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Non-typhoidal *Salmonella* prevalence and risk factors among outpatients at public health facilities in Gondar City, northwest Ethiopia

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Abstract

Non-typhoidal *Salmonella* is a major cause of foodborne illness, particularly in developing countries where poor hygiene and consumption of unsafe food or water are common, yet data on its prevalence and associated risk factors are limited in the study area. This study aimed to determine the prevalence and related risk factors of non-typhoid *Salmonella* among diarrheal patients attending health institutions in Gondar City. A cross-sectional study was conducted from September 2024 to August 2025, involving 432 patients with diarrhea who visited selected public health facilities. Stool samples were tested using standard microbiological and biochemical methods, and data on potential risk factors were collected through a structured questionnaire. Non-typhoid *Salmonella* was detected in 12 patients (2.8%). Significant predictors of infection included consuming raw vegetables and fruits [adjusted odds ratio (AOR)=4.00; 95% confidence interval (CI): 1.41-16.15], leaving food unrefrigerated for more than 2 hours (AOR=4.00; 95% CI: 1.31-18.46), contact with livestock (AOR=7.00; 95% CI: 1.91-26.90), and limited access to proper toilet facilities (AOR=11.00; 95% CI: 3.55-38.29). On the other hand, access to clean water was associated with a significantly lower risk of infection (AOR=0.12; 95% CI: 0.04-0.39). Although the prevalence was lower than global averages, the presence of multiple risk factors suggests ongoing transmission, indicating the need for improved hygiene, better food safety practices, and regular monitoring of non-typhoid *Salmonella* in the area.

Key words: non-typhoid *Salmonella,* diarrhea, prevalence, risk factors, stool culture, Ethiopia.

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Introduction

Salmonella is a common cause of intestinal infection, leading to illness, death, and economic loss globally (Siddiky *et al.*, 2022). It is the leading cause of foodborne illness worldwide, typically causing gastroenteritis with symptoms like diarrhea and vomiting (Liu *et al.*, 2023; Kumar *et al.*, 2025). This Gram-negative, rod-shaped bacterium belongs to the *Enterobacteriaceae* family (Cheni *et al.*, 2022) and is classified mainly into *Salmonella enterica* and *Salmonella bongori* species, with *Salmonella enterica* further divided into six subspecies (Elhadi *et al.*, 2013). Clinically, *Salmonella* is classified into typhoid and non-typhoid *Salmonella* (NTS) based on disease manifestation (Garedew *et al.*, 2018). The NTS commonly causes diarrhea but can lead to severe invasive infections in vulnerable groups (Marks *et al.*, 2017; Diab *et al.*, 2023).

Typhoid salmonellosis is caused by *Salmonella* Typhi or *Salmonella* Paratyphi A, while NTS gastroenteritis is mainly due to *Salmonella* Typhimurium (*S.* Typhimurium) and *Salmonella* Enteritidis (*S.* Enteritidis) (Amare *et al.*, 2024). Over 2000 NTS serovars infect humans and animals, with *S.* Typhimurium and *S.* Enteritidis responsible for over 40% of global human cases (Johnson *et al.*, 2018; Petrin *et al.*, 2023; Lamichhane *et al.*, 2024). In Sub-Saharan Africa, *S.* Typhimurium, *S.* Enteritidis, and *S.* Dublin predominate in invasive NTS infections (Marks *et al.*, 2017), while in Ethiopia, *S.* Uganda, *Salmonella diarizonae*, and *S.* Typhimurium are most common (Beyene *et al.*, 2024).

The NTS is a major public health issue, reflecting the "one health" link between humans, animals, and the environment (Mengistu *et al.*, 2020; Cuypers *et al.*, 2023). It causes about 93 million infections and 155,000 deaths annually (Balasubramanian *et al.*, 2019).), remaining a key cause of diarrhea (Woh *et al.*, 2021). Transmission primarily occurs through contaminated animal products but also *via* fresh vegetables and fecal-oral routes (Kim *et al.*, 2022; Zhang *et al.*, 2023; Hugho *et al.*, 2024).

In low-income countries, NTS risk is heightened by poor hygiene, unsafe water, overcrowding, and unsafe food handling practices (Ngogo *et al.*, 2020). In Ethiopia, factors like low education, crowded living conditions, poor water sources, and unsafe food storage increase infection risk (Dessale *et al.*, 2023). Behavioral risks include inadequate hand washing and consuming unpasteurized milk (Azanaw *et al.*, 2024). Prevalence in Ethiopia ranges from 1% to 13% among food handlers and diarrheal patients (Kahsay *et al.*, 2023).

