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Prevalence, antimicrobial resistance pattern, and associated factors of *Salmonella* serovars among human-animal interfaces in the Amhara National Regional State, Ethiopia

Getachew Mengistu[1,3*], Anwar Nuru[2], Baye Gelaw[3] and Feleke Moges[3]

Abstract

Background Salmonellosis in humans and animals poses significant public health concerns, leading to substantial economic losses and increased healthcare costs in both developed and developing countries. This study aimed to assess the prevalence, antimicrobial resistance pattern, and associated factors of *Salmonella* serovars in humans and animals (sheep, chicken, and cattle) in the Amhara National Region state of Ethiopia.

Methods A prospective cross-sectional study was conducted using a consecutive convenience sampling technique from September 2022 to January 2024 at public health facilities and 25 randomly selected animal farms surrounding each health facility in the Amhara National Regional State. Socio-demographic data and relevant clinical characteristics were collected using a standardized questionnaire. After enrichment with Selenite F broth, *Salmonella* serovars were isolated using MacConkey, Xylose Lysine Deoxycholate, and *Salmonella Shigella* agar. The *Salmonella* serovars were characterized through a series of biochemical tests. The antimicrobial resistance patterns were determined using the modified Kirby-Bauer disk diffusion technique. Data analysis was performed using SPSS version 29, and the results were summarized in tables and graphs.

Result The overall prevalence of *Salmonella* serovars among humans and animals was 12.1% (49/404; 95% CI: 9.2–15.6) and 2.7% (9/336; 95% CI: 1.3–4.8), respectively. Human isolates of *Salmonella* serovars exhibited high resistance rates to ampicillin (79.6%) and tetracycline (71.4%). Similarly, all animal isolates of *Salmonella* were found to be 100% resistant to both ampicillin and tetracycline. In contrast, a 2% resistance of *Salmonella* isolates was observed to ciprofloxacin among humans but 11% in animals. Data also showed that 71.4% and 88.9% of *Salmonella* isolates from humans and animals were resistant to three or more antibiotic classes, respectively. Close contact with patients experiencing diarrhea (AOR = 2.83; 95% CI: 1.05–7.629; $P = 0.04$) and with animals (AOR = 3.179; 95% CI: 1.574–6.419; $P = 0.001$) revealed a statistically significant association with the prevalence of culture-confirmed salmonellosis.

Conclusion The prevalence of culture-confirmed *Salmonella* serovars among humans and animals was significantly high. Many isolates of *Salmonella* from these groups showed resistance to three or more classes of antibiotics.

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*Correspondence:
Getachew Mengistu
gechomlt@gmail.com

Full list of author information is available at the end of the article

Individuals who come into contact with those experiencing diarrhea or who handle animals are at an increased risk of developing salmonellosis. To help reduce these infections, public health initiatives should focus on improving animal handling practices and promoting better hygiene in both the environment and personal habits through one health approach.

Keywords *Salmonella*, Animal, Human, Antimicrobial resistance, Ethiopia

Introduction

Salmonellosis among humans and animals is a major public health problem with a significant social and economic impact in developing and developed countries [1, 2]. The disease is especially catastrophic in developing countries, where conditions for endemic disease are still favorable [3]. Many animal species, especially chickens, pigeons, and reptiles, are potential reservoirs for the causative agent of salmonellosis, *Salmonella enterica* [1, 4]. *Salmonella* serovars, a foodborne infection, causes gastroenteritis, enteric fever, bacteremia, and other complications depending on the serovar and the host [5]. Typhoid fever, a global disease, causes 21.7 million illnesses and 217,000 deaths annually [6]. Likewise, non-typhoidal salmonellosis (NTS) is causing an estimated 93.8 million cases and 155,000 deaths annually. Of these, 80.3 million cases were foodborne [2]. Salmonellosis is a significant concern in developing countries due to its clinical symptoms, which can be mistaken for other febrile and diarrheal diseases, and the lack of adequate bacteriological laboratories. These factors are believed to result in many undiagnosed cases [7].

Antibiotic-resistant *Salmonella* infections are universal concerns, particularly in developing countries where there are unhygienic living conditions, close contact and sharing of houses between animals and humans, and consumption of raw or undercooked food items [8]. The widespread use of antimicrobials at suboptimal doses has led to multidrug-resistant strains, including resistance to quinolones and expanded-spectrum cephalosporins. Horizontal gene transfer enables the transmission of resistance genes among bacteria strains through mobile genetic elements, particularly plasmids [9–11]. The bacteria use various mechanisms to resist antibiotics, such as altering their permeability to the antibiotic, degrading the antibiotic, causing its efflux, or leading to its inactivation by enzymatic means [12]. The mutation and horizontal gene transfer of virulence factors are rising among *Salmonella* serovars. This trend is crucial for the emergence of multidrug-resistant (MDR) serovars, and it is also altering host adaptation and anatomical preferences and leading to new types of clinical manifestations and pathogenesis processes in infected hosts [13]. Multidrug-resistant in *Salmonella* is now defined by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) as resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole [6, 14].

Salmonella serovar prevalence varies seasonally. As high as 38.8% and as low as 6.4% prevalence was reported in autumn and winter, respectively, in Guangdong Province, China [15], and is further supported by research conducted in Norway by MacDonald and his colleagues [16]. Children aged < 5 years were the group affected mainly by *Salmonella* in Guangdong Province, accounting for 73%, of whom the infants (< 1 year) were 81.5% [15]. Different studies considered eggs and poultry products as significant sources of infection [17, 18]. Similarly, dairy products are the main risk for acquiring salmonellosis infection [19]. Consumption of contaminated meat was the primary vehicle for transmitting causative agents of salmonellosis, and outbreaks were also documented [20].

In Ethiopia, the of salmonellosis is associated with different factors, including a lower level of education status, low family economic status, improper waste disposal, poor handwashing practice, inappropriate disposal of feces, low access to clean potable water, and absence/irregular use of toilets [21, 22]. Thus, assessing prevalence and monitoring antimicrobial resistance among *Salmonella* isolates is crucial to reveal antimicrobial resistance patterns that will influence efficient antimicrobial therapy [12, 23–25]. The current study aimed to assess the prevalence, antimicrobial resistance patterns, and associated factors of *Salmonella* serovars in human and animal isolates from selected health facilities in the Amhara National Regional State of Ethiopia.

Materials and methods

Study setting

The study was conducted in selected health facilities in the Amhara National Regional State of Ethiopia and among animals near these facilities. Samples were collected from four health centers: Maraki Health Center in Gondar, Shibit Health Center in Bahir Dar, Dessie Health Center in Dessie, and Debre Markos Health Center in Debre Markos. These facilities were chosen based on their patient flow. Additionally, samples from local animal communities surrounding each health facility were collected randomly, specifically from the following species: sheep, chicken, and cattle. The geographic locations from which the *Salmonella* isolates were collected are illustrated in Fig. 1.

