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Isolation, Identification and Antimicrobial Resistance Profile of Salmonella in Raw cow milk & its products in Bishoftu city, central Ethiopia: implication for public health

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Abstract

Background *Salmonella* is a significant foodborne pathogen, with milk and milk products commonly implicated in its transmission. However, limited information is available regarding the direct link between antimicrobial use (AMU), dairy hygiene practices, and antimicrobial resistance (AMR) in *Salmonella* strains isolated from dairy products in Bishoftu town.

Methods Cross-sectional research was done from October 2023 to April 2024 to assess dairy farmers' antimicrobial usage (AMU) and hygiene practices and the occurrence of antimicrobial resistance (AMR) profiles of *Salmonella* isolated from raw cow milk and its products. A structured questionnaire was also used to assess the milk value chain's knowledge, attitude, and practices (KAP) regarding AMU, AMR, and hygiene practices. Salmonella isolation and identification was conducted using standard microbiological techniques and further confirmation was carried out using the OmniLog system. An antimicrobial susceptibility test was performed using the Kirby-Bauer disk diffusion technique..Data was analyzed using STATA version 14.2.

Results Among 41 dairy farmers interviewed, it was found that most of the respondents had sufficient knowledge (78%), desired attitudes (90%), and good practices (76%) regarding AMU and AMR. However, 36% of dairy farms had poor hygienic practices. Overall, 2% ($n=4$) of the samples tested positive for *S. enterica*. Of the 4 isolates, 3 were identified in dairy farm samples, whereas 1 was isolated from milk vendors. However, no *Salmonella* was identified in cheese or yoghurt samples obtained from the restaurants. Regarding the AMR profile, *S. enterica* isolates were resistant to amoxicillin (75%), streptomycin (75%), and tetracycline (50%). Resistant to two or more antimicrobials were identified in 75% of the isolates.

Conclusion This study indicated contamination of cow milk and its products with *S. enterica*. Therefore, appropriate control measures, including awareness creation among personnel and improving hygienic practices at the milk value chains is recommended to mitigate cross-contamination.

Keywords AMU and AMR, KAP assessment, Milk vendors, And S. enterica

Introduction

Foodborne illnesses seriously threaten global public health, safety, and the economy. Every year, an estimated 600 million infections and 420,000 fatalities occur because of food-borne diseases [31]. *Salmonella species*, prominent among foodborne pathogens, ranks as the

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third largest cause of mortality from diarrheal diseases globally [20]. It is responsible for an estimated 115 million human infections and 370,000 fatalities every year [48]. The World Health Organization (WHO) estimates that around one in ten individuals become sick from foodborne Salmonella infections each year, resulting in the loss of millions of healthy life years [38]. This problem is widespread, affecting countries globally, but developing countries face challenges due to inadequate food safety regulations, poor food handling practices, and limited financial resources [2, 6, 12].

Salmonella species are gram-negative, rod-shaped bacteria from the Enterobacteriaceae family and consist of two species, *Salmonella bongori* and *Salmonella enterica*, according to the White-Kauffmann system. This categorization is based on the surface structures (lipopolysaccharides, flagella, and capsular polysaccharides). The species *Salmonella enterica* has six subspecies: enterica, salamae, arizonae, diarizonae, houtenae, and indica, with around 2600 serovars (Vinueza, 2017;[20]. Among these, the subspecies *Salmonella enterica* is responsible for about 1500 serovars, of which 99% might cause infections in animals and humans [10]. *Salmonella enterica* is classified into two classes based on clinical features of human infections: Typhoidal Salmonella is specific to humans and causes typhoid fever, but Nontyphoidal Salmonella (NTS) has a wide range of hosts and causes several illnesses other than typhoid fever (Fanta, 2021). NTS serotypes are the leading cause of bacterial diarrhea and invasive infections, posing a significant risk to young children, the elderly, and those with weakened immune systems in developing countries [7].

Salmonella, a ubiquitous bacterium, poses significant public health concerns due to its ability to infect various animals and contaminate the environment. It is commonly found in the digestive systems of both domestic and wild animals [17] and shed in their feces, facilitating its widespread presence in animal waste, sewage, and contaminated materials (Pal et al., 2015; [1]). As a result, milk and dairy products are susceptible to contamination by infected animals or cross-contamination with fecal-containing pathogens during processing (Teklemariam *et al*., 2023). When humans consume contaminated food, they can develop salmonellosis, a diarrheal illness that can range from mild to severe, including abdominal cramps, fever, nausea, and vomiting. In extreme circumstances, *Salmonellosis* can cause dehydration and even death (Pal et al., 2015).

Milk is considered a highly nutritious food, but it can also be a vehicle for microbial hazards, particularly in developing countries where the hygiene and sanitation practices of dairy farms are inadequate (Kashima et al., 2013). The bacteria that are associated with raw milk include *Escherichia coli O157:H7, Salmonella enterica, Listeria monocytogenes, Campylobacter spp*., and *Staphylococcus aureus* (Verra et al., 2015). These can induce severe gastroenteritis in humans [59]. Infections with *Salmonella species* are particularly significant because they cause bacteremia in adults and children in developing countries [7].