Since many NTS infections come from contaminated food and the surrounding environment, it is important to know how people in the area are being exposed. Looking at everyday factors like how food is handled, the quality of water used, and whether produce is contaminated can show where food safety interventions are needed to reduce the spread of infection. Due to its significant burden, studying NTS prevalence and related risk factors is essential in places where the disease remains endemic and a major cause of diarrheal illness. Therefore, this study was designed to determine the prevalence of *Salmonella* infection and identify the key food-related and environmental risk factors contributing to its transmission and to inform practical food safety interventions.

Materials and Methods

Study design, area and period

A cross-sectional study was conducted from September 2024 to August 2025 among diarrheic outpatients at public health facilities in Gondar city, Northwest Ethiopia. Gondar, a historic tourist center, is located 747 km from Addis Ababa, the capital of Ethiopia, and 175 km from Bahir Dar, the capital of the Amhara Regional State, with a population of 621,168 (2016 estimate) (Amare *et al.*, 2024). The city lies at 1800-2200 m altitude, has an average annual rainfall of 1000 mm, a temperature of around 26°C, and relative humidity ranging from 30-70% depending on the season (Ejo *et al.*, 2016).

Study population, sample size determination and sampling procedure

The source population was all outpatients aged 6 months and older at public health facilities in Gondar city. The study populations include patients with diarrhea, excluding those suspected of salmonellosis who had used antimicrobials in the past 2 weeks.

The sample size was determined using a single population proportion formula, $n = (Z\alpha/2)^2 * p * (1-p) / d^2$ where, $Z\alpha/2 = 1.96$ for the standard scale of 95% level of confidence, level of precision (d) = 5% and p=0.15. Since epidemiological investigations of salmonellosis in Ethiopia showed that the highest prevalence of *Salmonella* infection was 15% reported by Mache (Beyene *et al.*, 2010). 10% non-response rate was considered. The calculated sample size was 196; because of the design effect of stratified sampling, the calculated sample size was multiplied by two, resulting in 392. By adding 10% (40) non-respondent rates, the final sample size became 432. The sampling process used to determine the final study sample is illustrated in Figure 1, outlining the identification of the target population and selection of participants through an appropriate sampling technique to ensure a representative sample.

Socio-demographic and specimen collection, handling and transportation

Data on socio-demographic and risk factors such as age, gender, education, marital status, family size, hygiene practices, food safety, animal contact, and raw food consumption were collected using a structured questionnaire. After obtaining consent, 3-5 mL of fresh stool samples were collected in labeled, leak-proof containers within an hour and promptly transported in an ice box to the University of Gondar Comprehensive Specialized Hospital (He *et al.*, 2023).

Isolation and identification of *Salmonella*

About 1-2 g of stool was inoculated into Cary-Blair medium and incubated aerobically at 37°C for 18-24 hours. Enriched samples were then streaked onto Xylose Lysine Deoxycholate agar (XLD, Oxoid, Ltd., United Kingdom) and re-incubated under similar conditions. Media performance was verified using known *Salmonella* positive controls. Suspected colonies appeared pale pink with black centers (indicating hydrogen sulfide production) and were subcultured for purity. Biochemical identification used triple sugar iron agar, Lysine decarboxilase, urea broth, sulfide indole motility tests, and citrate utilization tests (Oxoid, Ltd., United Kingdom), with typical *Salmonella* showing an alkaline slant, acid butt, hydrogen sulfide production, lysine decarboxylation, urea negativity, and citrate positivity.

Quality control

Questionnaires were clearly designed, translated into the local language, and back-translated to ensure accuracy. A 5% pretest was conducted at nearby health facilities, and necessary revisions were made. Data collectors received training on patient interaction, data collection, quality assurance, and analysis. Collected data were reviewed daily for completeness and accuracy. Fecal samples were processed following standard operating procedures, with strict adherence to bio-safety precautions. Contamination was minimized through chemical and thermal treatments like autoclaving and burning. Expiry dates of media and reagents were checked. The sterility of the media was tested by overnight incubation with 5% of the fresh batch. Reference strains (*Escherichia coli* ATCC 25922 and *S.* Typhimurium ATCC 14028) were used to validate media performance and biochemical tests (Amare *et al.*, 2024).

