Prevalence of enteric bacteria and enteroparasites in human immunodeficiency virus-infected individuals with diarrhoea attending antiretroviral treatment clinic, Arba Minch General Hospital, southern Ethiopia

A. A. Ayele[1], D. Tadesse[1], A. Manilal[1], T. Yohanes[1], M. Seid[1] and M. Shewangizaw Mekuria[2]

1) Department of Medical Laboratory Science and 2) Department of Public Health, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia

Abstract

In Ethiopia, only limited data are available regarding the prevalence of enteric bacterial pathogens and enteroparasites in human immunodeficiency virus (HIV) -infected individuals with diarrhoea. Hence, this study aims to assess the prevalence of enteric bacteria and enteroparasites, and also the antibiotic susceptibility patterns of bacteria in them. An institution-based cross-sectional study was performed in HIV patients with diarrhoea, who visited the Anti-Retroviral Therapy Clinic of the Arba Minch General Hospital between 1 March and 31 August 2019. Data pertaining to sociodemographic characteristics and other factors were collected using a structured questionnaire. Stool culture is of utmost importance in the case of HIV-infected individuals with diarrhoea. Stool samples were collected and examined for bacterial and parasitic pathogens following standard procedures. The antibiotic susceptibility test was performed as per the Kirby−Bauer disc diffusion technique. Data were analysed using SPSS software. A total of 180 individuals were included in the stool collection process. The prevalence rates of enteric bacteria and enteroparasites were 8.3% and 36.1%, respectively. Parasitic infections were more frequent than bacterial infections in these HIV-infected individuals; commonly identified enteroparasites were *Giardia lamblia* (8.9%) and *Cryptosporidium parvum* (8.3%). *Campylobacter* sp. was the most predominant enteric bacterial isolate (4.4%), followed by *Salmonella* (2.1%) and *Shigella* (1.1%) species. CD4 counts <200 cells/μL was significantly associated with both bacterial infections (adjusted OR 9.55, 95% CI 1.54−59.3, p 0.015) and parasitic infections (adjusted OR 3.53, 95% CI 1.3−17.9, p 0.03). Multidrug resistance was also detected in 100%, 75% and 60% of *Shigella*, *Campylobacter* and *Salmonella* sp., respectively. We found that enteroparasitic infections were more frequent than bacterial infections. Statistical analysis revealed that CD4 T-cell counts <200 cells/μL, quality of drinking water sources, hand washing habits after toilet and the presence of domestic animals were significantly associated with the prevalence of enteric pathogens.
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Corresponding author: A. Manilal, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia.
E-mail: aseermanilal@gmail.com

Introduction

Human immunodeficiency virus (HIV) is a retrovirus and an aetiological agent of acquired immunodeficiency syndrome (AIDS), the latter being an advanced stage of infection [1]. HIV predominantly infects and kills CD4 T cells, resulting in virus-induced immunosuppression, and ultimately AIDS [2]. Once

the CD4 T-cell counts drop to <200 cells/μL, the individual becomes highly vulnerable to opportunistic infections caused by various pathogens, such as protozoa, helminths, bacteria, viruses and fungi, and the aetiological agents of enteritis in HIV-infected individuals are too numerous to list [2].

The gastrointestinal tract is a crucial site in the pathogenesis of HIV infection, due to repressed immunological responses at the mucosal level that prevent intestinal idiopathic defence mechanisms [3]. Clinical manifestations in the gastrointestinal tract include odynophagia, dysphagia, nausea, vomiting, abdominal pain and eventually diarrhoea [4]. Diarrhoea is one of the hallmarks of HIV infection and is a significant cause of morbidity and mortality in later stages regardless of antiretroviral exposure [5]. There are numerous reasons for diarrhoea, the most common are related to opportunistic infections and antiretroviral medications [4]. It is estimated that more than 90% of HIV-infected individuals in developing countries and 50%−60% in developed countries have diarrhoea [6]. The WHO baseline scenario forecast for 2030 envisages that mortalities due to HIV/AIDS and diarrhoeal diseases in developing countries would remain around 1.7 million and 1.5 million, respectively [7]. The aetiological profile of infectious diarrhoea among HIV-infected individuals includes bacteria, parasites, fungi and enteric viruses [8]. Enteric bacterial pathogens, such as species of *Salmonella* (particularly enterica serotypes), *Shigella*, *Campylobacter* and *Escherichia coli*, are the most common [9,10]. Individuals with HIV infections are estimated to be at 20- to 100-fold increased risk of salmonellosis and associated bacteraemia, in more than 40% of cases [11]. In immunocompetent individuals, gastroenteritis with *Shigella* rarely develops into bacteraemia, whereas up to 50% of AIDS patients with shigellosis become bacteraemic [12]. The average occurrence of *Campylobacter* infections among AIDS patients is 40 times higher than that in non-infected individuals [13]. Incidence of enteroparasitic infections accounts for up to 95% of deaths in HIV-infected individuals in developing countries [14]. Enteroparasites of genera such as *Cryptosporidium*, *Microsporidia*, *Giardia*, *Entamoeba*, *Strongyloides* and *Isospora* are the common causes of severe and life-threatening diarrhoea in HIV-infected individuals [15]. For instance, infection rates by *Cryptosporidium* account for up to one-third of diarrhoea cases [16].

It has been reported that enteric bacterial and enteroparasitic infections are widespread in HIV-infected individuals in Ethiopia [17,18]. A survey of literature and examination of records indicates that studies so far have focused on the prevalence of either enteric bacterial or enteroparasitic infections [19−21]. Antimicrobial resistance is a growing concern across the globe [22] and it is not restricted to the enteric bacteria among HIV-infected individuals in Ethiopia. Antimicrobial susceptibility patterns of enteric bacteria isolated from diarrhoeic HIV-infected individuals exhibit regional variability and are consistently acquiring resistance to commonly used antibiotics [23]. Information pertaining to the possible risk factors (poor hygiene, ingestion of contaminated food and water, contact with infected domesticated animals and immune status) related to enteric infections in HIV-infected individuals is also scarce and the existing data obtained by research in the country give an ill-defined picture. To address these knowledge gaps, the present study is intended to estimate the prevalence of enteric bacterial pathogens and enteroparasites, and also to elucidate the antimicrobial susceptibility patterns of bacteria isolated from HIV-infected individuals with diarrhoea attending the Anti-Retroviral Therapy (ART) clinic of Arba Minch Hospital, southern Ethiopia.

