HYGIENIC PRACTICES, PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF SALMONELLA ISOLATED FROM MILK SUPPLY CHAIN IN AND AROUND BANBASI TOWN, BENISHANGUL GUMUZ, WESTERN ETHIOPIA

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ABSTRACT: Across-sectional study was conducted on Isolation, Identification and Antimicrobial susceptibility profile of *salmonella* and its Public health significance in milk supply chain of Banbasi town, western Ethiopia from December 2024 to March 2025 in dairy cows, with the objectives to isolate and identify *salmonella* from milk and milking environment, to assess the public health significance associated with risk factors and to estimate antimicrobial susceptibility patterns of salmonella isolates. A total of 384 samples were collected from Dairy cow and processed with Bacteriological methods. The Isolates were tested with a number of biochemical tests for confirmation and identification of salmonella. The study revealed that, 27.08% of salmonella prevalence was identified in collected milk swab samples in milk supply chain. Higher (38.28%) salmonella contamination was reported in milk container (bucket swab followed by milkers' hand swab (23.43%), and (19.53%) milk samples (P<0.05). There was significant (p< 0.05) association among age, parity, pregnancy status, body conditions, milking hygiene, teat lesion, udder washing, and drainage, with salmonella occurrence, which has significant difference (P<0.05). Whereas, breed, lactation stage, barn floor system, herd size, previous udder infection and blind teat had non- significantly associated with salmonella (P>0.05). Majority (90%) of drug resistance prevalence was reported in Penicillin G, followed by (86%) amoxicillin, cefoxitin (80%), 66% streptomycin, 50% sulphonamide and 50% gentamycin. Whereas higher (96%) of drug susceptibility was recorded in chloramphenicol (88%), followed by 66% ciprofloxacin, and 50% gentamycin. In this study, 34% mult- drug resistance were recorded in two (20%); three (10%); and five (4%) of antibiotic discs. The presence and consumption of raw milk may constitute a public health hazard and reduced milk quality due to salmonella. Thus health professionals should create awareness about milk handling practice, storage and milking process to Dairy farm owners, consumers, and milk collectors. And, regular resistance follow-up, using antimicrobials sensitivity tests helps to select effective antibiotics and to reduce the problems of drug resistance developments towards commonly used antimicrobials so as to reduce the problem encountered.
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INTRODUCTION

1.1 Background

Foodborne diseases are major public health problems in both developed and developing countries. More than 250 different foodborne diseases have been described. Most of these foodborne diseases are infectious diseases caused by a variety of bacteria. Foodborne bacterial diseases are a critical problem to the public health. Bacteria are commonly found in soil, water, plant, animals and animal products including milk, meat, cheese and yoghurt (CDC, 2018).

Cow milk has high water activity and nutritive value which serves as a kind medium for growth of microorganisms. Microbes commonly isolated and detected from milk and milk products pose a critical problem to human health. Bacteria which are commonly isolated from milk include *Escherichia coli, Staphylococcus aureus, Salmonella spp*. and *Listeria monocytogens*. Among these pathogens Salmonella attributes, the major part of foodborne diseases (Abeer *et al.,* 2012).

Salmonella species belong to Gram negative, rod shaped, facultative intracellular bacteria that potentially infect a wide variety of hosts. Salmonella is comprised of two species, Salmonella bongori and Salmonella enterica. Depending on the bacterial outer membrane somatic 'O' antigen, and fagellar 'H' antigen over 2700 different serovars of Salmonella has been characterized. Out of these 2700 serovars, nearly 1500 belong to the *Salmonella enterica subsp. Enterica*. *Salmonella enterica subsp. enterica* are the most common pathogenic and zoonotic bacteria causing diferent form of salmonellosis in human and animals (Guibourdenche *et al*., 2010).

Milk provided for human beings should not contain any pathogenic microorganisms. However, raw milk

and its products are considered as important sources of Salmonella. Milk and dairy products especially those produced from raw or unpasteurized milk have been attributed as potential vehicles for the transmission of Salmonella to humans. The contamination of raw milk by pathogenic microorganisms including Salmonella comes from feces of infected cattle, contaminated skin, infected udder, contaminated milking equipment, air, feed and water, and from milkers (Halawa *et al*., 2016). Salmonella is transmitted to human either through the fecal-oral route or through consumption of contaminated food (milk, eggs, and meats) and cause either typhoidal or non-typhoidal salmonellosis. In addition, milk is a potential source of multiple drug resistance, and is a potential public health concern. Antimicrobial resistance is one of the biggest global public health challenges (Halawa *et al*., 2016).

The Bacterial contamination of milk from an infected udder may render it unsuitable for human consumption by causing food poisoning or providing a vehicle for the spread of zoonotic diseases to humans (Mokgaotsi, 2019). Resistant bacteria from food animals may be passed through the food chain to humans resulting in resistant infections (Khairullah *et al.*, 2020).The major sources of pathogens and means of transmission include infected quarters and soiled udder, contaminated milking machines or milker's hands, udder washing trend, use of drying clothes, and tick infestation. Improvement of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country by controlling some of the major infectious disease through regular monitoring (Khairullah *et al*., 2020).

Antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products. Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat traced to dairy farms. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (Zelalem *et al.,* 2011).

1.2   Statement of the Problem

The handling and safety of milk and milk products are of great concern around the world and particularly in developing countries where the production takes place under un sanitized conditions and poor production practices (Mossie, 2019). In addition, there are challenges with continuous maintenance of the cold chain. Limited knowledge on the hygienic handling of milk and milk products, un-sanitized processing of milk by the smallholder of milk and milk products can be considered as indicators for the sub-standard production of milk and milk products in the country (Yilma, 2012).

Over the years, a number of studies have been carried out in Ethiopia that examine the occurrence of Salmonella in milk and feces of humans and cattle, as well as the development of patterns of resistance to various antibiotics in human and veterinary medicine (Abunna *et al*., 2017; Mitike, 2018). However, the studies available are sparse given the serious public health threat posed by the organisms. In addition, enough data was not available in Banbasi and its surrounding areas, which is one of the potential areas for milk production and consumption associated with increasing urbanization. Further studies on Salmonella prevalence in milk and its antibiotic resistance profile are also believed to complement the baseline data in Ethiopia and worldwide. In Ethiopia, currently there is a national interest to improve the dairy sector to enhance the benefits that would be gained from this sector. In line with this, animal disease researches that are directly related to dairy production help as an input for any intervention.

Different studies conducted in Ethiopia revealed fragmented substantial prevalence as well as antimicrobial susceptibility of *Salmonella* in veterinary and public health setups. According to some of study conducted in different part of Ethiopia, the prevalence rate of *Salmonella* in raw milk were reported, 10.42%, 12.9%, 8%, 12.5% and 10.76%, (Gebeyehu *et al.,* 2022; Fesseha *et al.,* 2020; Mustefa and Gebremedhin, 2018; Wabeto *et al*., 2017 and Zelalem *et al.,* 2011) respectively. Additionally, Lema, (2024), in Bishoftu town reported, 2.44% of Salmonella enterica from bulk milk samples, Liyuwork *et al*. (2013) in Addis Ababa, and Van *et al*. (2013) in the United States of America reported, a salmonella prevalence of 2.1% and 2.6% respectively. Similarly, the prevalence rate of Salmonella isolated from milk samples in Egypt, reported by Ahmed *et al*. (2014) (1.5%) and in Jigjiga town by Reta *et al*. (2016), (3%). Additionally, the prevalence rate reported by Murinda *et al*. (2002) in the USA was 2.24%, and Fufa *et al.* (2018) and Dadi *et al*. (2020) which was 0% and 0.7% at Meki and Sebata town Oromia, Ethiopia respectively. However, reports from coinciding study on lactating apparently healthy animals, personnel and

equipment used in the farms is limited especially in the current study area. The screening of milk and other dairy products for pathogenic organisms were play a vital role in curtailing human infection. Investigation of the prevalence and antimicrobial resistance of *Salmonella* from cattle and in contact human in dairy farms is of paramount importance to design methods to minimize the possible transmission of *Salmonella* between humans and cattle. Moreover, it is also important in combating the emergence of antibiotic resistant strains of *Salmonella* (Zelalem *et al.,* 2011).

