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ABSTRACT: Foodborne pathogens (FBPs) are transmitted principally through the consumption of contaminated food or drinking water and pose a remarkable public health risk, particularly in low-income countries. A cross-sectional study was conducted between November 2021 and January 2023 to estimate the prevalence, co-occurrence, and monthly patterns of FBPs in the physical exposures of children with diarrhea in Harar town and Kersa district, Ethiopia. Animal, food, and environmental samples were collected from direct or indirect contact sites of children with diarrhea. The isolation and identification of FBPs, including nontyphoidal *Salmonella* (NTS), diarrheagenic *E. coli* (DEC), and *Shigella*, was performed using selective and deferential culture media and a series of biochemical tests. Among the 438 analyzed samples, the overall prevalence of these pathogens was 18.3%, with 3.9% co-occurrence and 14.4% single pathogen occurrence rates. The highest prevalence was observed in wastewater (40.9%; AOR = 3.3; 95% CI: 1.1-10.1). The pathogen detection rate in food was 17.9% (AOR = 1.2; 95% CI: 0.4-3.6), with no significant difference between animal-sourced and other food categories. The occurrence rates of NTS, DEC, and *Shigella* in the meat samples were 13.9%, 5.4%, and 6.5%, respectively. Interestingly, DEC and *Shigella* were detected in cooked food. Moreover, *Shigella* was detected in drinking water (5%) and other water sources (10%). A significantly higher prevalence of FBPs was detected in poultry than in cattle and camel feces. This study revealed fluctuations in the monthly occurrence patterns of FBPs, with a peak of 37.1% during the dry season. In conclusion, the study revealed a high prevalence of FBPs, with no significant differences between rural and urban areas or food and water sources, highlighting the need for food safety measures in both settings. Further studies with larger sample sizes and advanced diagnostics are recommended to determine the relative contribution of each source.

KEYWORDS: Children, Diarrhea, Exposure sources, Foodborne pathogens, Ethiopia

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Introduction

Diarrheal diseases are major global public health concerns that disproportionately affect young children, elderly people and low-income countries. Diarrhea is the third leading cause of death in children under the age of five, with 443 832 deaths every year worldwide.[1] In Ethiopia, the incidence rate of diarrheal episodes in children under five (UFC) is 186.7 thousand per year, and it is the second leading cause of death among this age group, killing 150.7 children per 100 000 people annually.[2]

Foodborne bacterial pathogens (FBPs), such as nontyphoidal *Salmonella* (NTS), *Shigella, Campylobacter*, and diarrheagenic *Escherichia coli* (particularly enterotoxigenic *E. coli*, ETEC), are among the most prevalent causative agents of diarrhea in Ethiopia, collectively contributing to approximately 22.2% of all diarrheal diseases in children.[3-6] Animals and the environment serve as reservoirs for these zoonotic FBPs, and the close human–animal interactions, consumption of raw animal products, and poor food safety knowledge in Ethiopia further increase the public health impact of these pathogens.[7,8]

The situation in eastern Ethiopia is not different, and prevalence studies have reported 37% *E. coli*[9] and 16.7% *Salmonella*[10] in animals, as well as a prevalence of up to 24.4% for *Salmonella*, 15.4% for *Shigella*,[11] and 12.2% for pathogenic *E. coli*[12] in food items in this region. A meta-analysis also revealed a 14.8% pooled prevalence of bacterial FBPs in environmental samples in eastern Ethiopia.[5] Factors such as the practice of open defecation and poor hand-washing habits in this region can further exacerbate the risk of foodborne illness, particularly in rural areas.[13,14]

The increase in interactions among humans, animals, and ecosystems, driven by factors such as population growth, agricultural intensification, climate change, and globalization, coupled with increasing demand for animal protein, has led to an