Materials and methods

Study design

This study was carried out at the Arba Minch General Hospital, Arba Minch province, situated 505 km southwest of Addis Ababa, Ethiopia. An institution-based cross-sectional study was carried out among all the HIV-infected individuals with diarrhoea, attending the ART clinic of Arba Minch General Hospital between 1 March and 31 August 2019. The criteria for inclusion were: HIV-infected individuals aged ≥15 years and willing to participate in the study. The criteria for exclusion were all HIV patients who were severely sick and unable to provide stool samples, and HIV patients who underwent antibiotic/antiparasitic treatments for diarrhoea except cotrimoxazole prophylaxis between 15 February and 28 February 2019. This study was approved by the Institutional Review Board of the College of Medicine and Health Sciences, Arba Minch University (Ref. IRB/12036584/106/08/02/19).

Sample size determination and sampling technique

The sample size of bacteria was calculated using a single population proportion formula [24]. A prevalence of 0.13 was chosen from a previous study conducted in Ethiopia [25]. After considering a confidence interval of 95% ($z = 1.96$) and a 5% marginal error ($d = 0.05$), the sample size was calculated to be 184. During calculation, a 5% non-response rate (≈ 10) was applied and the final sample size became 194. A systematic sampling technique was used to obtain a representative sample and was further selected to recruit the study units. The sampling interval was calculated by dividing the total number of target patients by the sample size according to the latest annual report. The Kth value was inferred from the number of patients who attended the ART clinic during the study period and participants were selected by the lottery method.

Data collection and laboratory processing

Before data and sample collections, written consents were obtained from all the participants (or from children's parents if participant was an adolescent) after a clear briefing about the purpose of the study. A structured questionnaire was used to collect the sociodemographic data (sex, age, marital status, occupation, income, residential area and, educational level) and other factors (types of diarrhoea, previous history of antibiotic treatments, cotrimoxazole prophylaxis, diagnosis for opportunistic infections, latrine usage, hand washing practices, consumption of raw food and source of drinking water). Most recent CD4 T-cell counts (not more than 3 months old), details of diagnosis for opportunistic infections and use of cotrimoxazole prophylaxis were obtained from the medical records of patients.

Faecal sample collection and transportation

Sterile and leak-proof stool cups with a spoon, labelled with unique identification numbers, were provided for the collection of specimens. Each participant was instructed to collect a sufficient quantity of sample (≈ 5 g for loose stools or 10 mL for watery) aseptically. Immediately after collection, a direct microscopic examination using physiological saline was performed at Arba Minch General Hospital, ART clinic laboratory, and then transported in an ice-cold box to the Medical Microbiology and Parasitology Laboratory, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Arba Minch University. The stool samples were then processed within 2 hours of collection.

Bacteriological and parasitological processing

Culturing and identification process. All stool specimens were inoculated into Selenite F broth (Oxoid, Basingstoke, UK) and then incubated at 37°C for 24 hours. After the pre-enrichment period, the broth was subcultured onto MacConkey and xylose lysine deoxycholate agar media and incubated under aerobic conditions at 37°C for 24 hours. Growth of *Salmonella* and *Shigella* sp. was detected by their characteristic appearance on MacConkey and xylose lysine deoxycholate agar. Suspected colonies were further tested by a series of biochemical analyses to identify *Salmonella* and *Shigella* sp. [26]. Corresponding American Type Culture Collection strains were used as reference standards to validate the biochemical identification of *Salmonella* and *Shigella*. For the isolation of *Campylobacter* sp., campylobacter agar base with 10% sterile defibrinated sheep blood and rehydrated contents of *Campylobacter* Supplement-1 (Blaser-Wang) (FD006) were used. Agar plates were incubated under microaerophilic conditions (5%–10% O_2 and 10% CO_2 concentrations) at 42°C for 24–48 hours. Gram staining and biochemical tests were performed to identify *Campylobacter*

[26]. A standard reference strain of *Campylobacter jejuni* (ATCC 700819) was used as the quality control.

Antimicrobial susceptibility test

The antibiotic susceptibility profile was determined by the Kirby−Bauer disc diffusion technique according to the criteria set by the CLSI using Oxoid antibiotic discs [27]. Inocula were prepared by picking parts of similar test organisms (*Salmonella* and *Shigella*) with a wire loop and suspending in sterile normal saline. The density of suspension to be inoculated was determined by comparison with an opacity standard, McFarland 0.5 barium sulphate solution. The respective test organisms were uniformly seeded over the Müller−Hinton agar (Oxoid) and exposed to a concentration gradient of antibiotic diffusing into the agar medium from an impregnated paper disc followed by incubation at 37°C for 16−18 hours. For *Campylobacter* sp., Müller−Hinton agar supplemented with 5% sheep blood was used [28]. Antibiotic discs including ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), trimethoprimsulfamethoxazole (1.25/23.75 μg), gentamicin (10 μg), tetracycline (30 μg), doxycycline (30 μg), erythromycin (15 μg), azithromycin (15 μg), ceftazidime (30 μg), ceftriaxone (30 μg) and meropenem (10 μg) were used. Diameters of the zone of inhibition around the discs were measured and response was classified as sensitive, intermediate or resistant according to the standardized table supplied by CLSI [27,28]. A standard reference strain of *E. coli* (ATCC 25922) was used as quality control for the culture and to evaluate the potency of antibiotic discs. The multidrug resistance in this study corresponds to the resistance to three or more classes of antibiotics tested [29].

