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Original Article

Epidemiology of *Shigella*, *Salmonella*, and *Campylobacter* among diarrheic under-five-year-old children in central Ethiopia

Tizazu Zenebe[1], Ermiyas Endewunet[2], Aminu Seman[3], Tadesse Eguale[4], Tamrat Abebe[3]

[1] *Department of Medical Laboratory Science, Debre Berhan University, Ethiopia*
[2] *Department of Internal Medicine, Debre Berhan University, Ethiopia*
[3] *Department of Microbiology, Immunology and Parasitology, Addis Ababa University, Ethiopia*
[4] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia*

Abstract

Introduction: Diarrheagenic bacteria are among the major contributors to the global diarrheal burden. The absence of up-to-date data on the etiologies of diarrhea due to limited active surveillance and clinical laboratory capacity makes the burden more severe. *Shigella*, *Salmonella*, and *Campylobacter* are among the most common bacterial etiologies of childhood diarrhea. The present study aimed to provide epidemiological data on these bacterial etiologies in under-five-year-old children in Ethiopia.

Methodology: A cross-sectional health facility-based study was conducted from December 2020 to August 2021 in Addis Ababa and Debre Berhan, Ethiopia. Standard microbiological techniques including culture, antimicrobial susceptibility, and polymerase chain reaction (PCR) were used to characterize bacterial isolates.

Results: A total of 391 under-five-year-old children were tested. *Shigella* was the most common isolate in 10% (39/391), followed by *Campylobacter* in 7.2% (28/391). Age range of 0–12 months, poor childcare practice, and taking supplements early were associated with acquisition of the pathogens. The highest antibacterial resistance was observed for ampicillin among *Salmonella* and *Shigella* (100% and 87%, respectively). Extended spectrum ß-lactamase (ESBL) and carbapenemases production was observed in 8% and 3% of *Shigella* strains respectively. The majority of the bacterial isolates were susceptible to carbapenems.

Conclusions: The burden of bacterial pathogens continues to be a serious problem in Ethiopia. Detection of ESBL-and carbapenemase-producing *Shigella* strains could reveal the presence of resistant pathogenic strains in the area. Strengthening diagnostic laboratory capacity in healthcare facilities could reduce the burden.

Key words: *Shigella*; *Salmonella*; *Campylobacter*; under-five children; diarrhea; Ethiopia.

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Introduction

Diarrhea is among the leading causes of high mortality rates in sub-Saharan Africa in under-five-year-old children [1]. Bacteria, viruses, and parasites are the infectious causes of diarrhea [2]. Diarrhea due to bacterial pathogens continues to be a serious health problem in under-five-year-old children globally [3]. *Shigella*, *Salmonella*, and *Campylobacter* are commonly associated with diarrhea among under-five-year-old children in developing countries [4].

Shigella is a major contributor to the global diarrheal disease burden [5]. *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* are the four species of the genus *Shigella* [6]. The species distribution of *Shigella* varies globally. *S. sonnei* is the predominant species worldwide [7]; *S. flexneri* is more prominent across developing countries in Africa and Asia; and the less virulent *S. sonnei* predominates in higher-income countries [7]. There is an international shift in the etiology of bacillary dysentery from *S. flexneri* to *S. sonnei* in developing countries [7].

Salmonella infection is another major public health concern globally and has contributed to an increased economic burden on the health systems by causing bacterial enteritis in humans [8]. The genus *Salmonella* contains two species, *Salmonella enterica* and *Salmonella bongori* [9]. *S. enterica* is subdivided into six subspecies, and *Salmonella enterica* subsp *enterica* are the major contributor to human infections [9]. *S. Choleraesuis*, *S. Enteritidis*, and *S. Typhimurium* are commonly associated with diarrhea [9].

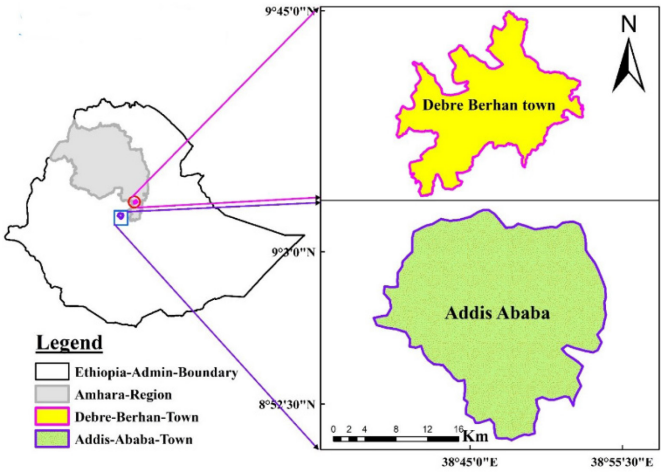
Campylobacter is also among the most frequently isolated bacteria from stools of infants with diarrhea in developing countries, mainly due to contaminated food or water [10]. In East Africa, *Campylobacter* infections have been recorded in both rural and urban areas,

particularly among children, and the prevalence varies between countries [11]. The genus *Campylobacter* contains 22 species; however, *C. jejuni* and *C. coli* are the most common causes of diarrhea [12].

The continuous increase of antimicrobial resistance (AMR) among enteric pathogens is another serious concern with impacts such as mortality and high public health costs [13]. AMR is the most serious global public health threat due to the emergence of extended spectrum ß-lactamase (ESBL)- and carbapenemase-producing pathogenic strains [14]. It is a multifaceted problem, and misuse of antibiotics in human beings, animals, and agriculture is the main factor responsible for the development and spread of resistant bacteria [13]. The presence of resistant pathogenic strains could be a possible threat for human health [15]. There is an overall decline in the total stock of antibiotic effectiveness due to resistance to all first-line and last-resort antibiotics globally, with varying resistance patterns by region and country [16]. Among the total estimated 13.7 million infection related deaths in 2019, there were 7.7 million deaths associated with 33 bacterial pathogens including diarrheagenic bacteria [17]. In addition, nearly 5 million people who died suffered from AMR-related illnesses [18].