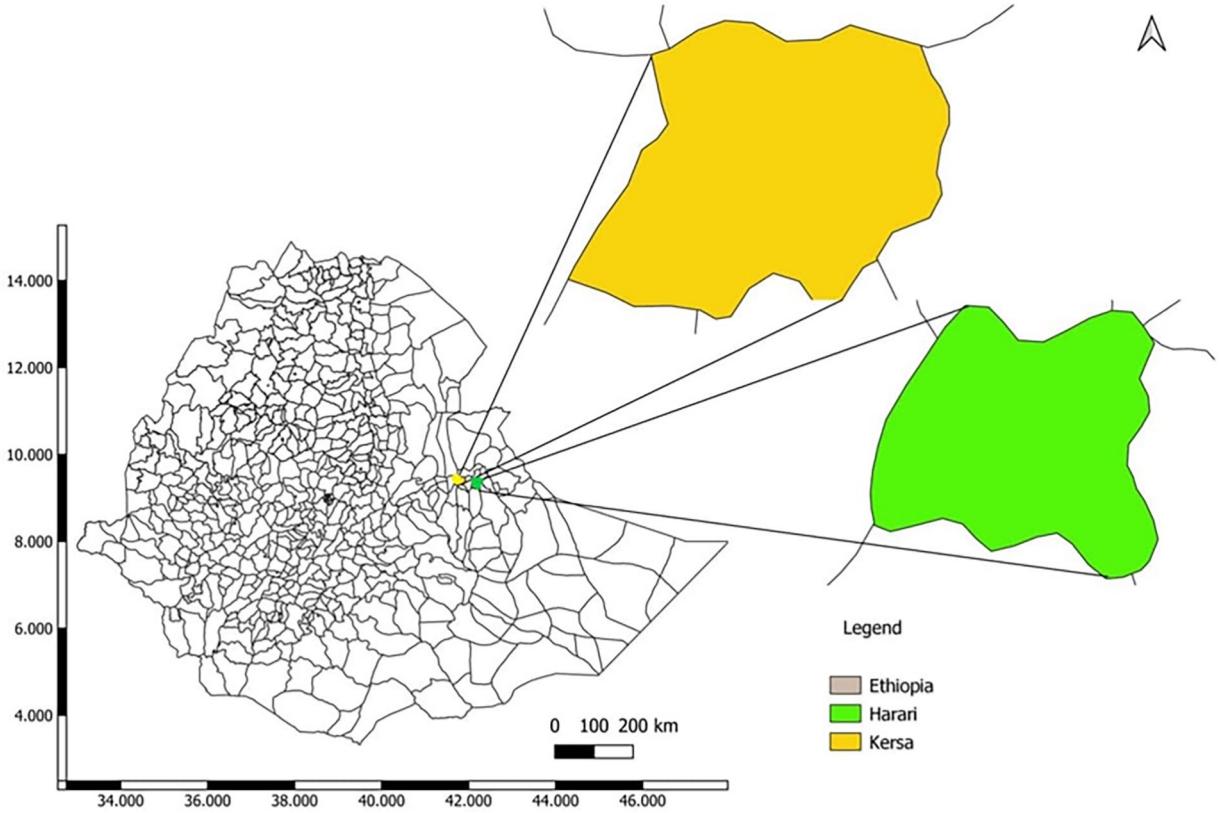

Figure 1. Maps of the study areas.

increase in the spread of zoonotic pathogens. In resource-limited settings, such as Ethiopia, where household livelihoods heavily depend on livestock, one health approach is essential for reducing the spread of zoonotic FBPs. The one health approach recognizes that human health is inextricably linked to the health of animals and the environment.[15,16] Despite Ethiopia's membership in the Global Health Security Agenda (GHSA), which promotes the one health approach, there is a paucity of comprehensive studies on the occurrence of foodborne diarrheal illnesses at the human–animal–environment interface in the country. The majority of previous studies have reported the prevalence of diarrheagenic FBPs separately, either in human patients, asymptomatic food handlers,[4] animals,[17,18] or food and environmental samples.[10,19,20] There is also a scarcity of data on the co-occurrence of multiple foodborne bacteria in animal, food, and environmental samples associated with individual diarrheic cases.

Investigating diarrheagenic FBPs in children, a significant portion of the population, and their environment in Ethiopia is important for assessing the health status of children. Moreover, identifying the principal bacterial FBPs and their co-occurrence in animal, food, and environmental samples associated with known diarrheic cases is crucial for designing targeted interventions and effective integrated disease prevention and control strategies. Therefore, this study aimed to estimate the prevalence, monthly occurrence patterns, and co-occurrence profiles of nontyphoidal *Salmonella*, diarrheagenic *E. coli*, and *Shigella* in animal, food, and environmental samples collected from the physical exposures of children with diarrhea through case-based tracing in Harar and Kersa, East Ethiopia.

Materials and Methods

Study area

The present study was carried out in the Harari region and Kersa district, East Ethiopia, between November 2021 and January 2023 (Figure 1). Harar is the capital of the Harari People National Regional State and is the only urban area in the region. The total population of the Harari region is estimated at 257 000, with 11% of its population aged between 0 and 4 years.[21] There are 7 hospitals, 8 public health centers, 32 health posts, and 25 clinics in the Harari region.[22]

The Harari region is known for its diverse agro-ecologies. The region shares borders with several districts of the east Hararghe Zone, including Kombolcha, Jarso, Gursum, Babile, Haramaya, and Fedis.[23] Harar town has two municipal abattoirs, where cattle, camels, and shoats (sheep and goats) are slaughtered. Additionally, shoats are slaughtered at different hotels and restaurants. In terms of waste management, Harar has a centralized sewage treatment facility. However, in certain areas, the waste disposal system is not connected to the main channel.

Kersa is one of the districts of the East Hararghe Zone, Oromia regional state. The district is located approximately 44 km west of Harar town. The district shares borders with the

Dire Dawa Administrative Council, Haramaya, Kurfa Chele, Bedeno, and Meta districts. Kersa boasts diverse topographies, including natural lakes within and around the district that serve various purposes for its residents.[4] Kersa district has a total population of 172 626. There are 6 health centers and 28 health posts that provide basic health services and no hospital at all in the district.[24] The majority of Kersa residents are farmers who are engaged in livestock rearing and crop cultivation. Like Harar, within the small towns of the Kersa district, there are animal farms and live animals, as well as poultry and dairy product marketing sites. In the case of drinking water, Kersa residents rely mainly on springs and wells, although tap water is accessible in towns and adjacent subdistricts. Some residents in the district also receive water from private ponds. On the other hand, the slaughterhouse in the Kersa district is not well equipped, and there is also no sewage treatment facility. The Kersa district lacks a properly equipped slaughterhouse, and there is also no sewage treatment facility.

Sample collection procedures

Prior to sample collection, children under 5 years of age (UFC) with acute diarrhea who were residents of Harar town or Kersa district were identified among those children seeking treatment at healthcare facilities. Case screening and diarrheic stool sample collection were conducted in collaboration with pediatricians, clinicians, and laboratory technicians working at Hiwot Fana Hospital, Kersa, and Adelle Health Centers; the full case identification procedure is described in a recently published article.[4]

After identifying the diarrheal cases, caretakers were interviewed to obtain their pertinent background and the case's recent exposure information, and samples were collected from the exposure environments of the cases. This included collecting animal, food, water, and wastewater samples through case-based contact sample tracing. The interview responses from each caretaker guided us in identifying specific sites or samples that had direct or indirect contact with the child shortly before the onset of diarrhea. These include a history of what the child has ingested, the source of the consumed food (self-produced, market, etc.), information regarding animal contact, specific household addresses, etc. The questionnaire also identified the source of drinking water, irrigational water, or recreational water used prior to the first signs of diarrheal illness. During face-to-face interviews with caretakers, we also identified any wastewater linked to the households, including wastewater discharged from nearby food processing factories (eg, abattoir waste) or marketplaces. Then, caretakers were requested for their agreement to visit households and the identified exposure sites for sample collection.