Antimicrobial resistance (AMR) has emerged as a significant worldwide public health problem, posing substantial challenges to effectively treating bacterial infections [50]. *Salmonella*, a common foodborne pathogen, is among the bacteria that have developed resistance to various antibiotics. The inappropriate use of antibiotics in livestock particularly, dairy farms have contributed to the rise of antimicrobial-resistant (AMR) Salmonella which can increase human health risks (Endrias [2, 61]). Some MDR *Salmonella* infections in humans have been connected to exposure to dairy farms or contaminated dairy products [1]. *Salmonellosis*, a costly disease affecting dairy producers, can lead to limited treatment options, prolonged illnesses, decreased milk yield, increased healthcare costs, and potentially fatal outcomes. Farmers must be aware of *Salmonella's* presence in seemingly healthy cows, as it poses significant food safety concerns [2], Bedhasa et al., 2022).

In nations with low and middle incomes, such as Ethiopia, there is a growing demand for animal protein, leading to the routine use of antibiotics for growth-promoting, therapeutic, and preventative reasons in livestock production [56]. Improper antibiotic use in livestock, particularly on dairy farms, along with inadequate waste management practices, can result in the release of resistant pathogens into the environment. This practice poses a significant risk as it can contribute to the emergence of antibiotic-resistant pathogens. This may lead to the development of antibiotic-resistant commensal organisms in livestock, posing a threat to public health [45].

Besides improper antibiotic use, the habit of consuming raw milk or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items have been reported as contributing factors to an outbreak of salmonellosis in human [57]. This suggests that milk and dairy products could be a source of *Salmonella* in Ethiopia in general and may be particularly significant in the central part of Ethiopia, where consumption of milk and milk products is high. Therefore, it is important to isolate pathogenic organisms, identify relevant risk factors, and regularly assess their AMR profiles.

In Ethiopia, despite multiple studies identifying *Salmonella* in milk and dairy products, including evidence of its prevalence among raw milk consumers [2, 13, 18, 53], there remains a critical gap in understanding the direct relationship between antimicrobial use (AMU), dairy hygiene practices, and the development of antimicrobial resistance (AMR) in *Salmonella* isolated from dairy products in the current study area. These gaps need for systematic surveillance and comprehensive investigation along the entire farm-to-fork continuum, encompassing routine examination of raw milk and milk product samples from restaurants, milk vendors, and dairy farms. Addressing these gaps is crucial to safeguarding consumer health, mitigating foodborne illnesses, and minimizing both direct and indirect economic losses associated with contaminated dairy products in Ethiopia. Hence, the objectives of this study were to isolate *Salmonella* from milk and milk products, to evaluate the AMR profile of *Salmonella* isolated from milk and other dairy products and to assess dairy farmers' AMU and hygienic practices in Bishoftu dairy farms.

Material and methods

Description of the study area

This study was conducted at dairy farms, milk vendors, and restaurants in Bishoftu town. Bishoftu town was purposefully selected because of its larger potential for dairy cattle density, which may pose a risk of *Salmonella* contamination in dairy products due to cross-contamination along the milk value chain. Bishoftu town is located in the east Showa zone of the Oromia region, situated approximately 45 km southeast of Addis Ababa (Fig. 1). The city is situated at 9° North latitude and 40° East longitude, with an altitude of 1850 m above sea level in the central highlands of Ethiopia. The town experiences an annual rainfall of 866 mm, with 84% occurring during the long rainy season from June to September, and the remainder in the short rainy season extending from March to May. The dry season extends from October to February. The mean annual maximum and minimum temperatures in the area are 26 °C and 14 °C, respectively, with a mean relative humidity of 61.3% [43].

Study population

The study population was all dairy farms and milk and milk product sellers found in Bishoftu town. A total of 41 dairy farms, 14 milk vendors and 28 restaurants selected randomly were included in the study. The study animals were apparently, healthy dairy cows in small-scale, medium-scale, and large-scale dairy farms located in the selected study areas. The study population included