Isolation and identification of enteroparasites. Stool specimens were obtained from all participants and examined for the presence of cysts, oocysts, eggs, trophozoites and larvae of enteroparasites by direct microscopic examination using physiological saline and a formol−ether concentration technique [30].

Data processing and analysis. The data were analysed using SPSS for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were performed. Bivariate and multivariate logistic regression analyses were carried out to measure the association between predictor variables and the outcome variable. Variables with a p-value <0.25 in the bivariate logistic regression model were further analysed in the multivariate logistic regression model for controlling the potential confounding factors. Crude odds ratio and an adjusted odds ratio (aOR) were used to determine the significance of the outcome predictors. A p-value ≤ 0.05 was considered statistically significant.

Results

Sociodemographic characteristics

As a whole, out of the 194 HIV-infected individuals who were the primary respondents, 180 turned up for stool collection during the stipulated study period, showing a response rate of 92.7%. Of them, 53.3% (n = 96) were female. A considerable proportion (38.3%) of these individuals was in the age range of 35−44 years. Detailed sociodemographic characteristics of the participants are shown in Table 1.

Prevalence and diversity of enteric bacteria and enteroparasites

A total of 15 enteric bacterial isolates from HIV-infected individuals with diarrhoea were tentatively identified up to the genus level. The overall prevalence of enteric bacterial isolates among HIV-infected individuals was observed to be 8.3%

(n = 15) (95% CI 5%−13%). After considering the colony morphology and biochemical characteristics, bacterial isolates were identified and sorted into three genera: *Campylobacter*, *Salmonella* and *Shigella*. Among the isolates, species of *Campylobacter* were the most commonly identified bacterial pathogen, accounting for over 4.4% (n = 8), followed by *Salmonella* 2.8% (n = 5) and *Shigella* 1.1% (n = 2). Only monobacterial infections were found.

According to microscopic examinations, 65 stool samples were found to be positive for enteroparasites. Isolates of parasites were tentatively identified and sorted into eight species: *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Cyclospora* sp., *Isospora belli* (protozoans); and *Strongyloides stercoralis*, *Ascaris lumbricoides* and *Taenia* sp. (helminths). The number and percentage of each parasite identified from stool samples are shown in Fig. 1. In the case of enteroparasites, the overall prevalence was 36.1% (95% CI 29.1%−43.6%). Of the five protozoans identified, the prevalences of *G. lamblia* and

TABLE 1. Sociodemographic, clinical and environmental characteristics of study participants

Variables	Category	Frequency[a] (%) (n = 180)	Enteric bacterial pathogen (%) (n = 15)	Enteroparasites (%) (n = 65)
Sex	Male	84 (46.7)	5 (33.3)	31 (47.7)
	Female	96 (53.3)	10 (66.7)	34 (52.3)
Age (years)	15−24	23 (12.8)	1 (6.6)	14 (21.5)
	25−34	48 (26.7)	4 (26.7)	10 (15.4)
	35−44	69 (38.3)	6 (40)	25 (38.5)
	>45	40 (22.2)	4 (26.7)	16 (24.6)
Residence	Urban	142 (78.8)	11 (73.3)	59 (90.7)
	Rural	38 (21.1)	4 (26.7)	6 (9.3)
Marital status	Married	107 (59.4)	7 (46.7)	32 (49.2)
	Unmarried	5 (2.8)	1 (6.6)	2 (3)
	Divorced	49 (27.2)	7 (46.7)	22 (34)
	Widowed	19 (10.6)	—	9 (13.8)
Occupation	Farmer	42 (23.3)	6 (40)	16 (24.6)
	Merchant	36 (20)	2 (13.3)	14 (21.5)
	Government employee	61 (33.9)	4 (26.7)	20 (30.7)
	Housewife	16 (8.9)	—	5 (7.7)
	Labour work	25 (13.9)	3 (20)	10 (15.4)
Education	Illiterate	46 (25.6)	6 (40)	17 (26)
	Literate	134 (74.4)	9 (60)	48 (74)
Income/month (Ethiopian Bir (ETB))	≤1000	61 (33.9)	6 (40)	24 (37)
	1001−2000	81 (45)	8 (53.4)	26 (40)
	>2000	38 (21.1)	1 (6.6)	15 (23)
Cotrimoxazole prophylaxis history	Yes	65 (36.1)	8 (53.3)	29 (44.6)
	No	115 (63.9)	7 (46.7)	36 (55.4)
Duration of diarrhoea	Acute	113 (62.8)	10 (66.7)	44 (67.7)
	Chronic	67 (37.2)	5 (33.3)	21 (32.3)
History of diarrhoea within 3 months	Yes	79 (43.9)	5 (33.3)	35 (53.8)
	No	101 (56.1)	10 (66.7)	30 (46.2)
Diagnosed for opportunistic infection	Yes	67 (37.2)	5 (33.3)	21 (32.3)
	No	113 (62.8)	10 (66.7)	44 (67.7)
Appearance of stool specimen	Watery	132 (73.3)	10 (66.7)	50 (77)
	Mucoid/bloody	18 (10)	4 (26.7)	5 (7.7)
	Loose	30 (16.7)	1 (6.6)	10 (15.3)
CD4 T-cell count (cells/μL)	<200	20 (11.1)	4 (26.7)	12 (18.4)
	200−500	68 (37.7)	8 (53.3)	29 (44.6)
	>500	92 (51.1)	3 (20)	24 (37)`
Source of water for drinking	Protected	149 (82.8)	9 (60)	57 (87.7)
	Unprotected	31 (17.8)	6 (40)	8 (12.3)
Where did you use latrine service	Private	112 (62.2)	8 (53.3)	41 (63)
	Public	68 (37.8)	7 (46.7)	24 (37)
Do you have a habit of consuming raw food	Yes	93 (51.7)	12 (80)	24 (37)
	No	87 (48.3)	3 (20)	41 (63)
Are there domestic animals in your house	Yes	79 (43.9)	12 (80)	29 (44.6)
	No	101 (56.1)	3 (20)	36 (55.4)
Hand washing practice after toilet	Yes	111 (61.1)	9 (60)	20 (30.7)
	No	69 (38.9)	6 (40)	45 (69.3)
Hand washing practice before meals	No	86 (47.8)	5 (33.3)	37 (57)
	Yes	94 (52.2)	10 (66.7)	28 (43)

