JEETHP TELEGECO

JEETHP contents list at www.telegeco.gr

Journal of Emerging Environmental Technologies and Health Protection

JEETHP - Journal site https://www.telegeco.gr/eISSN2623-4882.html

Cite as: Shuramo,,M., Sorsa ,M., Feyissa, N., Kelbesa ,K., Pal,M., Koliopoulos, T. (2023). Occurrence and Antimicrobial Resistance of Staphylococcus Aureus from Dairy Farna in Fiche and Hambiso Towns of North Shewa, Ethiopia, vol. 6, issue 1, pp. 17-34, Journal Emerging Environmental Technologies and Health Protection (JEETHP), ISSN 2623-4874, e-ISSN 2623-4882, https://www.telegeco.gr/JEETHP6I1A1.pdf

Occurrence and Antimicrobial Resistance of Staphylococcus Aureus from Dairy Farna in Fiche and Hambiso Towns of North Shewa, Ethiopia

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Abstract

Staphylococcus aureus belongs to the genus *Staphylococcus* and the family *Staphylococcaceae.* It is a non-motile, fermentative, and non-spore-forming bacterium that is facultative anaerobic. They develop quite large yellow or white colonies on nutrient rich agar media and are typically found in clusters that resemble a bunch of grapes when observed under a light microscope after Gram staining. *Staphylococcus aureus* is a desiccation and high osmotic condition tolerant bacteria that may thrive in potentially dry and stressful conditions. Human nares are the principal niche and greatest reservoir of *Staphylococcus aureus*; cows may be the second largest. The virulence factors produced by *Staphylococcus aureus* are essential for successful human and animal infection. It is one of the most significant bacteria in veterinary medicine, and it is widely known as a major cause of intramammary infections. *Staphylococcus aureus* is a common human pathogen that can cause a wide range of clinical infections, from minor skin infections to life-threatening infections. *Staphylococcus aureus* is a highly adaptable bacterium that develops resistance to the majority of antibiotics on the market. Drug resistance in *Staphylococcus aureus* has gradually risen over the last few decades as the pathogen have evolved and antibiotics have been abused. The treatment is ineffective; instead, control and prevention of *Staphylococcus aureus* should be prioritized. Eliminating conditions that expose the teat ends to bacteria and reducing the probability of cow-to-cow transmission are the most effective ways to prevent new infection and control *Staphylococcus aureus* in dairy animals. Contact precautions and adequate infection control techniques are used to prevent the spread of *Staphylococcus aureus* infection in humans.

Keywords: Antimicrobial resistance, KAP, Milk,North Shewa, Staphylococcus aureus, Public Health

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1. INTRODUCTION

The world faces an increase in food borne diseases (FBDs) as the result of unsafe food sources, improper serving, contamination from equipment, food handlers and keeping food in unsafe places which were suitable for micro-organisms [1, 2]. *Norovirus*, *Salmonella*, *Clostridiumperfringens*, *Campylobacter* and *Staphylococcus aureus* are the top known food borne pathogens [3]. There are more than 250 causative agents of food borne diseases, of which bacteria account for more than two-thirds of them [4].Among food borne bacteria *S. aureus* is one of the main causes of food borne diseases throughout the world [5].

Staphylococcus aureus (*S. aureus*) is a gram-positive, facultative anaerobe, non-motile, non-spore-forming, oxidase-negative, catalase-positive, and coagulase-positive bacteria [6]. *Staphylococcus aureus* is found as normal flora on the skin, anterior nares, nasopharynx, and mucous membranes of animals and humans [4]. It is distributed worldwide and also found in environmental sites like soil, water, air [7] and different foods of animal origin [8]. While about 20 to 30% of the human population carries *S. aureus*, the prevalence of *S. aureus* varies from host animal to host animal, and up to 90% of chickens, 42% of pigs, 29% of sheep, and between 14 and 35% of cows and heifers are carriers [9]. Staphylococcalfoodpoison (SFP) is one of the foremost prevalent causes of gastroenteritis worldwide. Diseases caused by *S. aureus*are skin infection (boils cellulitis, and staphylococcal scalded skin syndrome),bacteremia, osteomyelitis, endocarditis, pneumonia and septic arthritis [10].

Recently, antimicrobial resistance (AMR) is increasingly posing challenges to publichealth and animal health services [4]. Among them, *S. aureus* is the prominent drug resistant bacterial pathogens. It is the first bacteria reported developing resistance to the first formulated antibiotic penicillin [10]. The *S. aureus* could develop antimicrobial resistance by the acquisition of mobile genetic elements carrying resistance genes [11]. These antimicrobial resistant bacteria might be transmitted to occupational risk groups like farmers, meat packers, butchers, and hospital facility carers through handling or ingestion of contaminated materials [12].

