



Research Article

Isolation and Antimicrobial Susceptibility Profile of Non-Typhoidal Salmonella from Raw Bovine Milk and Assessments of Hygienic Practices in Gursum District, Eastern Hararghe, Ethiopia

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Abstract | Non-typhoidal Salmonella is a significant human and animal pathogen around the world. The majority of human non-typhoidal Salmonella infections are foodborne, however infections are also acquired through direct or indirect animal contact each year. Salmonella epidemiological data is required to establish effective control measures. A cross-sectional study was carried out between July 2022 and January 2023. The current study aimed to isolate non-typhoidal Salmonella from raw bovine milk and examine associated sanitary practices in Gursum District, Eastern Hararghe zone, Ethiopia. A total of 480 samples were obtained from bucket swabs, milk handlers' hands and milk. After pre-enrichment with buffered peptone water, the samples were moved to selenite cysteine broth and rappaport-vassilidis broth. Isolation was accomplished through plating on xylose lysine deoxycholate agar. Antimicrobial and biochemical susceptibility tests were performed. A total of 56 Salmonella isolates were recovered from bucket milk and hand swabs, including 22(13.7%), 20(12.5%), 22(13.7%) and 14(8.8%). Salmonella isolation from buckets was highest in Abadir and Funyan Bira kebeles, with an equivalent result of 8(20%). There is no statistically significant variation in the isolation of non-typhoidal Salmonella cases across all kebeles. The bulk of respondents (95% were female) and 83% of milk handlers in the research area were illiterate. According to knowledge, attitudes and practices of the community, some of them were milking the cow while sick 20% of the time, virtually all of them were washing the udder before milking 14% of the time and the bulk of them were cleaning their hands 85% of the time and milking equipment 90% of the time. All isolates were resistant to at least two antibiotics, including tetracycline, ampicillin and penicillin, at rates of 91.07%, 82.14 and 73.21 percent, respectively. Almost all isolates were chloramphenicol (100%), nalidixic acid (91.07%) and ciprofloxacin (91.07%) sensitive. The highest isolation was from milk and milk-contact surfaces at the market as a result of unsanitary handling from primary production to the market. The majority of Salmonella isolates were resistant to at least one of the antibiotics examined. Salmonellosis treatment may be challenging; thus antibiotic use must be limited.

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Introduction

Food-borne diseases are infectious or toxic disorders induced by the intake of contaminated foods infected with bacteria and/or their toxins, parasites, viruses, or chemicals (Gallo *et al.*, 2020). It is a global problem, with roughly 600 million cases of illness and 420,000 deaths caused by foodborne microorganisms each year. When compared to high-income countries, the problem was frequently more severe and less reported in low- and middle-income countries (Todd, 2020). There are numerous bacteria that cause food-borne illness in humans. Salmonellosis is one of the major zoonotic diseases all over the world, with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis and 155,000 deaths due to non-typhoidal Salmonella (Lima *et al.*, 2019).

Salmonella is a major cause of foodborne human salmonellosis globally (Sodagari *et al.*, 2020). Non-typhoidal Salmonella can be transmitted to humans by the ingestion of animal-derived goods such as bovine milk, eggs and poultry meat, as well as direct contact with animals or their habitats (Hu *et al.*, 2017). More than 2500 Salmonella *enteric* serovar have been identified, many of which can cause human illnesses. However, in human infections, NTS serovars, particularly *Enteritidis* and *Typhimurium*, were the most usually isolated serotypes (Abuaita *et al.*, 2021). A Non-typhoidal Salmonella can produce severe invasive disease with complicated extra-intestinal sickness, bacteremia and meningitis in youngsters, the elderly and immune impaired people (Naaz *et al.*, 2021). These infections could be quite dangerous, especially in people with impaired immune systems.

Nontyphoidal Salmonella is a leading source of foodborne illness, causing diarrhea, bacteremia and focal suppurative lesions. The majority of infections linked with humans and other mammals are caused by *S. enterica* *subsp. enterica*, *S. Enteritidis* and *S. typhimurium* (Olobatoke, 2017). Human disorders caused by milk-borne bacteria range from gastrointestinal disturbances such as diarrhea and vomiting to generalized and even life-threatening foodborne illnesses. They are not only important for public health but also for the economy (Sulaiman and Hsieh, 2017). Because it has a high nutritional value and acts as a suitable medium for the growth of numerous spoilage or pathogenic microorganisms, it

can cause product deterioration as well as infections and intoxications in consumers. Milk and milk derivatives play an essential role in feeding Ethiopia's rural and urban populations. It is manufactured on a daily basis, sold for cash, or easily processed. It is a cash crop in milk shed areas, allowing families to purchase other commodities and greatly contributing to household food security (Bereda *et al.*, 2012).

Because of a lack of refrigeration facilities at the farm and home level in poor countries of tropical regions with high ambient temperatures, raw milk is quickly contaminated with spoiled and harmful bacteria during milking, storage, transportation and processing (Kebede and Megerssa, 2018). Contaminated milk can harbor a variety of pathogenic and non-pathogenic microorganisms and as a result, milk could be a source of dangerous pathogens to consumers, leading to serious health problems. Bacteria such as Salmonella, *Escherichia coli*, *Klebsiella* and *Enterobacter* have been found in milk products in Ethiopia (Melini *et al.*, 2017).

World Health Organization estimates the distribution of non-typhoidal salmonellosis in Africa, allowing us to calculate the Global Burden of Foodborne Disease in Africa. This report anticipated 59,000 global deaths owing to NTS in 2010, including 32,000 deaths in Africa and 22,000 deaths due to invasive illness, predominantly in children (WHO, 2015). Nonetheless, data to understand NTS epidemiology exist in the majority of Sub-Saharan African nations. Every year, about 100,000 human cases are reported in the European Union (EU). According to the European Food Safety Authority (EFSA), the overall economic cost of human Salmonellosis could be as high as EUR 3 billion per year (Boqvist *et al.*, 2018). Because of the limited scope of studies and the lack of coordinated epidemiological monitoring systems in Ethiopia, estimating the burden of food-borne diseases is difficult. In Ethiopia, the prevalence of foodborne Salmonella infections has risen considerably in recent years (Belina *et al.*, 2021).

Globally, the occurrence of antibiotic-resistant strains of Salmonella has increased in recent decades due to the widespread use of antibiotics for treatment and prophylaxis in animals (Abate *et al.*, 2021). Animal health care in developing nations have been suboptimal due to an increased inclination for animal owners to carry pharmaceuticals in their homes and

engage inexperienced persons, such as farmers and animal attendants, to treat animals, resulting in multi drug resistance (Van *et al.*, 2020). Antimicrobials are licensed in human medicine for illness treatment and prevention (Masood *et al.* 2022). According to WHO, more than half of all medicines in poor countries are incorrectly prescribed, delivered, or sold and half of all patients fail to take them correctly. This, in conjunction with the use of antibiotics in animals, has led in the selection of antibiotic-resistant bacteria, which pollute animal food items and the environment (Mamo and Alemu, 2020). Although a few studies in Ethiopia have shown the incidence of Salmonella and antimicrobial susceptibility in humans, animals and food of animal origin, further research is needed on the subject (Kebede *et al.*, 2021).