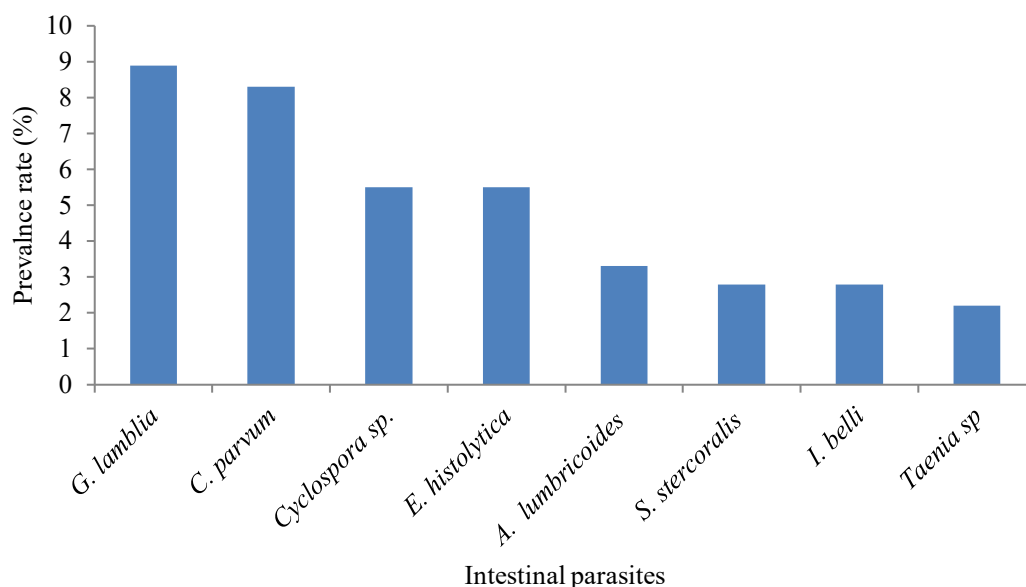[a]The total number of participants corresponding to 100 % is 180.

FIG. 1. Diversity, prevalence and distribution of intestinal parasites among study participants.

C. parvum were 8.9% and 8.3%, respectively. In the case of helminths, *A. lumbricoides* and *S. stercoralis* were the commonly isolated species. Dual infections were also observed. For instance, combinations of parasite species such as *C. parvum*−*Taenia* sp. (n = 2); *Entamoeba histolytica/dispar*−*S. stercoralis* (n = 2); *I. belli*−*A. lumbricoides* (n = 1) and *Cyclospora* sp.−*S. stercoralis* (n = 1) were recorded.

Enteric bacterial infections: associated factors
Different factors were analysed to find their possible association with enteric bacterial infection among HIV-infected individuals. In bivariate analysis, bacterial infections were found to be statistically significant in participants with CD4 T-cell counts ≤200 cells/µL (p 0.01), and in those using unprotected water sources (p 0.02), having the habit of consuming raw food (p 0.03) and maintaining domestic animals (p 0.01). All these groups of patients showed a higher prevalence of bacteria. In multivariate analysis, it was observed that CD4 T-cell counts <200 cells/µL (aOR 9.55, 95% CI 1.54−59.3, p 0.015), presence of domestic animals (aOR 6.7, 95% CI 1.63−27.4, p 0.08) and consumption of drinking water from unprotected sources (aOR 3.8, 95% CI 1.07−13.4, p 0.04) were also statistically significant (Table 2).

Enteroparasitic infections: associated factors
Of the various factors assessed by bivariate analysis, those such as age between 15 and 24 years (p 0.001), CD4 T-cell counts <200 cells/µL (p 0.005), CD4 T-cell counts between 200 and 500 cells/µL (p 0.03), history of diarrhoea (p 0.04) and handwashing practices after toileting (p 0.00) were found to be statistically significant. Risk factors involved in enteroparasitic infections with statistical significance in bivariate analysis were further subjected to multivariate analysis. Accordingly, CD4 T-cell counts <200 cells/µL (aOR 3.53, 95% CI 1.13−17.93, p 0.03), and handwashing practices after toileting (aOR 8.67, 95% CI 4.2−17.93, p 0.000) were found to be statistically significant (Table 3).

Antibiotic susceptibility pattern
Antibiotic susceptibility profiles of all bacterial isolates were confirmed using 12 antibiotics. The isolated enteric bacteria showed broad variations in their resistance/susceptibility. The highest degree of resistance was shown by isolates of *Salmonella* against three antibiotics tested and the range was 40%−80%. Resistance of the *Salmonella* isolates to erythromycin was 80% followed by 60% against ceftazidime. Isolates also exhibited 40% resistance against ampicillin, chloramphenicol, cotrimoxazole, gentamicin, tetracycline and ceftriaxone. Notably, a lower degree of resistance was displayed against ciprofloxacin (20%). The antibiogram of *Shigella* showed that all isolates were 100% resistant to ampicillin, gentamicin, erythromycin and ceftazidime. In addition, 50% of the isolates showed resistance to four antibiotics (tetracycline, ciprofloxacin, cotrimoxazole and ceftriaxone). On the other hand, all the isolates were susceptible to chloramphenicol, doxycycline, azithromycin and meropenem.