Statistical analysis

Data were coded and verified for accuracy before being entered into SPSS version 26 (Statistical Package for the Social Sciences). The data were cleaned, edited, and analyzed using SPSS version 26 for Fisher's Exact Test and R software for Firth's

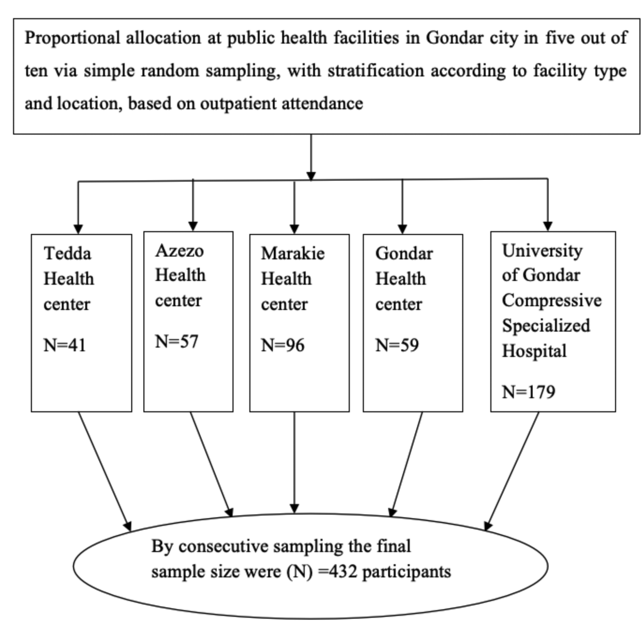

Figure 1. Sampling procedure to determine the study sample size.

Penalized Logistic Regression Model. Descriptive statistics were used to summarize the characteristics of the study population. Associations between variables and *Salmonella* positivity were assessed using Fisher's Exact Test. Due to the low prevalence (2.8%), Fisher's Exact Test was applied to examine associations between risk factors and NTS infection (Kim, 2017). Variables with p<0.20 in bivariate analysis were included in a multivariable Firth's Penalized Logistic Regression Model using R software to identify independent factors and reduce small sample bias (Suhas *et al*., 2023). Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were reported. Variables with a p-value less than 0.05 and a corresponding 95% CI were ultimately regarded as statistically significant.

Results

Socio-demographic characteristics of the participant

The study included 432 participants, slightly more male (52.1%). Ages ranged from 10 months to 80 years, with a mean of 22.4±19.8 years. Most were young children aged 1-9, mainly preschoolers not yet in formal education, and the majority lived in urban areas. Many participants were unmarried because most of them were children, while most adults were married. Few were employed, and household sizes were generally small to medium. *Salmonella* was detected across all groups, with higher prevalence in older adults aged 60 years and above, rural residents, and participants with no formal education levels (Table 1).

Prevalence and risk factors of non-typhoid *Salmonella*

The overall prevalence of non-typhoidal *Salmonella* among the study population was 2.8%. Consumption of raw fruits and vegetables was significantly associated with infection, while risk behaviors such as keeping food at room temperature and eating leftovers remained common. Additional contributing factors included water source, sanitation practices, and contact with domestic animals. The key tested risk factors, covering food handling, water source, animal contact, and sanitation, were presented, with low count or descriptive only variables excluded (Table 2), while detailed information on all contributing factors is provided in *Supplementary Table 1*. Fisher's Exact Test and Firth's Penalized Logistic Regression Model, appropriate when expected cell frequencies are below five, which applies to this study, revealed a significant association between *Salmonella* infection and several risk factors (p<0.05; 95% CI). Higher infection odds were linked to eating raw vegetables, unrefrigerated food, livestock contact, and open defecation, while access to clean water was associated with lower risk (Table 3).

Table 1. Demographic characteristics and Salmonella Positivity among 432 study participants at public health facilities in Gondar city, Northwest Ethiopia, 2024.