Statement of the problem

Non-typhoidal Salmonella is a major cause of foodborne infections and outbreaks worldwide, especially in developing countries. Raw bovine milk is one of the potential sources of transmission to humans, as it can be contaminated by fecal matter or environmental sources during milking, handling and/or storage. The consumption of raw or inadequately pasteurized milk and its products poses a significant public health risk, especially for children, pregnant women and immunocompromised individuals. Moreover, the emergence and spread of antimicrobial resistance among the isolates limits the treatment options and increases the morbidity and mortality of the infection.

Raw bovine milk and its products are frequently consumed in Ethiopia, particularly in rural regions. However, there is little information available in the country about the prevalence, molecular characterization and antibiotic susceptibility of Nontyphoidal Salmonella isolates from raw bovine milk. Furthermore, milk producers and consumers sanitary practices in regard to contamination with the bacteria are not extensively established. As a result, the purpose of this study is to isolate and identify the bacteria from raw bovine milk samples collected in Gursum area, Eastern Hararghe, Ethiopia, as well as to establish their antimicrobial susceptibility patterns. Furthermore, the study will evaluate the hygienic procedures of milk producers and consumers in the study area, as well as identify risk variables linked with Nontyphoidal Salmonella contamination. The findings of this study will provide valuable information for the

prevention and control of Nontyphoidal Salmonella infections and the rational use of antimicrobials in the study area and beyond.

Objectives

General objective: To assess the occurrence of NTS in raw bovine milk, milk handler and milk holding equipment, antimicrobial resistance and status of milk hygiene practices in Gursum district of Eastern Hararghe Zone, Ethiopia.

Specific objectives: To isolate and identify Nontyphoidal Salmonella from bovine milk and milk contacting surfaces in Gursum district of East Hararghe zone, Ethiopia.

- To assess antimicrobial susceptibility profile of the *Nontyphoidal* Salmonella isolates.
- To assess cow milk hygiene practices in relation to milk-borne pathogens.

Materials And Methods

Description of the study area

The research was carried out in the Gursum district of Oromia Regional State, Ethiopia, from July 2022 to January 2023. Gursum district was one of Oromia Regional State's 20 districts in the East Hararghe zone. The district's capital is Funyan Bira Town, which is 600 kilometers east of Addis Ababa. The district is located between 9° 7" and 9° 32" North latitudes and 42° 17" and 42° 38" East longitudes. Map of study area was indicated on (Figure 1). The district's overall land covering was estimated to be 71573 hectares and it was home to approximately 168476 people. The district's elevation spans from 1200 to 2950 meters above sea level, with an annual rainfall of 650 to 750 millimeters. The district's mean annual maximum and minimum temperatures were 25°C and 18°C, respectively (Tesgera *et al.*, 2017).

Gursum district shared boundaries with Somali Regional state to the east, Harari Regional state to the west, Jarso district to the north and Babble district to the south. The district has a population of roughly 168476 people. The Gursum district's agro-ecology was divided into three categories: Highland (5%), midland (45%) and lowland (50%). The two main cattle production systems were mixed-farm and extended production systems. The major crops farmed in the Gursum were sorghum, maize and groundnut and the principal animal feeds included crop leftovers,

grass and some modified forages. Gursum district has 110,864 cattle, 32,786 sheep, 73,331 goats, 14,566 donkeys, 8,735 camels, 32 horses, 52,881 poultry flocks, 5,192 traditional beehives, 2 transitional beehives and 2 contemporary beehives (Gursum Agricultural Office) (Baker, 2023).

calculated considering 3.3% previous prevalence of *Salmonella* spp. in raw bovine milk Reta *et al.* (2016) reported from Jigjiga. Hence, using 5% desired level of precisions at 95% confidence level, the required sample size was determined according to the formula described by Thrusfield (2018) as follows:

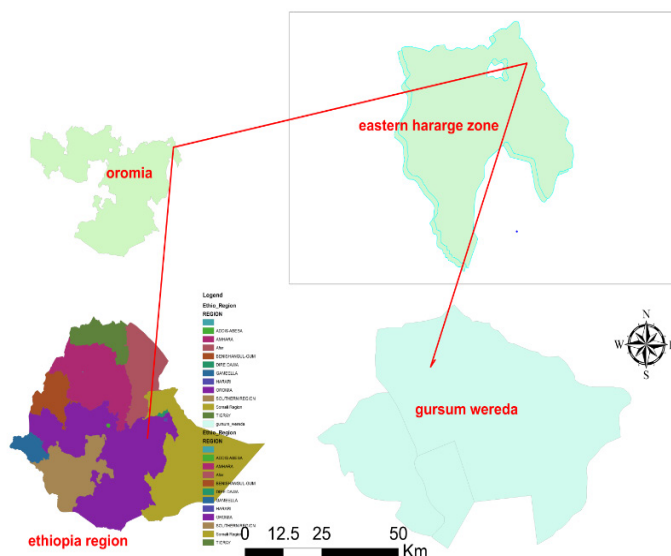


Figure 1: The map representing the study area from which study samples were taken.

Source: ArcGIS Pro, 2023 developed offline.

Study design

A cross-sectional study was conducted from July 2022 to January 2023. A total of 480 samples (160 milk, 160 bucket swabs and 160 hand swabs) were collected from four kebeles in Gursum District. Samples were processed for NTS isolation using standard microbiological methods and antimicrobial susceptibility testing was performed using the disc diffusion method.

Study population

The study population consisted of apparently healthy lactating dairy cows selected from the Gursum district's kebeles of Ibsa, Abadir, Haro Bate and Funyan Bira, while the target population consisted of all apparently healthy lactating dairy cows reared in the study area. Lactating cows receiving antibiotics were omitted from the study. We also removed milk samples from homes where the animal owners refused to participate in the study. Data on hygienic handling methods of raw cow milk were also acquired through face-to-face interviews with nursing cow owners' family members.

Sample size determination

The desired sample size of the current study was

$$n = \frac{Z^2 \times P_{exp} (1 - P_{exp})}{d^2}$$

$$n = \frac{1.96^2 \times 0.033(1 - 0.033)}{0.05^2}$$

$$= \frac{3.8416 \times 0.033(1 - 0.033)}{0.0025}$$

$$= 49.$$

Where, N = required sample size, Z= Confidence interval (95%), Pexp = Expected prevalence (3.3%), d = desired absolute precision (5%). As a result, the projected prevalence is set at 50%. As a result, the minimum required sample size (milk sample) was 49, although 160 raw cows' milk samples were obtained during the investigation to boost precision. In addition to raw milk samples, swab samples from milkers' hands and a bucket of home milk were obtained for NTS isolation. Milk from market equipment, a hand swab of the market and swab samples from the container had all been collected. The interview process was approximated based on formal survey studies for milk safety practice assessment in the house hold and market (Funyan Bira) sites of sample collection and sample size was calculated using Ashram (2007).

$$N = 0.25/SE^2 = 0.25/0.0025 = 100$$

Where: N= Sample size, SE (Standard Error) at 5% precision and 95% confidence level.

Sampling strategy and sample type

To collect raw cow milk, a multi-stage sample (District > Kebele > Household) using two stages of sampling technique (Kebele>household) was used. The Gursum district was chosen on purpose due of the ease of access to milk samples. According to the Gursum district agricultural office, Ibsa, Abadir, Haro Bate and Funyan Bira kebeles were chosen from among the district's 39 kebeles for their potential in cow milk production. Lactating cow homes were located using district data and the assistance of Development Agent (DA) workers. Finally, an equal number of homes were carefully recruited from each Kebele

using a systematic random sampling technique. Simultaneously, swab samples were obtained from milkers' hands and a pail of household milk and family members of the nursing cows owners were interviewed in the household. As a result, each Kebele received 40 raw cow's milk samples. Hand and bucket swabs were obtained from the same family and market (Table 1) and whenever there were more than one nursing cow in the household, a pooled milk sample was collected. Data on milk hygiene procedures were also collected using observational check lists created specifically for this study.