TABLE 2. Bivariate and multivariate analysis of factors associated with the prevalence of enteric bacterial among study participants

Variables		Enteric bacteria		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
		Yes	No				
Sex	Male	5	79	0.54 (0.18–1.66)	0.28		
	Female	10	86	1	1		
Age (years)	15–24	1	22	0.50 (0.05–4.74)	0.55		
	25–34	4	44	1	1		
	35–44	6	63	1.05 (0.28–3.93)	0.94		
	>45	4	36	1.22 (0.28–5.23)	0.78		
Residence	Urban	11	131	1	1		
	Rural	4	34	1.401 (0.42–4.67)	0.58		
Marital status	Married	7	100	1	1		
	Unmarried	1	4	3.57 (0.35–36.4)	0.28		
	Divorced	7	42	2.38 (0.78–7.21)	0.13		
	Widowed	0	19	0.00	0.99		
Educational status	Illiterate	6	40	2.083 (0.7–6.21)	0.19		
	Literate	9	125	1	1		
Occupation	Farmer	6	36	2.37 (0.627–9.000)	0.20		
	Merchant	2	34	0.84 (0.146–4.822)	0.84		
	Government employee	4	57	1	1		
	Housewife	0	16	0.00	0.99		
	Labour work	3	22	1.94 (0.40–9.39)	0.41		
Income level (ETB)	≤1000	6	55	4.04 (0.46–34.92)	0.20		
	1001–200	8	73	4.05 (0.49–33.65)	0.19		
	>2000	1	37	1	1		
CD4 T-cell count (cells/μL)	<200	4	16	7.42 (1.5–36.3)	0.01	9.55 (1.54–59.3)*	0.015
	200–500	8	60	3.95 (1.01–15.5)	0.05	4 (0.86–14.03)	0.063
	>500	3	89	1	1	1	1
COT prophylaxis	Yes	8	56	1	1		
	No	7	109	0.45 (0.15–1.30)	0.14		
Diarrhoea duration	Acute	10	103	1	1		
	Chronic	5	62	0.83 (0.27–2.54)	0.74		
History of diarrhoea	Yes	5	74	0.62 (0.20–1.88)	0.39		
	No	10	91	1	1		
Diagnosed for OI	Yes	5	62	0.83 (0.27–2.54)	0.74		
	No	10	103	1	1		
Stool consistency	Watery	10	122	2.38 (0.29–19.31)	0.42		
	Mucoid	4	14	8.28 (0.85–81.19)	0.07		
	Loose	1	29	1	1		
Drinking water source	Protected	9	140	1	1	1	1
	Unprotected	6	25	3.73 (1.22–11.4)	0.02	3.8 (1.07–13.4)*	0.04
Latrine usage	Private	8	104	1	1		
	Public	7	61	1.49 (0.52–4.32)	0.461		
Raw food consumption	Yes	12	81	4.15 (1.13–15.24)	0.032	3.5 (0.87–14.03)	0.08
	No	3	84	1	1	1	1
Presence of DA	Yes	12	67	5.85 (1.6–21.5)	0.01	6.7 (1.63–27.4)*	0.01
	No	3	98	1	1	1	1
HW after toileting	Yes	9	102	1	1		
	No	6	63	1.08 (0.37–3.18)	0.89		
HW before meals	Yes	5	81	1	1		
	No	10	84	1.93 (0.63–5.89)	0.25		

Abbreviations: 1, reference group; COT, cotrimoxazole; DA, domestic animals; HW, hand washing; OI, opportunistic infections.
Note: *p < 0.05.

Isolates of *Campylobacter* showed considerable resistance in the range of 50%–87.5% against tetracycline, ceftriaxone, cotrimoxazole, erythromycin and, ampicillin. However, 37.5% of the isolates were sensitive to ciprofloxacin, and chloramphenicol, gentamicin and ceftazidime had the lowest degrees of resistance (25%). All the isolates were 100% susceptible to azithromycin, doxycycline and meropenem (Table 4). The multidrug resistance in this study refers to the resistance to three or more groups of the 12 antibiotics tested. The most common antimicrobial resistance patterns in bacterial isolates are presented in Table 5. A particularly important result obtained is that all the isolates of *Shigella* were multidrug-resistant. Concerning the isolates of *Campylobacter* sp., only 75% were multidrug-resistant whereas, in the case of *Salmonella*, only 60% were found to be so.

Discussion

Although there is a decline in the occurrence of many opportunistic gastrointestinal tract infections after the introduction of ART, diarrhoea remains a major cause of morbidity and mortality among HIV/AIDS patients [31]. Improving the symptoms and preservation of the functional/nutritional status of HIV-infected individuals with diarrhoea is extremely important. In this context, it is important to determine the type of aetiological agents of the diarrhoea for appropriate therapy. The prevalence of acute gastroenteritis caused by enteric pathogens in HIV-infected individuals is not well studied or documented in many regions of Ethiopia because of limited surveillance, lack of laboratory facilities to diagnose the common bacterial agents, or both. Stool analysis is a convenient, reliable and inexpensive

TABLE 3. Bivariate and multivariate analysis of factors associated with the prevalence of enteroparasites among study participants

