *

Antimicrobial Susceptibility Profile

The isolated enteropathogenic bacteria from pediatric patients with diarrhea were 90.9%, 87.9%, 78.8% 72.7%, and 66.7% susceptible to piperacillin, amoxicillin-clavulanate, ciprofloxacin, cefepime, gentamycin and ceftriaxone respectively. However, 81.8% and 57.6% of isolated enteropathogenic bacteria were resistant to ampicillin and trimethoprim/sulfamethoxa-

zole, respectively. *Shigella sonnei* had the highest bacterial isolates resistant to many antibiotics, whereas *Y. enterocolotica* and *Shigella dysenteriae* were the bacterial isolates with the highest susceptibility to many antibiotics. The antimicrobial profile of *Shigella flexneri/boydii* isolates was 90% susceptible to amox icillin-clavulanate, 80% to piperacillin, and 70% to ciprofloxacin, but 80% of these isolates were resistant to ampicillin followed by 70% trimethoprim/sulfamethoxazole, 50% to chloramphenicol, 30% to gentamycin.

Regarding the antimicrobial profile of *EHEC O157: H7,* 90 % of isolates were susceptible to piperacillin, 80% to chloramphenicol, and 70% to gentamycin. However, 90% are resistant to ampicillin, and 60% to trimethoprim/sulfamethoxazole. *Salmonella* typhi isolates were 100% susceptible to amoxicillin-clavulanate, followed by 80% to ciprofloxacin, cefepime, and piperacillin, and 70% to gentamicin while 80% of them were resistant to ampicillin and trimethoprim/ sulfamethoxazole (Table 4).

Table 4: Antimicrobial susceptibility pattern of pathogenic enteric bacteria isolated from stool culture of pediatrics with diarrhea at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021(n=210).

Isolates	No	Patern	AMP	SXT	GM	FEP	AZM	CIP	C	CRO	MEM	PEP	AUG
Shigella f/b	10	S	-	2(20)	6(60)	6(60)	7(70)	8(80)	5(50)	5(50)	10(100)	9(90)	10(100)
		I	2(20)	2(20)	1(10)	3(30)	2(20)	2(20)	-	3(30)	-	1(10)	-
		R	8(80)	6(60)	3(30)	1(10)	1(10)	-	5(50)	2(20)	-	-	-
EHEC O157:H7	10	S	-	3(30)	7(70)	7(70)	5(50)	6(60)	8(80)	6(60)	10(100)	9(90)	6(60)
		I	1(10)	1(10)	2(20)	2(20)	3(30)	2(20)	-	1(10)	-	1	4(40)
		R	9(90)	6(60)	1(10)	1(10)	2(20)	2(20)	2(20)	3(30)	-	-	-
S. Typhi	5	S	1(20)	-	3(60)	3(60)	3(60)	4(80)	3(60)	3(60)	5(100)	4(80)	5(100)
		I	-	1(20)	1(20)	1(20)	2(40)	1(20)	-	1(20)	-	1(10)	-
		R	4(80)	4(80)	1(20)	1(20)	-	-	2(40)	1(20)	-	-	-
*S. E*nteritides	2	S	-	-	2(100)	2(100)	1(50)	2(100)	-	2(100)	2(100)	2(100)	2(100)
		I	1(50)	1(50)	-	-	1(50)	-	-	-	-	-	-
		R	1(50)	1(50)	-	-	-	-	2(100)	-	-	-	-
S. dysenteriae	2	S	-	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)
		I	1(50)	-	-	-	-	-	-	1(50)	-	-	-
		R	1(50)	1(50)	-	-	-	-	-	-	-	-	-
S. sonnei	2	S	-	-	-	2(100)	1(50)	-	1(50)	2(100)	2(100)	2(100)	2(100)
		I	-	-	1(50)	-	-	-	-	-	-	-	-
		R	2(100)	2(100)	1(50)	-	1(50)	2(100)	1(50)	-	-	-	-
S. Paratyphi A	1	S	-	-	1(50)	1(100)	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		I	-	-	-	-	-	-	-	-	-	-	-
		R	1(100)	1(100)	-	-	1(100)	-	-	-	-	-	-
Total Y. enterocolotica	1	S	-	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		I	-	-	-	-	-	-	-	-	-	-	-
		R	1(100)	1(100)	-	-	-	-	-	-	-	-	-
		S			1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		I	-	-	-	-	-	-	-	-	-	-	-