Table 1: Distribution of collected sample types from four Kebeles of the study area.

Sample type	Study areas				Total
	Haro Bate	Ibsa	Abadir	Funyan Bira	
Milk	40	40	40	40	160
Bucket swab	40	40	40	40	160
Hand swab	40	40	40	40	160
Total	120	120	120	120	480

Sample collections and transportation

Once the households of nursing cow owners in the Kebeles were located, the households recruited for the study were chosen systematically. At the home level, approximately 25 mL of milk was collected from a bucket of individual nursing cow milk in the household, as well as a hand swab from milkers. Milk samples were obtained from buckets from all identified and visited households and whenever more than one nursing cow was present in the household, a pooled milk sample was taken. Milkers' hand swabs and bucket swabs were collected from each cow milking individual in the specified household and milk container. Swab samples for bucket and hand swabs were collected before to milking by rotating and rubbing a sterile hardwood cotton swab against the sampled area numerous times horizontally and vertically. After swabbing, the swabs were moistened in a sterile test tube containing 10 mL of buffered peptone water (BPW), a pre-enrichment media.

All samples were processed for Salmonella isolation and antibiotic susceptibility testing. Meanwhile, volunteers involved in milk production (lactating cow owners) were asked about milk cleanliness and safety standards (Supplementary Figure 1A-1D). Each sample was identified with a permanent marker and placed in a sterile plastic container before being placed

in an ice box with an ice pack. The sampling site (a specific kebele), the sample type (such as individual cow milk from a family), or vendor at the marketing site and the date of sample collection are all identified by the labeling code. Finally, the samples were carried in an ice box filled with ice (Supplementary Figure 4D). All samples were transported to the Veterinary Microbiology Laboratory of Haramaya University in an ice box with ice packs on the same day of sample collection and analyzed upon arrival or within 24 hours of sampling at the microbiology laboratory of college veterinary medicine, Haramaya University.

Isolation of non-typhoidal Salmonella

Non-typhoidal Salmonella organisms identified from samples were isolated using the (ISO 6579, 2002) standard microbiology of food for detection of NTS. According to the guidelines, four processes are necessary to achieve the desired results. Non-selective pre-enrichment, selective enrichment, plating on selective media and biochemical confirmation are all phases in the isolation.

Non-selective pre-enrichment: During the investigation, 25 mL of milk sample was aseptically measured, combined with 225 mL of buffered peptone water, i.e. homogenized and incubated for 18hrs 2hrs at 37°C 1 °C (ISO 6579, 2002) to proliferate and regenerate injured cells. Primary enrichment improves NTS recovery (ISO 6579, 2002).

Selective enrichment media: Following the non-selective pre-enrichment stage, 100 L of pre-enriched material was transferred and mixed aseptically into a tube containing 10 ml of Rappaport Vasiliadis (RV) broth. The cells were then injected and cultured at 41.5 0.5 °C for 18 hours to promote Salmonella growth.

Plating on selective media: A loop of RV broth inoculum was transferred aseptically and streaked onto the surface of Xylose lysine e deoxycholate (XLD) agar plates prepared according to the manufacturer's instructions. The plates were incubated at 37 degrees Celsius for 18 to 24 hours. The plates were checked for the presence of Salmonella colonies. The plates were checked after incubation for the existence of usual and suspicious colonies. Due to the color change of the media, typical colonies of Salmonella produced on XLD-agar have a black center and a weakly translucent zone of reddish color (ISO 6579, 2002), whereas H₂S negative variations developed on

XLD agar are pink with a deeper pink center. Gram staining was done on Salmonella suspected colonies and only gram-negative rod results were evaluated for the following procedures. A single colony was subcultured on nutrient agar from the probable Salmonella colonies for additional biochemical confirmation tests.

Biochemical test of Non-typhoidal Salmonella isolates: Salmonella colonies isolated from non-selective (nutrient) agar and verified by gram staining were biochemically identified utilizing indole, Methyl red, Voges-Proskauer, urease, citrate utilization, triple sugar iron (TSI) and hydrogen sulphite synthesis tests (Supplementary Figure 3A-3D). Colonies found to be urease negative (no color change) and producing alkaline slant (pink) and acidic butt (yellow) on TSI with or without H₂S production (blackening for Salmonella and without H₂S for Shigella) are then tested for IMViC. Only colonies displaying the Salmonella unique IMViC pattern are regarded to be biochemically confirmed Salmonella isolates. Finally, a loop of pure colonies will be plated on TSA and incubated at 37°C for 24 hours to create a suspension for the antimicrobial susceptibility profile test.

Antimicrobial susceptibility test: The isolates were tested for antibiotic resistance using the disc diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2020). Clinical Laboratory Standards Institute (CLSI, 2020). Single colonies were transferred from nutrient agar plates into tubes containing 5 mL of (Oxoid, England) distilled water. The broth culture was incubated for 4 hours at 37 °C until it met the 0.5 McFarland turbidity criteria. To remove surplus inoculums, a sterile cotton swab was dipped into the solution, turned several times and firmly pressed on the inside wall of the tube above the level before being swabbed uniformly across the surface of Muller Hinton agar plate (Oxoid, England). The plates were allowed to dry for 30 minutes at room temperature. Antibiotic discs were properly put on the swabbed MHA plate, at least 15 mm apart from the plate's edge, to avoid inhibition zones from overlapping. The plates were then incubated for 24 hours at 37 °C.

The isolates' resistance to the following antibiotics was examined. Each Salmonella isolate was screened for eleven common antimicrobials. Ampicillin (AMP) 10µg, Gentamicin (CN) 10µg, Kanamycin

(K) 30µg, Erythromycin (E) 5µg, Tetracycline (TE) 30µg, Ciprofloxacin (CIP), 5µg Nalidixic acid (NA) 30µg, Chloramphenicol (C) 30µg, Vancomycin (VA) 30µg, Ceftriaxone (CRO) and Penicillin (PG) 10 µg were placed at least 15 mm apart from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated for 24 hours at 37°C. After incubation, the diameters of the inhibitory zones were compared to the diameters of the control organism. According to the Clinical Laboratory Standards Institute's (CLSI, 2020) interpretation standards, it is classified as resistant, moderate, or susceptible (Supplementary Figure 4A-4C).

Data management and analysis

The raw data collected during the study were imported into an Excel spreadsheet and analyzed using SPSS version 26. Descriptive statistics were used to summarize the prevalence of non-typhoidal Salmonella (NTS), expressed as frequencies and percentages. To assess associations between categorical variables, chi-square tests were employed and regression models were applied to identify significant risk factors for NTS contamination. The significance level for all statistical tests was set at $p < 0.05$. Chi-square tests were used to examine the relationships between risk factors (e.g., kebele location, milk handling practices and water source) and NTS occurrence. Statistically significant associations were determined at a 95% confidence interval, with a p-value of less than 0.05 considered significant. Regression models, particularly logistic regression, were utilized to quantify the strength and direction of these associations, providing odds ratios (OR) and confidence intervals (CI) for key risk factors. This approach allowed for a robust analysis of the factors contributing to NTS contamination in raw milk.