However, there is no recent study which shows the current prevalence associated risk factors, of *Salmonella* originated from cows' milk in Banbasi District. For the control and prevention of the disease, proper isolation and identification of the responsible bacterial agents are necessary. (Rahman *et al.*, 2021) .

1.3. Objectives of the study
1.3.1. General Objective
- Assessment of hygienic practices, prevalence and antimicrobial susceptibility profile of S*salmonella species* isolated from milk supply chain and its milking environments in and around Banbasi town, Benishangul Gumuz , western Ethiopia.

1.3.2 Specific objective

- To isolate and identify *Salmonella spp* from raw cows milk, udder, hand and container swabs using cultural and biochemical tests
- To estimate the prevalence of Salmonella in the study area

- To determine the antimicrobial susceptibility pattern of the Salmonella spp.
- To identify associated risk factors

2.   MATERIALS AND METHODS

2.1 Study Area

Bambasi district is one of the districts found in Benishangul Gumuz Regional State under Assosa zone. The district is located at 616 km on the main road from Addis Ababa to the regional capital, Assosa town and 45km in the North East direction of Assosa town. The geographical location of the study area is depicted using figure 1. The study will be conducted from November to April, 2024/2025 in Bambasi districts. The study was conducted in Bambasi Dairy farm milk supply chain (production channels). Bambasi district has 38 kebeles stretches over an area of 2210.16 k.m. square with human population of 62693. The region is found in the north west of the country between latitude of 9 and 110 N and longitude of 34 and 350 E and its altitude range is 1500-1900 meter above sea level. Annual rain fall is between 1350-1400 mm with uni modal type of rain fall that occurs between April and October. Annual temperature ranges between 21^0c - 35^0c. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 36,735 Cattle, 10732 Goat, 3739 Sheep, 4467 Equines, 41438 Poultry and 23423 beehives (CSA, 2015).
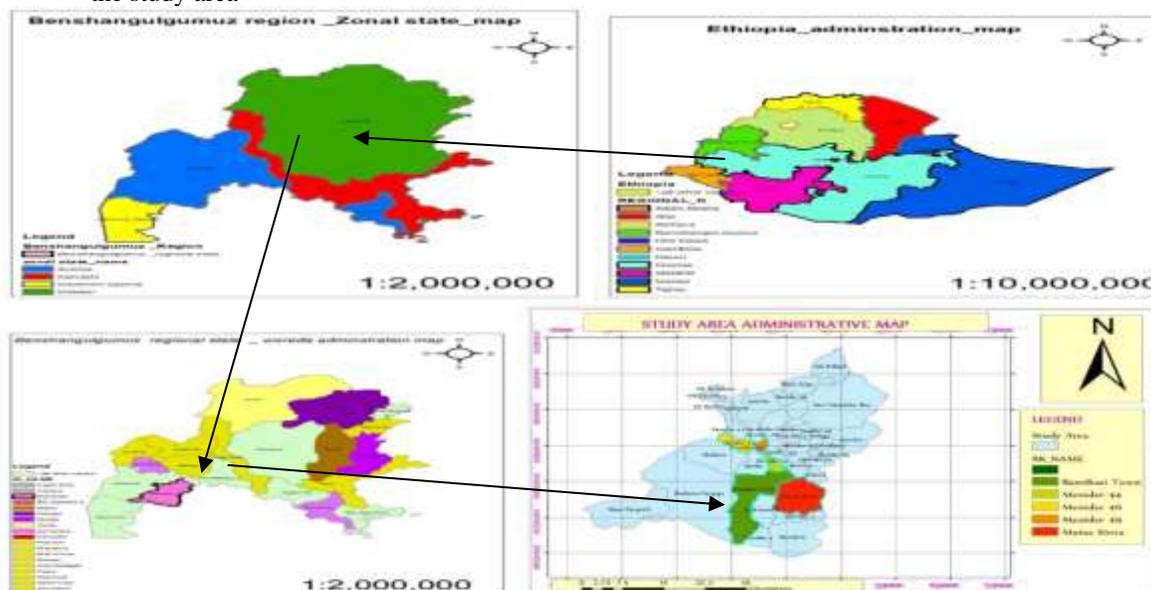

Figure 1: Administrative Map of Banbasi district

2.2 Study population

The study animals were apparently health lactating dairy cows, from Banbasi district, kept for supplying milk to consumers in Banbasi town and its surroundings. 128 milk samples, 128 milk handlers'(milkers') hand swab and 128 samples from milking containers or buckets were collected.

2.3 Study Design

A cross-sectional study was conducted from December 2024 to March 2025 to assess hygienic condition, antimicrobial susceptibility profile and isolate and identify *Salmonella* from raw cow milk supply chains.

2.4 Sample Size Determination

The sample size required for this study was determined by considering a 50% expected prevalence of Salmonella in the study, 5% desired absolute precision and 95% confidence level using the formula given in (Thrusfield, 2007) as there was no prior study in the area as follows.

$n = Z^2 \times Pexp(1-Pexp) \, d2$ Where: n=required sample size; Pexp=expected prevalence; d=desired absolute precision, Z=statistic for a level of confidence =1.96. Therefore a total of 384 samples were collected. Accordingly 128 pooled milk samples, 128 milk handlers' or milkers' hand swab and 128 samples from milking containers or buckets were collected.

Additionally, the questionnaire survey was entitled on dairy cow owners, handlers, and consumers or end users onto knowledge and attitudes of milk hygiene practices, and hence, the sample size required was calculated by using the formula given by Arsham (2002) as follows:

$$n = 0.25/SE2,$$

Where: n= sample size, SE (standard error) = 5%. The sample size was required for the **questionnaire survey** based on the above formula **is 100 respondents** were interviewed using proportional sampling from the market and from milking buckets at households. Of the total respondents, 50 dairy owners and 50 end users were interviewed by semi structural questionnaire.

2.5 Sampling Method

Purposive sampling techniques were used to select kebeles from Banbasi town based on their milk production potential. Simple random sampling technique was employed to selected small holder dairy farms, households and sampled animals in each kebeles. Based on the flock size, the farms were categorized as small scale (1–5 cows), medium scale (6–10 cows) and large scale (>10 cows) (Ababe *et al*., 2016). A total of 384 milk and milking environment sample were collected each containing 128 milk samples from udder, 128 samples from milkers' hand swabs or milk handlers and 128 samples from milking container (buckets) were collected.

2.6 Sample Collection procedures and Transportation

The samples were collected from milk and milking environment of raw milk , milking material swab and milk handler or milkers' hand, in and around Banbasi Town in lactating dairy cows along the marketing channels. The milk sample was taken from cows not treated previously with either intra mammary or systematic antimicrobials agents. Aseptic procedure was followed during milk samples collection in order to prevent contamination with microorganisms present on the skin udder and teats, on the hands of samplers and on the barn environment. Teat ends was cleaned and disinfected with ethanol (70%) before sampling. Strict foremilk (first jets) was discharged to reduce the number of contamination of teat canal (Quinn, *et al*., 2002). Sterile universal bottle with tight fitting cups was used. The universal bottle was labelled with permanent marker before sampling. To reduce contamination of teat ends during sample collection, the near teats was sampled first and then followed by the far ones (Hemling *et al*., 2002) .