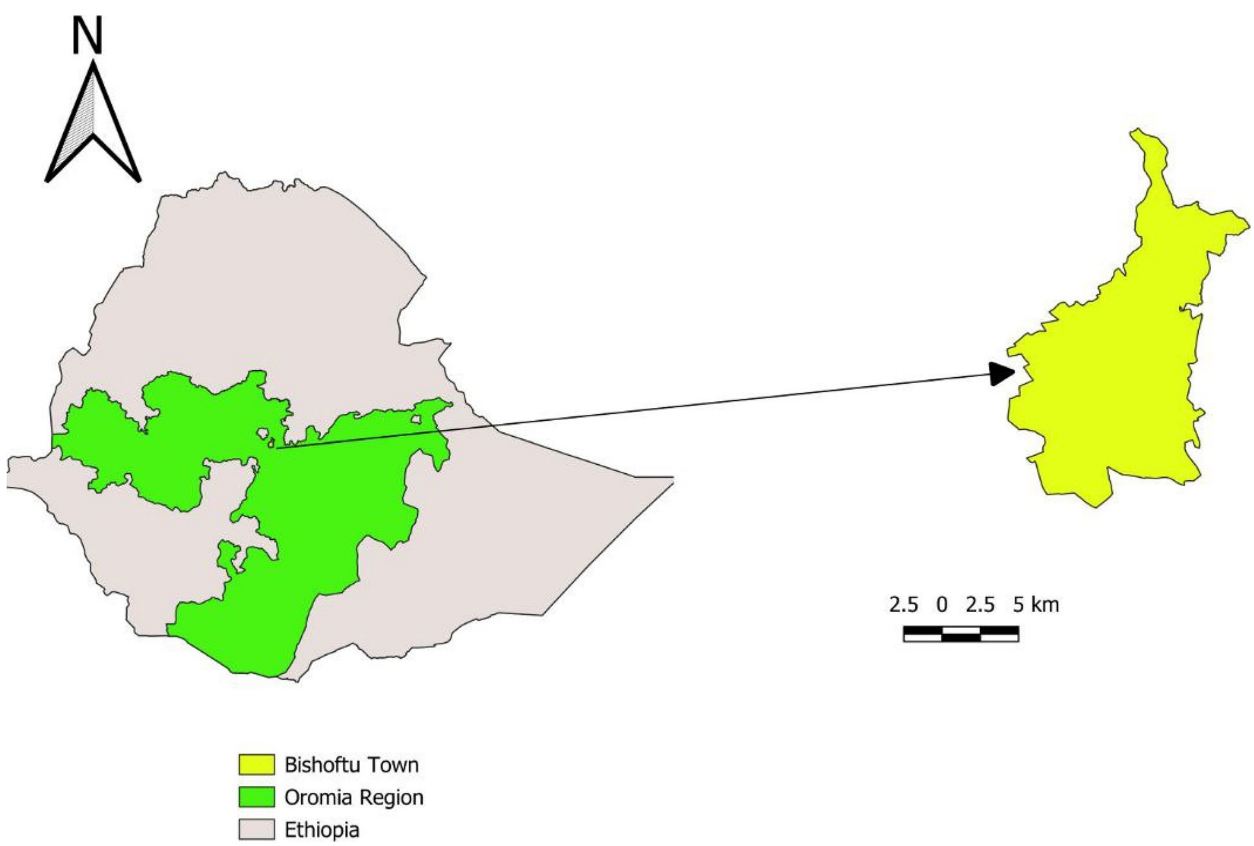

Fig. 1 Map of study Area (Bishoftu town, Oromia Region, Central highland of Ethiopia

crossbreeds and local breeds in small-scale, medium-scale, and large-scale dairy farms. Most of them (85%) were crossbreeds whereas a few were local (15%). Concerning management, (78%) of the herds were managed intensively while (22%) of herds were semi-intensive. The intensively managed cattle were kept indoors and received concentrate feeds in addition to hay and crop residues (such as corn stalks, wheat/barley straw and other leftovers from grain threshing). On the other hand, the semi-intensively managed cattle grazed freely on pasture but received supplementary feed in the morning and evening when they were milked. All cows were hand-milked twice daily, in the morning and evening.

Study design, source of sample and sampling method

A cross-sectional study design was used to assess AMU and hygienic practices of dairy farmers, and the occurrence of AMR *Salmonella* isolated from raw cow milk and milk products in the study area from October 2023 to April 2024. For this study, raw milk and milk products (cheese and yoghurt), floor swab, fecal samples and swabs from milk containers were gathered from various sources (milk vendors, restaurants, and dairy farms) in Bishoftu town. In addition, cheese and yoghurt samples were collected from restaurants, whereas bulk milk samples were collected from milk vendors.

A stratified random sampling method was used to collect samples from dairy farms. The farms were categorized based on their herd size into three strata; small-scale < 10 animals, medium-scale 10 to 50 animals, and large-scale > 50 animals using the classification made by Megersa et al., [39]. A simple random sampling technique was employed to select dairy farms, restaurants and milk vendors. Similarly, milk containers were selected by simple random sampling to collect appropriate raw milk and milk product samples. The milk and milk product samples were clearly labeled with the date of sampling, the type of sample, and the name of the farm and then held in an icebox with ice packs and transported to the Veterinary Public Health (VPH) laboratory of the College of Veterinary medicine and agriculture, Addis Ababa University (AAU-CVMA). In the laboratory, the samples were stored at 4 °C for a maximum of 24 h until they were transferred into an enrichment medium and inoculated onto a standard bacteriological media. After the isolation of *Salmonella*, the positive isolates were transported to Animal Health Institutes (AHI), Sebeta by standard transporting medium for confirmation.

Sample size determination

A simple random sampling technique was used. The necessary sample size was determined concerning the estimated prevalence of *Salmonella* and the desired minimum precision level, as outlined by Thrusfield (2007). The formula calculating the sample size,

$$n = Z^2\, P \frac{(1 - P)}{d^2}$$

Where n = required sample size, d = desired absolute precision, and P_{exp} = expected prevalence.

According to [25], the expected prevalence of *Salmonella* in this study is 4.8%, and the desired minimum level of precision is 5% at a 95% confidence level, with a z value of 1.96. Therefore, the minimum required sample size was 70. However, to increase the precision of the study, 200 samples were collected, including 41 bulk milk sample from dairy farms, 41 swab samples from milk containers, 21 fecal samples from apparently healthy cows, and 41-floor swab samples from cow environment and 14 cheese samples and 14 yoghurt samples from restaurants and 14 raw milk samples and 14 swab samples of milk container from open market milk vendors. The sample collection process was on a voluntary basis, and the willingness of the owners to provide the samples was considered at the farm level. In contrast, raw milk at milk vendors and cheese and yoghurt samples from restaurants were purchased. A structured questionnaire was also used to collect socio-demographic information and potential risk factors contributing to the antimicrobial-resistant profile of *Salmonella* isolated from milk and milk products.

Sample collection and transportation

Samples were obtained from various sources, including dairy cows (bulk milk, swab from milk containers, pooled floor swabs, and feces), raw milk from milk vendors, and cheese and yoghurt from restaurants. These samples were collected at the beginning of the day, with the timing arranged in advance with the farmers and milkers. The fecal samples were collected directly from the rectum and placed in a 50 ml universal screw-capped bottle containing 10 ml of peptone water as transport media. After milking, the milk samples were collected aseptically from the bulk tank and placed in a milk container. The raw milk cheese and yoghurt samples were purchased and collected in plastic bags.