Variables	Categories	Frequency n (%)	*Salmonella* +ve n (%)
Age	1-9	169 (39.1)	4 (2.37)
	10-19	50 (11.6)	1 (2.0)
	20-24	35 (8.1)	1 (2.86)
	25-44	105 (24.3)	3 (2.86)
	45-59	39 (9.0)	1 (2.56)
	60+	34 (7.933)	2 (5.88)
Gender	Male	225 (52.1)	6 (2.67)
	Female	207 (47.9)	6 (2.90)
Education	None	96 (22.2)	4 (4.17)
	Preschool	148 (34.3)	4 (2.70)
	Primary	81 (18.8)	2 (2.47)
	Secondary	58 (13.4)	1 (1.72)
	College/university	49 (11.3)	1 (2.04)
Residence	Rural	76 (17.6)	3 (3.95)
	Urban	356(82.4)	9 (2.53)
Occupation	Business	34 (7.9)	1 (2.94)
	Employed	49 (11.3)	1 (2.04)
	Agriculture	39 (9.0)	1 (2.56)
	Student	79 (18.3)	1 (1.27)
	Preschool	148 (34.3)	4 (2.70)
	Housewife	57 (13.2)	2 (3.51)
	None	26 (6.0)	0 (0)
Marital status	Divorced	15 (3.5)	1 (6.67)
	Married	162 (37.5)	3 (1.85)
	Single	55 (12.7)	2 (3.64)
	Widowed	9 (2.1)	1 (11.1)
	Not applicable	191 (44.2)	5 (2.62)
Family size	1-3 (small family)	199 (46.1)	6 (3.02)
	4-6 (medium family)	207 (47.9)	5 (2.42)
	>7 (large family)	26 (6.0)	1 (3.85)

Percentages for *Salmonella* positivity are calculated as (number positive ÷ total in category) × 100.

Discussion

This study reports the prevalence and risk factors of NTS among outpatients at public health facilities in Gondar City. The prevalence of NTS among outpatients at public health facilities in Gondar City among diarrheic patients in this study was 2.8%. The prevalence found in this study is lower than rates reported from several African countries: in Burkina Faso (17.9%) (Nikiema *et al.*, 2021), Southern Tanzania (16.5%) (Ngogo *et al.*, 2020), Tanzania Morogoro Regional Hospital children (5.3%) and adults (3.0%) (Cuco *et al.*, 2024), Kenya [12.6%) (Peter *et al.*, 2023) and 13.1%, (Muthumbi *et al.*, 2023)], Egypt (10.88%) (Diab *et al.*, 2023), and Uganda (5.2%) (Kivali *et al.*, 2024), Ethiopia [(3.8-15%) (Beyene *et al.*, 2010), (13.7%) (Garedew *et al.*, 2018)], Addis-Abeba 4.0% among males and 5.7% among female (Kebede *et al.*, 2021), Bahirdar and Gondar district (4.8%) (Abate and Assefa, 2021).

In contrast, the 2.8% prevalence in the current study aligns more closely with findings from Ethiopia, where several reports have shown isolation rates ranging from 1% to 10% among food handlers and diarrheal patients (Kahsay *et al.*, 2023), Harar hospital 2.8% (Teshale *et al.*, 2025), including comparable findings of 2.5% in Gondar (Beyene *et al.*, 2024) and 3.15% in Debre Markos (Dessale *et al.*, 2023).

The variation in *Salmonella* prevalence across studies may be due to differences in study design, population characteristics, settings (outpatients *vs*. community-based), diagnostic methods, and sample sizes; however, further investigation is required to confirm these factors. The lower prevalence observed in this study may reflect better hygiene, improved sanitation infrastructure, and effective public health and food safety measures in the study area as compared to rural settings, where such facilities are often limited. Additionally, urban residence and the religious fasting period during data collection may have reduced consumption of high-risk foods like raw meat, milk, and eggs, contributing to lower infection rates. Still, more research is needed to verify these factors. During the data collection period (June to August) in Gondar city, a widespread religious fasting season limited the intake of non-fasting foods like raw meat, milk, and eggs, commonly linked to *Salmonella,* leading to a likely reduction in infection rates.

Factors significantly associated with NTS infection included consumption of raw vegetables and fruits, allowing food to sit out for more than 2 hours, livestock contact, inadequate toilet access, and unsafe water use. However, because the number of positive cases was small, these associations should be interpreted with caution. Although Firth's Penalized Logistic Regression was applied to minimize small sample bias, the limited number of infections may still affect the statistical precision, robustness, and generalizability of the observed associations. Therefore, the identified risk factors should be considered preliminary indicators rather than definitive causal relationships. Beyond prevalence alone, these risk factors show how NTS spreads through the local environment, food system, and everyday conditions in Gondar City.