Results

Prevalence of isolated non-typhoidal salmonella

Overall, 56/480 (11.7%) of total samples from raw bovine milk, bucket swab and manual milkers tested positive for NTS in the study area. In the study area, NTS were isolated from cow milk (20.5%), bucket swab (22.5%) and milkers' hand swab (14.8%), respectively (Table 3). When compared to Ibsa 2 (5%) and Haro Bate 4 (10%) kebeles, Abadir and Funyan Bira had higher NTS isolation from bucket swab with equal values of 8 (20%). The study found no significant difference in NTS positive between cow

milk, bucket swab and hand swab collected from the four kebeles in the study area ($P > 0.05$). Bucket swabs have slightly higher odds of being positive than milk (OR = 1.12), but it is not statistically significant (Table 2).

Table 2: Logistic regression analysis of sample positivity.

Variable	Odds ratio (OR)	p value	95% confidence interval (CI)
Sample type (Ref: Milk)			
Bucket swab	1.12	0.09	(0.89 – 2.10)
Hand swab	0.82	0.22	(0.55 – 1.68)
Sample site (Ref: Ibsa)			
Abadir	1.24	0.35	(0.78 – 2.05)
Haro bate	0.94	0.77	(0.61 – 1.85)
Funyan bira	1.15	0.41	(0.72 – 1.98)

Table 3: Sample type and sample site-wise prevalence of NTS.

Type of sample	Sample site	No of sample	No of positive (%)	Total no of sample	Total no of positive (%)	p value
Milk	Ibsa	40	4(10.0)	160	20(12.5)	0.822
	Abadir	40	6(15.0)			
	Haro Bate	40	4(10.0)			
	Funyan Bira	40	6(15.0)			
Bucket swab	Ibsa	40	2(5.0)	160	22(13.8)	0.128
	Abadir	40	8(20.0)			
	Haro Bate	40	4(10.0)			
	Funyan Bira	40	8(20.0)			
Hand Swab	Ibsa	40	2(5.0)	160	14(8.8)	0.328
	Abadir	40	2(5.0)			
	Haro Bate	40	6(15.0)			
	Funyan Bira	40	4(10.0)			

Table 4: Antimicrobial susceptibility and resistance profile of NTS isolates.

Antimicrobial	Susceptible	Resistant	Odds ratio	p-value	95% CI for OR
Chloramphenicol	56 (100%)	0 (0%)	Reference	-	-
Nalidixic acid	51 (91.07%)	5 (8.93%)	1.45	0.32	(0.68-3.02)
Ciprofloxacin	51 (91.07%)	5 (8.93%)	1.40	0.35	(0.66-2.98)
Ceftriaxone	46 (82.14%)	10 (17.86%)	2.10	0.14	(0.82-5.35)
Gentamycin	41 (73.21%)	15 (26.79%)	3.25	0.07	(0.92-7.62)
Kanamycin	41 (73.21%)	15 (26.79%)	3.20	0.08	(0.89-7.55)
Vancomycin	41 (73.21%)	15 (26.79%)	3.30	0.06	(0.93-7.85)
Erythromycin	35 (62.5%)	21 (37.5%)	4.80	0.02	(1.35-9.75)
Tetracycline	5 (8.93%)	51 (91.07%)	15.32	<0.001	(5.52-35.21)
Ampicillin	10 (17.86%)	46 (82.14%)	9.12	<0.001	(3.68-21.42)
Penicillin	15 (26.79%)	41 (73.21%)	6.75	0.001	- 14.67)

Antimicrobial susceptibility of non-typhoidal salmonella

All the 56 NTS isolates were tested against eleven commonly used antimicrobials. All the isolates were found sensitive at list two or to more antimicrobials tested. The antibiotic sensitive profiles of the isolates showed that susceptibility the isolates were Chloramphenicol (100%), Nalidixic acid (91.07%), Ciprofloxacin (91.07%), Ceftriaxone (82.14%), Gentamycin, Kanamycin, Vancomycin (73.21%) and Erythromycin (62.5%) respectively. Those were Erythromycin (37.5%) Vancomycin, Gentamycin, Kanamycin and (26.79%) were similar results respectively. The high antimicrobials resistance of the isolates was recorded to tetracycline (91.07%), Ampicillin (82.14%) and Penicillin (73.23%) (Table 4).

Socio demographic characteristics of respondents

Out of 100 total respondents in the research area, 95% were female and 5% were male. The age groups were 15-30, with 53% being under 30-50 (38%) and above 51 (9%). In terms of marital status, single (6%), married (83%) and divorced (5%), while illiterate (83%), primary (16%) and secondary (1%) (Table 5).

Hygienic practices-related questionnaires

The research area's animal housing system consisted of 96% outside and 4% at home with family. The milking environment was classified as 74% under the roof, 21% in the shade and 5% in the open air. According to the varieties of milk used for human consumption, more than half (62%), ingest raw milk, 26% boiled milk and 12% fermented milk.

Table 5: Respondents' demographic characteristic of milk handlers (n=100).

Demographic characteristic	Category	Frequency	Percentage
Sex	Female	95	95.0
	Male	5	5.0
Age	15-30	53	53.0
	30-50	38	38.0
	Above 51	9	9.0
Educational level	Illiterate	83	83.0
	Primary	16	16.0
	Secondary	1	1.0
Marital status	Single	6	6.0
	Married	83	83.0
	Divorced	5	5.0

Table 6: Milk hygienic practices for NTS.

Hygienic related question	Frequency	Percent (%)
Animal housing system		
Home with family	4	4.0
Out side	96	96.0
Environment of milking		
Open air	5	5.0
Under the roof	74	74.0
Under the shade	21	21.0
Types of milk used for home consumption		
Raw	62	62.0
Boiled	26	26.0
Fermented	12	12.0
Milking frequency per day		
Once	13	13.0
Twice	87	87.0
Purpose of milk production		
Consumption	38	38.0
Sale	26	26.0
Both	36	36.0
Commonly milk product		
Butter	56	56.0
Fermented	15	15.0
Cheese	29	29.0
Water source for equipment cleaning		
Pond	67	67.0
River	30	30.0
Tap water	3	3.0
Types of smokers		
Nadhalo	51	51.0
Berryessa	49	49.0
Types of containers for milking		
Stainless	96	96.0
Traditional (okole)	4	4.0

The cow milking frequency per day is 87% twice per day and 13% once per day. At the farmer level, 38% of milk is produced for consumption, 26% for sale and 36% for both home use and sales. During the study period, the most common milk product utilized was 56% butter, 15% fermented milk and 29% cheese. Our study area's water sources for animal drinking were 67% pond, 30% river and 3% tape water. The majority of milk storage containers were 79% steeliness steel, 17% plastic and 4% traditional bucket. The milking equipment was cleaned using several traditional methods such as bakarkate (38%), hot water (37%) and both bakarkate and hot water (25%). Traditional uses of different trees as a smoker to container as a milk preservative were Berryessa 49% and nadhalo 51% (Table 6).