Terminological definitions

The study considered "exposure sources" to be any accessible sample sources that were identified by caretakers as having been ingested by or come into contact with children prior to the onset of diarrheal symptoms. These include food, animal, wastewater, and water sources. The term "exposure environment" in this study referred to the physical locations from which the exposure source samples were collected. The "direct exposure environment" is defined as the immediate physical environment where UFC with diarrhea have direct contact, such as their households. On the other hand, the "indirect exposure environment" referred to the physical locations where the family members, specifically the caretakers, of the UFC had contact and brought items used by the children. This can include nearby marketplaces, shops, restaurants, farms, etc., where any food items purchased for the children are identified as having potential contacts.

The present study analyzed human, animal, and environmental samples to determine the occurrence of FBPs in humans, animals, and their shared environment, considering the concept of one health. In this an integrated study approach, veterinarians, public health professionals, and environmental health professionals were involved in sample collection and analysis. The one health strategy is an important multisectoral approach for addressing the complex health challenges posed by zoonotic FBPs at the intersection of humans, animals, and the environment.[15,16,25] With increasing interactions between humans, animals, and the environment, as well as numerous other factors exacerbating the burden and spread of FBPs, the one health approach has emerged as a critical multisectoral and multidisciplinary collaboration and coordination strategy to prevent, detect, and effectively respond to zoonoses and other threats by integrating surveillance, data sharing, and targeted interventions across public health, veterinary, food safety, and environmental domains. Studies conducted in many countries, such as Colombia,[26] Mexico,[27] and Tanzania,[28] have shown evidence for the importance of implementing an integrated health strategy to address foodborne zoonoses.

Collection of samples from exposure environments: With the caretakers' consent to visit households and other contact sites, exposure sources were investigated by visiting the households and recent physical exposure, typically within 1 to 2 days after the collection of diarrheic stools from cases. During household visits, stored food and food leftovers were collected. Raw meat and milk; nonanimal-origin foods such as vegetables and fruit; and imported and locally prepared juices used by the cases were collected. Samples of other ready-to-eat (RTE) food leftovers, such as "injera with wot," mixed food items (meat or milk or egg-based locally prepared food such as sandwiches, soup, fetira, "jajura," etc.), drinking water, and wastewater, were also collected. Moreover, feces were aseptically collected from cattle, camels, sheep, goats, or poultry in the households, either directly from the rectum/cloaca or from fresh droppings. In cases where multiple animals of the same species were present in the household, only pooled fecal samples were collected.

In addition to samples collected from households, food samples, animal feces, wastewater, and water samples were collected from slaughterhouses, marketplaces, hotels and restaurants, grocery stores, butcher shops, nearby farms and lakes, etc., where the children had recent direct or indirect contact before becoming sick. The collected water source samples were drinking water, recreational water and irrigation water. The wastewater considered in this study was wastewater runoff from slaughterhouses (animal wastewater) or food preparation sites (mixed human and animal wastewater) and wastewater from humans only (eg, waste discharged from utensils and hand washing at hotels or restaurants that were linked to cases).

All the samples were collected aseptically into sterile stomacher bags (Fisher Scientific Biotech line), and three junior public health professionals and one veterinarian were involved in the collection of specific human-related data and animal samples, respectively, in consultation with a public health expert. The guiding questionnaire was first collected using Epi Info 7 software at the health facilities; hence, the locations and types of exposure samples to be collected were identified before going for households, food production sites, marketplace visits, etc., by checking the caretakers' responses from the tablet device ahead of time.

Sample preparation and laboratory analysis

All samples were collected aseptically in sterile stomacher bags, labeled, and immediately placed in a cooler box with ice packs. The samples were transported to the laboratory within 6 hours and processed on the same day at the microbiology laboratory of the College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia.

Sample preparation. Animal Feces: Approximately 5 g of animal feces was gently mixed using a sterile loop. One gram of the well-mixed feces was transferred into a sterile Falcon tube containing 10 ml of buffered peptone water (BPW) and incubated for 24 hours at 37°C.

Meat: 25 g of meat was measured aseptically, minced or cut into smaller chunks, and added to a sterile stomacher bag containing 75 ml of modified tryptic soy broth (mTSB). The samples were hand-stomached until well dispersed. The dispersed samples were then incubated at room temperature for 10 minutes, followed by incubation at 37°C for 4 hours and 42°C for 20 ± 1 hours. To analyze nonliquid food samples, 25 g of each well-dispersed sample was added to 225 ml of BPW in sterile stomacher bags. The samples were hand-stomached or massaged to ensure proper dispersion. The samples were incubated at room temperature for 10 minutes, followed by incubation at 37°C for 24 hours.

Milk: Twenty-five milliliters of the milk samples were added to 225 ml of BPW and mixed well by agitation. The samples were incubated at room temperature for 10 minutes, followed by incubation at 37°C for 24 hours.

Wastewater: A total of 25 ml of the wastewater sample was prepared in 225 ml of BPW in the same way as the liquid samples (Figure 2). For the nonturbid liquid samples (drinking water, including bottled water), the sample volume was increased to 125 ml, and the sample was prepared with double-strength BPW[29] but half the volume of the broth medium BPW, with a 1:1 sample-to-BPW ratio.