S: Susceptible,I: Intermediate, R: Resistant, AMP: Ampicillin, TMP-SXT: Trimethoprim/Sulfamethoxazole, GM: Gentamycin, FEP: Cefepime, AZM: Azithromycin, CIP: Ciprofloxacin, C: Chloramphenicol, CRO: Ceftriaxone, Tzp: piperacillin, AMC: amoxicillin-clavulanate, Shigella spp: shigella species, salmonella spp.: salmonella species

Multidrug-resistant Isolates

The overall prevalence of multi-drug resistance (MDR) was 42.4% (95% CI: 24.2, 60.6.) Ampicillin, trimethoprim/sulfamethoxazole, and chloramphenicol were the common antimicrobials responsible for the development of MDR. From the total number of *Shigella flexneri/boydii* isolates, six isolates were multidrug-resistant. Out of those isolates, two of them were detected as resistant to three antimicrobials, one isolate to four antimicrobials, and two of them to five antimicrobials. On the other hand, three of *EHEC O157:*

H7 isolates were multi-drug resistant, two of them were resistant to four antimicrobials, and one of them to five antimicrobials.

Concerning *Salmonella* Typhi isolates, three isolates were multi-drug-resistant. Out of those, two isolates were resistant to three antimicrobials, and one isolate was resistant to five antimicrobials. (Table 5).

Table 5: Resistance antibiogram of isolates from stool culture of pediatric patients with diarrhea at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021(n=210).

Number of antimicrobial resistances	Resistance antibiogram	**Resistance isolates**		
		***Shigella spp.* (N=14)**	*EHEC O157:H7* (n=10)	***Salmonella* Spp. (N=8)**
R1	AMP	-	110	-
	C	-	-	1
R2	AMP, TMP-SXT	3	4	1
	AMP, C	-	-	-
	AMP, GEN	2	1	-
R3	AMP, TMP-SXT, C	2(6)	-	2(6)
	AMP, TMP-SXT, AZT	-	-	-
	AMP, TMP-SXT, CRO	-	-	1(3)
	AMP, CIP, C		1(3)	
R4	AMP, TMP-SXT, CRO, AZT	-	1(3)	-
	AMP, TMP-SXT, C, CRO,	2(6)	-	-
	AMP, TMP-SXT, C, GM,	1(3)	-	1(2)
	AMP, TMP-SXT, C, GM, AZM,	2(6)	-	-
	CIP, AMP, TMP-SXT, AZM, C	-	1(3)	-
MDR No (%)	14(42.4)	7(21)	3(9)	4(11)

AMP: Ampicillin, TMP-SXT: Trimethoprim/Sulfamethoxazole, GM: Gentamycin, AZM: Azithromycin, CIP: Ciprofloxacin, C: Chloramphenicol, CRO: Ceftriaxone, None resistance, R1: Resistance for one antimicrobial, R2: Resistance for two antimicrobials, R3: Resistance for three antimicrobials, R4: Resistance for four antimicrobials, R5: Resistance for five antimicrobials

Discussion

The overall prevalence of pathogenic enteric bacteria among pediatric patients with diarrhea was 15.7% (95% CI: 10.8-20.6). This finding was consistent with previous studies that were conducted in Sudan (19%) (Osman *et al*., 2012), Central Africa (10.8%) (Lango-Yaya *et al*., 2017b), Jimma, Ethiopia (11.9%) (Lamboro *et al*., 2016), Arba Minch (17.55%) (Ameya *et al*., 2018), Bale Robe (11.2%) (Assefa *et al*., 2019), Felege hiwot referral hospital Northwest Ethiopia (14.9%) (Debas *et al*., 2011), Gonder (10.7%) (Alemu et al., 2019), Mekelle (14.2%) (Kahsay *et al*., 2015), Addis Ababa (14.5%) (Howe *et al.,* 2019), and Harar (18.2%) (Reda *et al*., 2011).