The knowledge, attitude and practices of milk handler about nontyphoidal salmonella

The KAPs of milk handlers has been assessed whether milk produced in study area was passed under hygienic condition. Almost all (100%) of milk handler have where knowledge ill lactating cow was not milked and treating lactating cow while ill. In order to minimize the microbial contamination of milk (86%) milkers doesn't wash the udder before milking and 14% used to wash. Almost all milkers were wash their hands before milking 85% and also cleaning milking equipment's ware 90% (Table 7).

Table 7: A questionnaire related to hygienic practices.

Questioner	No of re-spondents, Yes	No of re-spondents No
Milking while the animal was sick	20(20%)	80(80%)
Treating cow while sick	100(100%)	0.0
Wash udder before milking	14(14%)	86(86%)
Wash hand before and after milking	85(85%)	15(15 %)
Cleaning the equipment before milking	90(90%)	(10)(10%)

Discussion

Nontyphoidal Salmonella, as a major zoonotic pathogen, not only causes disease and death, but also causes a variety of socioeconomic damages. Salmonella infections in dairy cattle continue to be a big problem around the world. Furthermore, the danger of virus transfer to humans via the food chain is a significant burden in both poor and wealthy countries (Majowicz et al., 2010; Fufa et al., 2017). Salmonella was one of

the most common causes of food-borne sickness in the world (Busani *et al.*, 2006).

Isolation of non-typhoidal salmonella

In this investigation, the overall prevalence of NTS from cattle milk, bucket and hand of milkers was (11.7%). The findings agreed with prior studies by (Fufa *et al.*, 2017). Salmonella isolation from milk, hand swabs and milking equipment had (10.5%) near Modjo town. However, the results were lower (20%) than those of (Tadesse and Dabassa, 2012) in Kersa district raw cow milk. Differences in outcomes were caused by worker hygiene, equipment-cleaning methods, milking environment and farming system types.

The prevalence of Salmonella in cow milk samples in this investigation was (12.5%), which corresponded with a previous result from Gondar town of 12.8% (Deresse and Shaweno, 2015). Another report that was lower than our study was (5%) from bucket milk, 3% from England (Padungtod and Kaneene, 2006) and 4.4% from Asella dairy farm (Beyene *et al.*, 2016). Various investigations in various nations reported very low isolations of Salmonella from milk of 3.3%, Reta *et al.* (2016), 4% of Shahabi *et al.* (2016), 2.17% Junaidu *et al.* (2011) and 1.43% Lailler *et al.* (2005). On the other hand, reports from Iran (17.0%) by Hossein *et al.* (2013) and Egypt (29.0%) by Omar *et al.* (2018) are significantly higher than the current study.

The current investigation also discovered a high prevalence of NTS isolates (8.8%) in milker's hand swabs. Fufa *et al.* (2017) found a finding that was almost identical to a report from dairy farm workers in the Modjo town area: 3 (10.7%). However, this is lower than a recent study from Meki, Shewa which found 19% prevalence (Sheferaw *et al.*, 2021) and 13.63% among Addis Ababa dairy farm workers. The prevalence of Salmonella from hand swabs was 5.26%, according to the lower data. In the research area, NTS was isolated from milking buckets at a rate of 13.8%. The findings were similar to those reported by Fufa *et al.* (2017), who discovered that the frequency of NTS from bucket swabs in dairy farms was 9.5%. However, the study results were 5% lower than those reported in previous work from Addis Ababa (Zelalem *et al.*, 2017).

Antimicrobial resistant of nontyphoidal salmonella

Since the introduction of antimicrobial medications,

microbes have developed antimicrobial resistance. Failure of a treatment regimen, prophylactic use of antimicrobials, use of antimicrobials as growth promoters and the use of antimicrobials frequently used in human practice are all known to induce bacterial resistance. Antimicrobial resistance has been identified as a significant therapeutic issue in both veterinary and human medicine. In this work, the antimicrobial resistance of NTS isolated from cow milk was evaluated using the disk diffusion method against eleven antimicrobial agents (Ahmed and Baptiste, 2018). Overall, antimicrobial resistance was found to be moderately low (73.21%). Antibiotic sensitivity patterns may be used to investigate MDR bacteria in cow milk, which may create difficulties in people if they become infected with MDR bacteria from cow milk. The study's findings highlight the most frequent bacterial infections circulating in cow milk, as well as their broad spectrum of resistance to numerous antibiotics commonly used for therapeutic purposes.

NTS isolates were resistant to all antibiotics tested in this investigation. Tetracycline (91.07%) and Ampicillin (82.14%) were shown to have the highest resistance, followed by Penicillin (73.21%). Only 73.21% of the isolates tested positive for Penicillin resistance. The high resistance to these medications in gram negative bacteria could be attributed to the transfer of resistance genes from gram-positive bacteria, notably β -lactamase genes (Breijyeh *et al.*, 2020). The current investigation contradicted the findings of previous researchers (Siddiky *et al.*, 2022), who screened 56 isolates of NTS isolated from cow milk for different antibiotics and discovered low level Penicillin resistance. Meanwhile, they discovered the highest level of tetracycline antibiotic resistance.

Because these medications are extensively employed in the treatment of human patients and veterinary practice, the development of antimicrobial resistance by bacteria to these drugs constitutes a significant concern in both human and animal care. In Ethiopia, antimicrobial resistance in Nontyphoidal Salmonella isolates from animal and human sources has been observed (Pande *et al.*, 2015). As a result, illness control is a difficult challenge for protecting population use of cow milk. To lower the danger of disease management techniques such as cow milk equipment washing while also enhancing hygiene.

Salmonella isolates were shown to be resistant to Tetracycline, Ampicillin and Penicillin at rates of 91.07%, 82.14 and 73.21, respectively, in this investigation. This conclusion is consistent with the earlier order of 96.4%, 39.3% reported by [Fufa et al. \(2017\)](#) from milk, hand swab and bucket. [Diriba et al. \(2020\)](#) showed a lower antimicrobial resistance pattern of 67.8 for tetracycline. According to [Hailu et al. \(2015\)](#) of Alexandria, Egypt, 85.7% of Salmonella isolated from dairy calves were responsive to ampicillin and tetracycline. This finding contradicts the current study, which found that 96.4% and 39% of the isolates were resistant to tetracycline and ampicillin, respectively. The study rate was much higher than the published rate (91.07% and 82.14%). When compared to results described in America by [Blau et al. \(2005\)](#), who found 4.4% and 12.2% resistance levels, respectively, resistance rates to ampicillin and tetracycline are relatively high.

This study, on the other hand, revealed that all Salmonella identified were totally and or very susceptible to Chloramphenicol (100%), nalidixic acid and Ciprofloxacin (91.07%) and ceftriaxone (82.14%), with Vancomycin, Gentamycin and Kanamycin (73.21%) having the same sensitivity rate. This result, in conjunction with the findings of [Diriba et al. \(2020\)](#), demonstrated that Salmonella isolates were 100%, 80.65% and 70.9 percent sensitive to ceftriaxone, nalidixic acid and ciprofloxacin, respectively.

The study found that 70.9% of Salmonella species isolates were sensitive to ciprofloxacin, which is similar to the findings of [Abnet et al. \(2024\)](#), [Reta et al. \(2016\)](#), who found that 83.3%, 75% and 65.7% of Salmonella species isolates were susceptible to ciprofloxacin, respectively. Gentamycin was shown to be 73.21% effective in the study. Several researchers ([Jonathan, 2022](#); [Fufa et al., 2017](#)) disagreed with the result of being 100% sensitive to Gentamycin. Gentamycin sensitivity was observed to be 45.2% ([Diriba et al., 2020](#)). Antimicrobial-resistant non-typhoidal Salmonella in raw milk may be able to colonize the intestines of milk consumers. This makes treating diseases caused by NTS more complex. Evidence suggests that the global growth in antimicrobial resistance is mostly owing to the indiscriminate use of drugs in veterinary and public health treatment ([Lynch and Tauxe, 2009](#)).