The swab samples were collected before milking using a sterile wooden cotton swab and placed in a sterile test tube containing 10 ml of buffered peptone water as transport media. All samples were labeled and transported immediately to the Veterinary Public Health Laboratory of the AAU-CVMA for bacterial isolation. Finally, the suspected colony of Salmonella was confirmed at the Animal Health Institute (AHI), Sebata by using the

OmniLog system and antimicrobial susceptibility testing (AST) was performed on the isolates.

Bacteriological isolation of *Salmonella*

The bacteriological analysis was conducted following the microbiology of the food chain guidelines, specifically, the horizontal method outlined in ISO-6579–1, 2017 [40], for the detection, enumeration, and serotyping of *Salmonella*.

Biochemical characterization of *Salmonella* isolates

All potential *Salmonella* isolates underwent a series of biochemical tests for identification, including the Triple Sugar Iron (TSI) test, Indole test, Citrate utilization test, Methyl red test, Vogues Proskauer (VP) test, and urease test. Isolates meeting these criteria were then transferred and cultured on Nutrient Agar (NA) for antimicrobial sensitivity testing [40].

Identification of *Salmonella* using OmniLog

To identify *Salmonella,* the isolate to be identified was grown on Biolog Universal Growth (BUG) agar medium and then a single colony was suspended in a special "gelling" inoculating fluid (IFA). Then, 100 μL of the cell suspension was inoculated into a well of the GEN-III Micro Plate, and the Micro Plate was incubated to allow the phenotypic fingerprint to form. After incubation for 22 h at 33 °C the phenotypic fingerprint pattern was read by a combination of the Biolog MicroStation reader. The fingerprint data was imported into Omnilog Data Collection software, which searched an extensive database and made an identification call in seconds. The identification process of *Salmonella* involves four main steps. These steps were isolation of a pure culture on Biolog media, preparation of inoculum, inoculation of Micro Plates and load into the reader, and obtaining of ID results from the printer [14].

Antimicrobial susceptibility test

The pure isolates of *Salmonella* identified using OmniLog were subjected to AST using the Kirby–Bauer agar disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2022). A sterile cotton swab was used to swab the inoculum uniformly over the surface of the Mueller Hinton Agar (Criterion, C6421, USA) plate. The diameters of the clear zone of inhibition produced by diffused antimicrobial on lawn-inoculated bacterial colonies were measured to the nearest mm using a caliper. All eight zones of inhibition against eight antimicrobial agents for each isolate were recorded and compared with standards and interpreted as resistant, intermediate, or susceptible according to a published interpretive chart (CLSI, 2022).

Data analysis

The raw data generated from the laboratory work was arranged, organized, coded and entered an Excel spreadsheet 2010. Additionally, the KAP survey data gathered through the Kobo Toolbox server was retrieved as Excel files, carefully reviewed for errors, coded, and subsequently imported into the data analysis software. Data were analyzed using Stata/IC version 14.2. The laboratory results of *Salmonella* detected, and their AMR profile were mostly described in proportion.

Ethical clearance

This study was granted ethical approval by the College of Veterinary Medicine Animal Research Ethics Committee of Addis Ababa University, with reference number VM/ERC/02/09/16/2024. All procedures were executed by skilled professionals according to the guidelines and regulations established by the University's ethics committee. The welfare and well-being of the animals that participated in this study were ensured throughout the research. Before the commencement of the study, verbal consent was obtained from all farm owners for both the questionnaire interview and the collection of milk and fecal samples from their animals.

Results

Assessment of dairy farm workers regarding the KAP of AMU and AMR

The results of the average mean score analysis of participants' responses regarding their knowledge of AMU and AMR revealed that most respondents (78%, $n = 32/41$) demonstrated a good understanding of AMU and AMR. Conversely, 22% of respondents exhibited poor knowledge in this area. Three fourth (75%) of respondents identified antibiotics as effective against bacteria and most (97.6%) of respondents were aware of antibiotic resistance. More than half of participants (63.4%) recognized that antibiotic resistance can result in treatment failure and poor response to treatment and more than half of (53.7%) of participants were aware that an overdose or low-dose course of antibiotics can lead to AMR. Most (80.5%) of respondents understood that incomplete antibiotic courses could lead to antibiotic resistance, (87.8%) were aware of the antibiotic withdrawal period and (80.5%) were aware of antimicrobial side effects.

The attitude of AMU and AMR, the average mean score of respondents of dairy farm workers revealed that more than (90%) of respondents had a positive attitude toward AMU and AMR while 10% of respondents had a negative attitude toward AMU and AMR. Most (85%) of the respondents agreed that consulting a veterinarian before using antimicrobials is necessary. Majority (90%) of respondents disagreed with selling animal products or slaughtering animals during antimicrobial treatment without observing a waiting withdrawal period, while around 10% agreed with this statement. Most (87%) of respondents believed that adequate biosecurity, vaccination, and good management practices help reduce the use of antimicrobials and the majority (85%) of respondents disagreed that the use of antibiotics as growth promoters is necessary for livestock production.

Logistic regression analysis revealed that there was a significant association ($p < 0.05$) found between level of education, and knowledge and practice of the respondents. Moreover, statistically significant association ($p < 0.05$) was also found between the hygiene of feed and water storage and attitude (Table 1).