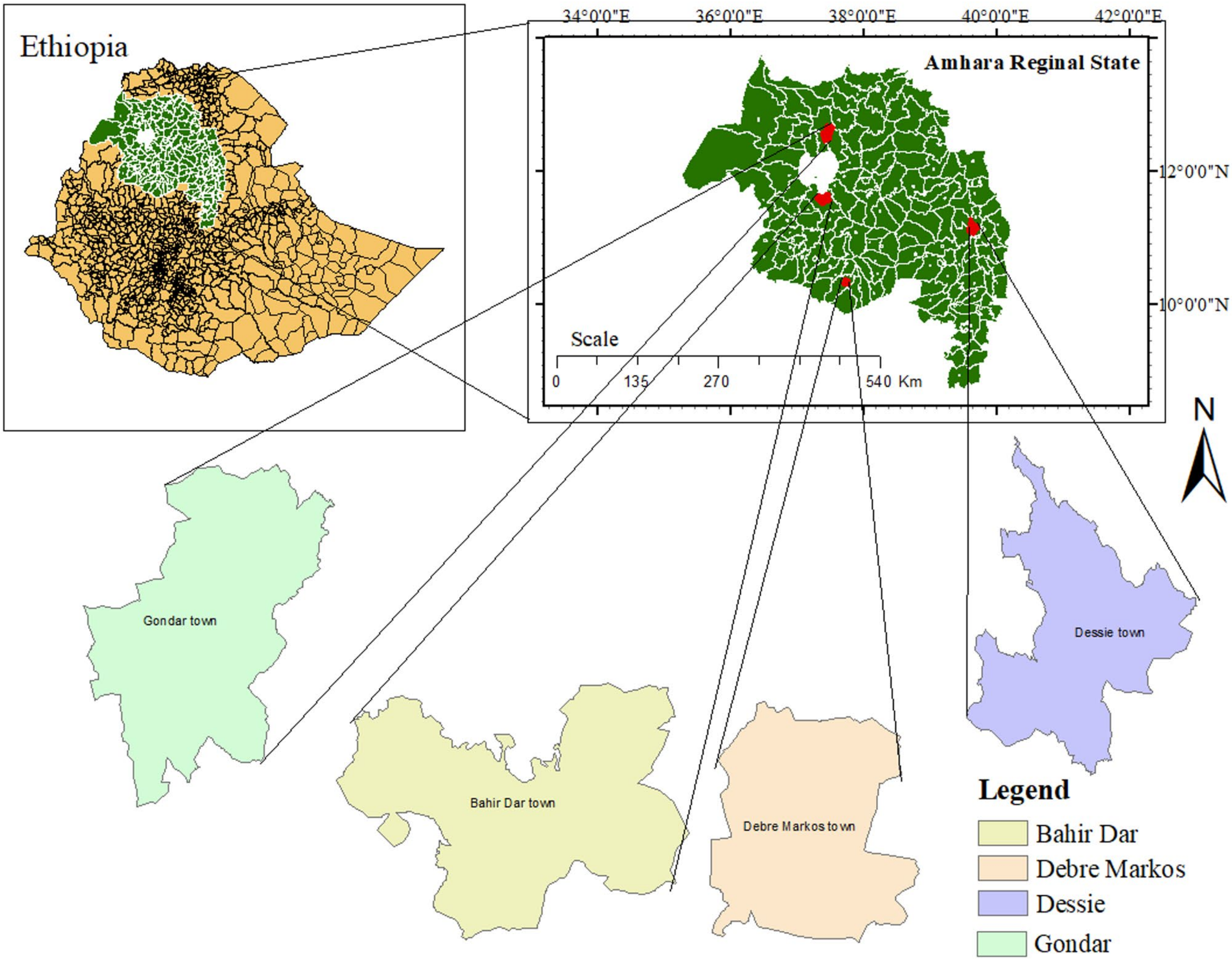

Fig. 1 A map showing study sites for prevalence, antimicrobial resistance pattern, and associated factors of *Salmonella* among human-animal interfaces in the Amhara National Regional State, Ethiopia

Study design and period

This study was conducted using a cross-sectional study design between September 2022 and January 2024.

Sample size determination

A. Sample size determination from human subjects.
   The sample size was determined by assuming a prevalence of 10.5% for *Salmonella* serovars among humans [26], along with a marginal error of 3% at a 95% confidence level. The calculations were conducted using Epi Info software (CDC, USA). Accordingly, the sample size was determined to be 404. An equal number of study participants were drawn from each study site (101 samples from each).
B. Sample size determination from animal subjects.
   The sample size was calculated based on an assumed prevalence of 16.7% for *Salmonella* serovars in animals [27], with a margin of error set at 4% and a confidence level of 95%. The calculations were performed utilizing Epi Info software (CDC, USA). Accordingly, the calculated sample size was 336. An equal number of animal samples were drawn from each study site (84 samples from each).
   In total, 740 samples were collected to isolate *Salmonella* serovars, with 404 samples from humans and 336 from animals.

Data collection

Using structured interview questionnaires and checklist observation, socio-demographic, behavioral, and environmental variables related to the nature of the organism and the disease were collected (Supplement 1).

Sample collection and processing

A standard technique for isolating and identifying *Salmonella* from human and animal samples was used. For human specimens, freshly passed stool specimens were collected, placed immediately in Cary Blair transport medium (Oxoid Ltd, Basingstoke, and Hampshire, England), and transported to the laboratory within 6 h of collection. For animal samples, a sterile cotton-tipped swab (2 × 3 cm) was first soaked in approximately 10 mL buffered peptone water (BPW, Oxoid Ltd., England). Then, rectal/fecal samples were collected using the pre-moistened swab. Cloacal swabs from chicken samples were collected by inserting pre-moistened sterile cotton swabs approximately 1–2 cm into the cloaca and rotating slowly to absorb the chyme. Rectum/fecal samples collected from animals were transported to the laboratory using Cary Blair transport medium (Oxoid Ltd., Basingstoke, and Hampshire, England) within 6 h of collection.

All samples were inoculated into Selenite F broth and incubated at 37 °C for 18–24 h to enrich the *Salmonella* isolates. Enrichment cultures were sub-cultured on standard culture media, including MacConkey, *Salmonella-Shigella* agar, and Xyline Lysine Dextrose agar. Colonies suspected of *Salmonella* serovars were selected and submitted to preliminary biochemical identification using Kligler Iron Agar, urease, and pyrrolidonyl aminopeptidase tests (PYR) (Oxoid, UK). Strains with a biochemical profile suggestive of *Salmonella* serovars were differentiated using arrays of biochemical tests, including H_2S, indole, methyl red, Voges-Proskauer, citrate (Simmons), PYR, urea, lysine decarboxylase, ornithine decarboxylase, motility, glucose, lactose, and sucrose fermentation. Copies of the pure culture of the isolated bacteria were preserved for future analysis and susceptibility testing.

Antimicrobial Susceptibility Test (AST)

The antimicrobial susceptibility of the *Salmonella* isolates was tested for antimicrobials by the modified Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Oxoid, UK). The selection of antibiotic disks and the protocol for disk-diffusion methods used to determine strain susceptibility were based on Clinical Laboratory Standard Institute (CLSI) guidelines [28]. The antimicrobial agents used in the study were as follows: ampicillin (AMP, 10 µg), azithromycin (AZM, 15 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CRO, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), imipenem (IMP, 10 µg, used only for human isolates), meropenem (MEM, 10 µg), sulphamethoxazole/trimethoprim in a 19:1 ratio (SXT, 25 µg), and tetracycline (TCY, 30 µg). The result of AST was recorded as resistant, intermediate, and susceptible. The antimicrobial resistance profile of the isolates was determined based on the host/specimen type to explore the nature of the bacteria and host adaptability.

Quality assurance

Specimens were collected and processed aseptically following the Standard Operating Procedure (SOP). Qualities of prepared culture plates and broth were monitored by incubating 5% of the prepared batch at 35–37 °C overnight. All new batches of media were checked with ATCC control species/serovars (*E. coli* ATCC 25922, *S. Typhi* ATCC 13311, and *S. Enteritidis* 13076) for supporting the growth of bacteria and susceptibility tests [28]. Muller Hinton media was prepared and dispensed at a depth of 4 mm to get accurate results from the antimicrobial susceptibility test. The inhibition zone of antimicrobial susceptibility tests was measured to the shortest zone of inhibition [28].