*Staphylococcus aureus*is a major health constraint that has public health and veterinary importancein Ethiopia [13;14]. It adversely affects public and animal health as a result the country might face economic loss due to a decrease in food quality and quantity, and an increment of treatment costs due to MDR [15].This study was carried out todetermine the occurrence,antimicrobialresistance of *Staphylococcus aureus*from dairy farms and knowledge, attitude and practice of farmers on milk contamination and antimicrobial resistance in Fiche and Hambisotowns of North Shewa, Ethiopia.

2. MATERIAL AND METHODS

The study was conducted in Fiche and Hambiso towns,North Shewa Zone, Oromia Regional State, Ethiopia from November 2021 to April 2022.

2.1. Description of the Study Areas

Fiche town: Fiche town is located in North Shewaof Oromia regional state. It is located 114 km northern of Addis Ababa. The town is located at latitude and longitude of 9°48′N and 38°44′E with an elevation ranging from 2,738 to 2,782 meters above sea level. The town has more than 65,000 inhabitants in 4 kebeles. The annual rainfall ranges from 1800-2200 mm and theaverage annual temperatureis 18.3 ^{o}C (high: 24 ^{o}C and low: 12.6 ^{o}C). The livestock population of the district is 94,064 cattle, 56,507 sheep, 16,875 goats, 18,281 equines and 39,948 poultry managed under extensive, semi intensive and intensive management system. The dairy population in the town include 300 female cross breed cows, 175 cross breed heifers and 90 cross breed female calves. The total land area of the district is about 67,020 hectares of which 336,220 hectares are used for crop production, 44,090 hectares for animal grazing, 12,976.4 hectares forest covered and 20,106 hectares for other activities [16].

Hambiso town: Hambiso town is capital town of Dagam district which located in North Shewa of Oromia regional state. It is located 126 km northern of Addis Ababa. It is located at a latitude and longitude of 9°48′N 38° and 24′E and an elevation between 2515 and 2547 meters above sea level. The annual rainfall ranges by average 1253.8 mm. Theannual temperature of town is high: 34^{o}C and low: 9^{o}C. The livestock population of the district is 124,064 cattle, 49,269 sheep, 33,973 goats, 19,229 equines and 79,505 poultry. In the town 304 recorded cross breed lactating cows, 113 cross breed heifers, 176 female cross breed calves are recorded. The total land area of the district is about 97,400 hectares of which 56,005 hectares are used for crop production 13,636 hectares for animal grazing, 2,824.6 hectares forest covered and others for other activities [17].

2.2. Study Design

A cross-sectional study was conducted from November 2021 to April 2022 GC to estimate occurrence and antimicrobial sensitivity of *staphylococcus aureus* in raw cow milk, milkers hands and milking buckets in Fiche and Hambiso towns of North Shewa, Ethiopia.

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2.3. Study Population and Study Animals

The study population was all healthy lactating cows of cross-breed in selected dairy farms which were kept under intensive and semi-intensive management systems. The herd size of dairy farms included in the study ranged from 2 to 27 with 1 to 11 lactating cows. During the study period total of 604 lactating cross breed cow were identified from 100 small and medium dairy farms.

The study animals included cross-breed (Local zebu X Jersey or Local zebu X Holstein-Fresian)dairy cows selected by simple random sampling techniques from the study population in the selected farms in Fiche and Hambiso towns. For smallholder dairy farms containing only one lactating cow the cow was taken as the sample animal.

2.4. Sampling Method and Sample Size Determination

The study towns were selected purposively based on their high potential in milk production, the absence of previous studies, and their proximity to the Ambo University Zoonotic and Food Safety Research Laboratory. Medium and smallholders dairy farms found in the towns were selected purposively based on the willingness of giving a sample, the presence of lactating cow and answering the questionnaire. Lactating dairy cows from each farm were selected by simple random sampling technique. The sample size was calculated according to Thrusfield [18] formula using 95% confidence interval (CI), 5% desired absolute precision, 15.3% expected prevalence

[19].N = Z2Pexp (1 − Pexp)/D2, where Z = 1.96, N = sample size, Pexp = expected prevalence, and D = absolute precision. Accordingly, the calculated sample size was 199. However to increase the precession of the estimate by adding 50%the sample size was set to 300 [20]. Additionally,38 milkers hand swabs and 52 milking buckets swab samples were collected from both towns. Over all 390 samples were collected for laboratory processing.