Prevalence of *Salmonella* based on bacteriological identification

A total of 200 samples were collected for bacterial examination from dairy farms, milk vendors, and restaurants. Of these, 2% (4/200) of the samples were found to be positive for *Salmonella (* 2.1% (95% CI: 0.7–6.3), 3/144 of the farm samples and 3.57% (95% CI: 0.44–23.7), and 1/28 of the milk vendor samples). However, no *Salmonella* was detected in any of the cheese or yoghurt samples collected from the restaurants. In general, of the 4 isolates, three were isolated from samples collected from dairy farms, whereas on were isolated from milk vendors. The study revealed a higher prevalence of *Salmonella enterica* at the farm level in comparison to milk vendors (Table 2).

Prevalence of *Salmonella* based on farm types

From the total of 41 dairy farms enrolled in this study (5 small scales, 34 medium scales and 3 large scales), the overall prevalence of *Salmonella* was 7.32%, with most samples testing negative (92.68%). Of these farms, 1 was small and the remaining 2 were medium-sized. From these, 1/41 (2.43%) and 2/41 (4.88%) *Salmonella* isolates were obtained from small-size and medium-size farms

Table 1 Association of KAP score with demographic characteristics

Variables	Category	Knowledge		Attitude		Practices	
		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Sex	Female	1.07(0.13–8.44)	0.95	1.52(0.28–8.43)	0.63	1.13(0.22–9.31)	0.88
Marital status	Single	0.55(0.05–6.42)	0.63	1.79(0.21–15.01)	0.53	1.86(0.25–13.87)	0.54
Level of education	High school	2.15(0.13–34.14)	0.58	1.38(0.13–14.35)	0.27	0.07(0.01–1.21)	0.06
	Graduated from college/university	24.15(1.87–311.97)	0.015	6.88(1.05–44.99)	0.04	0.75(0.12–4.66)	0.75
Management type	Semi-intensive	0.22(0.02–2.30)	0.205	1.64(0.19–14.04)	0.65	1.06(0.12–9.31)	0.95
Feed and water storage hygiene	Good	5.93(0.55–63.43)	0.141	2.04(0.28–14.87)	0.48	7.07(0.67–75.06)	0.10
	Poor	16.71(0.75–373.27)	0.076	0.07(0.01–0.94)	0.04	0.45(0.05–3.92)	0.46
Coefficient		0.47	0.47	0.61	0.59	2.55	0.33

Table 2 Prevalence of *Salmonella* from milk and milk products in Bishoftu town, Central Ethiopia

Sample source	Sample type	Number of samples examined	Pχositive *Salmonella*	Percentage (%)
Dairy farm	Bulk milk	41	1	2.44
	Fecal sample	21	1	4.67
	Pooled floor swab	41	1	2.44
	Swab of milk container	41	0	0
	Total	144	3	2.1
Milk seller	Raw milk	14	1	7.1
	Swab of milk container	14	0	
	Total	28	1	3.57
Restaurants	Cheese	14	0	0
	Yoghurt	14	0	0
	Total	28	0	0
Total		200	4	2
		Pearson $\chi^2(7)$ *P*-Value	4.6025 0.708	

respectively; and no *Salmonella* was isolated from large-scale dairy farms. The Pearson chi-squared test revealed 9.65 with a p-value of 0.047. This suggests a statistically significant association between sample type and the occurrence of the pathogen across dairy farms (Table 3).

Antimicrobial susceptibility profiles of *Salmonella*

The study revealed that the common antimicrobials used in the farms were oxytetracycline, fixed combinations of penicillin + streptomycin (pen strep), and sulfonamide in 100%, 100% and 65.9% of the farms, respectively. *Salmonella* isolates were subjected to an AST against 8 selected antimicrobial agents. Accordingly, 100%, 75%, and 75% of the isolates were found to be susceptible to gentamicin, amoxicillin-clavulanic acid, and ciprofloxacin, respectively. On the other hand, 75%, 75%, and 50% of the isolates were resistant to amoxicillin, streptomycin, and tetracycline (Table 4).

Multidrug resistance profile of *Salmonella*

Multidrug resistance (MDR) profile of *Salmonella* isolated from bulk milk samples, fecal samples and floor swabs collected from dairy farms and raw milk collected from milk vendors showed 75% ($n = 3/4$) of the isolates were resistant to more than two classes of antibiotics. *Salmonella* isolates from fecal samples showed high resistance to four classes of antibiotics while from bulk milk the isolates showed resistance to three classes of antibiotics. Additionally, raw milk collected from milk vendors showed resistance to a minimum of two classes of antibiotics as shown in Table 5.

Discussion

Globally, *Salmonella species* are recognized as prominent foodborne pathogens and rank as the third leading cause of death among diarrheal illnesses in human populations. The primary reservoir of this pathogen is in animals,

Table 3 Prevalence of *Salmonella* in small, medium and large-scale dairy farms

Sample type	Result	Farm size[a]			Total
		Small scale	Medium scale	Large scale	
Bulk milk	Positive	0	1(2.94%)	0%	
	Negative	5(100%)	33(97.06%)	3(100%)	
Swab of milk container	Positive	0	0	0%	
	Negative	5(100%)	34(100%)	3(100%)	
Floor swab	Positive	1(20%)	0	0%	
	Negative	4(80%)	34(100)	3(100%)	
Fecal sample	Positive	0	1(2.94)	0%	
	Negative	5(100%)	33(97.06%)	3(100%)	
Total	Positive	1(20%)	2(5.88)	0%	3(7.32%)
	Negative	4(80%)	32(94.12%)	3(100%)	38(92.68%)
	$\chi 2(4) = 9.6471$ *P*-value = 0.047				

[a] Number of small-scale farms = 5, medium scale = 34 and large-scale farm = 3

Table 4 Antimicrobial susceptibility profile of *Salmonella* isolated from cow milk and its product from the dairy farms and milk vendors in Bishoftu town, central Ethiopia