Statistical analysis

For statistical analysis, cleaned and coded data were entered into EpiData version 4.6 and exported to SPSS version 29.0 and Microsoft Excel 2013 (Microsoft Corporation, USA) statistical software. Tables, graphs, and percentages were used to display descriptive statistics. Binary logistic regression models were used to identify significant relationships between the predictors and the dependent variable. For the final analysis, multivariable backward stepwise logistic regression was fitted to predictor variables that showed a p-value < 0.25 significance in bivariable logistic regression, and variables with a p-value ≤ 0.05 were deemed statistically significant. The Hosmer and Lemeshow test was used to assess the model's fitness. An odds ratio of 95% CI was used to determine the strength of the association. Additionally, the antibiotic resistance patterns of *Salmonella* isolates were determined using WHONET software version 2024.

Result

Socio-demographic characteristics of the study population

A total of 740 samples were enrolled from humans ($n = 404$) and animals ($n = 336$), and the response rate was 100%. Among the human study participants, 373 (92.3%) were from urban areas, and 213 (52.7%) were male by sex. The age of the study participants ranged from one month to 76 years, with a mean of 17.4 years (SD ± 16.44 years). The frequency of children aged ≤ 5, 6–15, and > 15 years was 144 (35.6%), 62 (15.4%), and 198 (49.0%), respectively. Over half of the study participants (50.3%) could not read or write due to being uneducated or non-school-age children, as shown in Table 1. Regarding animal samples, most of them were collected from poultry (42.81%), having separate houses for the animals (86.85%), and no history of disease (68.50%) and antibiotics (73.70%) (Table 2).

Table 1 Socio-demographic characteristics and prevalence of *Salmonella* isolates among human study participants from September 2022 to January 2024

Socio-demographic Variables		**Salmonella isolates**		
		Positive *N* (%)	**Negative *N* (%)**	**Total *N* (%)**
Address	Debre Markos	15 (30.6)	86 (24.2)	101 (25.0)
	Bahir Dar	13 (26.5)	88 (24.8)	101 (25.0)
	Gondar	7 (14.3)	94 (26.5)	101 (25.0)
	Dessie	14 (28.6)	87 (24.5)	101 (25.0)
Age (Years)	≤ 5	10 (20.4)	134 (37.7)	144 (35.6)
	6–15	12 (24.5)	50 (14.1)	62 (15.4)
	> 15	27 (55.1)	171 (48.2)	198 (49.0)
Gender	Male	22 (44.9)	191 (53.8)	213 (52.7)
	Female	27 (55.1)	164 (46.2)	191 (47.3)
Residency	Urban	45 (91.8)	328 (92.4)	373 (92.3)
	Rural	4 (8.2)	27 (7.6)	31 (7.7)
Educational level	Unable to read & write	1 (2.0)	41 (11.5)	42 (10.4)
	Read & write only	5 (10.2)	20 (5.6)	25 (6.2)
	Grade 1–8 complete	15 (30.6)	50 (14.1)	65 (16.1)
	Grade 9–12 complete	10 (20.4)	45 (12.7)	55 (13.6)
	Above grade 12	6 (12.2)	50 (14.1)	56 (13.9)
	Not applicable*	12 (24.5)	149 (42.0)	161 (39.9)
Occupation	Farmer	3 (6.1)	21 (5.9)	24 (5.9)
	Trade/Merchant	5 (10.2)	33 (9.3)	38 (9.4)
	Employee	1 (2.0)	31 (8.7)	32 (7.9)
	Student	19 (38.8)	62 (17.5)	81 (20.0)
	Housewife	4 (8.2)	26 (7.3)	30 (7.4)
	Daily Labor	5 (10.2)	23 (6.5)	28 (6.9)
	Unemployed	0 (0.0)	10 (2.8)	10 (2.5)
	Not Applicable**	12 (24.5)	149 (42.0)	161 (39.9)
	Total	**49 (12.1)**	**355 (87.9)**	**404 (100.0)**

* Child who is six years of age or younger and is not enrolled in public or private school

**Child who is six years of age or younger and not applicable in any occupational work

Table 2 Characteristics and prevalence of *Salmonella* isolates among animal samples from September 2022 to January 2024

Associated factor Variables		**Salmonella isolates**		
		Positive *N* (%)	**Negative *N* (%)**	**Total *N* (%)**
Address	Debre Markos	4 (44.44%)	80 (24.46%)	84 (25.00)
	Bahir Dar	2 (22.22%)	82 (25.08%)	84 (25.00)
	Gondar	2 (22.22%)	82 (25.08%)	84 (25.00)
	Dessie	1 (11.11%)	83 (25.38%)	84 (25.00)
Type of Domestic Animal	Cattle	1 (11.11%)	60 (18.35%)	61 (18.15)
	Sheep	2 (22.22%)	127 (38.84%)	129 (38.39)
	Poultry	6 (66.67%)	140 (42.81%)	146 (43.45)
Separate house for the animals	Yes	7 (77.78%)	284 (86.85%)	291 (86.61)
	No	2 (22.22%)	43 (13.15%)	45 (13.39)
History of disease among the animals	Yes	3 (33.33%)	103 (31.50%)	106 (31.55)
	No	6 (66.67%)	224 (68.50%)	230 (68.45)
Use antibiotic history among animals	Yes	3 (33.33%)	86 (26.30%)	89 (26.49)
	No	6 (66.67%)	241 (73.70%)	247 (73.51)
	Total	**9 (2.70%)**	**327 (97.30%)**	**336 (100.00)**

Frequency distribution of possible risk factors

In the current study, nearly three-fourths (71.5%) of the study participants did not have contact with animals or did not have livestock in their houses. Additionally, a significant number of participants indicated that they had not been in contact with febrile patients (90.6%) or patients experiencing diarrhea (92.6%). Moreover, 88.9% indicated they had not traveled during the week before exhibiting signs of the infection. The majority of the study participants did not consume raw eggs (98.8%), raw meat (96.3%), and raw dairy products (96.3%). Data showed that 83.2% used pipe water for their household activities. At most, 98.5% of the study participants did not have comorbidity, whereas 67.3% had a family size of 3–5 members (Table 3).

Clinical characteristics of the study participants

Data showed that 123(30.4%) of the study participants suspected of *Salmonella* presented with watery diarrhea, 18.6% had dysentery, and 3.4% had bloody diarrhea. The majority of the patients (89.6%) had diarrhea for ≤ 5 days. In addition, 332(82.2%) of the patients had abdominal pain, whereas 43.1%, 42.3%, 39.1%, 31.9%, 30.0%, 23.5%, and 16.3% had fever, vomiting, headache, nausea, tenesmus, chills, and muscle aches, respectively. The actual diagnosis values of clinical features of a patient for salmonellosis are better explained by vomiting, nausea, 11–15 days of diarrhea duration, and blood and mucoid stool with receiver operating characteristic (ROC) areas of 0.59, 0.53, 0.53, and 0.52, respectively, as shown in (Table 4; Fig. 2).

Prevalence of *Salmonella* isolates

The overall prevalence of *Salmonella* serovars was 7.84% (95% CI: 6.1–9.9, *N* = 58). When analyzing human and animal samples separately, the prevalence of *Salmonella* serovars was 12.1% (95% CI: 9.2–15.6) among humans and 2.7% (95% CI: 1.3–4.8) among animals (Tables 1 and 2). Among the 49 patients infected with *Salmonella* serovars, 27 (55.1%) were female, and 22 (44.9%) were male, which gives an overall male-to-female ratio of 1:1.23. However, there were no statistically significant differences between male and female patients with *Salmonella* serovar infection (AOR = 1.620; 95% CI: 0.810–3.240) and a *p*-value of 0.172. On the other hand, the proportion of *Salmonella* serovar infection was 20.4%, 24.5%, and 55.1% among the age groups ≤ 5, 6–15, and > 15 years, respectively (Table 1; Fig. 3).