2.5. Questionnaire survey

A structured questionnaire was used to collect data about the management systems, associated risk factors for dairy farms of Fiche and Hambiso towns and use of antimicrobials in last year in the area. Major risk factors that were considered in the current study include cleaning conditions of the barn/milking environment, hygiene of milking cows, milk handlers, and milking buckets, milk storage conditions, drainage condition of the farming area, age, parity, and lactation stage of the cows. The knowledge, attitude and practice on factors causing milk contamination and AMR were determined.Under the knowledge, fourteen questions, under attitude, twelve questions and under practice, eleven questions Likert scalewere used for analysis. Each KAP question received three points for a correct/positive/good answer, two points for an uncertain/neutral/fair answer, and one point for a wrong/negative/poor answer.

2.6. Collection and transportation of samples

Milk samples were collected accordingly with at least 25 ml of raw milk per animal was collected into sterile universal bottles after discarding the first three milking streams [13]. The swab samples from milkers' hands and milking bucket was taken using sterile swabs in order to prevent contamination [21]. Samples of milkers' hand swabs were collected by rubbing sterile swabs onto the palms of both hands, the area between fingers, fingertips and rotating the swab 360 ^{o} between the bases of the fingers of both hands of the milkers [22]. Accordingly(2020)[22] the swabs of milking buckets were also taken by streaking an estimated area of about 10 cm^2 on the surface of the milk buckets. The swabs from the hands of the milkers' and milking buckets were kept in sterilized universal bottles containingof buffered peptone water.The collected samples were properly labelled (date of sample collection, type of sample, source of sample collection, and identification numbers of the samples) by permanent marker on the containers of the sample. The samples were transported under a cold chain to Ambo University Zoonotic and Food Safety Research Laboratory.

2.7. Isolation and identification of *Staphylococcus aureus*

Bacterial isolation and identification were carried out according to the techniques recommended by the International Organization for Standardization, [23]. Briefly, the collected raw milk samples and swabs were cultured on 5% sheep blood and the plates were incubated aerobically at 37°C for 24 hours. The bacterial growths were checked after 24 hours of incubation. The incubation period would be extended to 48 hours in cases there was nogrowth observed within 24 hours. The representative colony creating beta haemolysis was sub cultured on nutrient agar plates(Oxoid, UK) and incubated at 37°C for 24 hours to obtain pure isolates for further identification. Finally, the agent was identified based on biochemical tests such as catalase, mannitol salt agar, purple agar base, and coagulase tests [24].

2.8. Antimicrobial Sensitivity Testing

The *Staphylococcus aureus* isolate (n=69) was subjected to *invitro* antimicrobial sensitivity testing using Kirby-Bauer disc diffusion method. The antimicrobial discs used are those have animal and human health significance were taken into consideration. The isolates were inoculated in TSB and incubated at 37°C for 24 hours.The turbidity of the suspensions was adjusted to the turbidity visually comparable with that of 0.5 McFarland standards. The suspension was swabbed on the surfaces of Muller-Hinton Agar (MHA) plate by using sterile cotton swab. The antimicrobial discs were, then, placed on the agar plate using sterile forceps and pressed gently to ensure complete contact with the agar surface. The plates were held at room temperature for 15 minutes to allow drying of the flood. The discs were placed with a 3 cm gap between each other and 1.5 cm from the edge of the plates to prevent overlapping of the inhibition zones. The plates were read after 24hours incubation at 37°C under aerobic conditions. The value of inhibition zones was interpreted to classify the antimicrobial drugs as susceptible, intermediate or resistant according to procedures established by CLSI [25].

2.9. Data Management and Analysis

All laboratory finding, andcollected data were coded and entered into the Microsoft Excel spreadsheets. The data were cleaned and verified before entering into the STATA Data Editor View (Version 14) for statistical analysis (STATA Corporation. College Station, TX, USA). Descriptive statistics were used to summarize data in tables and graphs. The chi-square and logistic regression tests were used to determine the association of dependent (*S.aureus* prevalence, antimicrobial resistance patterns of *S.aureus* isolates and KAP questions) variables with independent variables (animal, farm and respondents characteristics). Uni variable and multi variable logistic regression analyses were done and OR and 95% confidence interval (CI) were estimated to determine statistical significance tests for the factors leading to the occurrence of *S. aureus* in the milk. Non collinear variables with a P value ≤ 0.25 in the uni-variable logistic regression analysis were considered for the multi variable analysis. The associations between different knowledge, attitudes, and practices' variables with different socio demographic variables were shown using the chi-squared test.Differences were considered statistically significant at $P < 0.05$.