Ethiopia is fifth among the 15 countries with high burden of pneumonia and diarrhea [1]. The diarrhea burden is more severe in under-five-year-old children in Ethiopia [19]. *Shigella* [20], *Salmonella* [21], and *Campylobacter* [22] have been reported among diarrheic patients in different areas and times in the country. However, access to quality-assured laboratory diagnosis has been a challenge in Ethiopia, as it is a low-income country, and this has resulted in delayed or inaccurate diagnosis and ineffective treatment [23].

Figure 1. Map of the study area: Addis Ababa and Debre Berhan, Ethiopia.



There is limitation in clinical laboratory capacity and active surveillance programs to obtain up-to-date data or monitor the emergence of pathogenic and resistant strains in Ethiopia. In addition, inappropriate antimicrobial utilization and management is a problem in Ethiopia [24]. Most common Gram-negative pathogens were found resistant to key antimicrobial agents described in the National Standard Treatment Guideline [25]. Due to this, there is a need to have up-to-date data on the epidemiology of pathogenic bacterial strains in under-five-year-old children in Ethiopia. Thus, the present study aims to provide up-to-date data on *Shigella*, *Salmonella* and *Campylobacter* in under-five-year-old children in central Ethiopia.

Methodology

Study design, setting, and population

A cross-sectional study was conducted in Addis Ababa and Debre Berhan, Ethiopia (Figure 1). The study was based at healthcare facilities and conducted from December 2020 to August 2022. Addis Ababa is the capital city of Ethiopia. Debre Berhan is located at a distance of about 130 km by road from Addis Ababa in the North Shoa Zone of Amhara regional state. According to the Central Intelligence Agency (CIA) World Factbook, the estimated population in Addis Ababa was 5.461 million in 2023 [26]; and the recent population estimate for Debre Berhan was 94,829 [27]. Three sub-cities of Addis Ababa and Debre Berhan were randomly selected using the lottery method and included in the study. One healthcare center from each selected sub-city of Addis Ababa and two healthcare centers from Debre Berhan were randomly selected to participate in the study. The study population included all children under five years of age who consulted with the pediatrics department of the selected healthcare facilities in Addis Ababa and Debre Berhan during the study period. Children under five years of age with diarrhea who had not received antimicrobials in the previous three weeks were enrolled in the study.

Study variables and data collection

Data on socio-demographic characteristics, clinical features, and other factors were collected from the parents/guardians using a standardized structured questionnaire (Supplementary Document 1). The socio-demographic data included age, gender, mother or guardian's occupation, and family income. The clinical features included initiation of illness, duration of diarrhea, stool frequency, type of diarrhea, dehydration status, fever, vomiting, nausea, thirst, abdominal distension, and history of previous treatment.

Sample size determination

The sample size was determined based on the prevalence rate of bacterial enteropathogens in a previous study conducted in Debre Berhan [28]. The sample size calculation was done considering 5% precision and 95% confidence level using the method described in [29]. The required sample size was determined to be 391. The sample size was allocated for each study area based on the estimated population of Debre Berhan town and selected sub-cities of Addis Ababa (165 for Debre Berhan and 226 for Addis Ababa).

Bacterial isolation and identification

The laboratory investigation was done in Tikur Anbessa Specialized Hospital microbiology laboratory (for Addis Ababa) and Debre Berhan Referral Hospital microbiology laboratory (for Debre Berhan). The stool samples were collected from the children in Cairy-Blair transport media (Oxoid Ltd, Basingstoke, UK).) and transported using cold chain [30] to the microbiology laboratory. The stool sample was inoculated onto MacConkey agar (Oxoid Ltd, Basingstoke, UK) and xylose-lysine-deoxycholate (XLD; Oxoid Ltd, Basingstoke, UK) media, and incubated at 37 °C for 18–24 hours for isolation of *Shigella* and *Salmonella* [31,32]. *Shigella* formed pink-red colonies on XLD, while *Salmonella* formed black centered pink-red colonies on XLD due to hydrogen sulphide (H_2S) production. *Shigella* and *Salmonella* produced colorless colonies on MacConkey agar. Conventional biochemical tests included oxidase, triple sugar iron agar, mannitol, urease, lysine decarboxylase, motility, indol, Simon's citrate, and hydrogen sulphide. In addition, Gram staining was conducted for the identification of bacterial isolates [30–32]. Positive control strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700063, and *P. aeruginosa* ATCC 27853) were used for confirmation of the biochemical test results. Confirmed bacterial isolates from each cultured plate were stored at – 80 °C in brain heart infusion broth containing 16% (v/v) glycerol. The *Campylobacter* stool samples were preserved at – 80 °C for use in polymerase chain reaction (PCR) assay.

Antimicrobial susceptibility tests

Antimicrobial susceptibility of all *Shigella* and *Salmonella* isolates was assessed using the disc diffusion method (Kirby–Bauer method) according to the Clinical and Laboratory Standards Institute guidelines (CLSI) [33], which are described in our previous work [34]. The antibiotics tested were ampicillin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ertapenem (10 µg), meropenem (30 µg), amoxicillin-clavulunate (20/10 µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), and cefepime (30 µg). All the antibiotics were from Oxoid Ltd (Basingstoke, UK). The results were interpreted using the CLSI guidelines [33]. *E. coli* ATCC 25922 was used as a reference strain.