The knowledge, attitude and hygienic practices of milk handler

Almost all (96%) nursing cows were housed in a barned housing arrangement where they were intermingled with diverse species, which favored contamination of the udder and teat, increasing the isolation of Salmonella from milk and milk-contacting surfaces. 62% of milk producers utilized raw milk without any temperature treatment, making it a potential source of contamination for both spoilage and dangerous bacteria. The majority of milking (74%) was done in open air, when the wind, sun and other variables lead to milk contamination. The findings agreed with the findings of [Hailu \(2013\)](#), who reported that food catering areas, including milk preparation and selling areas, should be sheltered (protected) from the sun, dust, wind, road traffic, garbage and waste, as such areas undoubtedly expose food (like milk) to contamination from microorganisms.

When it is unattainable to keep food preparation and selling areas clean or protect them from contaminant agents (such as dust, wind, road traffic, flies and other contaminant agents), the displayed food, including milk and its handling equipment should be properly covered or protected from contamination ([Amentie et al., 2016](#)). The movement of spoilage and harmful bacteria as a result of dust, debris and winds may trigger foodborne outbreak. In terms of equipment cleaning procedures used in the study region, the findings revealed that the majority of milk producers (27%), cleaned their milk handling equipment with warm water, detergent and *Lantana camara* leaves. Nonetheless, while the majority of milkers (85%) wash their hands and equipment (90%) before and after milking, just a few of them utilize warm water and soap to clean their hands and equipment. Instead, they use cool water without detergent. The study by [Worku et al. \(2014\)](#) found that the majority (69.7%) of respondents wash their milk handling equipment with cold water and detergents. Washing hands with cold water without detergent results in insufficient germ removal and is a major source of microbial contamination of milk ([Ahmed et al., 2022](#)).

Milk handling equipment is washed once a day across the supply chain, followed by drying (for 5- 10 minutes) and fumigation with smoke from burning stems of particular species of plant such as Bir'eensa (*Terminalia brownie*), nadhello, or Mi'eessaa (*Euclea schemperi*) used as decontamination, increasing

flavorsome and preserving milk from contamination. The findings were consistent with those of [Getaneh and Girma \(2023\)](#). According to the study, smoking milk containers in the preparation of homemade yogurt increased microbiological quality and taste when compared to non-smoked containers. Another study conducted in Kenya by [Wanjala et al. \(2016\)](#) revealed the usefulness of smoke containers in limiting microbial growth and so enhancing the keeping quality of camel milk. The primary goal of sanitizing is to destroy leftover bacteria on these surfaces soon before milking. Inadequate or incorrect cleaning and sanitizing allowed germs to linger on the equipment's surfaces and grow and reproduce, resulting in an increased number of various bacteria ([Ohnstad, 2013](#)).

According to personal observations, the majority of people did not adequately cover their milk handling equipment from flies, dust and other filth. This could have been a source of milk contamination from both spoilage and harmful microbes. In contrast, the majority of milk producers (79%) used aluminum cans; other researches have claimed that aluminum or stainless steel is favored to reduce contamination ([Bereda et al., 2012](#)). Stainless steel equipment is preferred for handling milk because it is readily cleaned to remove dust and bacteria from the container.

Through the supply chain, the bulk of the pastoral community's milk producers (67%) used pond, (34%) revere and (3%) tap water. The findings are consistent with the findings of Bereda and his colleagues, who reported that the majority (64.4%) of respondent milk producers in the Ezha district of Ethiopia. Water from non-tap sources is used by the majority of milk collectors and carriers who bring raw milk to Jigjiga (60%), Harar (60%) and Dire Dawa (66.7%) ([Amentie et al., 2016](#)). The source of water influences the bacteriological quality of milk ([Amenu et al., 2014](#)). The water used for milk hygiene activities must be drinkable. This finding suggests that the water used to clean equipment may be a source of spoilage and harmful microorganisms ([Ahmed et al., 2022](#)).

Conclusions and Recommendations

The investigation involved acquiring milk, hand and bucket swab samples for Nontyphoidal Salmonella isolation, with an overall incidence of 11.6%. The contamination was due to unsanitary handling

and dumping. A person who consumes raw milk on a regular basis is especially vulnerable to health problems. As a result, antimicrobial susceptibility testing on Nontyphoidal Salmonella isolate revealed high concentrations of multidrug resistance, potentially affecting health and indicating incorrect drug usage in the study area.

Therefore, based on the above conclusion, the following recommendations are forwarded:

- Unpasteurized milk, a type of dairy product, is not safe to consume due to potential contamination with harmful bacteria, hence, proper hygiene is crucial.
- Cook food thoroughly and keep it hot until served, using a food thermometer to ensure meat, poultry and eggs reach a safe internal temperature to kill germs.
- To avoid cross-contamination, refrigerate or freeze perishable foods within 2 hours, separate raw and cooked foods, use different cutting boards, plates and knives and wash well after each use.
- Further research is needed to assess risks, evaluate interventions and investigate mechanisms of multidrug-resistant Nontyphoidal Salmonella, an emerging public health threat affecting raw milk safety and quality.

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Novelty Statement

This study provides novel insights into the prevalence, isolation, and antimicrobial susceptibility profile of non-typhoidal Salmonella (NTS) in raw bovine milk, along with an assessment of hygienic practices in Gursum District, Eastern Hararghe, Ethiopia. Unlike previous studies that mainly focused on meat and animal feces, this research highlights raw milk as a potential reservoir of antimicrobial-resistant Salmonella, posing significant public health risks. To the

best of our knowledge, this is the first report on NTS contamination in raw bovine milk in the study area. Additionally, it provides up-to-date antimicrobial resistance patterns of NTS isolates, offering valuable data on emerging resistance trends. By linking poor hygienic practices with Salmonella contamination, this study presents crucial evidence for improving dairy hygiene, strengthening antimicrobial stewardship, and enhancing food safety policies in Ethiopia and beyond.

Author's Contribution

All authors have contributed equally to this article.

Supplementary Material

There is supplementary material associated with this article. Access the material online at:

Conflict of interest

The authors have declared no conflict of interest.