This finding was relatively lower than that of the study results reported from Hawassa, Ethiopia, (22.2%) (Mulatu *et al*., 2014) and Kenya (33.6%) (Shah *et al*., 2016). On the other hand, it was relatively higher than the studies reported from Southern Ethiopia (8%) (Hayamo *et al.,* 2020), Nekemte (9.2%) (Terfassa *et al.,* 2018), Hossana (9.3%) (Abebe *et al.,* 2018), and the Gaza Strip, Palestine (9.1%) (Elmanama *et al., 2013)*

In this study, the prevalence of *Shigella flexneri/boydii* was 30.3 %, which was relatively lower than the finding reported from Gondar, Ethiopia (64.7%) (Demissie *et al*., 2014), and Tanzania (90%) (Temu *et al*., 2007).But relatively higher than other studies reported from Indonesia (19.0%) (Orrett, 2008), Central Africa (4%) (Lango-Yaya *et al*., 2017a), and Sudan (5%) (Osman *et al*., 2012).

The prevalence of *EHEC O157:H7* isolates was 30.30%. This finding was comparable with the study findings revealed from Addis Ababa, Ethiopia (40%) (Howe *et al*., 2019), India (36.4%) (Manikandan *et al.,* 2013), and Iran (28%) (Heidary *et al*., 2014). However, it was relatively higher than the study results reported from Nigeria (6%) (Alkali *et al*., 2018).

The prevalence of *Salmonella* Typhi isolates was 15.16%. It was comparable with the result conducted in Sudan (10.5%) (Osman *et al*., 2012) and relatively higher than the study result revealed from Altamura Health Center, Southern Ethiopia, (4.76%) (Hayamo *et al*., 2020. Besides, this finding revealed that the

prevalence of *Salmonella enteritidis* was 6.06% (2/33). It was relatively lower than that of the study finding reported from China (20.8%) (Zeng *et al.,* 2021).

The prevalence of *Shigella dysenteriae* was 6.06%, which was consistent with the previous studies reported from central Africa (3%) (Zeng *et al*., 2021) and Tanzania (10%) (Temu *et al*., 2007). However, the result of the study was relatively lower than the findings conducted at Altamura Health Center Southern Ethiopia (52.38%) (Hayamo *et al*., 2020) and Gondar (17.65%) (Demissie *et al*., 2014). The prevalence of *Shigella sonnei* was 6.06%, which was comparable to the study conducted in Gondar (5.88%) (Demissie *et al*., 2014).

The above difference in magnitude and bacterial isolates might be due to differences in the geographical distribution of enteric pathogen, and socio-demographic factors such as residency, guardians' educational status, and household economic status (Siziya *et al*., 2013), the age of the child, the educational status of the caregiver, the practice of sanitation, personal hygiene, access to safe drinking water (Gebru *et al*., 2014), immune status, and nutritional status. There were reports that the low educational status of the guardians, lack of piped water supply (George *et al*., 2014), and consumption of contaminated food and raw milk can lead to the occurrences of bacterial diarrheal disease (Gally *et al*., 2017). Younger age (Arvelo *et al*., 2010) and poor hand-washing practices (George *et al*., 2014, Hayamo *et al*., 2020) are also highly associated with bacterial diarrheal disease occurrences.

Factors that are involved in the occurrence of diarrhea in pediatrics are complex, and the relative contribution of each factor varies as a function of the interaction between socioeconomic, environmental, and behavioral variables (Mengistie *et al*., 2013). In the current study, pediatrics who had guardians with untrimmed fingernails were more likely to be infected with pathogenic enteric bacteria compared to pediatrics who had guardians with trimmed fingernails. This finding was supported by the study result reported from Arba Minch, Ethiopia (Ameya *et al*., 2018).

A guardian who had no habit of washing their hands before feeding their children was five times more likely to be infected with pathogenic enteric bacteria. This findings is in line with the previous studies reported from Arba Minch (Ameya *et al*., 2018), Harar, Eastern Ethiopia (Reda *et al.,* 2011), and Addis Ababa, Ethiopia (Howe *et al*., 2019). Lack of hand-washing habit might increase contamination of hands after defecation that can increases the chance of bacterial diarrheal disease transmission from mothers/guardians to children (George *et al.,* 2014). (Mohammed *et al.,* 2022).