Milk samples were collected from each of clinically and sub clinically mastitic non-blind quarters of the selected lactating cows for bacterial isolation according to the (Quinn *et al*., 2022). After milking out and discarding the first two drops, about 2ml of milk will be tested on CMT paddle. After CMT test the sample taken from each quarter and about 20 ml of milk will be aseptically collected from mastitis positive result using sterile universal bottle.

About 20 ml of fresh whole milk samples was collected from sampling points (directly from the udder of lactating Cows. The samples were transported to the Regional Veterinary microbiology laboratory within 24 hrs for microbiological examination and the samples were kept at +4°C until microbiological analysis (ISO, 2018).

2.7. Questionnaire survey

 Semi- structured questionnaire was used to collect information from small-dairy holders with lactating cows. The questionnaire was made with pre-coded

response choices (closed-ended questions) with a few open-ended questions. Also, the questionnaire was used to collect information on possible risk factors for bacterial contaminations in milk. Risk factors considered in the current study was bacteriological quality of raw cow milk along extensive dairy farms (smallholder dairy farmers), conditions of the barn/milking environment, hygiene of milking cows' udder and milk handlers, hygiene of milking equipment with special emphasis to hygiene of milking procedures and milk handling practices, utensils used for milking, milk storage and uses of milk (for selling or domestic purposes). Furthermore, milk consumption behaviors and their awareness on the risk of zoonotic diseases that are associated with the consumption of raw milk was assessed. The questionnaire was administered through face to face interview. While administering questionnaires, direct observation on general cleanliness and hygienic conditions and practices.

2.8. Laboratory Analysis

2.8.1 Bacteriological Identification of Salmonella

Bacteriological examination was done according to microbiology of food chain (ISO, 2018). Accordingly, it involves three stage processes: pre-enrichment, enrichment and plating out to isolate Salmonella spp. In primary enrichment step; one ml of milk sample was measured aseptically, homogenized into 9 ml of buffered peptone water (HIMEDIA BM020, India) and incubated at 37°C for 24h. Then, 0.1ml aliquot was transferred and added to 10ml of Rappaport-Vassiliadis with soya (RVS). Finally, the tubes were vortexed and incubated at 41.5° for 24h. Lastly, the enriched milk sample was plated onto a XLD Agar (HIMEDIA M031, India). The secondary enrichment tubes were vortexed before plating on XLD agar. 10µl loopfull of bacterial culture was grown on XLD agar using streak method and incubated at 35°C for 24h. After the recommended incubation time; Typical Salmonella spp. colonies with characteristic growth morphologies of pink colonies with or without black centers considered as suspected salmonella colonies. Three to five typical colonies of Salmonella was picked and streaked onto Trypton soya agar and incubated at 37°C for 18–24h for the further biochemical identification.
Further biochemical tests were conducted to identify salmonella using ISO (2018). according, suspected colonies of salmonella were tested for indole, Methyl red, Vogas-Proskaur and citrate utilization (IMViC), triple sugar iron (TSI), urease, and sugar fermentation tests.

2.8.2 Antimicrobial susceptibility test

The antibiotic susceptibility tests of the Salmonella isolates was performed using Kibry-Bauer disk diffusion test on Muller Hinton agar (HIMEDIA M173, India). Pure colonies from trypton soya agar will be taken with a wire loop, transferred to a tube containing 5ml of saline water, and emulsified. The emulsified broth culture was incubated at 37°C as far as it reached the 0.5 McFarland turbidity standards. Sterile cotton head swab will be soaked into the emulsified broth and the bacteria was swabbed evenly over the surface of Muller Hinton agar plate within a sterile safety cabinet hood. The plates were put at room temperature for 15min to allow drying. nine Antibiotic discs with known concentration of antimicrobials (Chloroamphenicol (C) (25µg), amoxicillin ((10µg), Penicillin G (10) cefoxitin (CK-30µg), gentamycin (CN) (10µg), streptomycin (S) (10µg), kanamycin (K) (30µg), Ciprofoxacin (CIP) (5µg) and trimethoprim-sulphamethaxazole (TS) (300µg), all Oxoid, United Kingdom) was carefully placed on the plates with 90-150 mm size and the plates was incubated for 24h at 37°C. Following incubation, the diameters of clear zones produced by antimicrobial inhibition of bacterial growth was measured to the nearest millimeter for each disc using transparent straight-line ruler and then classified as resistant, intermediate, or susceptible according to published interpretive chart of clinical laboratory standard institute (CLIS, 2023/24). The MDR index was determined for each of the isolates examined using the formula: MDR index=X/Y; Where "X" is the number of antibiotics to which the strain display resistance, and "Y" is the total number of antibiotics to which the test strain had been evaluated for sensitivity (Kumar, 2014).

2.9 Data Analysis

The data generated from this study was entered and managed in Microsoft Excel Office 2016. All the data analysis was done using Statistical Package for Social Sciences (SPSS) software version 26. Descriptive statistics such as percentages and frequency distribution was used to describe the questionnaire survey and antimicrobial sensitivity test results. The association of the Salmonella isolates with the sample types, parity, age, breed, body condition, milking hygiene, pregnancy status, lactation length, previous udder infection, teat lesion, managements, floor system, and farm size was analyzed using Chi-square (χ^2) test. In all the analyses, P - value less than 0.05 (P<0.05) was considered as statistically significant.

3. RESULTS

3.1 Isolation and Identification Salmonella

Bacteriologically, 27.08% (104/384) of the samples tested were initially suspected to be presumptive colonies of Salmonella on Xylose-lysine-deoxycholate agar (XLD) with characteristic morphology of pink colony with black center. The bacteria was gram-negative medium sized rod-shaped), catalase (+), oxidase (-) and O-F (fermentative). Salmonella isolates were negative for Urease test.

The isolates were further subjected to sugar tests and able to ferment, Xylose, glucose, maltose, sucrose, and lactose. On TSI Acid production was indicated by the color change from reddish to yellow and the gas production was noted by the appearance of gas bubbles in the test tubes (Table 1).

Table 1. Results of biochemical tests performed on Salmonella Isolates

Xylose	Catalase	oxidase	Indole	MR	VP	Citrate	Urease	TSI	Isolate
+	+	-	-	+	-	+	-	R/y/H2S$^+$	salmonella

Key. (MR) =methyl red, Vp= voges –proskauer, (+)=positive reaction, (_) negative reaction, R/Y/H2S^{2+}= Slant red with alkaline environment; butt yellow with acidic environment.

Higher salmonella prevalence (38.28%) was recorded in milk container (bucket) swab followed by 23.43% in milkers' hand and 19.53% in *milk sample swab* isolated in sample types as Table 2.