Table 2. Prevalence and risk factors of non-typhoid Salmonella in relation to food consumption, handling practices, water, sanitation, and animal contact among 432 participants at public health facilities in Gondar city, northwest Ethiopia, 2024.

Risk factor category	Variable	Total (n)	NTS positive (n, %)	p	Note
Food handling	Food left unrefrigerated >2 hrs	168	7 (4.2%)	**0.014**	Higher risk
	Consumed leftovers	121	4 (3.3%)	–	Tested; low magnitude
Food consumption	Raw fruits/vegetables	132	8 (6.1%)	**0. 01**	Higher risk
	Other unspecified foods	168	3 (1.8%)	–	Low magnitude
Water source	Unsafe water	67	3 (4.5%)	**0.014**	Higher risk
	Safe piped water	365	9 (2.5%)	**0.014**	Lower risk
Animal contact	Contact with cows	44	3 (6.8%)	**0.002**	Higher risk
	Contact with both cows & chickens	36	2 (5.6%)	–	Low magnitude
Toilet and sanitation	Open defecation	52	3 (5.8%)	**<0.001**	Higher risk
	Hand washing station near toilet	214	6 (2.8%)	–	Tested; moderate magnitude

Percentages represent non-typhoid *Salmonella* (NTS) positive cases per category. Associations were tested using Fisher's exact test, with significant p-values in bold; results should be interpreted cautiously due to small sample sizes.

Table 3. Fisher's Exact Test and Firth's Logistic Regression Model analyses of risk factors associated with *Salmonella* infection (n=432) at public health facilities in Gondar city, northwest Ethiopia, 2024.

Variables	*Salmonella* Status	Yes (n)	No (n)	AOR (95% CI)	p
Consumption of raw vegetables and fruit	Positive	8	4	4.00 (1.41-16.15)	0.010
	Negative	124	296		
Allow food to sit out beyond 2 hours	Positive	9	3	4.00 (1.31-18.46)	0.014
	Negative	159	261		
Livestock contact	Positive	9	3	7.00 (1.91-26.90)	0.002
	Negative	124	296		
Open field defecation	Positive	5	7	11.00 (3.55-38.29)	0.000
	Negative	375	45		
Access to clean water	Positive	5	7	0.12 (0.04-0.39)	0.014
	Negative	360	60		

AOR, adjusted odds ratio; CI, confidence interval.

In our study, consumption of raw fruits and vegetables showed a significant association with NTS infections. Participants who ate raw fruits and vegetables had a higher likelihood of contracting NTS compared to those who did not consume them. Evidence from Ethiopia shows that *Salmonella* can enter the food chain through farm practices (Hailu *et al.*, 2024), transport or market handling (Degaga *et al*., 2022), or poor vendor hygiene (Zeynudin *et al*., 2025). These findings were comparable in Ethiopia (Gazu *et al.*, 2023), Hawasa, Ethiopia (Simion *et al.*, 2024), Addis Ababa, Ethiopia (Hailu *et al.*, 2024), Ireland, Europe (Ehuwa *et al.*, 2021), and Tennessee, United States (Mukherjee *et al.*, 2020). This aligns with the current study, emphasizing fresh produce as a major exposure route and that contamination can move along the pathway from production through transport and market handling to the point of sale.

In the current study, unsafe food handling practices such as leaving food unrefrigerated for more than 2 hours were significantly associated with NTS infections. Participants who left food unrefrigerated for extended periods had a higher risk of acquiring NTS compared to those who properly refrigerated their food. *Salmonella* multiplies quickly at room temperature, and previous Ethiopian studies show that proper refrigeration lowers contamination (Bedassa *et al*., 2023). This finding is consistent with a study conducted in south Ethiopia (Hayamo *et al.*, 2021), Dbre Markos town (Dessale *et al.*, 2023), South Africa (Mohamed and Habib, 2025), and Indonesia, Asia (Susanna *et al.*, 2020). Along with raw produce exposure, this denotes how household food handling can add to risks already present in the supply chain.

This study found that frequent or direct contact with livestock showed a significant association with NTS infections. Individuals exposed to livestock were more likely to be infected with NTS than those who had no such contact. Similar patterns have been reported in Ethiopia, where close interaction with animals increased the risk of infection (Beyene *et al*., 2024). Direct contact with animals was also linked to *Salmonella* infection in other Ethiopian populations (Mengistu *et al.*, 2025), and milking and handling areas can also act as ongoing reservoirs (Ayichew *et al.*, 2024). Activities like slaughtering or butchering increase risk (Tafere1 *et al.*, 2025). Evidence from Kenya shows that contamination in animals can move along the value chain into retail meat (Gichuyia *et al.,* 2023). Similar results were reported in Kilimanjaro, Tanzania (Hugho *et al.*, 2024). This suggests that livestock and their environments can transfer fecal bacteria to hands, surfaces, and food, making animal contact a major pathway for NTS transmission.