Regarding the antimicrobial resistance pattern of this finding, 81.8% and 57.6% of isolates were resistant to the tested trimethoprim/sulfamethoxazole and ampicillin, respectively. This was consistent with previous studies reported from Zambia (Chiyangi *et al*., 2017) and Addis Ababa, Ethiopia (Howe *et al*., 2019).

In this study, all isolates of *Shigella* species were susceptible to Amoxicillin Clavulanate, which was consistent with the study results reported from Tanzania, However, it is different from studies' results reported Addis Ababa Ethiopia (Howe *et al.,* 2019),and central Africa (Lango-Yaya *et al*., 2017. All *Salmonella* species isolated in this study were susceptible to amoxicillin Clavulanate. This was different from the study results reported from Central Africa (Lango-Yaya *et al.,* 2017) and Addis Ababa, Ethiopia (Howe *et al*., 2019). However, 85.7% of isolates of *Salmonella* species were resistant to ampicillin and trimethoprim/sulfamethoxazole. It was comparable to the findings reported from Ethiopia; Ambo, (Tosisa *et al.,* 2020), where 95.8% and 66.7% of isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole, respectively, Harar, 100% of isolates were resistant to ampicillin (Reda *et al*., 2011) and Mekelle, where 89% and 57.9% of isolates were resistant to ampicicillin and trimethoprim/sulfamethoxazole, respectively. A research in Central Africa also found that 84% and 96% of isolates were resistant to ampicillin and Trimethoprim/sulfamethoxazole, respectively (Lango-Yaya *et al.,* 2017).

Additionally, 71.4% and 57.1% of *Shigella species* isolates of this finding were resistant to trime-

thoprim/sulphamethoxazole and ampicillin, respectively. This finding was consistent with the studies reported from Ethiopia; Addis Ababa, 68.2% and 77.3% (Howe *et al*., 2019), 88.7%, 50%, Bahir Dar trimethoprim/sulphamethoxazole, and ampicillin resistance respectively (Adugna *et al*., 2015). The finding of this study report revealed that a relatively low rate of ampicillin-resistant *Shigella* species compared to the findings reported from Jimma, Ethiopia (100%) (Lamboro *et al.,* 2016) and, India (92.4%) (Manikandan and Amsath, 2013). Besides, the finding of this study revealed that a lower rate of trimethoprim/sulfamethoxazole-resistant *Shigella* species was isolated than the finding rfrom Tanzania (97.1%) (Temu *et al.,* 2007).

Regarding the antimicrobial profile of EHEC O157:H7 showed susceptibility to 90% Piperacillin, 80% Chloramphenicol, and 70% Gentamycin. This is comparable with findings from reported in Kenya (Shah *et al.,* 2016) and Addis Ababa, Ethiopia (Howe *et al.,* 2019). On the other hand, 90% and 60% of *EHEC O157: H7* isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole, respectively. Which was similar to the study reported in Ababa, Ethiopia (Howe *et al*., 2019), and, Kenya (Shah *et al*., 2016) and Addis. Besides, 90 % and 60% of *EHEC O157:H7* isolates were resistant to ampicillin, and trimethoprim/sulfamethoxazole respectively. This is similar to the study reported in Kenya (Shah *et al*., 2016). This study found relatively lower ampicillin resistance to *EHEC O157:H7* isolates than the finding reported from Nigeria, (41%) (Alkali *et al*., 2018).

In this study, 100% of *Yersinia enterocolitica* were resistant to ampicillin and trimethoprim/sulfamethoxazole. This result was inconsistent with the findings reported from the Gaza Strip, Palestine (Elmanama and Abdelateef, 2013). This disparity could be attributed to the types of antimicrobials examined and the largest number of isolates recovered in a study done in the Gaza Strip, Palestine.

The overall prevalence of multi-drug resistance (MDR) among the isolates was 42.4% (95% CI: 25-59.2). This result was relatively higher than the finding reported from Addis Ababa, Ethiopia (26.2%) (Howe *et al*., 2019), and this result was relatively lower than the finding reported from Nigeria (61.5%) (Alkali *et al*., 2018), Bahir Dar, Ethiopia accounting greater than 90% of Salmonella and 80% of Shigella spp. are multiple drug-resistant (Adugna *et al.,* 2015).