References

- Abate, L., Bachheti, A., Bachheti, R.K. and Husen, A., 2021. Antibacterial properties of medicinal plants: Recent trends, progress and challenges. Traditional herbal therapy for the human immune system, pp. 13-54. <https://doi.org/10.1201/9781003137955-2>
- Abuaita, B.H., Lawrence, A.L.E., Berger, R.P., Hill, D.R., Huang, S., Yadagiri, V.K. and O'Riordan, M.X., 2021. Comparative transcriptional profiling of the early host response to infection by typhoidal and non-typhoidal Salmonella serovars in human intestinal organoids. PLoS Pathog., 17(10): e1009987. <https://doi.org/10.1371/journal.ppat.1009987>
- Abnet S. M., Hamza M. Y and Amedine S. A. (2024). Isolation of Selected Bacterial Pathogens from Bovine Mastitis in Selected Dairy Farms Found in Dire Dawa Town, Eastern Ethiopia. European Journal of Clinical and Biomedical Sciences, 10(1), 1-14. <https://doi.org/10.11648/j.ejcb.20241001.11>
- Ahmed, M.O. and Baptiste, K.E., 2018. Vancomycin-resistant enterococci: A review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microbial Drug Resist., 24(5): 590-606. <https://doi.org/10.1089/mdr.2017.0147>
- Ahmed, Z.A., Kasim, M.H., Tegegne, B. and Salah, H.M., 2022. Assessment of hygiene practices and bacteriological quality of raw cow milk of selected dairy farm in Dessie, Ethiopia. Am. J. Aquacult. Anim. Sci., 1(1): 27-37. <https://doi.org/10.54536/ajaas.v1i1.1071>
- Amentie, T., Eshetu, M., Mekasha, Y. and Kebede, A., 2016. Milk postharvest handling practices across the supply chain in Eastern Ethiopia. J. Adv. Vet. Anim. Res., 3(2): 112-126. <https://doi.org/10.5455/javar.2016.c139>
- Amenu, K., Spengler, M. andré, M. and Zárate, A.V., 2014. Microbial quality of water in rural households of Ethiopia: Implications for milk safety and public health. J. Health, Popul. Nutr., 32(2): 190.
- Baker, A.M., 2023. Farmers' perception of climate variability, associated risks and local adaptation strategies in Gursum District, East Hararghe Zone, Ethiopia (Doctoral dissertation, Haramaya University).
- Belina, D., Hailu, Y., Gobena, T., Hald, T. and Njage, P.M.K., 2021. Prevalence and epidemiological distribution of selected foodborne pathogens in human and different environmental samples in Ethiopia: A systematic review and meta-analysis. One Health Outlook, 3(1): 1-30. <https://doi.org/10.1186/s42522-021-00048-5>
- Bereda, A., Yilma, Z. and Nurfeta, A., 2012. Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. Wudpecker J. Agric. Res., 1(11): 459-465.
- Beyene, T., Yibeltie, H., Chebo, B., Abunna, F., Beyi, A.F., Mammo, B. and Duguma, R., 2016. Identification and antimicrobial susceptibility profile of Salmonella isolated from selected dairy farms, abattoir and humans at Asella town, Ethiopia. J. Vet. Sci. Technol., 7(3): 320. <https://doi.org/10.4172/2157-7579.1000320>
- Blau, D.M., McCluskey, B.J., Ladely, S.R., Dargatz, D.A., Fedorka-Cray, P.J., Ferris, K.E. and Headrick, M.L., 2005. Salmonella in dairy operations in the United States: Prevalence and antimicrobial drug susceptibility. J. Food Prot., 68(4): 696-702. <https://doi.org/10.4315/0362-028X-68.4.696>
- Boqvist, S., Söderqvist, K. and Vågsholm, I., 2018. Food safety challenges and One Health within

- Europe. Acta Vet. Scand., 60: 1-13. <https://doi.org/10.1186/s13028-017-0355-3>
- Breijyeh, Z., Jubeh, B. and Karaman, R., 2020. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, 25(6): 1340. <https://doi.org/10.3390/molecules25061340>
- Busani, L., Scavia, G., Luzzi, I. and Caprioli, A., 2006. Laboratory surveillance for prevention and control of foodborne zoonoses. *Annali dell'Istituto Superiore di sanit *, 42(4): 401-404.
- Clinical and Laboratory Standards Institute (CLSI). (2020). Performance Standards for Antimicrobial Susceptibility Testing; 30th Edition. CLSI document M100. Wayne, PA: CLSI
- Deresse, B. and Shaweno, D., 2015. Epidemiology and in-hospital outcome of stroke in South Ethiopia. *J. Neurol. Sci.*, 355(1-2): 138-142. <https://doi.org/10.1016/j.jns.2015.06.001>
- Diriba, K., Kassa, T., Alemu, Y. and Bekele, S., 2020. *In vitro* biofilm formation and antibiotic susceptibility patterns of bacteria from suspected external eye infected patients attending ophthalmology clinic, Southwest Ethiopia. *Int. J. Microbiol.*, 2020: 1-12. <https://doi.org/10.1155/2020/8472395>
- Fufa, G., Shiferaw, D., Kinati, T. and Desalegn, M., 2017. The nexus between khat and other drug use among undergraduate students of Jigjiga University in Ethiopia; contributing factors and prevalence rates. *Public Health Res.*, 7(2): 49-54.
- Gallo, M., Ferrara, L., Calogero, A., Montesano, D. and Naviglio, D., 2020. Relationships between food and diseases: What to know to ensure food safety. *Food Res. Int.*, 137: 109414. <https://doi.org/10.1016/j.foodres.2020.109414>
- Getaneh, D. and Girma, M., 2023. Assessment of traditional way of value addition practices on processing of milk and milk products in selected districts of south Omo Zone. *Results Pastoral Spec. Supp. Regions Res.*,
- Hossein, G.Z., N. Mohit A. and Azad, N., 2013. Effect of temperature-humidity index on productive and reproductive performances of Iranian Holstein cows. *Iran. J. Vet. Res.*, 14(2): 106-112.
- Hailu, A., 2013. Cross breeding effect on milk productivity of Ethiopian indigenous cattle: Challenges and opportunities. *Sch. J. Agric. Sci.*, 3(11): 515-520.
- Hailu, D., Gelaw, A., Molla, W., Garedew, L., Cole, L. and Johnson, R., 2015. Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia. <https://doi.org/10.5455/jeos.20151102014711>
- Hu, Y., Cheng, H. and Tao, S., 2017. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. *Environ. Int.*, 107: 111-130. <https://doi.org/10.1016/j.envint.2017.07.003>
- Jonathan, A., 2022. Assessment of microbial contamination of raw cow milk and antimicrobial resistance of *Salmonella* spp. isolated in Ilala district, Dar es salaam, Tanzania (Doctoral dissertation, Sokoine Univ. Agric.,
- Junaidu, A.U., Salihu, M.D., Tambuwal, F.M., Magaji, A.A. and Jaafaru, S., 2011. Prevalence of mastitis in lactating cows in some selected commercial dairy farms in Sokoto metropolis. *Adv. Appl. Sci. Res.*, 2(2): 290-294.
- Kebede, L.G. and Megerssa, S.A., 2018. Assessment of dairy farmers hygienic milking practices and awareness on cattle milk-borne zoonoses in Bishoftu, Ethiopia. *J. Vet. Med. Anim. Health*, 10(2): 45-54. <https://doi.org/10.5897/JVMAH2017.0602>
- Kebede, R., Alemayehu, H., Medhin, G. and Egual, T., 2021. Nontyphoidal *Salmonella* and their antimicrobial susceptibility among diarrheic patients attending private hospitals in Addis Ababa, Ethiopia. *BioMed. Res. Int.*, 2021. <https://doi.org/10.1155/2021/6177741>
- Lailler, R., Sanaa, M., Chadoeuf, J., Fontez, B., Brisabois, A., Colmin, C. and Millemann, Y., 2005. Prevalence of multidrug resistant (MDR) *Salmonella* in bovine dairy herds in western France. *Prevent. Vet. Med.*, 70(3-4): 177-189. <https://doi.org/10.1016/j.prevetmed.2005.03.006>
- Lima, T., Domingues, S. and Da Silva, G.J., 2019. Plasmid-mediated colistin resistance in *Salmonella enterica*: A review. *Microorganisms*, 7(2): 55. <https://doi.org/10.3390/microorganisms7020055>
- Lynch, M.F. and Tauxe, R.V., 2009. Salmonellosis: Nontyphoidal. *Bacterial infections of humans: Epidemiol. Contr.*, pp. 677-698. https://doi.org/10.1007/978-0-387-09843-2_32
- Majowicz, S.E., Musto, J., Scallan, E., Angulo,