Table 2. Contamination rate of salmonella in various sample types

Sample Type	N=384	No. positive	Prevalence	OR	CHI2	P –value
Milk sample	128	25	19.53	1.36	7.90	0.03
Hand swab	128	30	23.43			
Container swab	128	49	38.28			
Total	**384**	**104**	**27.08**			

3.2. Risk Factors associated with the prevalence of salmonella

Age factors, parity, body conditions, pregnancy status, milking hygiene, floor type, teat lesion, udder washing before and after milking, and drainage system in the farms, were found to be statistically significant factors associated with the occurrence of salmonella (P<0.05). Higher occurrence of salmonella was identified in Dairy farm with small size (28.23%), followed by 22.89% in large size flock with (P>0.05; OR=0.13), which was statistically non-significant(P>0.05) and there was significant variation in the isolation rate of salmonella from 3-5 years age (21.73%), 25.09% in 5-9 years age and 33.33% in greater than 9 years age (P=0.02; OR=0.71), which significant(P<0.05). Higher salmonella infection rate were recorded in cross breed (31.30%) followed by local zebu breed (25.27%) with (OR=0.93; P>0.05). With regards to lactation stage, significance variation of salmonella contaminants were recorded in dry lactation period (33.06%) followed by 30% in late; 24.70% in mid and 21.6% in early lactation stage, with non-significant association (p>0.05; OR=1.09). Majority (38.09%) salmonella contaminants were investigated in poor condition while (16.41%) of salmonella rate in good body condition with significant difference (P<0.05; OR=0.33).

In the present findings, 27.63% of infection was reported in previous udder treatment followed by 21.2% which was non-significantly associated (P<0.05). 28.33% salmonella infection was investigated in non- blind teat while (22.07%) of contaminants were reported in blind teat dairy cows, which was not significant (P<0.05). Higher (60.71%) of the salmonella infection was isolated in teat lesion of lactating cows whereas, 24.43% of contaminants were isolated in non- teat lesion cows, which was significant (OR=5.15; P=0.000).

Consistently, significant variation of salmonella infection were investigated in non-pregnant lactating cows (30.55%) followed by 8.33% in pregnant dairy cows with (OR=0.20; p=0.001); and 40% infection was recorded in >6 parity, followed by 31.70% in 4-6 parity and 22.85% in 1-3 parity (OR=0.39; P=0.002) with significant variation(<0.05).

Majority (45.4%) of salmonella infection was isolated in poor milking conditions, while 15.4% was reported in good milking conditions with (OR=0.22; P=0.000); 28.44%, and 23.89% of the salmonella infection was isolated in concrete and muddy floor of the lactating cows barn, respectively with (OR=1.26), which was non- significant (P>0.05).

With regards to udder washing, and drainage, higher (65.21%) of salmonella isolation rate was in poor drainage and 10.78% were in good drainage of lactating cows, which was significant (P<0.05).

52.44% of salmonella infection were recorded in non- udder washing while 12.03% of salmonella isolation rate was identified in good udder washing dairy farms with OR=0.12; CHI2=75.83; P=0.00), which was significant (P<0.05) (Table 3).

Table 3: **Result of multivariate logistic regression of attribute risk factors with salmonella**

Factor	Categories	N=384	No (%) positives	OR	CHI2	P-value
Age(years)	3-5 (year)	23	5 (21.73%)	0.71	11.24	0.02*
	5-9 years	251	63(25.09%)			
	> 9 years	108	36(33.33%)			
Breed	Cross	115	36(31.30%)	0.93	0.49	0.78
	Zebu	269	68(25.27%)			
Parity	1-3	210	48(22.85%)	0.39	6.84	0.002*
	4-6	164	52(31.70%)			
	≥6	10	4(40%)			
Lactation Stage (m)	Early (≤3)	125	27(21.6%)	1.09	6.57	0.36
	Mid (4-6)	85	21(24.70%)			
	Late (7-9)	50	15(30%)			
	Dry (>9)	124	41(33.06%)			
Pregnancy Status	Yes	60	5(8.33%)	0.20	13.11	0.001*
	No	324	99(30.55%)			
Floor type	Concrete	159	42(26.41%)		0.77	0.67
	Muddy (soil)	225	62(27.55%)			
Milking hygiene	Good	240	29(12.08%)	0.12	75.83	0.000*
	Poor	144	75(52.08%)			
Previous udder Rx history	Yes	33	7(21.2%)	1.32	0.46	0.79
	No	351	97(27.63%)			
Blind teat	No	307	87(28.33%)	1.43	1.58	0.45
	Yes	77	17(22.07%)			
Teat lesion	No	356	87(24.43%)	5.15	18.74	0.000*
	Yes	28	17(60.71%)			
BCS	Good	195	32 (16.41%	0.33	23.60	0.000*
	poor	189	72 (38.09%)			
Herd size	Small	301	85(28.23)	0.76	1.03	0.59
	medium, large	83	19(22.89)			
Udder washing before milking	Yes	241	29(12.03%)	0.12	75.83	0.000*
	No	143	75(52.44%)			
Drainage	Yes	269	29(10.78%)	0.12	75.83	0.000*
	No	115	75(65.21%)			

3.3. Antimicrobial Susceptibility Test

Out of 104 *salmonella because of disc constraints, only* 50 isolates were subjected to antimicrobial susceptibility tests; among them, highest prevalence of antimicrobial resistance pattern were recorded in Penicillin (90%) followed by Amoxicillin (86 %), cefoxitin (80%), 66% in streptomycin, 50% in sulphonamides, and 50% in gentamycin , were drugs to which a large proportion of *salmonella* isolates' resistance. All 50 testes species of *Salmonella* isolates were highly susceptible to Chloramphenicol (88.63%), followed by Ciprofloxacilin (66%) and Gentamicin (50%) (Table 4).

Table 4: Antimicrobial susceptibility profile of salmonella isolates (n = 50).

Antimicrobial agents	Disc content (μg)	No. of Isolates	Resistance No (%)	Intermediate No (%)	Susceptible No (%)
Streptomycin	S-10	50	33(66)	2(4)	15(30)
Amoxicillin	Amx-10	50	43(86)	0(0)	7(14)
Trimethoprim-sulphamethaxazole (TS)	S-300	50	26(50)	5(10)	19(38)
Gentamycin	Gen-10	50	25(50)	0(0)	25(50)
Penicillin G	P-10	50	45(90)	5(10)	0(0)
Chloramphenicol	C-30	50	5(10)	1(2)	44(88)
Ciprofloxacin	Cip-5	50	17(34)	0(0)	33(66)
Cefoxitin	CK-30	50	40(80)	0(0)	10(20)

Key:%=percent, S=susceptible; I=intermidiate; R=resistance

3.3.1 Multi drug resistance of Salmonella isolates

Of all 50 salmonella isolates tested for antimicrobial susceptibility, 17(34%) isolates were developing multidrug resistance to different antibiotic discs. Ten isolates were found to be resistance to two antimicrobial drugs. From this isolates, (4) four isolates for (streptomycin and penicillin), three (3) isolates for amoxicillin and streptomycin, two isolates for (streptomycin, ciprofloxacin) and one isolates again for (amoxicillin, chloramphenicol).

Of the 5 isolates of Salmonella, three isolates were resistance to three antimicrobials, (amoxicillin, cefoxitin and streptomycin) and two isolate for (amoxicillin, chloramphenicol and gentamycin). Besides this, two isolates were resistance to five antimicrobials (amoxicillin, streptomycin, gentamycin, chloramphenicol, sulphonamides, cefoxitin) as indicated in Table 5.

Table 5. Multi drug resistance (MDR) pattern among Salmonella Isolates

No. AMR	AMR patterns	No. isolates	No. of isolates (%)
One	S	1	1(2)
Two	AMX, C	1	1(2)
	S, CIP	2	2(4)
	S, AMX	3	3(6)
	PEN, S	4	4(8)
THREE	AMX, GEN, C	2	2(4)
	PEN, FOX, S	3	3(6)
FIVE	AMX, C, GEN, S3, FOX	2	2(4)

Key; no= number; %=percent; AMX= amoxicillin; PG=penicillin, S3= sulfonamides; CHL= chloramphenicol; FOX= Cefoxitin

The MDR index was determined for each of the isolates examined using the formula: MDR index=x/y; Where "X" is the number of antibiotics to which the strain display resistance, and "Y" is the total number of antibiotics to which the test strain had been evaluated for sensitivity (Kumar, 2014).