3. RESULTS

3.1. Occurrence of *Staphylococcus aureus*

Out of the total 390 samples examined, 69 (17.69%) were positive to *S. aureus*. The result revealed that *S. aureus* is prevalent in 18.05% and 16.84% of the samples from Fiche and Hambiso respectively. About 17.33% of raw cow's milk, 21.05% of milkers' hand and 17.31% of milking bucket samples were positive for bacterium (Table 1).

Table 1: The overall isolation rate of *Staphylococcus aureus* from a different source of samples in Fiche and Hambiso, Central Ethiopia (November 2021 to April 2022).

Study towns	Sources of Samples	№ of Samples	№ of Positive Samples	%	95% CI
Fiche	Raw cow's milk	150	28	18.67	13.24-25.66
	Milkers' hand	18	4	22.2	09.00-45.21
	Milking bucket	22	5	22.7	10.12-43.44
Hambiso	Raw cow's milk	150	24	16	10.99-22.70
	Milkers' hand	20	4	20	08.07-41.60
	Milking bucket	30	4	13.3	05.31-29.68
Total of each sample	Raw cow's milk	300	52	17.33	13.47-22.02
	Milkers' hand	38	8	21.05	11.07-36.35
	Milking bucket	52	9	17.31	09.38-29.73
	Over all prevalence	69/ 390=17.69			14.23-21.79

3.2. Antimicrobial Sensitivity Profiles of *Staphylococcus aureus*Isolates

The findings of antimicrobial sensitivity profiles of *S. aureus* isolates using 13 types antimicrobial disks were as shown in figure 1. All the total of 69 *S. aureus* isolates were tested for antimicrobial sensitivity testing. The highest proportion of the isolates were susceptible to ciprofloxacin (82.6%), gentamycin (81.16%) and vancomycin (81.16%). In contrast, the highest-level of resistance of the isolates was seen to ampicillin (84.1%) and tetracycline (69.6%). From raw cow's milk 9 isolates of *S. aureus* was sensitive to all antimicrobial.

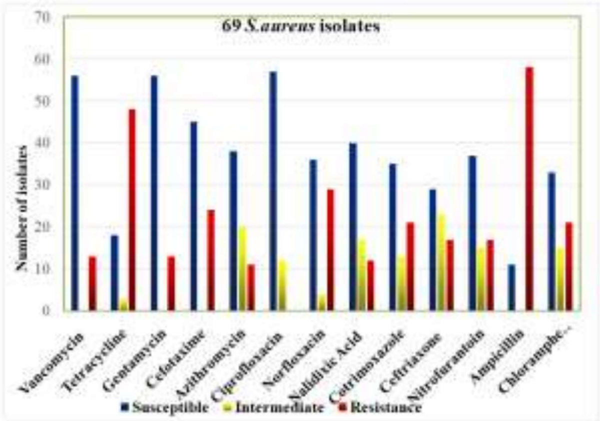

Figure 1: Results of antimicrobial sensitivity profiles of *S. aureus* isolates from milk, milkers hand and milking bucket swabs samples study area.

The finding of antimicrobial resistant highest numbers (62.32%) was sample taken from raw milk and lowest numbers (11.59%) antimicrobial resistant was taken from milkers' hand swabs (Table 2).

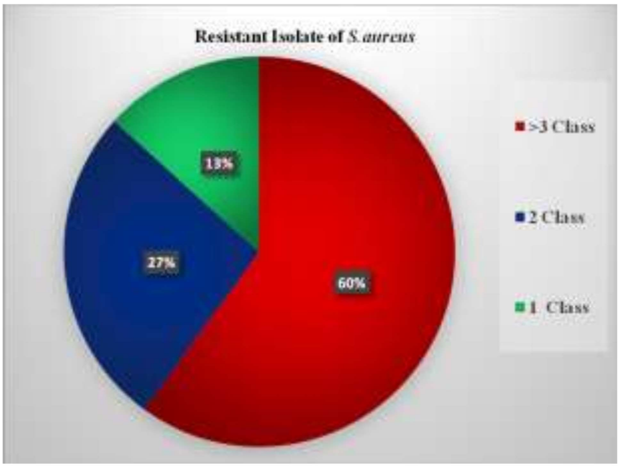

Figure 2: Percentages of *S. aureus* isolates resistant to the different antimicrobial class

Table 2: Number and percentages of resistant *S. aureus* to antimicrobials in milk, dairy farm workers hand swabs, and milking bucket swabs

Numbers of resistance antimicrobials classes	Types of samples			Total
	Raw cow milk №(%)	Milkers hand swab №(%)	Milking bucket swabs №(%)	№(%)
One	4 (50.00)	1 (12.50)	3 (37.50)	8 (13.33)
Two	9 (56.25)	2 (12.50)	5 (31.25)	16 (26.67)
MDR	30 (83.33)	5 (13.89)	1 (2.78)	36 (60.00)
Total	43 (62.32)	8 (11.59)	9 (13.04)	60 (100.00)

MDR=Multi drug resistance, №= Numbers of resistant *S. aureus* isolates

As shown in Figure 2, out of 60 isolates, 36(60%) *S. aureus* developed resistance to three or more classes of antimicrobial are multi drug resistance.