Phenotypic detection of ESBLs and carbapenemases production

The phenotypic detection of ESBLs and carbapenemases production was done following a previously described procedure [34]. Briefly, isolates resistant to cefotaxime (30 µg) and ceftazidime (30 µg) were tested for ESBL production using the combination disk method [33]. *Klebsiella pneumonia* ATCC 700603 (ESBLs positive) and *E. coli* ATCC 25922 (ESBLs negative) were used for quality control. The production of carbapenemase was tested using a modified carbapenem inactivation method [33]. *K. pneumonia* ATCC BAA-1705 (carbapenemase positive) and *K. pneumonia* ATCC BAA-1706 (carbapenemase negative) were used for quality control. The results from the ESBL confirmation and carbapenemase production were interpreted according to CLSI guidelines [33].

Detection of Campylobacter by PCR assay

Stool samples (after thawing) were pre-treated for total DNA preparation based on previously used procedures [35] to get cell pellets and total DNA. Briefly, 20 mg frozen sample was taken in a sterile tube containing 5 mL of ice-cold phosphate-buffered saline (PBS). After vortexing, it was centrifuged at 500 rpm for 4 minutes. The supernatant was transferred to new 5 mL sterile tube. The pellets were resuspended in 5 mL PBS buffer, mixed thoroughly and centrifuged at 500 rpm for 4 minutes and repeated twice. All the supernatants were centrifuged at 9000 rpm for 5 minutes. The pellet was washed 2 times by suspending it in 1.5 mL 70% ethanol and centrifuged at 13,000 rpm for 10 minutes each. Finally, the cell pellet was resuspended in molecular-grade water for extraction.

A previously used DNA extraction protocol [36] called the TE boil extraction method (T method) with minor modification was employed. Briefly, the pellet was suspended in 200 µL TE buffer (10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA), and the mixture was briefly mixed on a vortex mixer. The suspension was

boiled at 94 °C for 10 minutes in a dry block incubator (Thermo-Fisher Scientific, California, USA) and placed in a freezer at – 20 °C for 10 minutes; then placed at room temperature for 1 minute and centrifuged at 14,000 g for 5 minutes. Then, 100 µL of the supernatant was transferred into a nuclease-free Eppendorf tube and stored at – 20 °C until used for PCR assay. The procedure was optimized using Qiagen kit-based extraction method following the procedure recommended by the manufacturer (Qiagen, Hilden, Germany).

The PCR assay for the detection of *Campylobacter* species was done using the target genes, aspartokinase (*asp*), specific to *C. coli*; hipuricase gene (*hipO*), specific to *C. jejuni* [37]; and a universal 16S rRNA gene for the genus *Campylobacter* [38]. The primer sequences are presented in Table 1. The PCR assay was performed using a previously used procedure [34] with slight modification. Briefly, a single-plex PCR assay for the detection of the 16S rRNA target gene was performed. In the case of 16S rRNA-positive samples, a duplex PCR assay for the detection of *asp* and *hipO* genes was performed. The PCR reaction was carried out with 25 µL reaction mixture containing 12.5 µL Platinum™ II Hot-Start PCR Master Mix (2X) (Thermo Scientific, Waltham, USA), 0.8 µL of forward primer mix (0.4 µL each primer), 0.8 µL of reverse primer mix (0.4 µl each primer), 1 µL of template DNA, and 9.9 µL of molecular grade water. The PCR was carried out using one cycle of initial denaturation at 94 °C for 3 minutes; 30 cycles of denaturation at 94 °C for 30 seconds, annealing for 30 seconds at 60 °C, and extension at 72 °C for 30 seconds; followed by a final extension at 72 °C for 5 minutes. Extracted DNA samples of positive control (*C. jejuni* ATCC 3329) and previously identified known *Campylobacter* species (*C. jejuni* and *C. coli*) were used as positive control during optimization of the PCR assay. The PCR products were analyzed by gel electrophoresis on 1.7% (w/v) agarose gel in tris borate EDTA buffer (pH 8.2) stained with ethidium bromide (10 µg/mL). The gels were run for 1 hour at 120 V and visualized with the UV transilluminator system (BiORAD, Hercules, USA). The size of the products was confirmed by comparison with the molecular marker GeneRuler 100-bp DNA Ladder (Thermo Scientific, Waltham, USA).

Data analysis

The data were entered into an Excel spreadsheet and cross-checked for correctness before analysis. Data analysis was done using the Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp, Armonk, NY, USA). The data were analyzed using descriptive statistics and presented in tables and graphs. The difference between the variables was computed by using Chi square statistics and a p value < 0.05 was considered significant. Bivariate and multivariate analyses were done using a binary logistic regression model. Independent variables for the final model (multivariate logistic regression) were identified using a bivariate logistic regression model with $p < 0.25$. Model fitness was checked by the Hosmer and Lemeshow goodness-of-fit analysis (p value = 0.348). Multi-co-linearity of the independent variables was tested using the variance inflation factor (VIF) and tolerance tests. The results were interpreted with crude odds ratio (COR) and adjusted odds ratio (AOR).

Ethical considerations

The study was approved by the Addis Ababa University Institutional Review Board (protocol number: 025/20/DMIP) and the Ethiopian National Research Ethics Review Committee (Ref.No. RED/1.14/9428/21). Written informed consent was obtained from parents or legal guardian(s) of participants and witnessed consent was obtained in the case of illiterate parents or legal guardian(s).

Results

Profile of patient participants

A total of 391 children, aged < 5 years, participated in the study. Over half of the study participants were male (58.8%) and from Addis Ababa (57.8%), and the majority were in the age group 24–59 months (60.1%). The majority of the participants were first-born (47.6%), 35% were second-borne, 12.8% were third-borne, and 4.6% were born fourth or later. Regarding educational level of the mother or guardian, 11.8%

Table 1. Target genes for polymerase chain reaction (PCR) amplification, primers and their characteristics.