Antimicrobial Class	Antimicrobials tested	The number of isolates tested	Status of antimicrobial agent against the isolate		
			R (%)	I (%	S (%)
Tetracycline	Tetracycline	4	50	0	50
B-lactam	Ampicillin	4	0	50	50
	Amoxicillin	4	75	0	25
	AMC	4	0	25	75
Aminoglycosides	Gentamicin	4	0	0	100
	Streptomycin	4	75	0	25
Sulfonamides	TMP-SXT	4	25	25	50
Quinolones	Ciprofloxacin	4	25	0	75

Key R Resistant, *I* Intermediate, *S* Susceptible, *%* Percent; *TMP-SXT* Trimethoprim-Sulfamethoxazole, *AMC* Amoxicillin-Clavulanic acid

Table 5 Multidrug resistance profile of *Salmonella*

Antibiotics	Source of MDR	Frequency	Number of antibiotic classes	Percentage
AX, CIP, TE	Bulk milk	1	3	25%
AX, TE, S TMP-SXT	Fecal sample	1	4	25%
AX, S	Floor swabs	1	2	25%
S	Raw milk	1	1	
Overall MDR%		**3**		**3 (75%)**

AX Amoxicillin, *CIP* Ciprofloxacin, *TE* Tetracycline, *S* Streptomycin, *TMP-SXT* Trimethoprim-Sulfamethoxazole, *MDR* Multidrug resistance

with transmission to humans predominantly occurring through the consumption of animal-source foods including cow milk and its products [20]. Contamination of the environment and along the food chain with bacteria is often attributed to the presence of animal and human wastes that have been contaminated by bacterial pathogens [1].

The good knowledge demonstrated by the respondents about AMU and AMR is encouraging and it is consistent with the findings of [47] in Vietnam. In contrast, a significant proportion of livestock keepers in Ethiopia, as reported by Gemeda et al. [26], lacked knowledge about AMU and AMR. This disparity could be attributed to varying levels of awareness among livestock producers in different regions [23], however, Gebeyehu et al. [23] reported that the majority of farmers had a negative. This difference may stem from varying levels of knowledge and awareness about antimicrobial resistance, which can influence attitudes toward these issues. Encouragingly, all dairy farm workers (100%) stated that they did not add antimicrobials to animal feed, a result consistent with Hossain et al. [33] in Bangladesh, who reported that 98.1% of farmers refrained from such practices. This suggests adherence to appropriate antimicrobial usage practices. Additionally, all respondents reported completing the course of antibiotics within the last six months on their farms. This finding differs from a report in Ghana, where 63% of farmers tended not to complete antibiotic courses. Regional variations in antibiotic use practices may be influenced by factors such as awareness, education on proper antibiotic use, access to veterinary services, and the regulatory environment. Concerning the hygienic practices of dairy farms and milk vendors, the survey revealed that 60% of respondents practiced good hygiene,which is in agreement with Bedassa [11], who highlighted the type of milk container and water source used for cleaning milking equipment as significant risk factors for Salmonella contamination.

In the present study the isolation of of *Salmonella enterica* from bulk milk samples at dairy farms which is consistent with the previous research conducted in different locations; which is in agreement with the results reported by Liyuwork et al. (2013) in Addis Ababa, Ethiopia, and by Van et al. [56] in the United States of America where a prevalence rate of 2.1% and 2.6% were reported respectively. Similarly, the prevalence rate of *Salmonella* isolated from milk samples in Egypt, as reported by Ahmed and Shimamoto [5] was 1.5% and in Jigjiga town by Reta et al. (2016), was 3.3%, which is within the range of the current study's findings.Additionally, the prevalence rate reported by Murinda et al. [42] in the USA was 2.24%, further supporting the consistency of the present study's results. However, the prevalence of *Salmonella* isolated from bulk milk in this study is relatively higher than the report of Abunna et al. [3] and Dadi et al. [16] which was 0% and 0.7% at Meki and Sebata town Oromia, Ethiopia respectively. On the other hand, from Dire Daw (18.75%) by Tesfaye et al. (2013), Central Ethiopia (10%) by Geletu et al. (2023) and reports from Gondor (6.0%) by Ejo et al. [18] are much higher than the current investigation.The difference in the relative amount of the bacteria present in milk between the current study and previous research carried out in various study areas in Ethiopia could be explained by variations in the potential risk factors contributing to the occurrence of *Salmonella* in milk. Several factors, such as milking procedures, milk handling practices, hygiene and management practices, stocking density, use of contaminated utensils, housing type, animal movement, milking environment, ventilation, and production facilities in different areas, are examples of the main risk factors that influence the occurrence of *Salmonella* [2, 22, 28]. Furthermore, methods employed in the research areas may also be a factor in the variation in the relative isolation rate of *Salmonella.*

The isolation of *Salmonella* from the bulk milk could pose serious health risks to humans by causing *Salmonellosis* in high-risk populations like newborns, infants, the elderly, and people with immune-compromised, who are susceptible to *Salmonella* infections at a lower infective dose than healthy adults. Because dairy products are frequently consumed in Ethiopia without being properly boiled [25], it is a source of *Salmonella* infection.