Therefore, "8" is the number of antibiotics to which the strain display resistance, and "50" is the total number of antibiotics to which the test strain , MDR index=x/y=8/ 50=**0.16.**

3.4 Questionnaire survey

Semi structured questionnaire were interviewed to assess knowledge, attitudes and practices followed by dairy farms. The issues of public health significance arising from salmonella and possible sources of milk, general hygiene, hand washing before milking, use of towel, use of antiseptics for cleaning and milking, udder hygiene, frequency of cleaning, milkers' hand hygiene and milk container contamination with salmonella etc were assessed in Banbasi District (Table 6).

With respect to consumption of milk, 80 % of respondents, do have the habit of consuming milk at home, 40% of the society eat boiled milk, followed by (22%) raw, Ayib(20%), (18%) in form of ergo milk. Out of 50 consuming milk in different forms, 38 were acquiring illness after consuming raw milk and 62% no acquiring illness after consuming raw milk., with showing 40% abdominal pain and cramp, 34% both diarrhea and vomiting, followed by 18% diarrhea only and 8% vomiting only.

Consistently, 58% of the respondents indicated as there was milk borne disease, 42% of respondents and their family consume milk as common diet, 32% as required,14% frequently and 12% rarely. 70% of participant interviewer, indicated as there was time gap between taking and using the milk, while the rest was not. 42% of the participant interviewers keep milk in refrigerator (+4^{0c}) whereas, 58% of them kept their milk at room temperature.

Concerning the education level, majority (38%) of participants of the community of consuming milk in the district was un educated, followed by 32% of the respondent interviewers, consuming raw milk were those who read and write categories, (16%) elementary ; (10%) high school level and 4% of the consumers of the district were raw milk users in the area.

 56% of participants' did not know as salmonella was food born intoxication whereas 44% know salmonella as food born intoxication.

50% of respondents indicated, floor for housing dairy cows were natural floor categories, followed by 24% concrete floor, and 18% straw bedded floor. Besides this, 50% of the milking areas were natural floor, 24% concrete floor and 18% straw bedded floor.

With respect to dairy house cleaning, 50% of participants cleaning dairy house two times a day, 34% three times a day, and 10% one times a day. 60% participants clean their milking cows' udder before and after milking, 36% before milking, and 4% after milking, 48% of participants milking equipment's were plastic beaker, 32% aluminum cans, 16% clay pot, and 4% others.

48% of participants source of water to clean milking equipment's, teat and udder were well water, 34 %tap water, and 18% river were used for cleaning in the areas. Majority (70%) of participants did not wash the milk container with soap, detergent whereas 30% wash their milk container. Besides this, higher (68%) of participants wash their hand before milking while 32% didn't (Table6).

Table 6. Survey on Dairy farm owner associated with salmonellosis (N=50)

Factors	Categories	response rate	percent %
1. Do you consume the milk at home?	Yes	42	80
	No	8	20
2. If Yes, by what form?	Raw	11	22
	Ergo	9	18
	Ayib	10	20
	Boiled	20	40
3. Did one of your family members become ill after consuming raw milk?	Yes	19	38
	No	31	62
3. If yes, which of the following signs did he or she showed?	A. Diarrhea only	9	18
	B. Vomiting only	4	8
	C. Both diarrhea and vomiting	17	34
	D. Abdominal pain and cramp	20	40
4. Do you know any milk borne diseases?	Yes	29	58
	No	21	42
6. How often do you and your family consume the milk and its products?	A. Rarely	6	12
	B. As common diet	21	42
	C. Frequently	7	14

	D. As required	16	32
7. Is there any time gap between taking and using the milk?	Yes	37	70
	No	13	26
8. For how long do you keep milk at home?	A. 1-2hrs	22	44%
	B .2-8hrs	26	52%
	C.8-12hrs	2	4%
	D. more than 12hrs	0	0%
9. At what temperature do you keep milk?	A. < 4º c	21	42%
	B. Room temperature	29	58%
10. What is your level of education (who consumes raw milk)?	Read and write	16	32
	Elementary	8	16
	College	2	4
	High School	5	10
	illiterate	19	38
11. Do you know about salmonella food born intoxication?	Yes	22	44
	No	28	56
Floors used for housing dairy cows	A. concrete ground	12	24
	B. natural floor	29	50
	C. straw bedded floor	9	18
13. What about for milking areas?	A. concrete ground	12	24
	B. natural floor	29	50
	C. straw bedded floor	9	18
14. Frequency of cleaning cow house	A. Three times a day	17	34
	B. Two times a day	25	50
	C. One times a day	5	10
	D. Weekly	2	4
	E. Monthly	1	2
15. Frequency of cleaning milking cow udder ,	A. No cleaning	0	0
	B. Only before milking	18	36
	C. Only after milking	2	4
	D. Before and after milking	30	60
16. What kind of milking equipment did you use?	A. Aluminum cans	16	32
	B. Plastic beaker	24	48
	C. clay pot	8	16
	D. other	2	4
17. What is the source water do you use to clean your equipment, teat and udder?	A. River	9	18
	B. well water	24	48
	C. tap water	17	34
	D. stagnant water	0	0
18. Do you wash the milk container with soap, detergent?	Yes	15	30
	No	35	70
19. Do you wash your hand before milking?	Yes	34	68%
	No	16	32%
Hygienic practices of dairy farms and milk vendors	good	31	62%
	poor	19	38%

With respect to consumers, majority (50%) participants' milk consumers in the district consume milk in form of boiled, 24% yoghurt, 20% raw milk. And 6% cheese. 62% of participants had not have awareness about milk borne disease, and 38% had awareness about milk borne disease. 58% of consumers had not have awareness about milk borne disease while 42% had awareness. 62% of consumers did not have experience of illness after consuming milk and milk product, by consuming milk and milk products 52% of consumers showed stomach cramp; (30%) diarrhea, (16%) vomiting and 2% other clinical signs.

Majority (48%) of the milk in area was purchased from dairy farm owners followed by 28% in milk selling center; 20% in hotel/ café and 4% from other. Majority (62%) of the consumers use plastic for collecting milk , followed by (20%) metallic container; and 18% other traditional containers were used. 48% of the participants consumers milk stay at home prior to consumption for 1-2 hrs, followed by 28% for >2hrs; and 24% for <1hrs(Table 7).

Table 7. Results of Questionnaire survey at Consumer level on public Health significance of Salmonella (N=50*)*

Factors	Categories	Freq.	response rate, %
1. In what form do you consume milk? .	A. Boiled milk	25	50
	B. Yoghurt	12	24
	C. Cheese	3	6
	D. Raw milk	10	20
2. Do you have awareness about milk born disease?	Yes	19	38
	No	31	62
3. Do you know about Salmonella food born disease?	Yes	21	42
	No	29	58
4. Do you experience illness after consuming milk and milk product?	Yes	19	38
	No	31	62
5. If yes, what kind of clinical sign did you experience?	A. Diarrhea	15	30
	B. Vomiting	8	16
	C. Stomach cramp	26	52
	D. Other	1	2
6. Where do you purchase milk?	A. Farm	24	48
	B. Milk selling center	14	28
	C. Hotel /café	10	20
	D. Other	2	4
7. What kind of container do you use to collect milk?	A. Plastic	31	62
	B. Metallic	10	20
	C. Other	9	18
8. How long the milk stay at home prior consumption?	A. <1hr	12	24
	B. 1-2hr	24	48
	C. >2hr	14	28
9. At what temperature do you keep milk?	A. < 4 º c /refrigerator	31	62
	B. Room temperature	19	38

4. DISCUSSION
4.1 Prevalence of Salmonella

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, with transmission to humans predominantly occurring through the consumption of animal-source foods including cow milk and its products (Ferrari *et al.,* 2019). 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 (Abrar *et al*., 2020).