Isolation and identification

After overnight incubation, a loopful of aliquots from the enrichment was streaked in triplicate on selective and differential media: xylose lysine deoxycholate (XLD), Hektoen enteric agar (HE), and MacConkey agar (MAC) and aerobically incubated at 37°C. At the same time, 1 and 0.1 ml of the broth suspension were added to tubes sequentially containing 10 ml of tetrathionate (TT) and Rappaport Vassiliadis Soya (RVS) broth (Merck, Germany) and then plated on both XLD (GranuCult™ XLD) and HE (Oxoid CM0419B, UK) for *Salmonella* isolation. Characteristic colonies of each pathogen from the respective plates were picked and subjected to Gram staining. The isolation and identification of the pathogens were performed according to the modified ISO 6579:2002 for *Salmonella* and *Shigella* and the previously described procedure for *E. coli*.[30,31]

Specific biochemical reactions of presumptive colonies for each bacterial pathogen were examined on triple sugar iron (TSI) and lysine iron agar (LIA), urease and IMViC (Indole (I), methyl red (M), Voges proskauer (Vi), and citrate (C)) media, and the results were interpreted according to Andrews et al.[31] Additionally, biochemically confirmed *E. coli* isolates were plated on Sorbitol MacConkey (SMAC) agar (Oxoid CM0813). Most pathogenic *E. coli* strains do not ferment sorbitol,[32,33] and only biochemically confirmed, nonsorbitol fermenter (NSF) isolates were considered pathogenic *E. coli* or DEC (Figure 2).

Quality control: Throughout the study, data quality was maintained by adhering to an SOP.[4] The questionnaires used for case-based contact sample tracing were completed using an Epi Info™ 7, and the completeness and consistency of the data were checked daily. For laboratory activities, universal biosafety precautions were followed to ensure general safety. Each new batch and shipment of media was subjected to quality control testing, and the standard bacterial reference strains were cultured with test samples each time.[4]

Data analysis

The laboratory data were entered into Microsoft Excel, cross-checked, coded, and analyzed with SPSS version 22. Descriptive statistics were employed to compute and explain the study variables. The associations between explanatory variables were assessed using the Pearson chi-square (X^2) test, with statistically significant variances determined when $P \leqslant .05$ at the 95% confidence level. The prevalence of FBPs was determined by

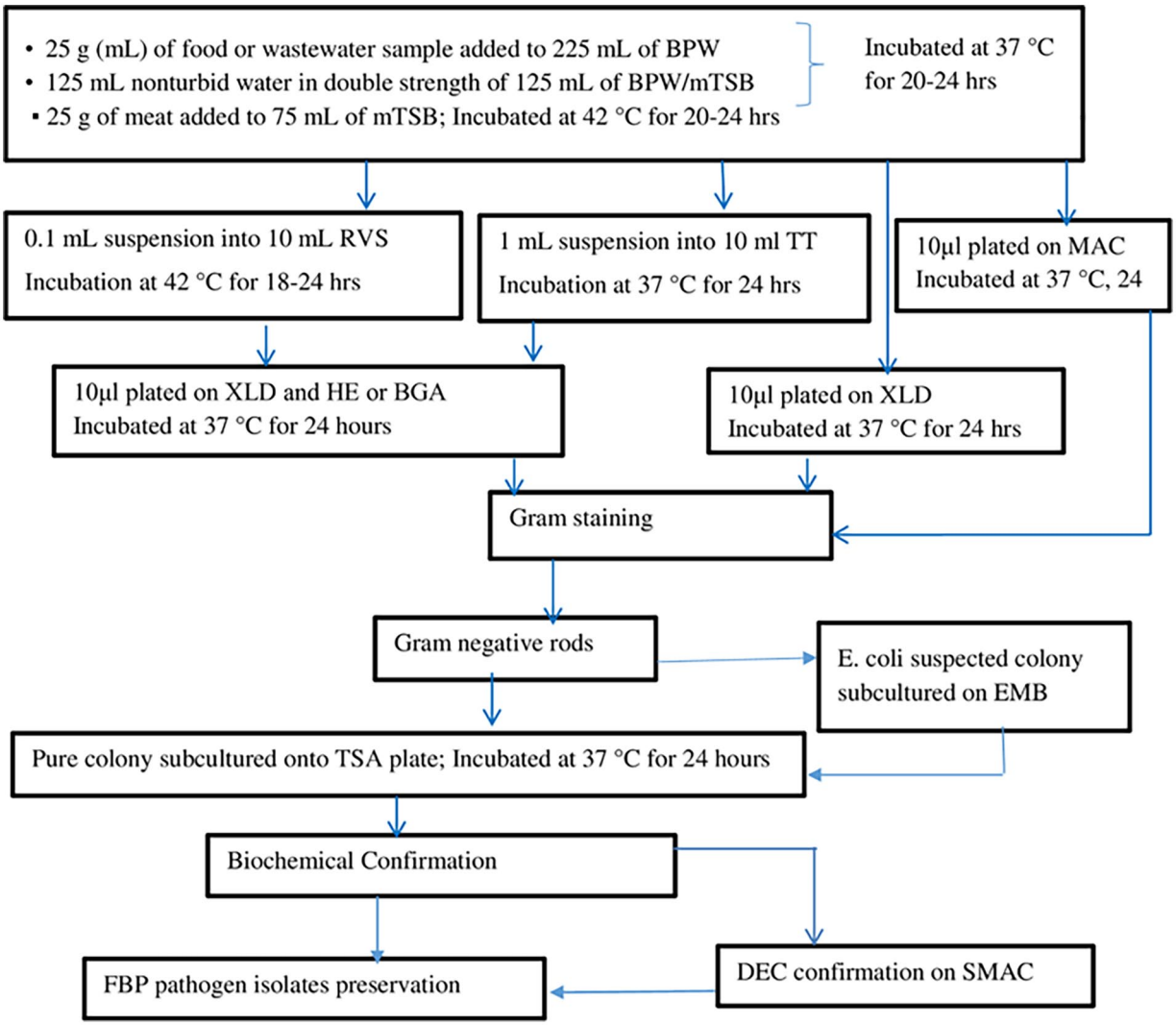

Figure 2. Environmental sample preparation and nontyphoidal *Salmonella*, diarrheagenic *E. coli*, and *Shigella* isolation and identification procedures.

dividing the total number of positive samples by the total number of samples analyzed and then multiplying by 100. When estimating the overall prevalence, we defined a sample as positive if it tested positive for at least one of the pathogens being investigated. The monthly rate of pathogen occurrence was determined by taking into account the total number of samples tested in a given month and the number of FBPs recovered from those samples. Furthermore, the term co-occurrence was used to describe instances where multiple bacterial pathogens were detected in a single sample. Binary logistic regression analysis was conducted to identify the relationships between individual independent variables (eg, sample sources and sample types) and the occurrence of diarrheagenic FBPs. The adjusted odds ratio was calculated to assess the association between the predictor and outcome variables.