About 4.76% *Salmonella enterica* was isolated in fecal samples. This finding is in consistent with prior research conducted by Geletu *et a*l. (2023), who reported a similar prevalence rate of 4.7% in central Ethiopia. Moreover, it aligns with the results documented by Gezahegn et al. [28] in the Bedele and Nekemte districts of western Ethiopia, where a prevalence rate of 2.97% was reported. Factors that could explain this consistency include the possibility that common problems with animal husbandry practices, sanitation, and hygiene could have an impact on the observed prevalence rates irrespective of geographical location.The fecal prevalence of *Salmonella* in fecal samples was found to be lower than that reported in previous studies conducted by Abunna et al. [2] in Modjo town, Ethiopia, who documented a higher prevalence rate of 12% prevalence rate which was reported by Khan et al. [37] in the Republic of Korea. Additionally, our results were lower than those reported by Hailu et al. [30] in Northern Ethiopia. The observed differences in prevalence rates between our study and previous research can be due to various factors, including variations in sampling methods, duration of sampling period, environmental conditions, animal management practices, animal husbandry, biosecurity measures, sanitation protocols and geographical variability. Additionally, variations in laboratory techniques and procedures can affect the accuracy and comparability of prevalence estimates across studies.

The prevalence of *Salmonella* isolated from floor swabs is in consistent with the previous studies by Gezahagn et al. (2023) in Bedelle and Nekemte towns in western Ethiopia and by Geletu et al. [25] in central Ethiopia, where the prevalence rate was reported to be 5% in both studies. The consistency of prevalence rates in these studies could be attributed to similar environmental conditions, management practices and biosecurity measures applied on dairy farms in these regions. Factors such as poor hygiene, animal overcrowding and inadequate cleaning and disinfection protocols could contribute to the presence of *Salmonella* on dairy farm floors.

The average prevalence rate of *Salmonella* isolated from raw milk at milk vendors is in agreement with the report of Tusa et al. [55] in Asella Town Oromia, Ethiopia with a prevalence rate of 3.3% and the finding of [34] in Iraq with the prevalence rate of 3%. However, the prevalence rate of the present finding was much lower than the report of Tesfay et al. (2013), in Bangladesh and the finding of Anukampa et al. [8] in India which was 41.7%, 45%, and 7.4% respectively.

The prevalence of *Salmonella* in raw milk varies across different milk vendors due to various factors. These include study design, sampling techniques, geographic locations, hygiene practices, and storage conditions. Larger sample sizes and advanced detection methods can yield higher prevalence rates. The prevalence of *Salmonella* in raw milk can fluctuate depending on regional and local practices, environmental factors, animal health, and farm practices. The variation in hygiene practices during milk production, handling, and storage can increase the risk of bacterial contamination. Inadequate sanitation, equipment cleaning, and improper storage conditions can also increase the risk. The health status of dairy animals and the presence of infectious diseases can also impact the prevalence of *Salmonella* in raw milk. Cross-contamination during milk handling and processing can introduce *Salmonella* from external sources.

In the present study, no *Salmonella* was isolated from cheese and yoghurt samples, which agreed with the findings of Ejo et al. [18] and Tesfaw et al. [53], who reported no *Salmonella species* found in cheese and yoghurt. The absence of *Salmonella* in cheese and yoghurt samples can be attributed to several factors. Proper storage and handling practices, including adequate refrigeration and hygienic handling, help to prevent contamination after processing. The sensitivity of the sampling and detection methods can also influence the absence of *Salmonella.*

In this study, an attempt was made to evaluate and compare the isolation rate of *Salmonella* in dairy farms of different herd sizes, namely small, medium, and large. The result showed out of 41 dairy farms (3 large-scale, 34 medium-scale and 5 small-scale dairy farms) the total prevalence rate of *Salmonella* was 7.32%. Our results showed that the isolation rate of *Salmonella* was significantly comparable between small and medium-sized farms. However, in this cross-sectional study, there was no *Salmonella* isolated from large-scale dairy farms. Several factors could contribute to this study's lack of *Salmonella* isolation from large dairy farms. Some possible reasons could be strict biosecurity measures: Large dairy farms may have stricter biosecurity protocols in place to prevent the introduction and spread of pathogens, including *Salmonella,* as compared to small-scale dairy farms. Management practices, such as regular cleaning and disinfecting facilities, can help reduce the spread of *Salmonella*.

Antimicrobial resistance is a growing worldwide issue in human and veterinary health, affecting both developed and developing countries. The growing use of antimicrobial drugs in food animal production and humans was a significant contributor to the establishment of bacterial resistance [24]. Antimicrobial susceptibility testing revealed 75%, 75%, and 50% resistance to amoxicillin, streptomycin, and tetracycline respectively. In comparison, 100% sensitivity to gentamycin was identified, followed by 75%, 75%, 50%, and 50% sensitivity to amoxicillin-clavulanic acid, ciprofloxacin, tetracycline, and ampicillin, respectively. Gebeyehu et al., [22] reported that antibiotic susceptibility test showed that *Salmonella* isolates were 100% resistant to ampicillin, while they were 100% sensitive to ciprofloxacin. Multi-drug resistance (MDR) was demonstrated in all isolates.