Target bacteria	Genus/Species	Target genes	Primer sequence	Amplicon size (bp)	Reference
Campylobacter	*Genus Campylobacter*	*16S rRNA*	F:5'- AGTTGGAAACGACTGCTAATACTC-3' R:5'- TTAATGGTTAAGCCATTAGATTTCAC-3'	450	[36]
	C. jejuni	*hipO*	F:5'-GACTTCGTGCAGATATGGATGCTT-3' R:5'-GCTATAACTATCCGAAGAAGCCATCA-3'	344	[35]
	C. coli	*asp*	F: 5'-GGTATGATTTCTACAAAGCGAG-3' R: 5'-ATAAAAGACTATCGTCGCGTG-3'	500	[35]

(46/391) were illiterate, 35.5% (139/391) were educated up to primary and junior school, 33.5% (131/391) were educated up to high school, and 19.2% (75/391) completed college or university. Detailed social-demographic data are summarized in Table 2.

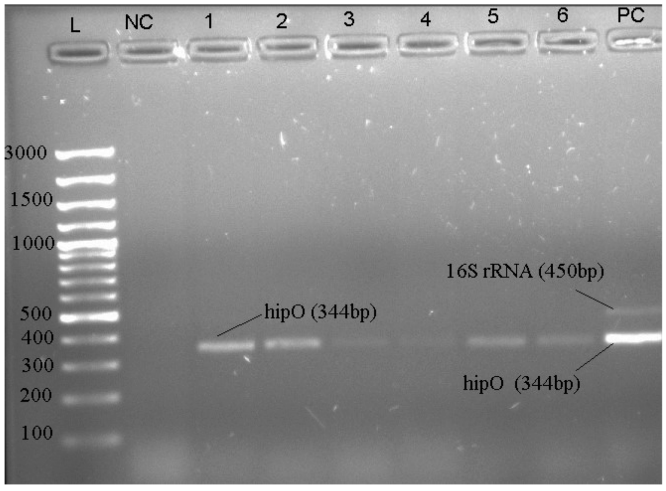
Clinical presentations of patients

The time of illness of the children was measured in 4 categories (Table 3). Most of the patients that were positive for *Shigella* and *Campylobacter* visited a healthcare facility within 1 day. The duration of diarrhea for all children was acute (< 1 week). The type of diarrheas observed in this study varied with the pathogen. Those who were infected with *Shigella* had bloody diarrhea (79.5%); those who were infected with *Campylobacter* had mucoid diarrhea (40%); and those who were infected with *Salmonella* had watery diarrhea (62.5%). Severe dehydration was observed in the children who were positive for *Shigella* (10.3%), and *Campylobacter* (6.7%).

Prevalence of Shigella, Salmonella, and Campylobacter

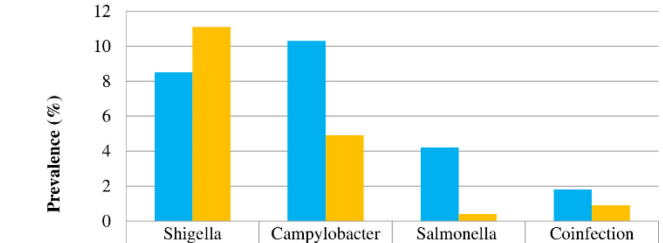
Among the 391 children included in the study, 10% (39/391) were infected with *Shigella*, 7.2% (28/391) with *Campylobacter*, 2% (8/391) with *Salmonella*, and the remaining 80% (316/391) were negative to these diarrheic bacterial pathogens. Only 3.8% (15/391) were positive for *Campylobacter jejuni*, and *C. coli* was not detected in the present study. The distribution of bacteria varied across the study areas (Figure 2). There was no significant difference in the presence of *Shigella*

Figure 3. Gel images of polymerase chain reaction (PCR) products of *Campylobacter jejuni*.



NC: negative control; 1–6: positive for hipO gene (344bp); PC: positive control.

Figure 2. Distribution of *Shigella*, *Salmonella*, and *Campylobacter* in under-five children in Addis Ababa and Debre Berhan, Ethiopia.



	Shigella	Campylobacter	Salmonella	Coinfection
Debre Berhan	8.5	10.3	4.2	1.8
Addis Ababa	11.1	4.9	0.4	0.9

in Addis Ababa (11.1%, 25/226) and Debre Berhan (8.5%, 14/165), and *Campylobacter* in Addis Ababa (2.7%, 6/226) and Debre Berhan (5.5%, 9/165) ($p > 0.05$). There was no statistically significant difference in the distribution of pathogenic bacteria (*Shigella*, *Salmonella*, and *Campylobacter*) based on gender and study area ($p > 0.05$). A sample gel image showing target genes for *Campylobacter* is presented in Figure 3. 1.3% of the samples indicated co-infections (4 *Shigella–Campylobacter*, and 1 *Salmonella–Campylobacter*). Among the *Campylobacter* isolates, only *Campylobacter jejuni* strains were detected.

Table 2. Socio-demographic data of the children included in the study.

Characteristics	Frequency
Location	
Debre Berhan	165 (42.2%)
Addis Ababa	226 (57.8%)
Gender	
Male	230 (58.8%)
Female	161 (41.2%)
Age	
0–12 months	64 (16.4%)
13–24 months	92 (23.5%)
24–59 months	235 (60.1%)
Number of the children in the family	
1	157 (40.2%)
2	143 (36.6%)
≥ 3	91 (23.3%)
Marital status of the mother or guardian	
Married	341 (87.2%)
Unmarried	32 (8.2%)
Divorced	14 (3.6%)
Widowed	4 (1.0%)
Occupational of the mother or guardian	
Employed	122 (31.2%)
Self employed	67 (17.1%)
Other	202 (51.7%)
Family income (per month)	
< $36 (low)	155 (39.6%)
$37–115 (middle)	169 (43.2%)
> $115 (high)	67 (17.1%)

Table 3. Clinical presentation of the diarrhea in under-five-year-old children (n = 62) who were positive for *Shigella, Salmonella*, and *Campylobacter* during their healthcare facility visit in Addis Ababa and Debre Berhan, Ethiopia.