3.2.1. Results on antimicrobials usage of dairy farms in the study areas

A total of 4118 and 5740 vials of antimicrobial were used during the year prior to this study for treatment and for prophylaxis purposes respectively.

Table 3: Antimicrobial and number of vials used in the last year in the veterinary clinics (Government or private) of the study area.

Study area	Antimicrobial used (Dosage in vials)					
	Oxytetracycline (10%)	Oxytetracycline (20%)	PenicillinStreptomycin	Intra mammary Infusion	Sulfa drugs	Total
Fiche	960	780	836	1350	192	4118
Hambiso	1440	1150	960	1850	340	5740
Total	2400	1930	1796	3200	532	9858

3.3. Questionnaire Survey

As depicted in Table 4, the demographic characteristics of the interviewees revealed that majority (80%) are males. Most of the farm managers (75%) were within age group between 20 and 60 years. The education level of the respondents indicated only about 9% of the respondents had an education level of college diploma and above.

Table 4: Demography of the Respondents

Demographic Characteristics	Category	Residential Area		Total
		Fiche	Hambiso	№ (%)
		№ (%)	№ (%)	
Sex	Male	40(80)	39(78)	79(79)
	Female	10(20)	11(22)	21(21)
Age	20-39	19(38)	14(28)	33(33)
	40-59	15(30)	27(54)	42(42)
	≥60	16(32)	9(18)	25(25)
Education	Illiterate	9(18)	8(16)	17(17)
	Elementary school	21(42)	19(38)	40(40)
	High school	13(26)	21(42)	34(34)
	College and above	7(14)	2(4)	9(9)

3.3.1. Risk factors associated with prevalence of Staphylococcus aureus in raw co*w's milk*

The univariable logistic regression analysis revealed that milk from old animals, cows with many calving level and late lactation stage were with significantly high contamination with *S.aureus* than other categories of the factors ($p<0.05$, Table 5).

Table 5: The result of univariable logistic regression analysis of contributing risk factors for the occurrence *S. aureus* in the study area

Variable	Category	Tested	Positive	OR	95% CI	P.value
Town	Fiche	150	28	1	-	-
	Hambiso	150	24	0.83	0.46-1.51	0.542
Age	Young	90	8	1	-	-
	Adult	159	21	1.56	0.66-3.68	0.310
	Old	51	23	8.42	3.38-20.95	0.000
Parity	Prim	120	8	1	-	-
	Few	101	14	2.25	0.90-5.61	0.081
	More	79	30	8.57	3.67-20.04	0.000
Lactation stage	Early	69	5	1	-	-
	Middle	79	13	2.52	0.85-7.48	0.095
	Late	152	34	3.69	1.37-9.89	0.010
Body condition	Good	50	16	1	-	-
	Moderate	250	36	0.36	0.18-0.71	0.004
Drainage condition	Good	50	23	1	-	-

	Poor	250	29	0.15	0.08-0.30	0.000
Management system	Intensive	270	45	1	-	-
	Semi-Intensive	30	7	1.52	0.62-3.76	0.363
Previously exposed to mastitis	Yes	249	43	1	-	-
	No	51	9	1.03	0.47-2.27	0.948
Use drying towel separate for each	Yes	253	34	1	-	-
	No	47	18	3.99	2.00-7.97	0.000
Previously treated with antimicrobial	Yes	230	47	1	-	-
	No	70	5	0.30	0.11-0.79	0.014
Type of floor in house	Concrete	270	45	1	-	-
	Soil	30	7	0.66	0.27-1.62	0.363
Organoleptic change present on milk	Yes	61	13	1	-	-
	No	239	39	0.72	0.36-1.45	0.359
Source of water used for cleaning	Tape	200	40	1	-	-
	Under ground	100	12	0.55	0.27-1.09	0.088

CI=Confidence Interval, OR=Odd Ratio, Pos. =Positive

The variables, which were found significantly associated with the isolation rates of *S.aureus* from raw milk samples were checked for non-collinearity (p≤0.25) and in turn analysed by using multi variable logistic regression model. The output of the multivariable logistic regression analysis indicated that the risk factors more parity and late lactation stages were found significantly associated with the prevalence of *S.aureus* from raw milk (p<0.05) (Table 6).