Results

In this study, a total of 438 environmental samples, 217 from Harar town and 221 from Kersa district, were analyzed to determine the occurrence of foodborne bacteria in animal, food, and environmental samples collected from the physical exposures of children with diarrhea. The overall prevalence of bacterial FBPs was 18.3%, with 3.9% and 14.4% co-occurrence and single pathogen occurrence rates, respectively. Among the sample types and sources, the highest pathogen prevalence was observed in wastewater (40.9%; AOR = 3.3; 95% CI: 1.1-10.1) and in samples collected from abattoirs (34.9%; AOR = 2.7; 95% CI: 1.4-4.9). The prevalence of FBPs was 14.5% in Kersa district and 22.1% in Harar town, with no significant difference ($P > .05$) between the settings (Table 1).

This study revealed fluctuations in the monthly occurrence patterns of bacterial FBPs in environmental samples throughout the study period. From November 2021 to January 2022, the monthly occurrence of pathogens linearly increased, peaking at 37.1%. Nevertheless, the occurrence pattern declined to zero in November 2022 and January 2023 (Figure 3).

The prevalence rates of NTS, DEC, and *Shigella* in the exposure environment were 11.6%, 5.5%, and 5%, respectively. The corresponding prevalence rates in the urban setting (Harar town) were 13.8%, 6.9%, and 5.5%, respectively (Figure 4).

Table 1. Prevalence of foodborne bacteria in exposure environments of children with diarrhea in Harar town and Kersa district, Ethiopia (N = 438).

TYPE AND SOURCE OF SAMPLE		CO-OCCURRENCE (%)	SINGLE PATHOGEN (%)	TOTAL POSITIVE (%)	COR (95% CI)	AOR (95% CI)	P VALUE
Type	Animal	5 (3.1)	13 (7.9)	18 (11)	0.9 (0.3-2.5)	0.8 (0.3-2.2)	.60
	Food	6 (3.6)	24 (14.3)	30 (17.9)	1.5 (0.6-4.2)	1.2 (0.4-3.6)	.70
	Wastewater	6 (9.1)	21 (31.8)	27 (40.9)	4.8 (1.7-14)	3.3 (1.1-10.1)	.03
	Water Source	0	5 (12.5)	5 (12.5)	1	1	–
Source	Abattoir	9 (6.8)	37 (28)	46 (34.9)	3.6 (2.1-6.1)	2.7 (1.4-4.9)	.02
	Household	1 (1.1)	5 (5.4)	6 (6.5)	0.5 (0.2-1.2)	0.4 (0.2-1.0)	.05
	Market	7 (3.3)	21 (9.8)	28 (13.1)	1	1	–
Site	Harar town	9 (4.2)	39 (18)	48 (22.1)	1.7 (1.0-2.8)	1.1 (0.6-1.9)	.82
	Kersa (Rural)	8 (3.6)	24 (10.9)	32 (14.5)	1	1	–
Total		**17 (3.9)**	**63 (14.4)**	**80 (18.3)**		–	–

Water sources–drinking water, recreational water and irrigation water.

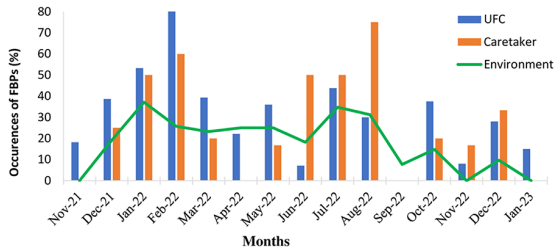

Figure 3. Monthly occurrence patterns of foodborne bacteria in the exposure environments of children with diarrhea between November 2021 and January 2023 in eastern Ethiopia.

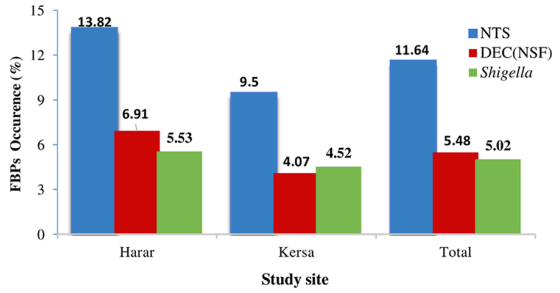

Figure 4. Prevalence of nontyphoidal *Salmonella*, diarrheagenic *E. coli*, and *Shigella* in the exposure environments of children with diarrhea by study site.

As shown in Table 2, the distribution of FBPs did not significantly differ among food types and sources, but the highest *Shigella* isolation rate (11.5%, $P = .04$) was detected in food samples collected from abattoirs. This study revealed that 5.3% of the milk samples were positive for both NTS and DEC, but *Shigella* was not detected in the milk samples. The pathogen occurrence rates in meat were 14% for NTS, 5.4% for DEC, and 6.4% for *Shigella* spp. Moreover, DEC and *Shigella* were detected in 3.7% and 7.41% of cooked food, respectively, but NTS was not identified in cooked food.