Antibiotic susceptibility pattern of *Salmonella* serovars among human-animal interfaces

The antimicrobial susceptibility patterns of *Salmonella* isolates (*n* = 58) from both humans and animals are displayed in Fig. 4. The data indicate that *Salmonella* serovars have a higher rate of drug resistance among human isolates, with resistance levels recorded at 79.6%

Table 3 Frequency of possible associated factors among human study participants from September 2022 to January 2024

Associated factor Variables		Salmonella Isolates		
		Positive *N* (%)	Negative *N* (%)	Total *N* (%)
Contact with animals	Yes	22 (44.9)	93 (26.2)	115 (28.5)
	No	27 (55.1)	262 (73.8)	289 (71.5)
Consumption of raw egg	Yes	1 (2.0)	4 (1.1)	5 (1.2)
	No	48 (98.0)	351 (98.9)	399 (98.8)
Consumption of raw meat	Yes	2 (4.1)	13 (3.7)	15 (3.7)
	No	47 (95.9)	342 (96.3)	389 (96.3)
Consumption of raw dairy products	Yes	0 (0.0)	15 (4.2)	15 (3.7)
	No	49 (100.0)	340 (95.8)	389 (96.3)
Consumption of raw vegetables	Yes	13 (26.5)	74 (20.8)	87 (21.5)
	No	36 (73.5)	281 (79.2)	317 (78.5)
Contact with febrile individual	Yes	6 (12.2)	32 (9.0)	38 (9.4)
	No	43 (87.8)	323 (91.0)	366 (90.6)
Diarrheic patient at home	Yes	8 (16.3)	22 (6.2)	30 (7.4)
	No	41 (83.7)	333 (93.8)	374 (92.6)
Travel history for the last 7 days	Yes	7 (14.3)	38 (10.7)	45 (11.1)
	No	42 (85.7)	317 (89.3)	359 (88.9)
Drinking water supply	Pipe	38 (77.6)	298 (83.9)	336 (83.2)
	Surface water	8 (16.3)	29 (8.2)	37 (9.2)
	Well	3 (6.1)	15 (4.2)	18 (4.5)
	Hand-pump	0 (0.0)	12 (3.4)	12 (3.0)
	River	0 (0.0)	1 (0.3)	1 (0.2)
Comorbidity	Yes	0 (0.0)	6 (1.7)	6 (1.5)
	No	49 (100.0)	349 (98.3)	398 (98.5)
Family size	< 3	7 (14.3)	71 (20.0)	78 (19.3)
	3–5	36 (73.5)	236 (66.5)	272 (67.3)
	> 5	6 (12.2)	48 (13.5)	54 (13.4)
Total		**49 (100.0)**	**355 (100.0)**	**404 (100.0)**

Table 4 Frequency of clinical features among human study participants from September 2022 to January 2024

Clinical feature Variables		**Salmonella isolates**		
		Positive *N* (%)	**Negative *N* (%)**	**Total *N* (%)**
Consistency of stool	Watery	15 (30.6)	108 (30.4)	123 (30.4)
	Mucoid	8 (16.3)	90 (25.4)	98 (24.3)
	Bloody	3 (6.1)	11 (3.1)	14 (3.4)
	Mixed (blood and mucus)	11 (22.4)	64 (18.0)	75 (18.6)
	Loose	12 (24.5)	82 (23.1)	94 (23.3)
Duration of diarrhea (days)	≤ 5	43 (87.8)	319 (89.9)	362 (89.6)
	6–10	3 (6.1)	26 (7.3)	29 (7.2)
	11–15	3 (6.1)	4 (1.1)	7 (1.7)
	> 15	0 (0.0)	6 (1.7)	6 (1.5)
Nausea	Yes	13 (26.5)	116 (32.7)	129 (31.9)
	No	36 (73.5)	239 (67.3)	275 (68.1)
Vomiting	Yes	13 (26.5)	158 (44.5)	171 (42.3)
	No	36 (73.5)	197 (55.5)	233 (57.7)
Chills	Yes	15 (30.6)	80 (22.5)	95 (23.5)
	No	34 (69.4)	275 (77.5)	309 (76.5)
Tenesmus	Yes	18 (36.7)	103 (29.0)	121 (30.0)
	No	31 (63.3)	252 (71.0)	283 (70.0)
Abdominal pain	Yes	42 (85.7)	290 (81.7)	332 (82.2)
	No	7 (14.3)	65 (18.3)	72 (17.8)
Headache	Yes	26 (53.1)	132 (37.2)	158 (39.1)
	No	23 (46.9)	223 (62.8)	246 (60.9)
Muscle aches	Yes	8 (16.3)	58 (16.3)	66 (16.3)
	No	41 (83.7)	297 (83.7)	338 (83.7)
Fever	Yes	25 (51.0)	149 (42.0)	174 (43.1)
	No	24 (49.0)	206 (58.0)	230 (56.9)
Total		**49 (100.0)**	**355 (100.0)**	**404 (100.0)**

for ampicillin and 71.4% for tetracycline. In contrast, all animal isolates (100%) demonstrated resistance to both ampicillin and tetracycline.

On a more positive note, *Salmonella* serovars showed high susceptibility rates among human isolates for ciprofloxacin (98.0%) and ceftriaxone (91.8%). Among animal isolates, susceptibility was also high for chloramphenicol, gentamicin, and ciprofloxacin, with each showing a susceptibility rate of 88.9%.

Patterns of multidrug resistance among *Salmonella* isolates

The current study revealed a total of 34 antibiotic resistance patterns among *Salmonella* isolates from humans and 9 patterns from animals. Among the 49 *Salmonella* serovars isolated from humans, the rate of MDR was 6.1%, corresponding to 3 isolates. In contrast, no MDR *Salmonella* serovars were found in animals, based on the current definition of MDR for this group, which includes resistance to trimethoprim-sulfamethoxazole, ampicillin, and chloramphenicol (Tables 5 and 6).

Associated risk factors of *Salmonella* infection

In this study, after adjusting for confounding factors, contact with animals and patients with diarrhea were identified as significant predictors of salmonellosis among the suspected patients. Study participants who had contact with patients with diarrhea were 2.8 times more likely to develop salmonellosis compared to those who did not have contact (AOR = 2.83; 95% CI: 1.05–7.629; $P = 0.04$). Similarly, individuals with animal contact were 3 times more likely to acquire *Salmonella* bacterium and develop salmonellosis disease than those people who did not have contact with animals (3.179; 95% CI: 1.574–6.419; $P = 0.001$). On the contrary, associated factors such as raw egg consumption, raw meat consumption, raw vegetables consumption, and contact with febrile individuals were not significantly associated. Nevertheless, a relatively higher proportion of *Salmonella* isolates was observed among individuals who consumed foods with possible sources of infection and had contact with infected febrile patients (Table 7A and B).

Discussion

Zoonotic bacterial diseases are among the most significant public health problems worldwide [29]. One of the most common pathogens involved in this type of infection is *Salmonella* bacteria [30, 31]. In this study, the overall prevalence of *Salmonella* among humans was 12.1% (95% CI: 9.2–15.6), which is in line with previous