As observed in this study, a lack of sanitation, such as defecating in open fields instead of using pit latrines, was significantly linked to NTS infections. Individuals who practiced open defecation were at a greater risk of contracting NTS compared to those who used pit latrines. Regional evidence shows that inadequate sanitation and unimproved water sources contribute to environmental reservoirs of *Salmonella* in water, soil, and wastewater (Kebenei *et al.*, 2025), while limited access to safe water allows repeated fecal contamination of domestic supplies (Dekker et al., 2018). This observation is in line with findings from a study conducted in Guinea-Bissau and Senegal (Im *et al.*, 2016), Kenya (Okullo *et al.*, 2017), Tanzania (Ngogo *et al.*, 2020), Rwanda (Nyamusore *et al.*, 2018, Indonesia, Asia (Susanna *et al.*, 2020), and Iraq, the Middle East (Abdulhaleem *et al.*, 2019). This speculates how poor sanitation contributes to contamination of soil, water, and domestic environments, sustaining ongoing exposure.

The current analysis revealed that access to clean water significantly reduced risk, underscoring the role of safe water in preventing NTS transmission. Participants with dependable sources of safe water were less likely to be infected with NTS than those lacking such access. This result is consistent with findings from a study in Debre Markos town, Ethiopia (Dessale *et al.*, 2023), the southern highland of Tanzania (Ngogo *et al.*, 2020), Iraq, the Middle East (Abdulhaleem *et al.*, 2019), and a review on *Salmonella* in water (United States of America) (Liu *et al.*, 2018). This indicates safe water not only reduces the risk of direct ingestion but also lowers the chance of contaminating food, hands, and utensils during preparation.

The findings highlight how NTS spreads through linked food-related, animal, and environmental pathways, emphasizing the need for interventions along the food chain. Consumption of raw produce, unsafe food handling, livestock contact, and poor sanitation contribute to ongoing exposure. These observations align with evidence from Ethiopia and other countries showing contamination from farm practices, transport, market handling, and inadequate hygiene. Public health actions like better produce washing, safe food handling, cold storage, market hygiene, access to clean water, veterinary checks, and community education can help reduce NTS in similar urban settings.

Limitations

The study was conducted only within the city and health institutions, which limits the generalizability. The lack of a diarrhea-free control group in the outpatient setting may have led to underestimating NTS prevalence and exposure effects. Social desirability bias may have caused participants to overreport hand washing, potentially overstating its protective role. Information bias and recall bias were also present, as parents reported on behalf of children. Due to limited resources, NTS was identified only to the species level without the use of molecular methods. Data collection occurred from June to August 2025; a period when seasonal dietary habits and rising living costs likely reduced consumption of high-risk foods like raw egg, milk, and meat, potentially lowering exposure of participants in the study area. Relying on Fisher's exact test limits the strength and precision of the associations.

Conclusions

The prevalence of NTS among diarrhea patients attending health institutions in Gondar city was relatively low, but several preventable factors still contributed to infection. Significant predictors included consumption of raw vegetables, leaving food unrefrigerated, livestock contact, poor sanitation, and lack of safe water**.** The identified risk factor associations should be interpreted cautiously, as they are preliminary due to the low number of NTS-positive cases and require confirmation through larger, multi-site studies. Recommendations should focus on integrated efforts across the food chain, including improved hygiene during food production, transport, marketing, and household handling, as well as strengthening community awareness and safe food handling practices. Regular NTS screening and safer interactions with livestock may help reduce the risk of NTS infection by promoting safer practices among food operators and increasing awareness among consumers.

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Online supplementary material:
Supplementary Table 1. Prevalence and risk factors of non-typhoid *Salmonella* in relation to food consumption, handling practices, eating locations, water, sanitation, and animal contact among 432 participants at public health facilities in Gondar City, northwest Ethiopia, 2024.

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Availability of data and materials: the data supporting these research findings are included in the article. Additional information is available from the corresponding author upon request.

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