Variables		Intestinal parasite		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
		Yes	No				
Sex	Male	31	53	1.07 (0.58–1.96)	0.84		
	Female	34	62	1	1		
Age (years)	15–24	14	9	5.91 (1.99–17.57)	0.001		
	25–34	10	38	1	1		
	35–44	25	44	2.16 (0.92–5.06)	0.08		
	>45	16	24	2.53 (0.99,6.49)	0.05		
Residence	Urban	59	83	1	1		
	Rural	6	32	0.26 (0.10–0.67)	0.005		
Marital status	Married	32	75	1	1		
	Unmarried	2	3	1.56 (0.25–9.80)	0.63		
	Divorced	22	27	1.91 (0.95–3.84)	0.07		
	Widowed	9	10	2.11 (0.78–5.68)	0.14		
Educational status	Illiterate	17	29	1.05 (0.52–2.11)	0.49		
	Literate	48	86	1	1		
Occupation	Farmer	16	26	1.26 (0.55–2.87)	0.58		
	Merchant	14	22	1.30 (0.55–3.07)	0.54		
	Government employee	20	41	1	1		
	Housewife	5	11	0.93 (0.28–3.05)	0.91		
	Labour work	10	15	1.37 (0.52–3.58)	0.52		
Income level	≤1000	24	37	0.99 (0.43–2.28)	0.99		
	1001–200	26	55	0.72 (0.33–1.61)	0.43		
	>2000	15	23	1	1		
CD4 T-cell count (cells/μL)	<200	12	8	4.25 (1.55–11.65)	0.005	3.53 (1.13–17.93)*	0.03
	200–500	29	39	2.11 (1.08–4.11)	0.03	1.53 (0.70–3.31)	0.28
	>500	24	68	1	1	1	1
COT prophylaxis	Yes	29	36	1	1		
	No	36	79	0.57 (0.30–1.06)	0.07		
Types of diarrhoea	Acute	44	69	1	1		
	Chronic	21	46	1.4 (0.74–2.65)	0.30		
History of diarrhoea	Yes	35	44	1.88 (1.02–3.5)	0.04	2.02 (0.97–4.20)	0.61
	No	30	71	1	1	1	1
Diagnosed for OI	Yes	21	46	0.72 (0.38–1.36)	0.31		
	No	44	69	1	1		
Stool consistency	Watery	50	82	1.22 (0.53–2.82)	0.64		
	Mucoid	5	13	0.77 (0.21–2.77)	0.69		
	Loose	10	20	1	1		
Drinking water source	Protected	57	92	1	1		
	Unprotected	8	23	0.56 (0.24–1.34)	0.19		
Latrine usage	Private	41	71	1	1		
	Public	24	44	0.94 (0.50–1.77)	0.86		
Raw food consumption	Yes	24	69	0.39 (0.21–0.73)	0.003		
	No	41	46	1	1		
Presence of DA	Yes	29	50	1.05 (0.57–1.93)	0.88		
	No	36	65	1	1		
HW after toileting	Yes	20	91	1	1	1	1
	No	45	24	8.53 (4.26–17.05)	0.00	8.67 (4.2–17.93)*	0.00
HW before meals	Yes	37	49	1	1		
	No	28	66	0.56 (0.304–1.039)	0.07		

Abbreviations: 1, reference group; COT, cotrimoxazole; DA, domestic animals; HW, hand washing; OI, opportunistic infections.
Note: *p < 0.05.

way (easy work-up) of diagnosing the aetiological agents causing secondary enteroparasitic infections [32], especially in the Ethiopian context. In fact, stool examination is the first route. Most of the bacterial, viral, fungal and parasitic pathogens can be diagnosed by this process. The results of this study brought out some relevant pieces of information pertaining to the prevalence and diversity of enteric bacteria and enteroparasites in HIV-infected individuals with diarrhoea in the Arba Minch province of Ethiopia. It is important to note that this is the first study in this context carried out in the southern region of Ethiopia and hence assumes considerable significance. The overall prevalence of enteric bacteria among HIV-infected individuals with diarrhoea was 8.3% and is comparable to the results of a previous study in another region of Ethiopia (12.6%) [23]. Also, the diversity of enteric bacteria observed in this work are similar to those in some previous studies [23,33].

Among the three bacterial pathogens belonging to the three genera isolated, *Campylobacter* sp. was the most predominant and this is similar to the information obtained from several studies reported from Ethiopia and South Africa [23,34]. However, this rate of isolation is much lower than that obtained from a study from another part of Ethiopia (13.1%) [35]. The preponderance of *Campylobacter* sp. has been attributed to direct contact with infected household pets or the consumption of contaminated animal products, as campylobacteriosis is primarily a zoonosis [35]. In the case of *Salmonella* sp., the prevalence was found to be 2.8% and relatively similar to the results of several studies conducted in Ethiopia (5.1%), Uganda (4%) and Senegal (1.4%) [23,36,37]. The prevalence of *Shigella* sp. (1.1%) was also comparable to data obtained from earlier studies conducted in Ethiopia (1.3%) and Senegal (2.8%) [23,37]. Nevertheless, contrary to our results, previous studies from

TABLE 4. Antibiotic susceptibility patterns of enteric bacterial isolates

Antibiotics (μg)	Salmonella sp. (n = 5)			Shigella sp.(n = 2)			Campylobacter sp. (n = 8)		
	S	I	R	S	I	R	S	I	R
AMP	2	1	2	0	0	2	1	0	7
CHL	3	0	2	2	0	0	4	2	2
CPR	3	1	1	1	0	1	4	1	3
COT	3	0	2	0	1	1	2	1	5
GEN	3	0	2	0	0	2	4	2	2
ERY	1	0	4	0	0	2	2	0	6
AZT	2	3	0	2	0	0	6	2	0
TTC	1	2	2	1	0	1	4	0	4
DOX	4	1	0	2	0	0	8	0	0
CTR	2	1	2	1	0	1	2	1	5
CZM	2	0	3	0	0	2	3	3	2
MER	5	0	0	2	0	0	8	0	0

Abbreviations: I, intermediate; R, resistant; S, susceptible. Antibiotic abbreviations: AMP, ampicillin; AZT, azithromycin; CHL, chloramphenicol; COT, cotrimoxazole; CPR, ciprofloxacin; CTR, ceftriaxone; CZM, ceftazidime; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; MER, meropenem; TTC, tetracycline.

TABLE 5. Antibiotic resistance patterns of enteric bacterial isolates

Resistance pattern	Antibiotics	Salmonella sp. n (%) (n = 5)	Shigella sp. n (%) (n = 2)	Campylobacter sp. n (%) (n = 8)
R0	None	—	—	
R1	AMP	—	—	—
	CIP	—	—	—
	ERY	1 (20)	—	—
R2	AMP, CIP	—	—	
	AMP, ERY	1 (20)	—	1 (12.5)
	ERY, CIP	—	—	1 (12.5)
R3	AMP, CIP, ERY	—	1 (50)	—
	AMP, CIP, SXT	1 (20)	—	1 (12.5)
R4 and above	AMP, CIP, TTC, ERY SXT/CTR/ CHL/GEN	2 (40)	1 (50)	5 (62.5)

Abbreviations: R0, no resistance at all; R1, resistant to one antibiotic; R2, resistant to two antibiotics; R3, resistant to three antibiotics; R4 and above, resistant to four or more antibiotics. Antibiotic abbreviations: AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTR, ceftriaxone; ERY, erythromycin; GEN, gentamicin; SXT, sulfamethoxazole; TTC, tetracycline

another region of Ethiopia (4%) and Uganda (6%) reported higher prevalence rates of *Shigella* sp. [19,36]. The incidence of enteric bacteria may be considered an indicator of poor hygiene and sanitation, as well as of consumption of contaminated water and food. Our results imply that stool analysis for bacterial identification is a very important aspect and high alert from ART clinicians is warranted in this regard. In other words, clinicians should always keep a high index of suspicion in the diagnosis of enteric infections.