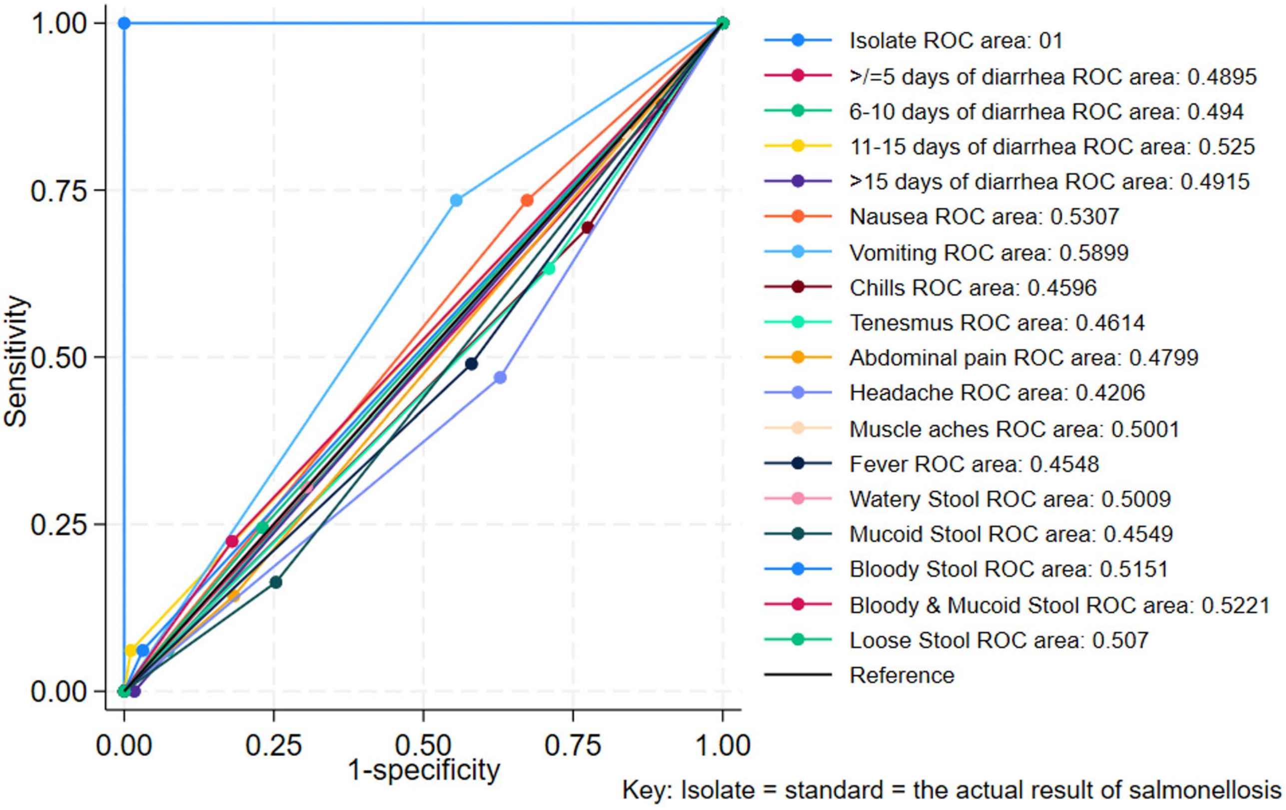

Fig. 2 ROC curve of clinical features of the patients for the diagnosis of salmonellosis from September 2022 to January 2024

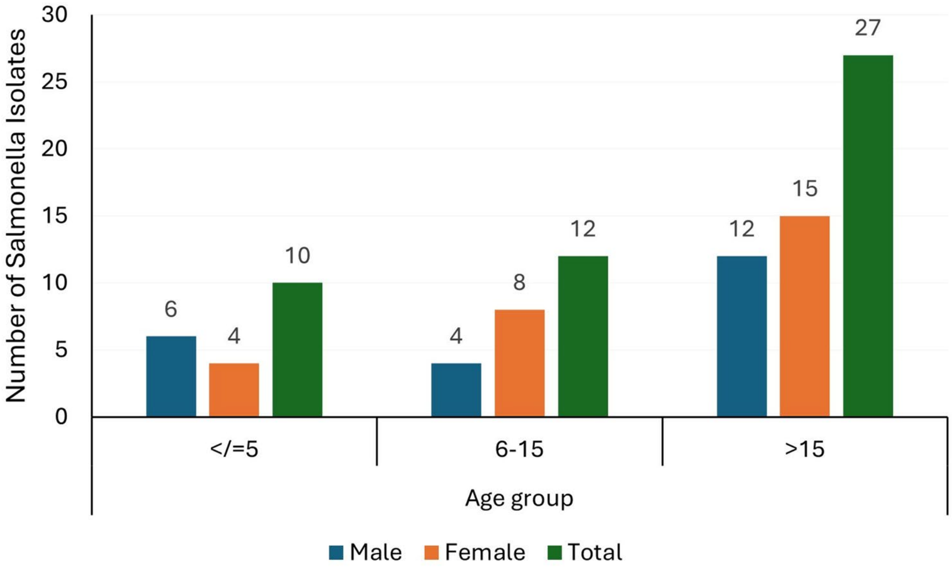

Fig. 3 Age and gender distribution of patients who were positive for *Salmonella* species ($n = 49$), Amhara, Ethiopia, from September 2022 to January 2024

reports from Butajira (10.5%) [32], Arba Minch (12.6%) [22], and Jigjiga (11%) [33]. On the other hand, a higher prevalence was reported in Injibara (25.7%) [34] and Southwest Ethiopia (20.5%) [35]. Lower prevalence was also reported from Debre Markos (3.15%) [36], Bahir Dar (7.8%) [37], and Dessie (5.21%) [38]. The disparity may arise from several factors, including the use of enrichment media in this study, seasonal variations, differences in hygiene and sanitation, variations in age groups, and discrepancies in case selection criteria. Other studies have identified these factors as causes of variation in *Salmonella* prevalence [39, 40].