Table 6: Results of multivariable logistic regression analysis of factors associated the contamination of the milk with *S. aureus* in the study area.

Variable	Category	Tested	Positive	AOR	95% CI	P.value
Parity	Prim	120	8	1	-	-
	Few	101	14	2.29	0.71-7.39	1.65
	More	79	30	5.26	1.77-15.66	0.003
Lactation stage	Early	69	5	1	-	-
	Middle	79	13	2.75	0.75-10.06	0.126
	Late	152	34	3.17	1.03-9.78	0.044
Body condition	Good	50	16	1	-	-
	Moderate	250	36	1.01	0.32-3.23	0.980
Drainage condition	Good	50	23	1	-	-
	Poor	250	29	0.24	0.09-0.66	0.005
Use drying towel separate for each animal	Yes	253	34	1	-	-
	No	47	18	1.77	0.53-5.90	0.352
Previously treated with antimicrobial	Yes	230	47	1	-	-
	No	70	5	0.77	0.23-2.53	0.665
Source of water used for cleaning	Tape water	200	40	1	-	-
	Under ground water	100	12	0.67	0.28-1.65	0.392

AOR= Adjusted odd ratio, CI= confidence intervals, Pos.=Positive

3.3.2. The response of the dairy farmers and/or workers on the public health aspects of raw milk, hygienic practice during milking, and milk handling

As indicated in Table 7, majority of the respondents (77%) had knowledge on milk borne diseases (77%). However, all of them (100%) had not been using antiseptic during milking (100%), andmost of farmers (90%)were not storing milk in refrigerators till consumption or selling.

Table 7: Knowledge of the farmers'/ farm workers on the public health aspect of raw milk and hygienic practice during milking and milk handling

Questionnaire	Levels	Number	%
Knowledge on Milk Borne Diseases	Yes	77	77.0
	No	23	23.0
Training on milk quality handling and hygiene	Yes	43	43.0
	No	57	57.0
Usage of antiseptic during milking	Yes	00	0.00
	No	100	100.0
Storage of milk in refrigerator	Yes	10	10.0
	No	90	90.0
Milking containers and hand towels were washed with soap or detergent	Yes	98	98.0
	No	2	2.0
Milking utensils	Plastic	83	83.0
	Aluminium	17	17.0
Milk consumption habit	Raw	47	47.0
	Boiled	53	53.0

Table 8: Chi-square analysis of knowledge of the farmers'/ farm workers on the public health aspect of raw milk and hygienic practice during milking and milk handling

Questionnaire		Characteristics of the respondents										
		Town		Sex		Age			Education			
		Fiche	Hambiso	Male	Female	20-40	40-60	≥60	Illiterate	Elementary School	High School	Above High School
Disease may acquired from foods like milk	Nº (%)	13(56.5)	10(43.5)	20(87)	3(13)	8(34.8)	9(39.1)	6(26.1)	10(43.5)	11(47.8)	2 (8.7)	0(0.0)
	Yes (%)	37(48.1)	40(51.9)	59(76.6)	18(23.4)	25(32.5)	33(42.9)	19(24.7)	7 (9.1)	29(37.7)	32(41.6)	9(11.7)
	χ2	0.508		1.140		0.101			21.090			
	p-val	0.635		0.388		0.951			0.000			
Training on milk quality handling and hygiene	Nº(%)	23(40.4)	34(59.6)	44(77.2)	13(22.8)	16(28.1)	24(42.1)	17(29.8)	12(21.1)	24(42.1)	19(33.3)	2(3.5)
	Yes (%)	27(62.8)	16(37.2)	35 (81.4)	8 (18.6)	17 (39.5)	18(41.9)	8(18.6)	5 (11.6)	16 (37.2)	15(34.9)	7(16.3)
	χ2	4.937		0.261		2.211			5.886			
	p-val	0.026		0.609		0.331			0.116			
Storage of milk in refrigerator	Nº (%)	47(52.2)	43(47.8)	72(80)	18(20)	29(32.2)	36(40)	25(27.8)	17(18.9)	36(40)	32(35.6)	5 (5.6)
	Yes (%)	3(30)	7(70)	7(70)	3(30)	4(40)	6(60)	0 (0.0)	0(0.0)	4(40)	2(20)	4(40)
	χ2	1.778		0.542		3.800			14.394			

Alu = Aluminium, Nº= Number, Pla = Plastic

3.3.3. Knowledge, Attitude and Practices (KAP) of farmers/farm workers on factors contributing milk contamination and antimicrobial resistivity (AMR)

The responses of the farmers to all knowledge related questions were summarized in Table 9. Majority of the respondents agree that personal hygiene, washing hands with soup, drying after hand washing will reduce milk contamination. However, 88% of them disagreed that the use of use antimicrobials for minor and unidentified cases like cough, diarrhoea, nasal discharge, skin infection, poor body condition, external and internal parasites was not important (Table 9).