In this study, the overall prevalence of salmonella among 384 samples from apparently healthy Dairy cows were found to be 104/384 (27.08%), with higher 38.28% salmonella prevalence in milk container /(bucket) swab, followed by 23.43% in milkers' hand swab, and 19.536% in milk samples, which was statistically significant (P=0.03; OR=1.36). The present findings were agreed with the earlier report of Abdi Bedassa *et al*., (2023) in Ethiopia reported, salmonella contaminants prevalence in raw milk samples at the production (19.7%) and at milk collection (21.3%) levels. Besides this, it was comparable with the previous findings of salmonella reported from Dire Daw (18.75%) by Tesfaye *et al*. (2013), Central Ethiopia (10%) by Geletu *et al*. (2023). However, this investigation were higher as compared to the reports from Gondor (6.0%) by Ejo *et al*. (2016).

As compared to the present study, Seblewebgel A *et al*., (2024) in Woilta Sodo reported that, overall salmonella contaminants in milk supply chain, was 19.6%. The highest salmonella contaminants in hand swab was (28.8%) and lowest salmonella prevalence was (14.2%) in bulk milk samples. And the prevalence was 24.3% in the milking environment, 18% in the milk supply chain samples and 15.5% in the diarrheic patient samples.

As compared to the present findings, higher salmonella findings were reported by Abdi Bedassa, (2021) in Oromia region, a total of 480 samples of raw milk, pasteurized milk, and cottage cheese cultured individually on both XLD and HE selective media, 40 % (192/480) and 67.5% (324/480) salmonella isolates on XLD and HE agar plates, respectively.

From sampling point of view, 6.51%, 7.81 %, and 12.76% of salmonella prevalence were reported in milk samples, milkers' hand swab, container (bucket) swab respectively, which was significant(P<0.05). This report was consistent with the findings of Isayas A *et al*., (2023) who reported, (9.3%) Salmonella prevalence from direct udder milk samples by bacteriological methods. And consistently, Lema T, (2024), in Bishoftu town, central Ethiopia, reported, 2.1% of salmonella in dairy farms, 3.57% in milk vendors, and 2.08% in café/ restaurants, by bacterial examination. And Lema T, (2024), in Bishoftu town, central Ethiopia, indicated that the overall prevalence of Salmonella enterica was 2%; of which 2.44%, 0%, 4.76%, and 2.44% were from bulk milk, swab of milk container, fecal sample and floor swab at dairy farms, 3.5% from raw milk and swab of milk container at milk vendors and 0% and 0% cheese and yogurt from restaurant, respectively.

This findings in agreed with the prevalence reports of 10.42% (A. Gebyhu *et al*.,2022), 12.9% (H. Fesseha *et al*.,2020), 10.76% (Z. Addis *et al*., 2011), 8% (B. A. Mustefa & E.Z.Gebremdhin., 2018), and 12.5% (B.A.Mustefa &E.Z.Gebremdhin, 2018; W.Wabeto *et al*.,2017). This could be attributed to the agro ecological similarity of the study area and the similarity of the protocol of bacterial isolation and identification techniques.

The present study among the sample types (6.51%) salmonella prevalence was reported, which was agreed with the earlier reports of Lema T, (2024), in Bishoftu town, reported as 2.44% of Salmonella enterica from bulk milk samples at dairy farms which is consistent with the previous research conducted in different locations. Specifically, it aligns closely with the results reported by Liyuwork *et al*. (2013) in Addis Ababa, Ethiopia, and by Van *et al*. (2013) 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 *et al*. (2014) 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*. (2002) 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 Fufa et al. (2018) and Dadi *et al*. (2020) which was 0% and 0.7% at Meki and Sebata town Oromia, Ethiopia respectively.

Even though the current study isolated and identified only 6.51% of the Salmonella from the bulk milk, compared to previous studies, this 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 and it is a source of Salmonella infection (Gelatu *et al*.,2022).

The prevalence rate of Salmonella isolated from container swab in the present study was 12.76 %. This finding is consistent with previous studies by Gezahagn *et al*. (2023) in the town of Bedelle and Nekemte in western Ethiopia and by Gelatu et al. (2022) in central Ethiopia, where the prevalence rate in dairy farms 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, and poor container (bucket) washing habit, animal overcrowding, and inadequate cleaning and disinfection protocols could contribute to the presence of Salmonella contamination on container, udder teat and dairy farm floors.

4.2 Associated risk factors for salmonella

In the present study, significant prevalence of risk factors were associated with salmonella infection in the dairy farms. Nine potential risk factors were estimated using logistic regression analysis: sample type, age, parity, body conditions, pregnancy status, milking hygiene, teat lesion , udder washing, and drainage system in the farms, were significantly associated with the occurrence of salmonella contaminants (P<0.05).

The current study found that the age of the animals was statistically significant and that there were strong relationships between age categories and the rate of Salmonella detection. Furthermore, the study found that the rate of Salmonella isolation was higher in animals over the age of 9 years than in other age groups (33.33%) followed by 5-9 years age in (25.09%) and 3-5 years age in (21.73%) (CHI2=18.64; P=0.02; OR=0.71). This finding was agreed to the findings of Isayas A *et al*.,(2023) in selected District of Wolaita zone, South Ethiopia, who reported, 6.5% salmonella infection in 3-6 years age, 7.5% in 7-9 years and 44.4% in greater 9 years age of Dairy cows' milk, which was significant (P<0.05). This finding was supported by

(Biffa *et al*., 2005), who discovered a strong association between age and the prevalence of bacteria.

A significant difference in the distribution of Salmonella isolates was observed between body condition categories (P=0.00*; OR=0.33). According to the current study's findings, animals with poor body conditions had more Salmonella (38.09%) in their milk than animals with good body conditions (16.41%). This could be because the body condition of animals is reduced when they are infected with parasites, feed, and water deprivation, and stress create favorable conditions for pathogens to multiply in the body of animals (JH Gillespie 1981; JFL Lues, MR *et al*.,2006). This could also be associated with an animal's immune system's weakened ability to defend against infection-causing agents as they age (Fufa *et al.,* 2013). This finding was comparable with the findings of Isayas A. *et al*., (2023) in selected District of Wolaita zone: higher salmonella infection in poor body condition(17.6%), followed by 6.1% in medium and 2.9% in good body conditions, which was significant (P<0.05).

In the current study, we also attempted to compare the rate of salmonella isolation among farms with the varying breeds, lactation stages, and parity. Accordingly, the prevalence of Salmonella isolates was high in cross breed (31.30%) than in local zebu (25.27%); highest in the dry period(33.06%); late lactation stage (30%) and followed by mid (24.70%) and early (21.6%) lactation stage; as well as highest in those animals with many parities (40%) and followed by moderate (31.70%), and few (22.85%) parities. This could be partly due to the types of animal breeding (physiological difference), lactation stage (as lactation stage increased so did Salmonella prevalence), and parities. Also, milk handling, and management practices and the likelihood of contamination are considered high in the late lactation stage and many parity animals since Salmonella are ubiquitous (RJ. Erskine, 2001; OM. Radostits *et al*., 2007). Besides this, Isayas A *et al*.,(2023) in Wolaita zone reported, 19.1% salmonella infection in late lactation stage, 5.9% in mid lactation and 2.8% in early lactation stage, which was significant(P<0.05) And 17.3% of salmonella infection was reported in cross breed and 5% in local zebu breeds, which has significant difference(P<0.05). Comparably, Isayas A et al, (2023) reported, higher (44.4%) salmonellosis was recorded in many parties, followed by (7.3%) moderate and (6.7%) in few parties , which was significantly associated (p<0.05).