Symptoms	*Shigella* (n = 39)	*Campylobacter* (n = 15)	*Salmonella* (n = 8)
Time of illness (days)			
1	12 (30.8%)	5 (33.3%)	1 (12.5%)
2	14 (35.9%)	5 (33.3%)	1 (12.5%)
3	10 (25.6%)	3 (20%)	5 (62.5%)
≥ 4	3 (7.7%)	2 (13.3%)	1 (12.5%)
Duration of diarrhea (days)			
2	10 (25.6%)	8 (53.3%)	2 (25%)
3	12 (30.8%)	2 (13.3%)	4 (50%)
≥ 4	17 (43.6%)	5 (33.3%)	2 (25%)
Stool frequency (times per day)			
2	5 (12.8%)	5 (33.3%)	3 (37.5%)
3	8 (20.5%)	4 (26.7%)	1 (12.5%)
≥ 4	26 (66.7%)	6 (40.0%)	4 (50.0%)
Type of diarrhea			
Watery	4 (10.3%)	4 (26.7%)	5 (62.5%)
Mucoid	3 (7.7%)	6 (40.0%)	3 (37.6%)
Bloody	31 (79.5%)	1 (6.7%)	0 (0.0%)
Loose	1 (2.6%)	4 (26.7%)	0 (0.0%)
Dehydration status			
Mild	5 (12.8%)	10 (66.7)	6 (75.0%)
Moderate	15 (38.5%)	0 (0.0%)	1 (12.5%)
Severe	4 (10.3%)	1 (6.7%)	0 (0.0%)
None	15 (38.5%)	4 (26.7%)	1 (12.5%)
Fever			
Yes	12 (30.8%)	1 (6.7%)	2 (25.0%)
No	27 (69.2%)	14 (93.3%)	6 (75.0%)
Vomiting			
Yes	18 (46.2%)	6 (40.0%)	3 (37.5%)
No	21 (53.8%)	9 (60.0%)	5 (62.5%)
Nausea			
Yes	5 (12.8%)	1 (6.7%)	2 (25.0%)
No	34 (87.2%)	14 (93.3%)	6 (75.0%)
Increased thirst			
Yes	13 (33.3%)	4 (26.7%)	2 (25.0%)
No	26 (66.7%)	11 (73.3%)	6 (75.0%)
Abdominal distension			
Yes	5 (12.8%)	2 (13.3%)	1 (12.5%)
No	34 (87.2%)	13 (86.7%)	7 (87.5%)

Factors associated with acquisition of pathogenic bacteria

Age, childcare, and time at which supplement food was introduced were associated with the acquisition of pathogenic bacteria (*Shigella*, *Salmonella*, and *Campylobacter*; Table 4). Children in the age group 25–59 months were less likely (AOR = 0.329, CI = 0.115, 0.942) to acquire pathogenic bacteria compared to children in age group 0–12 months. The children who were cared for by grandmothers, close family, and daycare (AOR = 2.094, CI = 1.091, 4.019); and their mothers (AOR = 4.569, CI = 1.287, 16.228) were 4 times and 2 times more likely to acquire the pathogenic bacteria compared to children who were cared for by house workers, respectively. Children who began supplemental food at the age of 6–12 months were 3 times more likely (AOR = 2.615, CI = 1.256, 5.444) to acquire the pathogenic bacteria compared to children who began supplemental food before 6 months of age.

AMR profile of Shigella and Salmonella

A high resistance to ampicillin was recorded in *Shigella* (87%) and *Salmonella* (100%). The isolates were also resistant to tetracycline (Table 5). Resistance to quinolone and sulphonamides was observed in isolates of both pathogenic bacterial genera. *Shigella* isolates resistant to third (8%) and fourth generation (5%) cephalosporin and carbapenem (3%) were also documented in this study. However, ciprofloxacin (75–90%), amoxicillin–clavulanate (82–87%), chloramphenicol (92–100%), and cephalosporin and carbapenem were effective against the majority of the bacterial isolates. ESBL-producing *Shigella* strains (8%) and carbapenem-producing *Shigella* strains (3%) were identified. However, ESBL-and carbapenemase-producing *Salmonella* strains was not found in the present study.

Table 4. Factors associated with pathogenic bacteria (*Shigella*, *Salmonella*, and *Campylobacter*) in the children included in the study.

Variables	Pathogenic bacteria		Univariate analysis		Multivariate analysis	
	Yes (%)	No (%)	COR (95%CI)	*p* value	AOR (95%CI)	*p* value
Gender						
Male	30 (13.0%)	200 (87.0%)	1.00	0.305	1.00	0.152
Female	27 (16.8%)	134 (83.2%)	0.744 (0.423, 1.309)		0.647 (0.356, 1.174)	
Age						
0–12 months	5 (7.8%)	59 (92.2%)	1.00		1.00	
13–24 months	14 (15.2%)	78 (84.8%)	0.472 (0.161, 1.384)	0.255	0.370 (0.117, 1.173)	0.091
25–59 months	38 (16.2%)	197 (83.8%)	0.439 (0.165, 1.167)		**0.329 (0.115, 0.942)**	0.038
Location						
Debre Berhan	27 (16.4%)	138 (83.6%)	1.00	0.393	1.00	0.657
Addis Ababa	30 (13.3%)	196 (86.7%)	1.278 (0.727, 2.246)		1.156 (0.610, 2.192)	
Child care						
ǂHouse worker	36 (20.1%)	143 (79.9%)	1.00		1.00	
Mother	18 (10.9%)	147 (89.1%)	2.056 (1.116, 3.787)	0.021	**2.094 (1.091, 4.019)**	0.013
Others*	3 (6.4%)	44 (93.6%)	3.692 (1.084, 12.573)		**4.569 (1.287, 16.228)**	
Introduction of supplement food						
< 6 months	15 (23.1%)	50 (76.9%)	1.00		1.00	
6–12 months	41 (13.4%)	266 (86.6%)	1.946 (1.002, 3.781)	0.076	**2.615 (1.256, 5.444)**	0.022
> 12 months	1 (5.3%)	18 (94.7%)	5.400 (0.665, 43.865)		5.685 (0.660, 48.997)	
Hand washing facility near toilet						
Yes	11 (10.1%)	98 (89.9%)	1.00	0.122	1.00	0.088
No	46 (16.3%)	236 (83.7%)	0.576 (0.286, 1.158)		0.519 (0.245, 1.102)	
Location of disposal container						
Kept indoor	49 (16.3%)	252 (83.7%)	1.00	0.086	1.00	0.083
Kept outdoor	8 (8.9%)	82 (91.1%)	1.993 (0.907, 4.382)		2.048 (0.910, 4.610)	