Table 9: The response of the farmers for each knowledge and knowledge's question of milk contamination and AMR causing factors.

Table 9: The response of the farmers for each knowledge and knowledge's question of milk contamination and AMR causing factors.

Knowledge questionnaire	Disagree № (%)	Uncertain №(%)	Agree № (%)
Sickness after consuming a raw milk was signs of milk contamination	23(23)	13 (13)	64(64)
Keeping personal hygiene can reduce disease caused by food borne	0(00)	0(00)	100(100)
Bacteria may be occurs in our hands, nose, our saliva and under nails	0(00)	1(1)	99(99)
Wearing earrings, rings, watches and others can promote the risk of milk contamination	0(00)	12(12)	88(88)
Disease such as diarrhoea, vomiting and others can interrupt the work	1(1)	25(25)	74(74)
Washing hands before milking used to reduce chance of milk contamination	0(00)	0(00)	100(100)
Drying the hands after washing used to reduce chance of milk contamination	0(00)	17(17)	83(83)
Consuming raw milk has risk	11(11)	40(40)	49(49)
Administered the drugs by themselves without authorization/training has risk	0(00)	0(00)	100(100)
Withdraw the treatment of animals without finish the complete dose has cause antimicrobial resistance	10(10)	3(3)	87(87)
Refers to use antimicrobial for minor and unidentified cases was not important	88(88)	7(7)	5(5)
Do you know the frequent use of the same drug have negative impact on curing of sick animals	31(31)	43(43)	26(26)
Treatanimals without having a good knowledge about drugs have cause drug resistance	28(28)	44(44)	28(28)

Table 10: The response of the farmers for each attitude and attitudes question of milk contamination and AMR causing factors.

Attitudes questionnaire	Disagree №(%)	Uncertain №(%)	Agree №(%)
Wearing properlymay reduce milk contamination	00(00)	1(1)	99(99)
Using refrigerator to store milk is good for milk	00(00)	1(1)	99(99)
Speaking, eating and drinking at milking area increase risk of contamination	10(10)	27(27)	63(63)
Water quality can have direct effect on milk contamination	00(00)	1(1)	99(99)
Hand washing before work decreases the likelihood of milk contaminated	00(00)	1(1)	99(99)
Cleaning of the equipmentdecreases the likelihood of food being contaminated	00(00)	00(00)	100(100)
Putting the milk without cooling was cause milk contamination	00(00)	2(2)	98(98)
Treatment of animals without prescription do not causeantimicrobialresistance during treatments	66(66)	9(9)	25(25)
Treating animals with same antimicrobial frequently may causeantimicrobial resistance	3(3)	40(40)	57(57)
Stoptreatment with out finishing course when the animals stopped sign of disease may causeantimicrobial resistance	00(00)	11(11)	89(89)
Injecting the over left drugs present at home until the animals taken to veterinary clinics was good	57(57)	20(20)	23(23)
Allow the injection of many drugs at once was better than injecting specific treatment	26(26)	16(16)	58(58)

The responses of the farmers to the attitude's questions were summarized in Table 10. Accordingly, about 90% of them have negative attitude on the responsibility of interruption of antimicrobial treatment without completing full dose in animals play role in drug resistance development.

The result of the respondents' practice on the factors contributing to the occurrence of milk contamination and AMR was shown in Table 11. All of the farm workers or farmers do not go to health centre or hospital for check up every six months, only 41% of the farmers wait the withdrawal periods after animals treated with drugs.

Table 11: The response of the farmers for each practice and practices question of milk contamination and AMR causing factors.

Questionnaire	Never№(%)	Sometimes №(%)	Always №(%)
Go to health centre or hospital for check up every six month	100(100)	00(00)	00(00)
Handle raw milk when your hands have wounds	80(80)	20(20)	00(00)
Consume or sell your milk when animals sick?	74(74)	13(13)	13(13)
Mix evening and morning milk when you took to markets?	00(00)	00(00)	100(100)
Wash your hands and container with soap or detergents	00(00)	1(1)	99(99)
Treatment and vaccinate your cows in order to prevent zoonosis	00(00)	12(12)	88(88)
Consults professional before giving drugs for animals	00(00)	10(10)	90(90)
Do you follows treatment correctly and finish the duration of treatments foranimal	00(00)	24(24)	76(76)
Wait the withdrawal periods after treated with drugs	41(41)	18(18)	41(41)
Purchase drug for your dairy cow with prescription	00(00)	27(27)	73(73)
Do you treat animals with out having sufficient **knowledge**	92(92)	8(8)	00(00)

The result of the knowledge, attitudes and practices assessment revealed that 52%, 52% and 38% of the respondents from Fiche; whereas 42%, 54% and 34% of respondents from Hambiso are unknowledgeable, negative attitudes and poor practices respectively on factors causing milk contamination and antimicrobial resistan

ce.