Salmonella Typhi, *Salmonella enteritidis*, and *Salmonella Paratyphi* which were resistant to many first-line antibiotics tested. Moreover, 60% of Salmonella typhi isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, Chloramphenicol, Ceftriaxone, Cefepime, Gentamycin, 50% of *Salmonella enteritidis* were resistant to ampicillin, trimethoprim/sulfamethoxazole,chloramphenicol and Ceftriaxone, and 100% of *Salmonella* paratyphi A isolate was resistant to trimethoprim/sulfamethoxazole, Ceftriaxone and ampicillin. This result was in line with the studies reported from central Africa (Lango-Yaya *et al.,* 2017), and Jimma, Ethiopia (Lamboro *et al*., 2016). This study also detected 3 (30%) MDR *EHEC O157:H7* isolates. Among them, 2 (20%) isolates were resistant to four antimicrobials and 1(10%) was resistant to five antimicrobials. This result was relatively lower than the study result reported from Nigeria (61.5%) (Alkali *et al*., 2018). Furthermore, *Shigella flexneri/boyd*ii, *Shigella sonnei*, and *Shigella dysenteriae* had multiple drug resistance to seven antibiotics. A comparable study finding was reported from Tanzania (Temu *et al*., 2007).

There are several different factors responsible for differences in antibiotic resistance. Some of them like infrequent use of these antibiotics (Lango-Yaya *et al*., 2017a), high rate of self-medication, and lack of treatment compliance of patients to adhere to dosage regimens. The other factors like variation in prescription practice among healthcare providers (Okeke *et al.,* 2007; Temu *et al.,* 2007), indiscriminate use by purchasing without prescription (Mengistu *et al.,* 2014) and, the variation in the number of isolates (*Alkali et al.*, 2018). Inappropriate prescription of antibiotics induces resistance and increased infectious disease mortality in both developing and developed countries (Dandekar and Dandekar, 2010).

The study provides crucial insights into identifying pathogenic enteric bacteria up to the species level and their strong association with drug resistance. However, this study unable to perform additional confirmation of EHEC 0157:H7 using serology and molecular Polymerase chain reaction (PCR) and Campylobacter due to lack enriched and selective media.

Conclusion

The prevalence of pathogenic enteric bacteria was high in the current study. Untrimmed fingernails of guardians, and pediatrics of parents/guardians with no habit of washing hands before feeding, were factors associated with isolated pathogenic enteric bacteria. The most frequently identified isolates were *Shigella flexneri/boydii* and *EHEC O157:H7*. The current study points out that piperacillin, amoxicillin-clavulanate, and ciprofloxacin can be used as part empirical of drugs for enteric bacteria. The overall prevalence of multi-drug resistance was high. Therefore, routine stool bacterial cultures and antimicrobial susceptibility testing should be implemented for all pediatric patients with diarrhea. Further longitudinal study with serological and molecular PCR techniques by using different selective/enriched media should be conducted to identify pathogenic enteric.

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Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

FT and DA conceived and designed the study. FT, GK, TS, SM, and DA participated in collecting scientific literature, and critical appraisal of articles for inclusion, analysis, and interpretation of the findings. FT participated in data collection, writing up results, drafted the manuscript, and prepared the manuscript for publication. All authors have read and approved the final version of the manuscript

List of Abbreviations.

AOR: Adjusted Odd Ratio; CI: Confidence Interval COR: Crude Odd Ratio; HFSUH: Hiwot Fana Specialized University Hospital; EHEC: Entero-Hemorrhagic Escherichia coli, MMAC: Mannitol MacConkey Agar SMAC: Sorbitol MacConkey Agar SS: Shigella –Salmonella medium TCBS: Thiosulfate Citrate Bile Salt Sucrose Medium XLD: Xylose-Lysine-Deoxycholate medium.

References

Abebe,W.,Earsido,A.,Taye, S.,Assefa,M.,Eyasu, A. & Godebo, G. 2018. Prevalence and antibiotic susceptibility patterns of Shigella and Salmonella among children aged below five years with Diarrhoea attending Nigist Eleni Mohammed Memorial Hospital, South Ethiopia. *BMC Pediatrics*, 18, 1-6.