∗ Others = including grandmother, close family member, and day care; ǂHouse worker refers to a paid female employee in a home working as a maid (including looking after the baby). These values written in **bold** indicate statistical significance at 95% confidence interval. AOR: adjusted odds ratio; CI: confidence interval; COR: crude odds ratio.

Discussion

Identification of the etiological agent may not be required for patient management when the diarrhea is self-limiting. However, in severe or prolonged cases, with symptoms consistent with invasive disease, or in patients with potential complications, the etiological agents need to be identified for effective treatment [39–41]. Moreover, public health personnel utilize epidemiological data to identify and track the causes of outbreaks of diarrheal diseases. In this era of new emerging bacterial strains, updating the epidemiology of pathogenic bacteria will help in effective patient management. In this study, the prevalence of *Shigella*, *Salmonella*, and *Campylobacter*; factors associated with the acquisition of the pathogenic bacteria; and up-to-date resistance profile of *Shigella* and *Salmonella* were determined.

A prevalence of 6.6%, 4.8%, and 9% for *Shigella*, *Salmonella*, and *Campylobacter*, respectively, among diarrheic under-five-year-old children was reported in Ethiopia [20–22]. In this study, the prevalence of *Shigella*, *Campylobacter*, and *Salmonella* were 10%, 7.2%, and 2% respectively. This finding showed a slight increase in the prevalence of *Shigella*, and decrease in prevalence of *Salmonella* and *Campylobacter* compared to the above reports [20–22]. This slight discrepancy may be due to difference in the diagnosis method and study design. Meta-analysis studies [20–22] estimate the pooled prevalence regardless of difference in local prevalence, unlike cross-sectional studies (present study). A recent cross-

Table 5. Antimicrobial resistance profile of *Shigella* and *Salmonella* isolated from the under-five-year-old children included in the study.

Group of antibiotics		*Shigella* (n = 39)		*Salmonella* (n = 8)	
		R%	S%	R%	S%
Penicillin	Ampicillin	34 (87.0%)	5 (13.0%)	8 (100%)	0 (0.0%)
	Amoxacillin–Clavulanate	7 (18.0%)	32 (82.0%)	1 (13.0%)	7 (87.0%)
Quinolone	Ciprofloxacin	4 (10.0%)	35 (90.0%)	2 (25.0%)	6 (75.0%)
Aminoglycosides	Gentamicin	3 (8.0%)	36 (92.0%)	1 (13.0%)	7 (87.0%)
Folate synthesis inhibitors	Trimethoprim–Sulfamethazole	15 (38.0%)	24 (62.0%)	5 (63.0%)	3 (37.0%)
Phenicols	Chloranphenicol	3 (8.0%)	36 (92.0%)	0 (0.0%)	8 (100%)
Tetracycline	Tetracycline	33 (85.0%)	6 (15.0%)	7 (88.0%)	1 (12.0%)
3rd generation cephalosporin	Ceftazidime	3 (8.0%)	36 (92.0%)	0 (0.0%)	8 (100%)
	Cefotaxime	3 (8.0%)	36 (92.0%)	0 (0.0%)	8 (100%)
4th generation cephalosporin	Cefepime	2 (5.0%)	37 (95.0%)	0 (0.0%)	8 (100%)
Carbapenem	Meropenem	1 (3.0%)	38 (97.0%)	0 (0.0%)	8 (100%)
	Ertapenem	1 (3.0%)	38 (97.0%)	0 (0.0%)	8 (100%)

R: resistance; S: sensitive.

sectional study reported 6.6% prevalence of *Campylobacter* [42], 7.6% prevalence of *Shigella*, and 0.38% prevalence of *Salmonella* [43] in Ethiopia; which are similar to the present finding. Other cross-sectional studies conducted in Guinea-Bissau [44] and Georgia [45] showed similar prevalence of *Shigella*, *Salmonella*, and *Campylobacter*. The results of this study revealed a higher epidemiological profile of *Shigella* and *Campylobacter*, compared to *Salmonella*, among the studied children in Ethiopia. The distribution of these pathogenic bacteria did not vary with gender and study area in the present study. This indicates that these pathogens should be considered as important targets for infection prevention and control measures in Ethiopia, regardless of gender and locality.