Therefore, the lower prevalence in local zebu breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscles, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and Mcdaniel, 1985). In addition to physical barriers, the difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001). This may be due to an absence of dry period therapy and birth related influences. During a dry period, due to low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply (Aylate *et al*., 2013). Radostits *et al*. (2000) suggested that, the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat.

A cow with many categories of parity had the highest Salmonella isolation rate (40%). When compared to animals with a moderate level of parity, those with fewer categories of parity have a more effective defense mechanism (Erskine, 2001). This might be due to the increased opportunity and contamination of salmonella infection and the prolonged duration of infection (Markos *et al*.,2023). The higher early occurrence of salmonella with parity in the current study is comparable with the previous reports of Mulugeta and Wassie, (2013) in Wolaita Sodo town, Mekibib *et a*l., (2010) in Holota town and Haftu *et al*., (2012) in northern Ethiopia. The association might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without salmonella control program (Radostits *et a*l., 2007). The reason could be the increased chance of infection time and the prolonged duration of infection on the animal body, particularly in a herd lacking appropriate disease prevention and control programs (O. M. Radostits *et al*., 2007).

It is well known that raw milk for public consumption and the consumption of raw milk-based products pose some risks if not properly pasteurized. Contamination of raw milk can occur from the environment, particularly during milking and milk handling, as well as from water and milking equipment and facilities (Bille *et al*., 2010). Salmonella occurrences appeared to be high in the milk samples tested here, and the possibility of this organism growing in improperly handled milk and products made from raw milk poses a public health risk, particularly to vulnerable members of the population.

Occurrence of salmonella was significantly associated with milking hygienic practice (P=0.000*). Cows at farms with poor milking hygiene standard are severely affected (52.08%) than those with good milking hygiene practices

(12.08%) with (P=0.000; OR=0.12). This findings were agreed with the findings of (Mulugeta and Wassie, 2013; Lakew *et al*., 2009; Sori *et al*., 2005). This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis (Radostitis *et al*., 2007).

Majority (27.55%) of salmonella infection was isolated in muddy floor of dairy cows followed by 26.41% in concrete floor system, with (OR=1.08; CHI2=0.77; P>0.05), which was 1.08 times more contaminates with salmonella as compared to the concrete floors. This finding was consistent with earlier report of Isayas *et al*., (2023) in selected districts of wolaita zone. That was, (10.32%) salmonella infection in poor husbandry hygiene, and zero infection was reported in good husbandry hygiene of dairy cows and the farm's husbandry hygiene and management system, with no significant association with salmonellosis (p > 0.05). The findings of a high prevalence of salmonella in farms with muddy (soil) floors when compared with concrete floor types shows the occurrence of salmonella is significantly associated with the housing (bedding) type or condition of the farm. This is due to association with poor sanitation and cows which were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of salmonella pathogens. The main sources of infection are udder of infected cows transferred via milker's hand, towels and environment (Radostitis *et al*., 2007). Salmonellosis was generally considered to be moderately prevalent and was one of the diseases of dairy cows in the study area that could have an impact on dairy production and have serious health impact. In the present study, majority (65.21%) of salmonella infection was recorded in poor drainage and 10.78% in good drainage of Dairy cows, which was significant (P=0.000, OR=0.12). Consistently, 52.44% of salmonella infection were recorded in poor udder washing system followed by 12.03% of salmonellosis in good udder washing dairy farms with OR=0.12; P=0.000), which was significant (P<0.05). This study revealed that dairy cows house with poor drainage was 0.12 times more likely to be harbor salmonella than well drainage housing systems. And also poor udder washing system was 0.12 times more likely harbor salmonella as compared to good udder washing system. The association can be attributed to poor sanitation practices and the housing of cows in dirty and muddy common barns with bedding materials that promote the survival and transmission of pathogens (Bizunesh *et al*., 2022). Similarly, Abdi Bedassa *et al*., (2023) in Ethiopia reported, salmonella contamination in raw milk samples at the production and at milk collection levels and indicated the temperature of water used for cow udder washing, the practice of mixing milk lots, the type of milk container, use of refrigeration, and milk filtration.

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 (Fufa *et al*., 2017; Gebeyehu *et al.,* 2022; Gezahegn *et al*., 2023).

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, and large. Our results showed that the isolation rate of Salmonella was significantly comparable among small, and large-sized farms. Higher salmonella contamination was reported in small herd size (28.23%), followed by 22.89% in large herd size (P>0.05; CHI2=1.03; OR=0.76). These findings indicated that, the contaminants in small herd size was 0.76 times more likely contaminate the large herd size. This result was in a greed with the previous findings of Lema T, (2024), in Bishoftu town, central Ethiopia, who reported (5 small scales, 34 medium scales and 3 large scales), and 7.32% salmonella prevalence. Of these farms, 2.43% and 4.88% Salmonella isolates were obtained from small-size and medium-size farms respectively; and no Salmonella was isolated from large scale dairy farms, which was statistically significant association between sample type and the occurrence of the pathogen across dairy farms. This variation might be due to crowding of dairy farms, poor biosecurity measures, poor drainage, poor sanitary, hygiene and cleaning system of the herd/ flock size.

4.3 Questionnaire survey results

The consumption of raw milk and its different forms of product is common in Ethiopia, which is not safe from consumers' health point of view as it may lead to transmission of various diseases. It may be contaminated at the site of production and during processing, the cow itself, unclean milk containers and the milk handlers. The hygienic condition or quality of milk has serous implication on public health safety. The questionnaire results mainly gave broad understanding of the milking and hygienic practice.

In this study among the farmers, 22 % had a habit of drinking raw milk and 62 % of them didn't have awareness about food born disease associated with

consumption of raw milk. This results is agree to a study done by Tsige, (2018) around Arsi Negelle town, which is 21.7% of the raw milk consumption and 62% of them have no awareness about milk borne disease among farmers. Though the results showed relatively a lower percentage of raw milk consumption, still these individuals are at a greater risk of contracting food born intoxication infection than those who do not consume raw milk. Similar to the consumer farmers, 20% consumer drink raw milk and yoghurt (24%), 6% cheese, 50% boiled milk and have no awareness of milk borne disease.

Consumers are last group of food chain and therefore they are risk of any mal practice occurring in the chain. Also 38% of consumer's farmers kept milk at room temperature. This lacks of refrigeration facilities at farm and house hold level with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Tsige, 2018).

Regarding the hygienic practices of dairy farms and milk vendors, the survey result showed that 62% of respondents had good hygienic practices. However, the type of housing, barn cleaning, drainage system, unhygienic milking methods, type of milk container, type of water used for washing hands and milk equipment were identified as risk factors for the occurrence of Salmonella in raw cow milk. This finding was in line with the finding of Bedassa (2021) which identified the source of water used for washing milking equipment and, type of milk container as the most risk factors for the occurrence of Salmonella.

In the present study, 68% of the respondents wash their hands before milking, while 32% did not wash their hands, which was agreed with the findings of the Agenes J., (2022) in Ilala district Darsalam, Tanazania , who reported, Hand washing before milking cows was found to be about 65.2% which is insufficient for sustaining milk quality. This result is lower than the reports in Mvomero and Njombe districts Tanzania by (Mdegela *et al*., 2009) and that in Jimma (>94%) by (Yilma, 2012). The majority of farmers fail to follow milking protocols by skipping or failing to perform some of the most important procedures during milking. The amount of microorganisms in the milk is also known to be affected by general cleanliness at milking time. In general, animal attendants' unsanitary behaviors may lead to microbial contamination of the cow's milk. Previous studies in Tanzania had similar findings (Karimuribo *et al*., 2005; Mdegela *et al*., 2009; Swai and Schoonman 2011; Shija, 2013; Kanyeka, 2014).