Adugna, A., Kibret, M., Abera, B., Nibret, E. & Adal, M. 2015. Antibiogram of E. coli serotypes isolated from children aged under five with acute diarrhea in Bahir Dar town. *African Health Sciences*, 15, 656-664.

Alemu, A., Geta, M., Taye, S., Eshetie, S. & Engda, T. 2019. Prevalence, associated risk factors and antimicrobial susceptibility patterns of Shigella infections among diarrheic pediatric population attending at Gondar town health care institutions, Northwest Ethiopia. *Tropical diseases, travel medicine, and vaccines*, 5, 1-8.

Alkali, B., Mohammed, K., Opaluwa, S., Najim, Z., Ochei, J. & Kakako, S. 2018. Resistance pattern and plasmid profile of E. coli isolated from diarrhoeic children in selected health centers in Sokoto, Nigeria. Journal of Advances in Microbiology, 11, 1-7.

Ameya, G., Tsalla, T., Getu, F. & Getu, E. 2018. Antimicrobial susceptibility pattern, and associated factors of Salmonella and Shigella infections among under-five children in Arba Minch, South Ethiopia. Annals of clinical microbiology and antimicrobials, 17, 1-7.

Arvelo, W., Kim, A., Creek, T., Legwaila, K., Puhr, N., Johnston, S., Masunge, J., Davis, M., Mintz, E. & Bowen, A. 2010–control study to determine risk factors for diarrhea among children during a large outbreak in a country with a high prevalence of HIV infection. *International Journal of Infectious Diseases*, 14, e1002-e1007.

Assefa, A. & Girma, M. 2019. Prevalence and anti microbial susceptibility patterns of

Salmonella and Shigella isolates among children aged below five years with diarrhea attending Robe General Hospital and Goba Referral Hospital, South East Ethiopia. *Tropical diseases, travel medicine, and vaccines*, 5, 1-11.

Bartram, J., Brocklehurst, C., Fisher, M. B.,Luyendijk, R., Hossain, R., Wardlaw, T. & Gordon, B. 2014. Global monitoring of water supply and sanitation: history, methods and future challenges. *International journal of environmental research and public health*, 11, 8137-8165.

Cheesbrough, M. 2005. District laboratory practice in tropical countries, part 2, Cambridge University Press.

Chiyangi, H., Muma, J. B., Malama, S., Manyahi, J., Abade, A., Kwenda, G. & Matee, M. I. 2017. Identification and antimicrobial resistance patterns of bacterial enteropathogens from children aged 0–59 months at the University Teaching Hospital, Lusaka, Zambia: a prospective cross-sectional study. *BMC Infectious Diseases,* 17, 1-9.

Clsi 2018. Performance Standards for Antimicrobial Susceptibility Testing.

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, 249-56.*

Demissie,A., Wubie, T., Yehuala, F. M., Fetene, M. & Gudeta, A. 2014. Prevalence and antimicrobial susceptibility patterns of Shigella and Salmonella species among patients with diarrhea attending Gondar Town Health Institutions, Northwest Ethiopia. *Sci J Pub Health*, 2, 469-75.

Elmanama,A.A.& Abdelateef, N. 2013. Antimicrobial resistance of enteric pathogens isolated from acute gastroenteritis patients in Gaza strip, Palestine. *The International Arabic Journal of Antimicrobial Agents,* 2.

Gally, D. L. & Stevens, M. P. 2017. Microbe Profile: Escherichia coli O157: H7–notorious relative of the microbiologist's workhorse.Micro biology, 163, 1-3.

Gebru,T.,Taha, M. & Kassahun, W. 2014. Risk factors of diarrhoeal disease in under-five children among health extension model and non-model families in Sheko district rural community,Southwest Ethiopia:a comparati ve cross-sectional study. *BMC Public Health*, 14, 395.

Gelchu, T. & Abdela, J. 2019. Drug therapy problems among patients with cardiovascular disease admitted to the medical ward and had a follow-up at the ambulatory clinic of Hiwot Fana Specialized University Hospital: The case of a tertiary hospital in eastern Ethiopia. *SAGE Open Medicine*, 7, 20503 1211 98 604 01.