However, acquisition of pathogenic bacteria by children under five years of age who had diarrhea varied depending on age, child care, and time when supplement food was introduced. Children in the age group 25–59 months were less likely to acquire pathogenic bacteria compared to children of age group 0–12 months. In agreement with the present finding, a study conducted in Poland found that the prevalence of *Salmonella* and *C. jejuni* varied by age; prevalence of *Salmonella* was 27.3% in the 0–12 months age group, and 9.1% in the 25–36 months age group; while the prevalence of *C. jejuni* was 30.8% in 0–12 months age group, and 7.7% in 25–36 months age group. Thus, the prevalence of pathogenic bacteria decreased with increase in age [46]. The possible justification for this could be that the children of age below one year have low immunity compared to older children [47]. In addition, risk exposure through complementary feeding, in particular at the beginning (at 6 months), can result in frequent bacterial infections, and lead to a high prevalence of the pathogens [47]. However, contrary to the present finding, a study in Mozambique found that prevalence of any enteric infection was positively associated with age and ranged from 71% in children who were 1–11 months old to 96% in children who were 24–48 months old; thus, the incidence of infection increased with age [48]. The Mozambique study further concluded that the overall enteric infection (viral, bacterial, and parasitic) increased with age; however, among bacterial pathogens, only *Shigella* infections increased with age (and not *Salmonella* and *Campylobacter* infections). This discrepancy may be due to risk factors associated with individual pathogens and the actual variation in the presence of pathogens in the study area. For example, a study conducted in Ethiopia found that significant risk factors for a *Campylobacter*-positive stool were keeping animals inside ($p = 0.027$, OR 3.5), owning cattle ($p = 0.018$, OR 6.5), and *Campylobacter*-positive poultry feces ($p < 0.001$, OR 1.34) [49].

The routes for feco-oral infections from one host to another include direct or indirect transmission via contamination [50]. In the present study, children who were cared for by others (grandmothers, close family, and day care) had 4 times more risk of acquiring the pathogenic bacteria compared to children who were cared by a house worker. This could be due to the difference in the risk factors presented by the groups (grandmothers, close family, and day care) to the child. Child contact with vulnerable groups (old age), unconditional contact with relative (with poor hygiene condition), and staying in infection risk area (day care) may have contributed to the difference. In addition, when a house worker is present in the family, the work load in the family will be lowered and hygiene can be enhanced; thereby improving infection prevention [51]. Children who began their supplement food at the age of 6–12 months were more likely to acquire the pathogenic bacteria compared to children who began supplement food at an age lower than 6 months. The World Health Organization (WHO) recommends that complementary food should begin after 6 months of age [52], and healthcare professionals also inform the mothers/guardians to follow this recommendation. As a result, mothers/guardians may feed their children more frequently when they are above 6 months of age, compared to feeding children at an age lower than 6 months. Consequently, there was a lower possibility of transmission of infection to children who were less than 6 months of age, compared to older children. The hygienic practices adopted by the mothers/guardians while feeding the children also makes a difference and the prevalence of hygienic practices during complementary feeding of their children aged 6–24 months in Ethiopia has been studied [53].

Most (98%) antimicrobials prescribed in Ethiopia are from the national essential medicine list [54] and the antibiotics are grouped into access, watch, and reserve categories [55]. Access antibiotics are easily accessible and are widely used empiric treatment options; watch antibiotics are used in the highest priority cases; reserve antibiotics are reserved for the treatment of infections that are confirmed or suspected to be due to multi-drug-resistant organisms, and are treated as "last-resort" options. In Ethiopia, high resistance to ampicillin and tetracycline in *Salmonella* and *Shigella* isolates were reported in different studies [43,56]. This study also confirmed the presence of high resistance to ampicillin and tetracycline in *Salmonella* and *Shigella* strains. This

indicates that these drugs may not be used for treating such pathogenic strains. In the present study, resistance to commonly used antimicrobials such as quinolone and sulphonamides was observed in both *Shigella* and *Salmonella*. This occurrence of bacterial strains that are resistant to both access and watch antibiotics groups could lead to limitation in treatment options. However, in the present study, ciprofloxacin (75–90%), amoxicillin–clavulanate (82–87%), chloramphenicol (92–100%), and cephalosporin and carbapenem were relatively effective against the majority of the bacterial isolates. Such varied and increasing AMR in *Shigella* and *Salmonella* could be a major concern for selecting empirical treatment of acute infectious diarrhea.

Shigella isolates that were resistant to third and fourth generation cephalosporin, and to carbapenem, were found in the present study, and this finding was similar to previous reports [43,56]. ESBL-producing Enterobacteriaceae have been reported in East Africa, including in Ethiopia [57]; however limited data is available for ESBL-producing *Shigella* strains in the region. The prevalence of ESBL-and carbapenemase-producing *Shigella* strains in the present study was 8% and 3%, respectively. A systematic review of studies in Asia found a 23.9% pooled prevalence rate of ESBL-producing *Shigella* strains [58], which is higher than that observed in the present study. This difference could be due to variations in the study design. The detection of ESBL-producing *Shigella* strains could indicate future challenges to treatment practices. Thus, updated and local AMR data for the identified pathogens is needed for optimal antimicrobials utilization.

Conclusions

Shigella, *Salmonella*, and *Campylobacter jejuni* continue to be a burden for children under five years age in Ethiopia. The predominant bacterial pathogen identified in our study was *Shigella*. The burden of these pathogenic bacteria may be affected by age, child care, and time when food supplementation is started in children. Some of the commonly used antibiotics may not be effective against the pathogens. Detection of ESBL-producing strains in the present study suggests the presence of possible threat of resistant strains in the area. Treatment practices should be updated to follow local AMR data for the identified pathogens. Strengthening the clinical laboratory capacity and initiating active surveillance programs could help to provide up-to-date data continuously for effective treatment practice and patient management.

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Corresponding author

Tizazu Zenebe Zelelie, PhD.
Department of Medical Laboratory Science, Debre Berhan University, Ethiopia.
Tel: +251912372837
Fax: 011 681 20 65
Email: tizazuzenebe@yahoo.com

Conflict of interests

No conflict of interests is declared.