The prevalence of NTS in animal feces collected from abattoirs was 10.8%, whereas that of *Shigella* and DEC was 5.4%. The occurrence rates of NTS, DEC, and *Shigella* in poultry were 1.8%, 14.7%, and 8.8%, respectively. However, none of the shoats' feces tested positive for each pathogen. The occurrence rate of NTS in wastewater was 33.3%, with a significantly higher prevalence in abattoir-linked wastes ($P \leq .05$) than in those from households or marketplaces. *Shigella* and DEC were detected in 7.5% and 9.1% of the wastewater samples, respectively. Notably, *Shigella* was detected in drinking water at a prevalence of 5% (Table 2).

Discussion

Diarrheagenic foodborne bacterial pathogens (FBPs) pose a significant health risk and can be contracted by contaminated food, exposure to environmental samples, and contact with animals. The present study assessed the occurrence of NTS, DEC, and *Shigella* in the exposure environments of children with diarrhea in Ethiopia. Bacterial FBPs were detected in all sample types, with an overall occurrence rate of 18.3%. The pathogen occurrence rates were 11.6% for NTS, 5.5% for DEC, and 5% for *Shigella* in the environment.

The overall FBP and NTS occurrence rates are higher than the pooled prevalence reported for FBPs (8.1%) and for *Salmonella* (7.4%) in environmental samples in Ethiopia.[5] Another study reported a lower prevalence of 3.8% for pathogenic *E. coli*.[34] The higher prevalence and detection of FBPs in all sample types could be due to the ability of these pathogens to tolerate various environmental stressors.[35] This higher prevalence might also be attributed to the fact that all samples analyzed in the current study were collected from direct or indirect exposures of diarrheic cases through case-based tracing of suspected sources of the pathogen(s) causing diarrhea. However, higher prevalences of 13%[5] and 10%[36] for pathogenic *E. coli*

Table 2. Occurrence of NTS, DEC and *Shigella* in animal, food and environmental samples in eastern Ethiopia.

SAMPLE TYPES AND SOURCES	NO. ANALYZED	NO. POSITIVE (%)		
		NTS	*SHIGELLA*	DEC
Household associated food	37	1 (2.7)	0	0
Market associated food	79	9 (11.4)	3 (3.8)	6 (7.6)
Abattoir associated food	52	9 (17.3)	6 (11.5)	2 (3.9)
***P* value**		**0.1**	**0.04**	**0.19**
Animal sourced food (ASF)	119	15 (12.6)	6 (5)	6 (5)
Other	49	4 (8.2)	3 (6.1)	2 (4.1)
***P* value**		**0.41**	**0.78**	**0.79**
Meat	93	13 (13.94)	6 (6.5)	5 (5.4)
Milk	19	1 (5.3)	0	1 (5.3)
Other food	56	5 (8.9)	3 (5.4)	2 (3.6)
***P* value**		**0.43**	**0. 52**	**0.88**
Cooked/semi-cooked food	27	0	2 (7.4)	1 (3.7)
Raw food	141	19 (13.5)	7 (5)	7 (5)
Total	**168**	**19 (11.3)**	**9 (5.4)**	**8 (4.8)**
***P* value**		**0.04**	**0.61**	**0.78**
Feces from Abattoir	37	4 (10.8)	2 (5.4)	2 (5.4)
Feces from Household	31	0	1 (3.2)	2 (6.5)
Feces from Market	96	5 (5.2)	2 (2.1)	5 (5.2)
***P* value**		**0.15**	**0.61**	**0.92**
Cattle and camel	74	5 (6.8)	2 (2.7)	4 (5.4)
Poultry	34	4 (11.8)	3 (8.8)	5 (14.7)
Shoat (sheep and goats)	56	0	0	0
Total	**164**	**9 (5.5)**	**5 (3.1)**	**9 (5.5)**
***P* value**		**0.05**	**0.05**	**0.01**
Abattoir waste	36	19 (52.8)	4 (11.11)	6 (16.7)
Household waste	13	2 (15.4)	0	0
Marketplace waste	17	1 (5.9)	1 (5.9)	0
Total	**66**	**22 (33.3)**	**5 (7.6)**	**6 (9.1)**
***P* value**		**0.00**	**0.41**	**0.06**
Drinking water	20	0	1 (5)	0
Other water sources	20	1 (5)	2 (10)	1 (5)
Total	**40**	**1 (2.5)**	**3 (7.5)**	**1 (2.5)**
***P* value**		**0.31**	**0.55**	**0.31**

Other food: vegetables and fruits, juices, other RTE food, food-leftovers, including injera with wot, soup, etc.; abattoir-associated food: meat collected from or linked to slaughterhouses and slabs.
The *P* value is bolded to indicate the level of significance.

have been reported in previous studies. The disparity in the occurrence of FBPs among various studies could be ascribed to distinct risk factors because, epidemiologically, infectious diseases have important geographical heterogeneity in both disease risk and burden.[37]

The co-occurrence of FBPs were 3.1%, 3.6% and 9.1% in the animal, food and wastewater samples, respectively, demonstrating a potential risk of being exposed to multiple pathogens in the area, which could lead to severe coinfections. Pathogen co-occurrences can result in synergistic interactions, which differ from the effects of a single contaminant.[38,39]

The study revealed no significant variation in the prevalence of FBPs between Harar town and Kersa district, highlighting the high burden of the pathogens in both urban and rural settings. Diarrheagenic pathogens remain a significant concern in resource-limited countries such as Ethiopia[40] because of poor environmental sanitation, irrespective of the setting. However, Asfaw et al.[13] reported that the risk of FBD is aggravated in rural areas where there is generally less awareness regarding the causative agents, transmission routes, and mitigation of foodborne infections.