George, C. M., Perin, J., De Calani, K. J. N., Norman, W. R., Perry, H., Davis Jr, T. P. & Lindquist, E. D. 2014. Risk factors for diarrhea in children under five years of age residing in peri-urban communities in Cochabamba, Bolivia. *The American journal of tropical medicine and hygiene*, 91, 1190-1196.

Gutmans, J. 2023. The microbiological background of infectiousdiarrhea. 10.13140/RG.2.2.18227.91682.

Hayamo, M., Alemayehu, T., Tadesse, B., Mitiku, E. & Bedawi, Z. 2020. Shigella and Salmonella, Antibiotics Susceptibility Pattern and Associated Risk Factors among Diarrheic Children in Southern Ethiopia: a cross-sectional study.

Heidary, M., Momtaz, H. & Madani, M. 2014. Characterization of diarrheagenic anti microbial resistant Escherichia coli isolated from pediatric patients in Tehran, Iran. Iranian Red Crescent Medical Journal, 16.

Howe, R., Ayenew, Z., Biazin, H., Gebre-Selassie, S., Yeshitila, B., Assefa, A. & Abebe, T. 2019. Bacteriaenteric pathogens and antimicrobial susceptibility profile among pediatric patients with diarrhea: a cross sectional study in selected health facilities, addis ababa,

Kahsay, A. G. & Teklemariam, Z. 2015. Prevalence of Shigella among diarrheic children under 5 years of age attending Mekelle health center, north Ethiopia. *BMC research notes,* 8, 1-4.

Kosek, M.,Bern,C. & Guerrant, R. L. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organizatio*n, 81, 197-204.

Lamboro,T.,Ketema, T. & Bacha, K. 2016. Prevalence and antimicrobial resistance in Salmonella and Shigella species isolated from out patients, Jimma University Specialized Hospital, Southwest Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology*.

Lango-Yaya, E., Djeintote, M. & Djimeli, C. 2017. Contribution to the Study of Antibiotic Resistance on Salmonella and Shigella Strains Isolated in Central African Republic. *J Microbiol Exp*, 4, 00105.

Magiorakos, A.-P., Srinivasan, A., Carey, R., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S.,

Hindler, J., Kahlmeter, G. & Olsson-Liljequist, B. 2012. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18, 268-281.

Manikandan, C. & Amsath, A. 2013. Antimicrobial resistance of enteric pathogens isolated from children with acute diarrhea in Pattukkottai, Tamil Nadu, India. *International Journal of Pure and Applied Zoology*, 1, 139-145.

Mengistie,B.,Berhane,Y.&Worku,A.2013. Prevalence of diarrhea and associated risk factors among children under five years of age in Eastern Ethiopia: A cross-sectional study. Open Journal of Preventive Medicine, 3, 446.

Mengistu, Mulugeta, Gebru, Tsehaynesh, Abraham, Lema & Aseffa 2014a. Prevalence and antimicrobial susceptibility patterns of Salmonella serovars and Shigella species. J Microb Biochem Technol, 6, S2-006.

Mengistu, G.,Mulugeta,G. Lema,T.& Aseffa, A. 2014. Prevalence and antimicrobial susceptibility patterns of Salmonella serovars and Shigella species. *J Microb Biochem Technol,* 6, S2-006.

Mohammed, J., Shiferaw, A., Zeleke, A., Eshetu, Y., Gebeyehu, Z., Ayehu, A. & Adem, Y. 2022. Prevalence and Associated Risk Factors of Intestinal Parasites among Diarrheic Under-Five Children Attending Bahir Dar and Han Health Centers, Northwest Ethiopia: A Cross-Sectional Study. *J Parasitol Res*, 2022, 7066529.

Mokomane, M., Kasvosve, I., Melo, E. d., Pernica, J. M. & Goldfarb, D. M. 2018. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management.Therapeutic advances in infectious disease, 5, 29-43.

Moss, C., Kuche,D., Bekele, T. H.,Salasibew, M., Ayana, G., Abera, A., Eshetu, S., Dangour, A. D. & Allen, E. 2020. Precision of Measurements Performed by a Cadre of Anthropometrists Trained for a Large Household Nutrition Survey in Ethiopia. Current developments in nutrition, 4, nzaa139-nzaa139.