The overall prevalence pertaining to the enteroparasites was 36.1%. A similar trend was observed in previous studies reported from Ethiopia (35.8%) and Senegal (31.1%) [20,37]. At

the same time, these results are not consistent with a couple of studies performed in different regions of Ethiopia itself [21,38]. The discrepancy in the prevalence rates of enteroparasites may be attributed to the variations in sociodemographic characteristics among the study populations, endemicity of parasites, effectiveness of interventions in curbing opportunistic infections, sample sizes as well as general hygiene level. In our study, commonly isolated parasites were protozoans, which was comparable with the results of earlier studies from different regions of Ethiopia and Cameroon [20,38,39]. Among protozoans, *G. lamblia* was the type predominantly identified and its isolation rate (8.9%) was similar to that in a couple of the earlier reports from different regions of Ethiopia [20,38]. At the same time, higher rates of prevalence were also reported from Kenya (16.6%) [40]. The rate of prevalence of *C. parvum* (8.3%) observed in the present study was almost the same as that revealed by earlier work in Ethiopia (8%) and Cameroon (7.1%) [41,42]; but was much lower than the extent observed in a study conducted in another province of Ethiopia (15.4%) [43]. All of these variations could be correlated to the types study design and laboratory techniques employed. The isolation rate of *Entamoeba histolytica/dispar* was 5.5%, which was also in line with the results of a study in Cameroon (7.8%) [42]. Regarding the helminths, the isolation rate of the prominent species *A. lumbricoides* was 3.3%, and this is comparable to the results of previous research conducted in Ethiopia (2.5%) [41]. However, we only collected a single stool sample from each patient for the diagnosis and some species of parasites may have been overlooked. Also, even after a proper diagnosis and completion of treatment of diarrhoea, symptoms may persist, because of the possible presence of secondary infections [4]. The high prevalence of parasitic infections despite the availability of ART observed in this study compels the need for including routine stool examinations in the follow up of patients attending the ART clinic and, if required, blood cultures too. Our findings warrant that ART clinicians should not underestimate the relevance of stool examination while treating HIV-infected individuals presenting with diarrhoea, especially those with lower CD4 T-cell counts.

The present set of results indicate that enteric bacterial infections had a significant statistical association with certain variables. For instance, the prevalence of enteric bacteria was strongly and significantly associated with low CD4 T-cell counts, i.e. <200 cells/μL. The extent of enteric infections depends on the degree of immune suppression, which in turn is determined by CD4 T-cell counts. Our findings are also consistent with the results of earlier studies, which reported that patients with CD4 T-cell counts <200 cells/μL account for a considerable number of cases with a higher rate of bacterial infections [44]. The presence of domestic livestock and poultry

in close proximity to inmates increases the potential for faecal contamination within the household, and subsequent transmission [45]. We found that the prevalence of enteric bacteria was also prominent among those who rear domestic animals and they were 6.7 times (95% CI 1.63–27.4, p 0.008) more susceptible to become infected than individuals who have had no such contacts. It can be inferred that risk factors associated with bacterial infections must be given due consideration during the policy-making meant for interventions in the study area.

Cell-mediated immunity is the main defence mechanism against infections caused by enteroparasites. Similar to the case of enteric bacteria, the prevalence rates of enteroparasites were strongly associated with low CD4 T-cell counts, i.e. <200 cells/µL, and patients in this category were 3.53 times (95% CI 1.13–17.93, p 0.03) more vulnerable to acquiring enteroparasitic infections than patients having CD4 T-cell counts >500 cells/µL. This parallels the outcome of a study conducted earlier in India, which reported that CD4 T-cell counts <200 cells/µL promote the prevalence of enteroparasites [44]. A study from Cameroon also demonstrated such trends [46]. Further, the prevalence of enteroparasites was strongly associated with poor handwashing habits after toileting, and individuals with this lifestyle were 8.7 times (95% CI 4.2–17.93, p 0.00) more susceptible in acquiring the infections than their counterparts with good handwashing practices. High incidence of diarrhoea in HIV-infected individuals in developing countries could be the result of poor hygiene, inadequate supply of clean water and difficulty in accessing treatment [47].

A disturbing finding is that patients with lower CD4 T-cell counts (i.e. <200 cells/µL) were at significantly greater risk of developing both bacterial and parasitic diarrhoea. It is well-acknowledged that patients' high adherence to ART markedly increases their CD4 T-cell counts, slows down the progression of the disease and reduces their susceptibility to opportunistic infections [48]. Results related to the associated risk factors substantiate the implementation of critical measures by healthcare professionals, for immune restoration in patients with lower CD4 T-cell counts (by means of ART). In fact, opportunistic infections are rare in individuals with a preserved immune system. Results of the present study infer that the effective way of reducing the impact of diarrhoeal diseases and the risk of contracting infections rests in improving immune status, maintaining hygiene and sanitation, and using potable water.