The results revealed that wastewater harbors 3.3 times more FBPs (AOR = 3.3; 95% CI: 1.1-10.1) than water sources do. Moreover, NTS, DEC, and *Shigella* were significantly more prevalent in abattoir-associated waste ($P \leqslant .05$) than in waste collected from households and marketplaces. Wastewater is an ideal tank for FBPs because it contains many contaminants from domestic residences, commercial properties, and agriculture.[41,42] The present study also analyzed untreated wastewater runoff from abattoirs or food preparation facilities, as well as waste generated from utensils and hand washings at different sites linked to reported cases. Consequently, the discharge of abattoir waste into the drainage channel during and after the slaughtering process may contaminate other samples, such as meat, thereby increasing the prevalence of pathogens. Similarly, contaminated abattoir environments contribute to the greatest number of positive samples.[43] Furthermore, a recent study reported a high prevalence of FBPs, for instance, 22.7% *S. enterica* in abattoir-sourced samples.[44] Conversely, Tassew et al.[45] reported a 12.5% rate of food contamination by FBPs at the abattoir level.

The likelihood of pathogens being present in the environment was 1.2 times greater when contaminated food was involved (AOR = 1.2; 95% CI: 0.4-3.6) compared to water sources, indicating the presence of inadequate food safety measures. However, a higher prevalence of food contamination by pathogenic organisms, reaching up to 43.3%, was also previously reported in Ethiopia.[45] Pathogenic bacteria can contaminate food at any point throughout the whole food chain, with a greater burden in low-income regions such as Ethiopia.[46]

The food contamination rates of NTS, DEC, and *Shigella* were 11.3%, 4.8%, and 5.4%, respectively. In contrast, a systematic review reported a prevalence of 7.7% for *Salmonella*[47] and 18.1% for *E. coli*[48] in different animal-sourced foods in Ethiopia. DEC and *Shigella* were identified in 3.7% and 7.4% of the cooked food samples, respectively, whereas none of the cooked food samples tested positive for NTS. The detection of these pathogens in cooked food indicates contamination of the food after cooking or due to undercooking practices. Similarly, a prior study conducted in Ethiopia reported the public health hazards of cooked foods.[49]

The prevalence of NTS in meat is higher than that reported in prior studies: 4.1%,[50] 4.7%,[44] 5.7%,[51] and 1.2%[45] in Ethiopia but comparable to previous findings in Africa.[52] Similarly, the prevalence of *Shigella* (6.5%) in meat was higher than that reported by Al-Asmari et al.[53] and Tassew et al.[45] but comparable to the findings of other studies.[54,55] Conversely, a 0% *Shigella* in meat was reported in Ethiopia.[56] The lack of separate rooms for evisceration and the practice of horizontal slaughtering in current abattoirs could increase carcass contamination in abattoirs compared with marketplaces and households. The occurrence of DEC in meat is comparable to the rates reported by Zelalem et al.[57] and Mersha et al.[58] However, this rate is lower than that reported by Tassew et al.[45] (26.6%) and higher than that reported in other studies in Ethiopia.[50,59]

Among the 19 milk samples analyzed, NTS and DEC were detected in 5.3% of the milk samples. These findings are lower than the prevalences of 33.8%[60] and 58%[61] reported for *E. coli* and 19.7% reported for *Salmonella*[62] from Ethiopia. On the other hand, comparable *Salmonella* prevalence was previously reported,[63] although a lower prevalence of 3.3% was also documented in another study in Ethiopia.[61] In contrast to our findings of zero *Shigella* in milk samples, it has been reported in milk at rates of 17.5%[61] from Ethiopia, 37%,[64] and 7%[65] in two studies from Egypt. Our findings may suggest a lower likelihood of *Shigella* carriers contaminating milk samples with fecal matter.

Foodborne bacteria were also detected in other food categories, including RTE foods, fruits, vegetables, and mixed food leftovers, with a prevalence of 3.6% for DEC, 8.9% for NTS and 5.4% for *Shigella*. However, prior studies have shown higher prevalences of *Salmonella* (24.4%[11] and 57.5%[66]), *E. coli* (72.5%[66]) and *Shigella* (15.4%,[11] and 14.1%[20]) in vegetables and RTE foods in Ethiopia. Nevertheless, the occurrence rate of *Shigella* in this food category is comparable to that reported by Pakbin et al.[67] who reported a prevalence of 4.8% in vegetable-based salads.

In general, the study revealed that meat, milk, and other food handling practices contribute to the overall burden of FBD in Ethiopia, suggesting the need for food safety measures. The variation in prevalence among studies might be associated with educational level, personal hygiene practices, and knowledge of safe food handling and preparation.

The prevalence of the three FBPs was significantly higher in poultry ($P \leqslant .05$) than in cattle and camels. Similarly, previous studies reported a higher prevalence of *Salmonella* in

poultry than in cattle or shoats.[68] On the other hand, the prevalence of NTS in poultry is higher compared to that reported in previous studies in Ethiopia,[17,69] but it is lower than the 16.7% reported by Abdi et al.[10] in Ethiopia. Studies have also reported varying prevalence rates of pathogenic *E. coli* in feces in poultry (7.8%[34] and 4.2%[70]) and cattle (1.9%,[59] 4.7%,[71] and 37%[9]) in Ethiopia.

Despite its zoonotic importance, no studies have reported *Shigella* in animals in Ethiopia. In fact, children can easily develop shigellosis by ingesting food contaminated with the feces of apparently healthy or diarrheic animals.[72-75] Likewise, the current prevalence is comparable to that of *Shigella* reported in poultry feces (6.7%) in Nigeria.[76] However, a study conducted in Northwest China reported a higher prevalence (6.2%) in fecal samples collected from diarrheic calves.[77] Nevertheless, other studies have reported a zero prevalence of *Shigella*.[55,72]

In contrast to previous studies,[36,78] neither *Salmonella* nor DEC nor *Shigella* was detected in shoat feces. Similarly, a 0% prevalence of NTS in goats and *Shigella* in sheep and goat feces has been reported in Etiopia.[56] The 0% *E. coli* prevalence in our study can be attributed to the fact that the exclusive focus of this particular study was DEC; notably, sheep and goats are not natural hosts for *Shigella*. However, the occurrence of FBPs in animals can vary among studies for several reasons, including variations in the sample size taken into account, the study area, and nonuniform protocols for sampling and identification of pathogens.