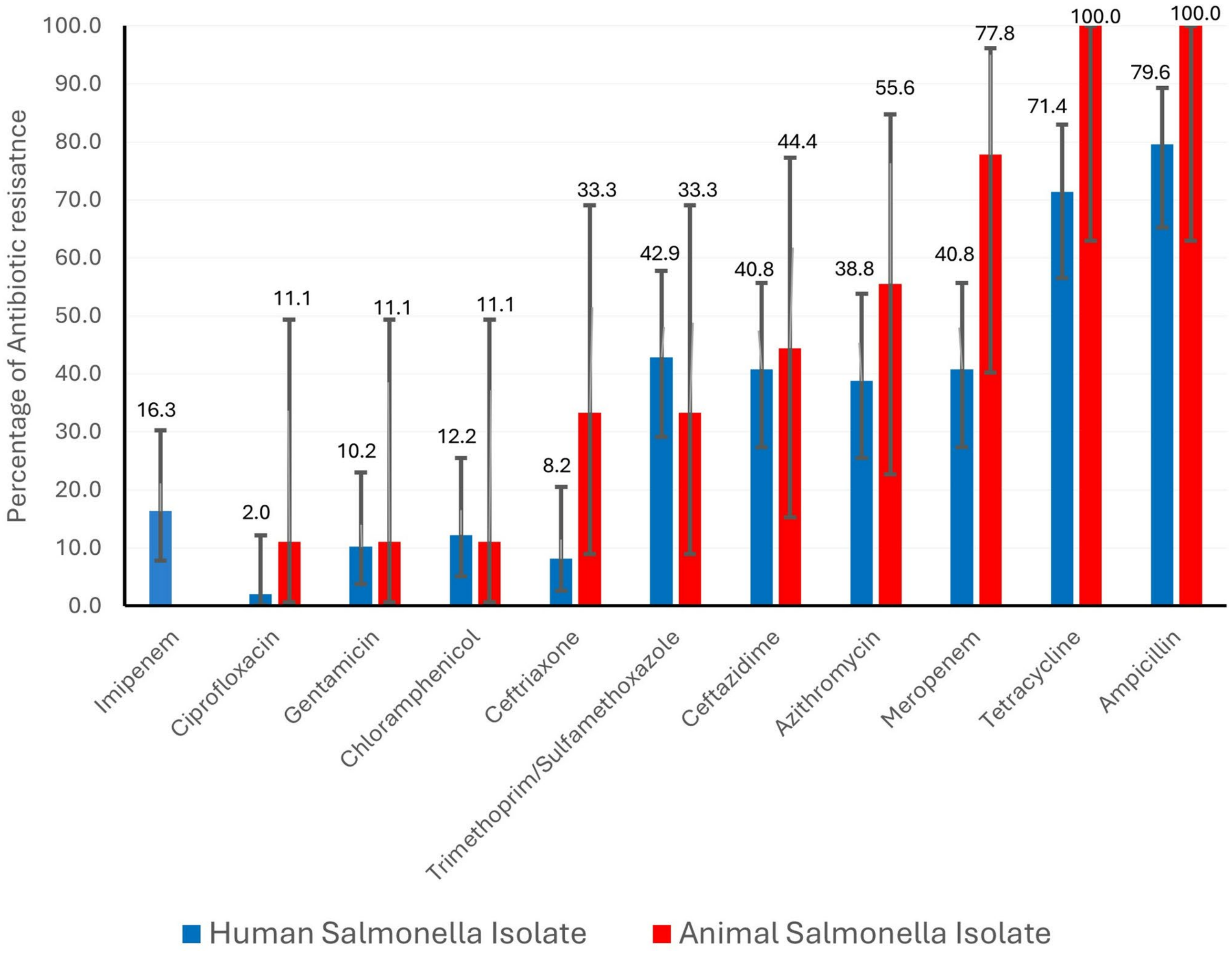

Fig. 4 Percentage of antibiotic resistance with its 95% CI among human and animal *Salmonella* isolates from September 2022 to January 2024

The prevalence of *Salmonella* among animals was 2.7% (95% CI: 1.3–4.8), which is lower than previous reports from Gondar from chicken droppings in poultry farms (16.1%) [41] and Addis Ababa from poultry farms (6.4%) [42]. On the other hand, a similar prevalence was reported in Bishoftu among cattle (2.5%) [43] and in Adama and Modjo towns among poultry farms (2.9%) [44]. Previously, a lower prevalence of *Salmonella* serovars was reported in Estonia in poultry (0.1%) [45]. Variation of *Salmonella* prevalence due to variation in the sample type, location, breed, and/or age of the animals was previously reported by a study conducted by Abdi et al. [27].

In the present study, the drug resistance rate of human *Salmonella* isolates for ampicillin was 79.6% (95% CI: 65.2–89.3), which is relatively higher than the previous report from Debre Berhan (0.0%) [46], Addis Ababa (2.7%) [47], and Hawassa (0.0%) [48]. On the other hand, a similar prevalence of resistance was reported from Addis Ababa (80.0%) [49] and Dilla University Student Cafeteria (80.9%) [50]. However, the current resistance rate for ampicillin was relatively lower than the findings of Dessie [38], Hossana [51], Hawassa [52], and Jimma [53], which reported 100% resistance.

Likewise, the resistance rate of human *Salmonella* isolates for tetracycline in the current study was 71.4% (95% CI: 56.5–83.0), which is higher than the study conducted in Bahir Dar (39.4%) [37], University of Gondar student's cafeteria (37.5%) [54], Debre Berhan (40.0%) [46], and Addis Ababa among diarrheic patients attending health facilities (5.6%) [47]. Furthermore, equivalent findings were reported from Debre Markos (71.4%) [36] and Southeast Ethiopia (79.3%) [21]. Nevertheless, the findings in the current study were relatively lower than the findings from Eastern Ethiopia (88.2%) [55] and Dessie (85.0%) [38].

This high level of resistance to tetracycline and ampicillin in human *Salmonella* isolates could be due to the ease of availability and usage of these drugs from drug shops/pharmacies and lack of awareness of administering

Table 5 Antimicrobial resistance profile of *Salmonella* isolates from human samples from September 2022 to January 2024

Profile	Resistance profile Human Isolates	Number of Salmonella isolates	MDR
	-	2	0
1	SXT	1	0
1	GEN	1	0
1	CAZ	1	0
1	AMP	1	0
2	CAZ, MEM	1	0
2	AMP, TCY	3	0
2	AMP, GEN	3	0
2	AMP, CAZ	1	0
3	GEN, TCY, SXT	2	0
3	AMP, TCY, SXT	2	0
3	AMP, GEN, TCY	2	0
3	AMP, AZM, TCY	2	0
4	AZM, GEN, TCY, SXT	1	0
4	AMP, GEN, TCY, SXT	3	0
4	AMP, CHL, TCY, SXT	1	1
4	AMP, CAZ, GEN, TCY	1	0
4	AMP, CAZ, GEN, MEM	1	0
5	AZM, CAZ, GEN, IPM, MEM	1	0
5	AMP, CHL, GEN, TCY, SXT	1	1
5	AMP, CAZ, GEN, MEM, TCY	1	0
5	AMP, AZM, GEN, MEM, TCY	2	0
5	AMP, AZM, CAZ, MEM, TCY	1	0
6	AMP, CAZ, CRO, MEM, TCY, SXT	1	0
6	AMP, AZM, GEN, IPM, MEM, TCY	1	0
6	AMP, AZM, CRO, GEN, MEM, TCY	1	0
6	AMP, AZM, CAZ, IPM, MEM, TCY	1	0
6	AMP, AZM, CAZ, GEN, MEM, SXT	1	0
6	AMP, AZM, CAZ, CRO, TCY, SXT	1	0
7	AMP, AZM, CAZ, GEN, MEM, TCY, SXT	1	0
7	AMP, AZM, CAZ, GEN, IPM, MEM, TCY	1	0
7	AMP, AZM, CAZ, CIP, MEM, TCY, SXT	1	0
8	AMP, CAZ, CRO, CHL, GEN, MEM, TCY, SXT	1	1
8	AMP, AZM, CAZ, GEN, IPM, MEM, TCY, SXT	4	0

AMP—Ampicillin, AZM—Azithromycin, CAZ—Ceftazidime, CHL—Chloramphenicol, CIP—Ciprofloxacin, CRO—Ceftriaxone, GEN— Gentamicin, IMP—Imipenem, MEM—Meropenem, SXT—Trimethoprim/Sulfamethoxazole, TCY—Tetracycline,

Table 6 Antimicrobial resistance profile of *Salmonella* isolates from animal samples from September 2022 to January 2024

Profile	Resistance profile	Number of Salmonella isolates	MDR
	Animal Isolates		
2	GEN, TCY	1	0
4	AMP, CAZ, MEM, TCY	1	0
5	AMP, CAZ, GEN, MEM, TCY	1	0
5	AMP, CAZ, CRO, MEM, TCY	1	0
5	AMP, AZM, MEM, TCY, SXT	1	0
5	AMP, AZM, CIP, GEN, TCY	1	0
6	AMP, AZM, CRO, MEM, TCY, SXT	1	0
6	AMP, AZM, CRO, GEN, MEM, TCY	1	0
7	AMP, AZM, CAZ, GEN, MEM, TCY, SXT	1	0

AMP—Ampicillin, AZM—Azithromycin, CAZ—Ceftazidime, CIP—Ciprofloxacin, CRO—Ceftriaxone, GEN— Gentamicin, MEM—Meropenem, SXT—Trimethoprim/Sulfamethoxazole, TCY—Tetracycline,

antimicrobials. In the current study areas, self-medication, dropping prescribed antimicrobials before their entire course of therapy, and sharing medication with other people are standard practices in the communities [56, 57].