Routine determination of bacterial profiles and their antibiotic sensitivity patterns could help the patients in getting definitive therapy, and thereby shortening the duration of diarrhoea and associated complications. Besides, antibiotic susceptibility patterns may differ from region to region and with time; hence periodic updates pertaining to the susceptibility profiles are much needed for the rational use of antibiotics. Regarding the resistance profile of *Salmonella* sp., 80% of the isolates were found to be resistant to erythromycin. This is more or less comparable to the results of a study performed in another part of Ethiopia [49]. On the other hand, the *Salmonella* sp. were susceptible to azithromycin. It is known that *Salmonella* isolates are intrinsically resistant to erythromycin through active efflux [50], but naturally susceptible to azithromycin [51]. It is important to note that 60% of the isolates were resistant to ceftriaxone. Hence, it is envisaged that the use of ceftriaxone must be restricted in the study area. On the other hand, the low rate of resistance manifested against ciprofloxacin is an encouraging finding from a public health perspective. The resistance profiles of *Shigella* sp. were alarming as all isolates showed maximum resistance against ampicillin, erythromycin and ceftazidime (i.e. 100%). Our results are in line with the findings of a previous work from western Ethiopia, which reported 100% resistance against ampicillin [19], and another study from Ethiopia reported a higher resistance rate against erythromycin and ampicillin [49]. In contrast, azithromycin was found to be more potent than erythromycin against *Shigella* sp. in our study. The resistance exhibited by *Shigella* sp. to ciprofloxacin and ceftazidime is of serious concern as these antibiotics are currently recommended as the first-line and second-line treatments, respectively, by WHO [52]. Regarding the *Campylobacter* isolates, there was a higher degree of resistance to ampicillin, i.e. 87.5% followed by 75% towards erythromycin, 62.5% to ceftriaxone and 62.5% to cotrimoxazole. This is comparable to a study conducted in the southern part of India [53]. The resistance exhibited by *Campylobacter* isolates in our study was more severe than that reported by another study in southern Ethiopia, documenting a decreased resistance to erythromycin (55%) and ampicillin (30%) [49]. A slight increase (37.5%) in ciprofloxacin resistance was observed in this study. *Campylobacter* is increasingly acquiring resistance to the macrolide and fluoroquinolone antimicrobials (e.g. ciprofloxacin); this rising resistance is a menace. Recently, fluoroquinolone-resistant *Campylobacter* was listed as a high-priority pathogen that requires research and development of new antibiotics [54].

The antibiogram of all the enteric bacterial isolates closely resemble those reported from various regions of Ethiopia [19,49]. Therefore, to avoid the possible emergence of resistance, antibiotic susceptibility patterns must be periodically inspected to choose appropriate regimens. In contrast, a notable result of the present study is that all the isolates of three enteric bacteria showed higher sensitivity to doxycycline, azithromycin and meropenem, indicating the need for judicious use of broad-spectrum antibiotics.

Extensive use of antibiotics or anti-motility drugs may lead to serious complications by prompting the emergence of multidrug-resistant bacteria or chronic carriers. The emergence of drug-resistant bacteria is a great concern to clinicians treating HIV-infected individuals with diarrhoea. In most Ethiopian hospitals, antibiotic treatments are not streamlined as per the microbiological culture data. As a corollary to this, bacterial species are envisaged to acquire resistance to the currently practiced antibiotic regimens and this trend has become a major concern in Arba Minch, as we detected a high prevalence of multidrug resistance among *Shigella*, *Salmonella* and *Campylobacter* sp. This is in agreement with the results of a previous study from southern Ethiopia revealing that more than 90% of *Shigella*, *Salmonella* and *Campylobacter* sp. were multidrug-resistant [49]. It is envisaged that unrestricted, frequent and inappropriate usage of antibiotics could be the reason for the emergence of multidrug-resistant enteric bacteria. Therefore, it is high time for the medical fraternity to be vigilant regarding the guidelines to achieve an overall reduction of antimicrobial resistance.

Shortcomings of the present work include a confined cross-sectional study design with a limited number of participants/sample size and shorter tenure. In view of the lack of facilities, antisera serotyping was not performed to differentiate among *Salmonella* isolates. Due to the inadequacy of advanced techniques/chemicals, some of the bacterial pathogens were not identified.

Conclusion

This is the first report on the prevalence of enteric bacteria, enteroparasites and antibiotic susceptibility patterns in HIV-infected individuals with diarrhoea in the Arba Minch province of Ethiopia. The results of our study have serious implications for the management of enteric infections among HIV-infected individuals in this region. We found that enteroparasitic infections were more frequent than bacterial infections. Therefore, frequent stool analysis, careful and proper diagnosis followed by subsequent treatment are recommended. Nevertheless, all the isolates of three enteric bacteria were susceptible to doxycycline, azithromycin and meropenem. Resistance to ciprofloxacin, tetracycline, erythromycin, ceftriaxone and cotrimoxazole are emerging. Another alarming fact is that the majority of the isolates of *Shigella*, *Salmonella* and *Campylobacter* sp. were resistant to most of the antibiotics currently in use. Hence, enhanced surveillance is needed to evaluate these trends. Statistical analysis revealed that CD4 T-cell counts <200 cells/μL, quality of drinking water sources, hand washing habits after toilet use, and the presence of domestic animals were significantly associated with the prevalence of enteric pathogens. This information highlights the need for prompt and accurate diagnosis of diarrhoeal aetiology, and pathogen-specific therapy to minimize the associated morbidity. Also, there must be a high index of suspicion from the clinician to look for the possibilities of secondary infections. In addition to non-invasive stool culturing, in the absence of a proper diagnosis, invasive studies can also be recommended. Health promotional messages should be given to maintain an extreme level of personal hygiene always, and enhanced alertness during handling pets or bovids. Even lactose-free diets can be recommended to curb diarrhoea. Finally, the provision of safe potable water to eliminate the transmission of diseases must be ensured.

Authors' contributions

AA, AM, DT and TY conceived and designed the project. AA performed the experiments. AA, DT, AM and TY coordinated data collection. AA, MSM, DT and MS analysed the data. AM drafted the paper and all authors have read and approved the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical approval

This study was ethically approved by the Institutional Review Board of the College of Medicine and Health Sciences, Arba Minch University (Ref. IRB/12036584/106/08/02/19). Before data and sample collections, written consents were obtained from all the participants or their parents (if adolescents) after a clear briefing about the purpose of the study. Strict confidentiality was maintained during the interview process, and anonymity was kept during data processing and report writing.

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