Furthermore, all farmers reported udder washing before milking using either bare hands or a piece of cloth. In this study, the sole cleaning agent used for cleaning the udder was water with no detergents. This result has an agreement with the study in Tanzania (Mdegela et al., 2009; Gwandu et al., 2018) and Ethiopia (Tegegne and Tesfaye, 2017) in which farmers did not use detergents for udder cleaning.

48% of respondents in the community were commonly used plastic containers for collecting and storing milk during the current study which is consistent with findings of (Schoder *et al*., 2013; Gwandu *et al*., 2018) in Tanzania which revealed that farmers used **plastic containers** for milk collection. 100% of the dairy cow owners utilized water and soap for cleaning milk handling equipment which is in agreement with the reports of (Tegegne and Tesfaye, 2017). Cleaning the equipment with soap and good quality water is likely to eliminate milk residue, including microorganisms, thereby affecting the milk's microbiological quality. In addition, it was observed in the current study that milk was stored at room temperature for a long time, prompting growth of microorganisms over time. Similarly, (Kivaria *et al*., 2006a) reported that the high microbial load in milk is due to a lack of cold chain. In general, unhygienic milk handling may have contributed to microbial contamination in milk. However, there was no statistical significant association between microbial contamination in milk and most of the unhygienic practices that were observe in this study (P>0.05).

We were observed in hand washing before milking, hand washing between milking different cow and milking sick cow respectively. This is in agreement with the study on bacteriological milk quality by Tegegne and Tesfaye., (2017) in Ethiopia who showed that there was a significant effect on hand washing prior to milking in total bacterial count. The findings are in contrast with those from (Kivaria *et al.,* 2006a) who reported that cleaning frequency of milk container, milk storage time, milk storage containment and mixing fresh with previous milk significantly influenced the microbial quality of marketed milk.

4.3 Antimicrobial Susceptibility test result

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 (Gebremedhin *et al*., 2021). In the current investigation, Salmonella isolates (n= 50) were evaluated against eight frequently used antimicrobials using (CLSI, 2022) guidance. Antimicrobial susceptibility testing revealed 90%, 86%, 66, 50% and 50 % resistance to penicillin, amoxicillin, streptomycin, gentamycin,

and sulphonamides respectively. In comparison, 96% sensitivity to cefoxitin, was identified, followed by 88%, 66%, and 50% sensitivity to chloramphenicol, ciprofloxacin, and gentamycin, respectively. .

Agenes J., (2022) in Ilala District, Dar Salam, Tanzania reported, comparably, the isolated Salmonella spp showed resistance to penicillin (100%), ampicilin (100%) and amoxicillin/clavulanic acid (100%). In addition, comparably, Seblewngel A *et al*., (2024) in Woilta sodo , Ethiopia, reported that, among the Salmonella isolates tested, 89.7%, 88.5%, 77.8%, 77.8%, 66.7% and 63.3% were resistant to amoxicillin, ceftazidime, ampicillin, streptomycin, doxycycline, and tetracycline, respectively. All the isolates were susceptible to norfloxacin, and 81% of them were susceptible to ciprofloxacin.

The current findings revealed that 34% of the isolates were resistant to two or more classes of antibiotics, which was lower than the report of Fesseha *et al*. (2020), 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 isolates. 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 (Farhan *et al.,* 2024), self-medication due to easy access to antimicrobials without prescription in the public health sector, and administration of sub therapeutic doses.

As compared to the present findings, Seblewngel A *et al*., (2024) in Woilta sodo, Ethiopia, indicated higher multidrug resistance (MDR) was detected in 88% of the isolates and it was higher at the farm than milk collectors and retailers.

The current investigation found 86 % amoxicillin resistance, which was greater than the findings of Beyene *et al.* (2016) and Fesseha *et al*. (2020) 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 Abeba, as reported by Zewdu and Cornelius (2009), 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*. (2018) and Obaidat and Stringer (2019), which were 80% and 89.3%, respectively.

On the other hand, our data suggested a greater resistance rate than the studies by Abra *et al*. (2020),

Gelatu *et al*. (2022), and Beyene *et al*. (2016) 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 (2017), (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. (2018) in the United States, which was 28% lower than the report of Fesseha *et al*. (2020), 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 (Fesseha *et al*., 2020). 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 50%. As compared to the present findings, higher prevalence was reported by Addis *et al*. (2011) (73.3%) and Tadesse and Anbessa (75%). Besides this, higher susceptible of gentamycin was reported by Tesfaw *et al.* (2013), Abunna *et al*. (2017) and Beyene *et al*. (2020) who documented a resistant rate of 100%.

Additionally, the susceptibility rate of ciprofloxacin was 66% which was lower than the report of 83.3% documented by Addis *et al*. (2011). 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.

5.   CONCLUSION AND RECOMMENDATIONS

The present study has shown that salmonella was prevalent (27.08%) in dairy farms. Higher salmonella prevalence was recorded in milking container swab (38.28%); followed by milkers' hand swab (23.43%), and milk samples (19.53%), which was significant(P<0.05) in Banbasi town. Age, breed, parity, pregnancy status, milking hygiene, teat lesion, body conditions, and were potential risk factor associated with salmonella contaminants in the recent studies. And also udder washing conditions before and after milking, drainage system, milkers' hand and milk containers were the determinants of Salmonella milk contaminants. The prevalence of salmonella indicates

the higher public health risk due to the widespread consumption of raw milk and its products. The study also revealed in adequate knowledge of milk borne disease and occurrence of antimicrobials resistance salmonella. In addition, the large proportion of MDR *salmonella* isolates may impede effective control of Salmonella udder infection in cows as well as it present a public health risk due to the spread of drug-resistance zoonotic *Salmonella*. It was observed that *Salmonella* isolates were highly sensitive to cefoxitin, chloramphenicol, Ciprofloxacin, and Gentamycin. Whereas, the highest rate of resistance among the isolates was against penicillin followed by amoxicillin. In general, the study has revealed the possibility of the public health risk posed by *Salmonella* in Banbasi town. Education programs increase knowledge and raise awareness of farm workers, milk product handlers, and milk selling centers on the important of good hygiene help to increase the good practices of food handlers, which could significantly, reduce contamination levels, So based on this conclusion the following recommendations are forwarded;

- Raw milk intended for human consumption must be subjected to pasteurized or heat treatment at least equivalent to pasteurization temperatures.
- Education programs increase knowledge and raise awareness of farm workers, milk product handlers, and milk selling centers on the important of good hygiene help to increase the good practices of food handlers, which could significantly, reduce contamination levels,
- Hands of milkers' and milk containers should be be effectively cleaned with detergents and soap
- Udder washing should be practiced before and after milking
- Milk should be maintained in a cold chain starting from production until consumption
- Awareness should be created among community for implementation of better control and subsequent reduction of SFP
- Mastitis control strategy should be initiated and promoted in the study area;
- Monitoring, rational use of drugs, and periodic assessment of the antimicrobial sensitivity of drugs prior to use are recommended.
- Hygiene measures during milking procedure should be practiced that may reduce the transmission of the disease
- There should be regular antimicrobial sensitivity test to select effective and alteration of antibiotics to reduce the

problems of drug resistance development towards commonly used antibiotics.

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