In this study, low resistance was observed to ciprofloxacin (2.0%; 95% CI: 0.1–12.2) among human *Salmonella* isolates, which is comparable to the reports from Addis Ababa (4.3%) [50], Butajira (5.9%) [37], and Nekemte (6.67%) [57]. However, the high resistance rate of *Salmonella* isolated from humans for ciprofloxacin was reported in previous studies, such as 65% in Adama [58] and 57.2% in Dire Dawa [59]. Contradictorily, no resistance to ciprofloxacin was also reported in Dessie [38], Debre Berhan [46], Addis Ababa [47], and Hawassa [48]. Like ciprofloxacin, the current study observed a relatively minimal level of *Salmonella* resistance to ceftriaxone among human isolates (8.2%; 95% CI: 2.6–20.5), which is relatively lower than the study from the University of Gondar student cafeteria (43.7%) [54] and Adama (45.0%) [58]. On the other hand, a parallel resistance rate was observed in studies conducted in Addis Ababa (4.3%) [49] and Nekemte (3.33%) [60]. On the contrary, findings from Debre Berhan [46], Hossana [51], Butajira [32], Dire Dawa [59], and Jimma [53] did not show any resistance of human *Salmonella* isolates to ceftriaxone. This low level of resistance to ciprofloxacin and ceftriaxone may be due to new strains of *Salmonella* serovars circulating in the study population without genetic markers conferring resistance to antimicrobials or loss of genetic markers responsible for those antibiotics' resistance because of proper utilization of the antibiotics by health professionals.

In this study, high levels of resistance to ampicillin (100.0%; 95% CI: 62.9–100) were observed among *Salmonella* isolates from animal samples, which is higher than the previous studies from Adama and Modjo among poultry farms (0.0%) [44], Ambo and Holeta among beef (5.0%) [61], and Jimma among apparently healthy slaughter cattle (57.6%) [62]. However, comparable results were reported from Central Ethiopia among poultry farms (92.0%) [63], Libya among poultry slaughterhouses (100%) [64], and Pakistan among beef (90.0%) [65]. In a similar manner to ampicillin, 100.0% (95% CI: 62.9–100) tetracycline resistance was observed among the animal *Salmonella* isolates in this study, which is higher

Table 7A Bivariable and multivariable logistic regression analysis of factors associated with *Salmonella* infection among suspected patients at public health facilities in the Amhara region from September 2022 to January 2024

Socio-demographic Variables		Positive Frequency (%)	Negative Frequency (%)	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Age (Years)	≤ 5	10 (20.4)	134 (37.7)	0.473(0.221–1.011	0.53		
	6–15	12 (24.5)	50 (14.1)	1.520(0.718–3.216)	0.274		
	> 15	27 (55.1)	171 (48.2)	1			
Gender	Male	22 (44.9)	191 (53.8)	1		1	
	Female	27 (55.1)	164 (46.2)	1.429(0.784–2.605)	0.244	1.620(0.810–3.240)	0.172
Residency	Urban	45 (91.8)	328 (92.4)	1			
	Rural	4 (8.2)	27 (7.6)	0.891(0.361–3.229)	0.891		
Educational level	Unable to read & write	1 (2.0)	41 (11.5)	0.203 (0.024–1.757)	0.148	0.062 (0.006–0.638)	0.019
	Read & write only	5 (10.2)	20 (5.6)	2.083 (0.571–7.607)	0.267	0.955 (0.231–3.943)	0.949
	Grade 1–8 complete	15 (30.6)	50 (14.1)	2.5 (0.897–6.966)	0.08	0.98 (0.309–3.11)	0.973
	Grade 9–12 complete	10 (20.4)	45 (12.7)	1.852 (0.623–5.504)	0.268	0.938 (0.285–3.091)	0.917
	Above grade 12	6 (12.2)	50 (14.1)	1		1	
	Not applicable	12 (24.5)	149 (42.0)	0.671 (0.239–1.882)	0.448	2.625 (0.314–21.946)	0.373
Occupation	Farmer	3 (6.1)	21 (5.9)	4.429 (0.431–45.516)	0.211	8.641 (0.707-105.601)	0.091
	Trade/Merchant	5 (10.2)	33 (9.3)	4.697 (0.519–42.487)	0.169	5.988 (0.606–59.18)	0.126
	Employee	1 (2.0)	31 (8.7)	1		1	
	Student	19 (38.8)	62 (17.5)	9.5 (1.215–74.282)	0.032	12.592 (1.453-109.101)	0.021
	Housewife	4 (8.2)	26 (7.3)	4.769 (0.501–45.36)	0.174	15.208 (1.451-159.425)	0.023
	Daily Labor	5 (10.2)	23 (6.5)	6.739 (0.737–61.663)	0.091	11.559 (1.101-121.337)	0.041
	Unemployed	0 (0.0)	10 (2.8)	0 (0–0)	0.999	0 (0–0)	0.999
	Not Applicable	12 (24.5)	149 (42.0)	2.497 (0.313–19.912)	0.388	2.625(0.314–21.946)	0.373
	Total	**49 (100.0)**	**355 (100.0)**				

than in the study conducted from Addis Ababa among slaughtered cattle (39.3%) [66], Ambo and Holeta among beef (15.0%) [61], and Jimma among apparently healthy slaughter cattle (42.4%) [62]. However, a closer tetracycline resistance rate of *Salmonella* among animal isolates was reported from the study conducted in Debre Zeit and Modjo among chickens (82.0%) [67]. This increase in resistance could be due to the unregulated increase in the prescription of antimicrobials [68], often purchased and used by unskilled practitioners in the veterinary and public health sectors, especially ampicillin and tetracycline/oxytetracycline [69]. There is also a lack of compliance and monitoring for antimicrobials at all healthcare levels in Ethiopia. Additionally, using antimicrobial drugs at sub-therapeutic or prophylactic levels in food animals can lead to the selection of antimicrobial resistance genes in *Salmonella* and other pathogens that affect humans and animals [27].

In the current study, a low level of animal *Salmonella* isolate resistance was recorded to chloramphenicol (11.1%; 95% CI 0.6–49.3), gentamicin (11.1%; 95% CI 0.6–49.3), and ciprofloxacin (11.1%; 95% CI 0.6–49.3). This low level of resistance was equivalent to the study conducted in Jimma (15.2%) [62] for chloramphenicol, Addis Ababa (7.1%) [66] for gentamicin, and China (5.9%) [70] for ciprofloxacin. However, the current resistance rate of animal *Salmonella* isolated to chloramphenicol, gentamicin, and ciprofloxacin was higher than the studies conducted in Adama and Modjo [44] and Central Ethiopia [63], which did not document any resistance of isolates against the drugs. Contrastingly, the result of this study was relatively lower than the finding from Vietnam (62.98%) [71] for chloramphenicol, Libya (69.0%) [64] for gentamicin, and Pakistan (61.4%) [65] for ciprofloxacin. In contrast to earlier research, the comparatively low resistance rates observed in this study may be attributed to the lesser or minimal use of these antibiotics on Ethiopian farms than in other countries. According to this research and previous reviews, none of the farms in the current study, along with a few in prior studies conducted in Ethiopia [44, 72, 73], reported the use of these antibiotics.

The prevalence of MDR among human *Salmonella* isolates in this study was 6.12% (95% CI = 1.4–17.4, *N* = 49), which is similar to the reports from Addis Ababa (5.6%) [47] and Jimma (5.2%) [53]. However, the MDR level of *Salmonella* among human isolates in the current study was lower than in the study conducted in Debre Berhan (40.0%) [46], Butajira (27.5%) [32], and Hawassa (40.0%) [52]. Moreover, no MDR animal *Salmonella* isolates were recorded in this study, which is in agreement with the study conducted in Addis Ababa among slaughtered cattle (0.0%) [66]. However, significant levels of MDR *Salmonella* isolates were recorded in Gondar (73.9%) [41]

Table 7B Bivariable and multivariable logistic regression analysis of factors associated with *Salmonella* infection among suspected patients at public health facilities in the Amhara region from September 2022 to January 2024