Mulatu,G.,Beyene,G.&Zeynudin,A. 2014. Prevalence of Shigella, Salmonella, and Campylobacter species and their susceptibility patterns among under-five children with diarrhea in Hawassa town, South Ethiopia. *Ethiopian journal of health sciences,* 24, 101.

Omar, M. H. 2015.Prevalence of enteric bacteria associated with diarrhea in children less than five years of age and their sensitivity to antibiotics in Unguja Island-Zanzibar. The Open University of Tanzania.

Oppong, T.,Yang, H., Amponsem-Boateng, C., Kyere, E. D., Abdulai, T., Duan, G. &Opolot, G. 2020. Enteric pathogens associated with gastroenteritis among children under 5 years in sub-Saharan Africa: a systematic review and meta-analysis. *Epidemiology & Infection*, 148.

Orrett, F. A. 2008. Prevalence of Shigella serogroups and their antimicrobial resistance patterns in southern Trinidad. Journal of health, population, and nutrition, 26, 456.

Osman, M. M., Hassan, A. N. & Holie, M. A. 2012. Bacterial etiology of diarrhoeal diseases in children under 5 years old in Ombadda Hospital-Sudan. *Sudanese Journal of Public Health,* 7, 93-97.

Reda, A. A.,Seyoum, B., Yimam, J., Fiseha, S. & Jean-Michel, V. 2011. Antibiotic susceptibility patterns of Salmonella and Shigella isolates in Harar, Eastern Ethiopia. *Journal of Infectious Diseases and Immunity,* 3, 134-139.

Shah, M.,Kathiiko, C.,Wada, A.,Odoyo, E.,Bundi, M., Miringu, G., Guyo, S., Karama, M. & Ichinose, Y. 2016. Prevalence, seasonal variation, and antibiotic resistance pattern of enteric bacterial pathogens among hospitalized diarrheic children in suburban regions of central Kenya. *Tropical medicine and health,* 44, 1-8.

Siziya, S., Muula, A. S. & Rudatsikira, E. 2013. Correlates of diarrhea among children below the age of 5 years in Sudan. *African Health Sciences*, 13, 376-383.

Temu, M., Kaatano, G., Miyaye, N., Buhalata, S., Shushu, M., Kishamawe, C. & Changalucha, J. 2007. Antimicrobial susceptibility of Shigella flexneri and S.dysenteriae isolated from stool specimens of patients with bloody diarrhea in Mwansa, Tanzania. Tanzania *Journal of Health Research*, 9, 186-189.

Terfassa, A. & Jida, M. 2018. Prevalence and anti biotics susceptibility pattern of Salmonella and Shigella species among diarrheal patients attending Nekemte Referral Hospital, Oromia, Ethiopia. International journal of microbiology, 2018.

Tosisa,W.2015. Prevalence And Antibiotic Resistance of Enteric Bacterial Pathogens Isolated from

Childhood Diarrhea in Ambo Town Public Health Institutions. Addis Ababa University.

Tosisa, W., Mihret, A., Ararsa, A., Eguale, T. & Abebe,T. 2020. Prevalence and antimicrobial susceptibility of Salmonella and Shigella species isolated from diarrheic children in Ambo town. BMC Pediatrics, 20, 1-8.

Weinstein, M. P. & Lewis, J. S. 2020. The Clinical and Laboratory Standards Institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of clinical microbiology*, 58, 10.1128/jcm. 01864-19.

WHO 2019. Malnutrition https://www.who.int/health-topics/malnutrition#tab=tab_1.

Youssef, M., Shurman, A., Bougnoux, M.-E., Rawashdeh, M., Bretagne, S. & Strockbine, N. 2000. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunology & Medical Micro biology, 28, 257-263.

Zeng, X., Lv, S., Qu, C., Lan, L., Tan, D., Li, X. & Bai, L. 2021. Serotypes, antibiotic resistance, and molecular characterization of non-typhoidal salmonella isolated from diarrheic patients in Guangxi Zhuang Autonomous Region, China, 2014–2017. Food Control, 120, 107478.