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Annex – Supplementary Items

Supplementary Document 1. Questionnaire for assessment of factors associated with diarrhea in Addis Ababa and Debre Berhan, Ethiopia (English version)

I. Patient information

1. Name of health facility _____
2. Identification number (card number) _____
3. Address_____

II. Socio-demographic information for under-five-year-old children.

S.No	Questions (for child and caregiver)	Response
1.	Age of the child	_____ (in month) or _____ (in year)
2.	Gender of the child	☐ Male ☐ Female
3.	Relation of the respondent to the child?	☐ Mother ☐ Guardian (caregiver) ☐ Other (specify)
4.	Age of the mother or guardian (caregiver)	_____
5.	Gender of the guardian (if not mother)	☐ Male ☐ Female
6.	How many persons are in the household?	_____
7.	How many children are in the family?	_____
8.	Birth order	_____
9.	Educational level of the mother or guardian	☐ Illiterate ☐ Primary ☐ Junior secondary ☐ Preparatory ☐ College or University
10.	Educational level of the father	☐ Illiterate ☐ Primary ☐ Junior secondary ☐ Preparatory ☐ College or university
11.	Marital status of the mother or guardian	☐ Single ☐ Married ☐ Other (specify)
12.	Ethnic group	☐ Amhara ☐ Oromo ☐ Tigray ☐ Other (specify)
13.	Occupation (Mother)	☐ Employed ☐ Self-employed ☐ Other (specify)
	Occupation (Father)	☐ Employed ☐ Self-employed ☐ Other (specify)
14.	Family income per month (in Birr)	_____

III. Clinical features

15.	Time when illness started	_____
16.	Duration of diarrhea in days	_____
17.	Stool frequency per day	
18.	Type of diarrhea	☐ Watery ☐ Mucoid ☐ Bloody ☐ Loose
19.	Child dehydration status	☐ Mild ☐ Moderate ☐ Severe ☐ None
20.	Other clinical features	
	Fever	☐ Yes ☐ No
	Vomiting	☐ Yes ☐ No
	Nausea	☐ Yes ☐ No
	Increased thirst	☐ Yes ☐ No
	Abdominal distension	☐ Yes ☐ No
	Other (specify)	_____
21.	Previous treatment	☐ Yes ☐ No
	If yes when?	_____

IV. Behavioural and health related factors related to under-five-year-old children

22.	How is the child cared daily at home?	☐ By house worker ☐ By mother ☐ In day care ☐ Other (specify)___
23.	Feeding practice	☐ Exclusive breast milk ☐ Breast milk & solid food ☐ Solid food only ☐ Formula Milk
24.	Duration of breastfeeding	☐ < 1year ☐ 1–2 years ☐ > 2years
25.	Beginning age of supplementary feeding	☐ < 6 moths ☐ 6–12 moths ☐ > 12 months
26.	What method do you use to feed your child?	☐ Hand ☐ Cup or spoon ☐ Bottle
27.	When do you wash your hand?	☐ Before food preparation ☐ Before and after feeding ☐ After latrine ☐ After cleaning the child's bottom
28.	What do you use for hand washing?	☐ Soaps ☐ Water only ☐ Others
29.	Does your child eat by himself/herself?	☐ Yes ☐ No
30.	Does your child wash his/her hands before feeding?	☐ Yes ☐ No

31.	How does the child dispose his/her feces?	☐ With diaper ☐ Latrine ☐ Ground ☐ On baby potty (popo) ☐ Under clothes
32.	Where do you dispose your child's stool?	☐ Child use latrine ☐ Put into latrine ☐ Throw in garbage ☐ Bury ☐ Leave on the ground
33.	Where do you dispose the water used for washing your child's stool?	☐ Put in latrine ☐ Throw on the ground ☐ Throw in the garbage ☐ No water used
34.	What vaccines has your child taken?	☐ Measles vaccine ☐ Rota vaccine ☐ Other (specify)
35.	Nutritional status of the child	☐ Under nutrition ☐ Normal
36.	Is there any family member who travelled abroad?	☐ Yes ☐ No ☐ If yes, when? _____

V. Environmental exposure variables

37.	House ownership	☐ Private ☐ Rented ☐ Other (specify)
38.	Is a separate kitchen available?	☐ Yes ☐ No
39.	Ownership of latrine	☐ Shared ☐ Private ☐ Public
40.	What type of toilet does the household mainly use?	☐ Pit latrine with slab ☐ Pit latrine without a slab or pour-flush latrine ☐ Open field ☐ Other (specify)_____
41.	Is the hand washing facility available near the latrine?	☐ Yes ☐ No
42.	How often is the latrine cleaned?	☐ Every time it is soiled ☐ Every day ☐ 1–2 times a week ☐ Not cleaned
43.	Where is your refuse container (bin) located?	☐ Indoors ☐ Outdoors ☐ Do not own a bin
44.	Does your bin have a properly fitting lid?	☐ Yes ☐ No
45.	If your waste is not collected, how do you dispose of your waste?	☐ Burn ☐ Bury ☐ Dump ☐ Other (specify)____
46.	How often is your waste/refuse collected by the local authority?	☐ Daily ☐ Weekly ☐ Monthly ☐ Never ☐ Other (specify)_____
47.	Are there any domestic animals in the compound?	☐ Yes ☐ No
48.	What types of animals are in the house or the yard?	☐ Cattle ☐ Goats ☐ Dog ☐ Chicken ☐ Other specify
49.	Where do you mainly get your drinking water from?	☐ Tap water ☐ Packed water ☐ Protected spring/well water ☐ Unprotected spring/well water ☐ Other
50.	Time to obtain drinking water (shift)	☐ No shift (daily) ☐ Shift ☐ if shift, how long?
51.	What treatment of water do you use?	☐ Filtering ☐ Boiling ☐ None ☐ Other (specify)___
52.	What kind of utensils do you use for storing water?	☐ Storage containers without lid ☐ Storage containers with lid
53.	Do you always clean and empty the storage container before replacing with fresh water?	☐ Yes ☐ No

Data collector name_____ Date _____ Signature_____