The current findings revealed that 75% of the isolates were resistant to two or more classes of antibiotics, which was lower than the report of Fesseha et al. [21], who documented the MDR rate of 96.4% from selected dairy farms in Hawasa town. However, these findings were higher than those previously reported by Tesfaw et al. (2013), who documented a 50% MDR of *Salmonella* isolate. The possible reasons for the high AMR level of *Salmonella* might be due to the increasing rate of irrational use of antimicrobials in dairy farms, frequent usage both in livestock and public health, use of counterfeit drugs in animal husbandry [19], self-medication due to easy access to antimicrobials without prescription in the public health sector, and administration of sub therapeutic doses. Solomon et al. [51] also reported that among the nine *Salmonella* isolates, eight, six, six, five, and four isolates were found resistant to ampicillin, cefoxitin, tetracycline, Co-trimoxazole, and doxycycline hydrochloride, respectively. Furthermore, six of the *Salmonella* isolates showed multidrug resistance. According to Tadesse et al. [52], the antimicrobial test showed that *Salmonella* species were 100% resistant to ampicillin, 59.3% to trimethoprim-sulfamethoxazole, 59.3% to tetracycline, and 55.6% to amoxicillin/clavulanate. From the total antimicrobial tested bacteria, 81.5% (22/27) were resistant to three and above classes of antibiotics (drug classes). Another report by Ayichew et al., [9] revealed complete resistance (100%) to clindamycin, erythromycin, vancomycin, amoxicillin, and ceftazidime, with 75% resistant to ampicillin. Conversely, 92%, 83%, and 67% of the isolates were susceptible to norfloxacin, ciprofloxacin, and trimethoprim, respectively. Notably, all isolates were classified as multidrug-resistant (MDR).

The current investigation found 75% amoxicillin resistance, which was greater than the findings of Beyene et al. [13] and Fesseha et al. [21] in Asella and Hawasa Town, Ethiopia, who reported resistance rates of 58.3% and 25%, respectively. The observed high resistance to streptomycin is not surprising, as these antimicrobials are commonly used in all farms to manage bacterial infection. The streptomycin resistance in the current study is consistent with previous results in Addis Ababa, as reported by Zewdu and Cornelius [61], who recorded a resistance rate of 75% among food items and personnel. However, the results of our research's resistance rate were lower than those reported by Ketema et al. [36] and Obaidat and Stringer [44], which were 80% and 89.3%, respectively.

On the other hand, our data suggested a higher resistance rate than the studies by [1], Geletu et al. [25], and Beyene et al. [13] who documented a 60%, 46% and 41.7% resistance rate respectively. The resistance profile towards tetracycline was 50%, which is comparable with the findings of Mulaw [41], (52.8%) among lactating cows in dairy farms in Bahir Dar Town, Ethiopia. However, it is interesting to note that the tetracycline resistance rate found in the current study exceeds that of Xu et al. [60] in the United States, which was 28% lower than the report of Fesseha et al. [21], who recorded a resistant rate of 96.4%. This difference in resistance rates might be due to the increasing use of inappropriate antimicrobials on dairy farms. Such methods provide selection pressure, which increases the survival and growth of bacterial strains containing resistance genes [21]. As a result, such action may contribute to the variations in resistance profiles found among studies. The growing frequency of antibiotic resistance highlights the critical need for extensive antimicrobial management procedures to prevent the emergence and spread of resistant bacterial strains.

The present results showed that *Salmonella* isolates were susceptible to gentamycin and with a susceptibility rate of 100%. This was consistent with the reports of Tesfaw et al. (2013), Abunna et al. [2] and Beyene et al. (2020) who documented a resistant rate of 100% but, higher than 73.3% and 75% reported by Addis et al. [4] and Tadesse and Anbessa, respectively. Additionally, the susceptibility rate of ciprofloxacin was 75% which was lower than the report of 83.3% documented by Addis et al. [4]. The variation in ciprofloxacin effectiveness in Ethiopian dairy farming might be related to drug type, different bacterial strains, resistance gene evolution, and limited use in Ethiopian animal production. The misuse of antimicrobials in livestock may lead to the emergence and spread of pathogens that are harmful to human, animal and environmental health [32]. One of the major contributors to the rise of AMR is antibiotic misuse [23], which is linked to an antimicrobial knowledge gap.

Conclusion

The present study revealed the occurrence of contamination of cow milk and its products with *Salmonella enterica* along the milk value chain at farms and milk vendors. The isolations of the bacteria from bulk milk, fecal samples, and floor swabs of the cow environment were found to be the potential sources of milk contamination at the farms and milk vendors. The presence of *Salmonella enterica* in bulk milk at the farm level and milk vendors indicates that there was cross-contamination of milk possibly because of Salmonella shedding in cattle feces, poor animal hygiene and housing conditions, contact with contaminated water or feed, fecal contamination of milking equipment or milk storage tanks, unsanitary milking practice, poor hygiene of milk handlers/vendors, improper storage and incomplete or improper antibiotic treatment. The study has also revealed the possibility of a public health risk posed due to *Salmonella enterica* in the study area. In general, the assessment of AMR profile of *Salmonella* in dairy farms is critical for safeguarding public health, ensuring food safety, minimizing economic losses, and promoting the overall well-being of animals, the environment, and humans. By understanding the prevalence and dynamics of *Salmonella* in dairy environments, proactive measures can be taken to prevent contamination, reduce risks, and protect both the dairy industry and consumers. Therefore, creating public awareness about good milk handling practices, milk-borne diseases, and their prevention for dairy farmers and consumers should be implemented.

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Authors' contributions
L.T.: Conceptualization; Investigation; Formal analysis; Data Collection; Writing-original draft, Writing-review & Editing T.B.: Conceptualization, Investigation; Data Collection; Writing-review & Editing F.A: Conceptualization; Methodology; Writing-review & Editing, Fund Acquisition; Supervision; Writing-review & editing.

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Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
This study was approved, and ethical clearance was obtained from the Institutional Review Board of the College of Veterinary medicine and Agriculture. Sciences, Addis Ababa University (VM-ERC 02/09/16/2024).

Consent for publication
All authors consent to publication.

Competing interests
The authors declare no competing interests.

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