Interestingly, *Shigella* was detected in 5% of the drinking water and 10% of the lake water analyzed in this study. Similarly, NTS and DEC were also detected in 5% (1/20) of the lake water used for both recreational and irrigation purposes, as was drinking water for animals. These findings indicate unsanitary fecal contamination of water. Moreover, bacterial FBPs are potential waterborne pathogens.[79] In fact, because of poor hand washing habits after toileting and touching animal excreta, pathogens can enter the water and food chain during food processing or preparation.[4] Similarly, Bedada et al.[49] reported a 0% prevalence of *Salmonella* in drinking water in Jimma. In contrast, a prevalence of 0% for *Shigella*[49,80] and 3.3% for *Salmonella*[80] in drinking water has been reported in Ethiopia. The occurrence of DEC in water in the present study is partially in agreement with a previous report[70] but lower than the prevalence reported in another study in Ethiopia.[79]

Similar to our current results, different studies have detected *Shigella* in nonprimate hosts and various environmental samples.[20,45,67,81,82] Distinct groups of *Shigella* strains such as *S. flexneri*, as described by Connor et al.[82] can persist in particular locations and environments for extended periods, ranging from tens to hundreds of years. These strains can thrive in the local environment without relying on humans. Eventually, they reemerge from the environment and cause disease. In general, members of the family Enterobacteriaceae are dominant bacteria that contaminate unprotected water sources.[80]

The occurrence of some diarrheagenic FBPs is seasonal, with peak times varying by year and geographic location.[83] Likewise, the fluctuations in the monthly occurrence patterns of bacterial FBPs in exposure sources and diarrheic cases may indicate that when pathogen occurrence is high in the environment, the likelihood of acquiring FBPs through contaminated food, water, animal contact, etc., in the vicinity and developing an infection increases, and vice versa.[35] The highest monthly occurrence of bacterial FBPs was observed in January 2022, which may be attributed to a shortage of water for adequate food sanitation and personal hygiene practices during the dry season.[84] In many villages within and around the present study area, it is customary to use shared water sources (water points) for animal drinking, irrigation, food preparation, and other purposes. However, irregularities in the number of cases admitted to health facilities could result in unforeseen fluctuations in pathogen occurrence in general, and this was evident when the number of cases decreased in some months, leading to a decline in pathogen occurrence patterns in the environmental samples as well.

The second highest monthly occurrence of FBPs in the exposure environments of children with diarrhea was observed in July 2022 (summer). A higher prevalence during the summer season could be attributed to high temperatures and rains that increase pathogen proliferation and contamination of surface waters and food crops.[85]

Limitations of the Study

Although the present study provides valuable insights into the occurrence of important bacterial FBPs in Ethiopia using a one-health approach, this study has some limitations. First, as samples were collected through case-based tracing, the included sample sizes from different exposure sources were not proportional. For example, food leftovers were often not accessible during sample tracing, as they are typically not stored or only stored for a few hours in our study area. There were also variations in the number of traced exposure samples per child case, as some families were reluctant to provide samples from households. This was due to insufficient awareness and concerns related to social issues and the earlier COVID-19 pandemic. Furthermore, the study did not include genetic analysis to assess the relatedness of bacterial isolates from various sources.

Conclusion and Recommendations

The present study employed a one health approach to identify foodborne bacterial pathogens (FBPs), such as nontyphoidal *Salmonella*, diarrheagenic *E. coli*, and *Shigella*, in the exposure environments of children with diarrhea in Ethiopia. This study analyzed animal, food, and environmental samples in a shared laboratory and identified these important zoonotic FBPs across all sample types.

The overall occurrence rate of FBPs was statistically highest in wastewater and slaughterhouse environments, suggesting that

both are major contributors to the high occurrence of bacterial FBPs in the study area. Interestingly, the study did not find statistically significant differences in the occurrence of bacterial FBPs between rural and urban areas, indicating the importance of these foodborne bacteria in both settings. Similarly, no significant differences were detected in contamination rates between animal-sourced foods and other food categories or between food and water sources. Notably, the detection of DEC and *Shigella* in cooked food samples indicates post-cooking contamination, highlighting the need for improved food safety measures. The results of the present study also revealed a higher FBPs prevalence in poultry and a high occurrence rate of pathogens during both dry and rainy seasons.

Therefore, the findings of this study demonstrate the need to consider effective control strategies for FBPs in animals, animal products, as well as nonanimal-sourced foods, and the environment to reduce the burden of foodborne bacteria-associated diarrheal disease. Specifically, enhancing the management of poultry droppings and wastewater, especially abattoir-associated waste, is advisable to reduce the risk of diarrhea in children. In another way, the study suggests the necessity of collaboration among medical, veterinary, and environmental health professionals, as well as other relevant sectors, in managing foodborne zoonoses, including the sharing of resources to facilitate joint surveillance and integrated intervention strategies. Further studies with larger sample sizes and advanced diagnostic techniques are recommended to better understand the relative contributions of different exposure sources through source attribution analyses.

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Author Contributions

In general, the authors collectively contributed to the activities of the study. Additionally, TG and TH were responsible for funding acquisition, while DB performed laboratory activities, data analysis, and manuscript drafting. CH and AJ mainly handled the sample collection and transportation process.

Ethics Approval

The project was granted ethical approval from the Institutional and National Health Research Ethics Review Committee (IHRERC).

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