- F.J., Kirk, M., O'Brien, S.J. and International Collaboration on Enteric Disease "Burden of Illness" Studies, 2010. The global burden of nontyphoidal *Salmonella gastroenteritis*. Clin. Infect. Dis., 50(6): 882-889. <https://doi.org/10.1086/650733>
- Mamo, D.B. and Alemu, B.K., 2020. Rational drug-use evaluation based on World Health Organization core drug-use indicators in a tertiary referral hospital, Northeast Ethiopia: A cross-sectional study. Drug, Healthc. Patient Saf., pp. 15-21. <https://doi.org/10.2147/DHPS.S237021>
- Masood, F., Thebeau, J.M., Cloet, A., Kozii, I.V., Zabrodski, M.W., Biganski, S. and Wood, S.C., 2022. Evaluating approved and alternative treatments against an Oxytetracycline-resistant bacterium responsible for European foulbrood disease in honey bees. Sci. Rep., 12(1): 5906. <https://doi.org/10.1038/s41598-022-09796-4>
- Melini, F., Melini, V., Luziatelli, F. and Ruzzi, M., 2017. Raw and heat-treated milk: From public health risks to nutritional quality. Beverages, 3(4): 54. <https://doi.org/10.3390/beverages3040054>
- Naaz, H., Suravaram, Tadi, L. and Wajid, M., 2021. Invasive non-typhoidal salmonella sepsis in top-up fed five-month-old infant: Case report. J. Clin. Diagn. Res., 15(2): <https://doi.org/10.7860/JCDR/2021/47924.14539>
- National Committee for Clinical Laboratory Standards (NCCLS). (2020). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Ninth Informational Supplement. NCCLS document M100-S29. Wayne, PA: NCCLS
- Ohnstad, I., 2013. Effective cleaning of the milking machine. Livestock, 18(1): 28-31. <https://doi.org/10.1111/j.2044-3870.2012.00174.x>
- Olobatoke, R.Y., 2017. Public health burden of non-typhoidal *Salmonella* strains in sub-Saharan Africa. Int. Res. J. Publ. Environ. Health.
- Omar, D., Al-Ashmawy, M., Ramadan, H. and El-Sherbiny, M., 2018. Occurrence and PCR identification of *Salmonella* spp. from milk and dairy products in Mansoura, Egypt. Int. Food Res. J., 25(1): 446-452.
- Padungtod, P. and Kaneene, J.B., 2006. *Salmonella* in food animals and humans in northern Thailand. Int. J. Food Microbiol., 108(3): 346-354. <https://doi.org/10.1016/j.ijfoodmicro.2005.11.020>
- Pande, V.V., Gole, V.C., McWhorter, A.R., Abraham, S. and Chousalkar, K.K., 2015. Antimicrobial resistance of non-typhoidal *Salmonella* isolates from egg layer flocks and egg shells. Int. J. Food Microbiol., 203: 23-26. <https://doi.org/10.1016/j.ijfoodmicro.2015.02.025>
- Reta, M.A., Bereda, T.W. and Alemu, A.N., 2016. Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. Int. J. Food Contam., 3(1): 1-9. <https://doi.org/10.1186/s40550-016-0027-5>
- Shahabi, N., Tajik, H., Moradi, M. and Forough, M., 2016. Antibacterial properties of *Zataria multiflora* Boiss. essential oil nanoemulsion formed by emulsion phase inversion. J. Food Microbiol., 3(3): 45-56.
- Sheferaw, D., Abebe, R., Megersa, B., Amenu, K., Abunna, F., Regassa, A. and Wako, F., 2021. Dairy cattle lameness prevalence, causes and risk factors in selected farms of southern Ethiopia. Ethiop. Vet. J., 25(2): 27-42. <https://doi.org/10.4314/evj.v25i2.3>
- Siddiky, N.A., Sarker, S., Khan, S.R., Rahman, T., Kafi, A. and Samad, M.A., 2022. Virulence and antimicrobial resistance profile of non-typhoidal *Salmonella enterica* serovars recovered from poultry processing environments at wet markets in Dhaka, Bangladesh. PLoS One, 17(2): e0254465. <https://doi.org/10.1371/journal.pone.0254465>
- Sodagari, H.R., Wang, P., Robertson, I., Habib, I. and Sahibzada, S., 2020. Non-typhoidal *Salmonella* at the human-food of animal origin interface in Australia. Animals, 10(7): 1192. <https://doi.org/10.3390/ani10071192>
- Sulaiman, I.M. and Hsieh, Y.H., 2017. Foodborne pathogens in milk and dairy products: Genetic characterization and rapid diagnostic approach for food safety of public health importance. In: Dairy in human health and disease across the lifespan. Academic Press. pp. 127-143. <https://doi.org/10.1016/B978-0-12-809868-4.00009-1>
- Tadesse, T. and Dabassa, A., 2012. Prevalence and antimicrobial resistance of *Salmonella* isolated from raw milk samples collected from Kersa district, Jimma Zone, Southwest Ethiopia. J. Med. Sci., 12(7): 224. <https://doi.org/10.3923/jms.2012.224.228>

- Tesgera, T., Regassa, F., Giro, B. and Mohammed, A., 2017. Study on prevalence and identification of ixodid ticks in cattle in Gursum district, East Hararghe Zone of Oromia Regional State, Ethiopia. *J. Parasitol. Vector Biol.*, 9(4): 27-33.
- Thrusfield, M., 2018. *Veterinary epidemiology*. John Wiley and Sons.
- Todd, E., 2020. Food-borne disease prevention and risk assessment. *Int. J. Environ. Res. Publ. Health*, 17(14): 5129. <https://doi.org/10.3390/ijerph17145129>
- Van, T.T.H., Yidana, Z., Smooker, P.M. and Coloe, P.J., 2020. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *J. Glob. Antimicrob. Resist.*, 20: 170-177. <https://doi.org/10.1016/j.jgar.2019.07.031>
- Wanjala, N.W., Matofari, J.W. and Nduko, J.M., 2016. Antimicrobial effect of smoking milk handling containers' inner surfaces as a preservation method in pastoral systems in Kenya. *Pastoralism*, 6(1): 1-7. <https://doi.org/10.1186/s13570-016-0064-y>
- Worku, T., Negera, E., Nurfeta, A. and Welearegay, H., 2014. Milk handling practices and its challenges in Borana Pastoral Community, Ethiopia. *Afr. J. Agric. Res.*, 9(15): 1192-1199. <https://doi.org/10.5897/AJAR2013.8247>
- World Health Organization, 2015. WHO estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015. World Health Organization.
- Zelalem, A., Endeshaw, M., Ayenew, M., Shiferaw, S. and Yirgu, R., 2017. Effect of nutrition education on pregnancy specific nutrition knowledge and healthy dietary practice among pregnant women in Addis Ababa. *Clin. Mother Child Health*, 14(3): 265. <https://doi.org/10.4172/2090-7214.1000265>