Possible associated factor Variables		Frequency (%)	Frequency (%)	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Contact with animals	Yes	22 (44.9)	93 (26.2)	2.295 (1.246–4.227)	0.008	3.179 (1.574–6.419)	0.001
	No	27 (55.1)	262 (73.8)	1			
Consumption of raw egg	Yes	1 (2.0)	4 (1.1)	1.828(0.200 -16.697)	0.593		
	No	48 (98.0)	351 (98.9)	1			
Consumption of raw meat	Yes	2 (4.1)	13 (3.7)	1.119(0.245–5.116)	0.884		
	No	47 (95.9)	342 (96.3)	1			
Consumption of raw dairy products	Yes	0 (0.0)	15 (4.2)	0.00(0.00–0.0)	0.999		
	No	49 (100.0)	340 (95.8)	1			
Consumption of raw vegetables	Yes	13 (26.5)	74 (20.8)	1.371(0.692–2.717)	0.366		
	No	36 (73.5)	281 (79.2)	1			
Contact with febrile individual	Yes	6 (12.2)	32 (9.0)	1.408(0.557–3.563)	0.470		
	No	43 (87.8)	323 (91.0)	1			
Drinking water supply	Pipe	38 (77.6)	298 (83.9)	1		1	
	Surface water	8 (16.3)	29 (8.2)	2.163 (0.922–5.074)	0.076	2.222 (0.771–6.41)	0.139
	Well	3 (6.1)	15 (4.2)	1.568 (0.434–5.668)	0.492	1.468 (0.351–6.139)	0.599
	Hand-pump	0 (0.0)	12 (3.4)	0 (0–0)	0.999	0 (0–0)	0.999
	River	0 (0.0)	1 (0.3)	0 (0–0)	1	0 (0–0)	1
Family size	< 3	7 (14.3)	71 (20.0)	1			
	3–5	36 (73.5)	236 (66.5)	1.547(0.660–3.627)	0.315		
	> 5	6 (12.2)	48 (13.5)	1.268(0.401–4.005)	0.686		
Contact with Diarrheic Patient	Yes	8 (16.3)	22 (6.2)	2.953(1.235–7.062)	0.015	2.83 (1.05–7.629)	0.04
	No	41 (83.7)	333 (93.8)	1			
Live Outside residences for the last 7 days	Yes	7 (14.3)	38 (10.7)	1.390(0.584–3.312)	0.457		
	No	42 (85.7)	317 (89.3)	1			
Comorbidity	Yes	0 (0.0)	6 (1.7)	0.0(0.0–0. 0)	0.999		
	No	49 (100.0)	349 (98.3)	1			
Total		**49 (100.0)**	**355 (100.0)**				

and Ambo and Holeta (30.0%) [61] previously. This low level of MDR in the current study among human and animal *Salmonella* isolates may be due to current changes in the definition of MDR among *Salmonella* isolates [74]. In other ways, a high level of resistance (71.4% and 88.9%) to three or more classes of antibiotics was observed among human and animal *Salmonella* isolates in the current study, respectively.

In the present study, participants who had contact with diarrhea patients were 2.8 times at higher risk of *Salmonella* infection (AOR = 2.83; 95% CI: 1.05–7.629; *P* = 0.04) than their counterparts. This finding was comparable with the study conducted in Ethiopia [4, 75] and other countries, such as China [76] and Denmark [77]. Another factor that increased the odds of acquiring salmonellosis was contact with animals (3.179; 95% CI: 1.574–6.419; *P* = 0.001). This was supported by a wide range of evidence showing that contact with animals increases the likelihood of zoonotic *Salmonella* infection [22, 38, 78–82]. Other variables, such as the consumption of raw eggs, raw meat, and raw vegetables, did not show any significant association, even though the burden was higher among those who consumed them. Education on personal hygiene and hand washing after handling diarrheal patients and animals can help prevent and control *Salmonella* bacteria through one health approach [55]. Ethiopia established a National One Health Steering Committee (NOHSC) and Technical Working Groups (TWG) on October 2, 2018, as part of its One Health initiative. This initiative includes a second phase of a five-year strategic plan for the period of 2022–2026 [83]. However, the creation of awareness about One Health principles and their importance for the community and responsible bodies remains limited. Additionally, weak encouragement and collaboration among regional One Health task forces further restrict the community's and responsible entities' awareness.

Limitations of the study

The study intentionally excluded certain animals not typically part of the traditional Ethiopian diet—such as equines and pork—which may harbor *Salmonella* bacteria and pose public health risks. Additionally, due to budget constraints, the researchers could not conduct comprehensive molecular testing that would have offered valuable insights into the genetic relationships between

Salmonella strains found in humans and those obtained from animals. This limitation hinders a deeper understanding of the transmission pathways of this disease in the region.

Conclusion and recommendation

The prevalence of *Salmonella* infection among humans and animals was notably high compared to the worldwide frequency. Most *Salmonella* isolates from humans and animals were highly resistant to ampicillin and tetracycline. More than two-thirds of the *Salmonella* isolates from human and animal samples were resistant to three or more antibiotics in different classes of antibiotics. Ciprofloxacin and ceftriaxone are promising antimicrobial options for treating *Salmonella* infections in humans. Conversely, for animals, chloramphenicol, gentamicin, and ciprofloxacin are considered effective antimicrobials for treating *Salmonella* infections.

After controlling for confounding variables, our findings revealed that contact with animals and exposure to diarrheic patients were significant predictors of salmonellosis in suspected human cases. Routine implementation of culture-based bacterial detection and antibiotic sensitivity testing is crucial for antimicrobial prescriptions. The results indicated that it is essential to enhance the One Health approach within both human and animal health systems to effectively manage zoonotic diseases like *Salmonella*. This is crucial for preventing drug resistance, especially through an antimicrobial stewardship program that is currently inadequately implemented in Ethiopia. Moreover, it is prudent to undertake more extensive longitudinal studies.

Abbreviations

AOR	Adjusted Odds Ratio
AST	Antimicrobial Susceptibility Test
CDC	Centers for Disease Control and Prevention
CLSI	Clinical Laboratory Standard Institute
MDR	Multidrug-resistant
NOHSC	National One Health Steering Committee
NTS	Non-typhoidal Salmonellosis
ROC	Receiver Operating Characteristic
SOP	Standard Operating Procedure
TWG	Technical Working Groups
WHO	World Health Organization

Supplementary Information

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Supplement 1: Data collection tools

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Author contributions

The following are the contributions of the authors GM - Perform Conceptualization, Design of the work, Writing - original draft, Writing - review and editing, Investigation, Software, Data curation, Supervision, Formal analysis, Project administration, Validation, Funding acquisition, Resources, and VisualizationAN - Perform Design of the work, Writing - review and editing, Investigation, Data curation, Supervision, Project administration, Validation, and VisualizationBG - Perform Conceptualization, Design of the work, Writing - review and editing, Data curation, Supervision, Project administration, Validation, Funding acquisition, and VisualizationFM - Perform Conceptualization, Design of the work, Writing - review and editing, Investigation, Software, Data curation, Supervision, Formal analysis, Project administration, Validation, Resources, and Visualization.

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Data availability

Data is provided within the manuscript file.

Declarations

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki, and ethical clearance was obtained from the Institutional Review Board of the University of Gondar (Ref. number V/P/RCS/05/839/2021) and Amhara Public Health Institute (Ref. number 03/1363) before the actual data collection. Written permission to conduct the study was obtained from Bahir Dar, Gondar, Debre Markos, and Dessie town health offices. Then, a support letter was written to health institutions to obtain permission for the data collection process. Written informed assent and consent were obtained from the participants and their parents or guardians. Study participants were told that their participation was entirely voluntary and that they had the right to withdraw from the study at any moment. Anonymity and confidentiality concerns were also addressed. All study participants' results were promptly shared with their clinicians so that the proper course of action could be taken.

Consent for publication

Not applicable.

Clinical trial

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Medical Laboratory Science, College of Health Sciences, Debre Markos University, P.O. Box 269, Debre Markos, Ethiopia
[2]Department of Paraclinical Sciences, College of Veterinary Medicine and Animal Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia
[3]Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia

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