Table 12: **The association of the respondents on KAP question of milk contamination and AMR causing factors.**

Characteristics of the respondents		Knowledge		OR	P	Attitude		OR	P	Practice		OR	p
		Good Knowledge	%			Positive	%			Good	%		
Town	Fiche №(%)	24/50	48.00	Ref		24/50	48.0	1.084	0.841	31/50	62	Ref	
	Hambiso№(%)	29/50	58.00	1.50	0.317	23/50	46	Ref		33/50	66	1.190	0.677
Sex	Male № (%)	45/79	56.96	2.15	0.128	39/79	49.4	1.584	0.360	51/79	64.6	1.121	0.822
	Female № (%)	8/21	38.1	Ref		8/21	38.1	Ref		13/21	61.9	Ref	
Age	20-40 № (%)	17/33	51.5	0.98	0.971	17/33	51.5	1.889	0.242	19/33	57.6	Ref	

	40-60 №(%)	23/42	54.8	1.12	0.826	21/42	50	1.778	0.267	27/42	64.3	1.326	0.554
	≥60 № (%)	13/25	52	Ref		9/25	36	Ref		18/25	72	1.895	0.260
Education	Illiterate № (%)	1/17	5.9	Ref		10/17	58.8	1.786	0.486	10/17	58.8	Ref	
	Elementary School № (%)	23/40	57.5	21.65	0.004	18/40	45	1.023	0.976	25/40	62.5	1.167	0.794
	High School № (%)	20/34	58.8	22.86	0.004	15/34	41.1	0.987	0.986	23/34	67.6	1.464	0.535
	Above High School№ (%)	9/9	100			4/9	44.4	Ref		6/9	66.7	1.400	0.696

№= Number

4. DISCUSSIONS

The present study was conducted to estimate the prevalence and antimicrobial susceptibilitypatterns of *S. aureus* isolated from raw cow's milk, milkers hand swabs, and milking bucket swabs in Fiche and Hambiso towns of North Shewa.

The total prevalence of *S. aureus* in currently studied Fiche and Hambiso towns was 17.69%, which was lower than prevalences reported from other part of Ethiopia like from Gamo [26] which reported a prevalence of 42.6%, from Shire [27] which was reported of 32.81%, and from Egypt [28] which the reported prevalence was 41.1%. The present prevalence was comparable to the 16.6% prevalence of *S. aureus* reported from Sululta and Mukaturi town, Ethiopia [19], from Sebeta town [13] which was reported of 19.6%, from Bishoftu (Lakecha *et al.*, 2021)[29] which was reported of 21.46%. The prevalence of *S. aureus* in this study was higher than the other reports from Ethiopia, like Ambo and Guder town (Megersa, 2015)[30]which was13.9% and Holeta, Central Ethiopia [31] which was 10.69%. The main reasons for the variation of *S. aureus* prevalence from studies to studies could be the due to the difference in standard of management in the farm, difference of epidemiology [32], sample size, and standard of laboratory process undertaken [33].

The study finds 17.33% of raw cow milk was positive with *S. aureus*. This finding was lower than finding from Addis Ababa [34] which 24.6% of milk was contaminated by *S. aureus*. The finding on contamination of raw milk was higher than finding from Holeta, Central Ethiopia[31], which only 8.64% of raw milk was positive with *S. aureus*. Higher prevalence was identified from the milker's hand which was 21.05%. This finding was line with report finding from Bishoftu [29], and Sululta and Mukaturi[19] which 25.53%, and 25% of milkers hand was positive with *S. aureus*respectively. This finding was higher than finding from Central Ethiopia [35] which 10% of *S.aures* was identified from pooled hand swabs.

The current study carried out on antimicrobial susceptibility test indicated that the highest numbers of *S. aureus* isolates were susceptible to ciprofloxacin (82.6%), gentamycin (81.16%), vancomycine (81.16%), cefotaxime (65.2%), nalidixic acid (58%), azithromycin (55%), nitrofurantoin (53.6%), norfloxacin (52.2%) and cotrimoxazole (50.7%). This result was contrast with finding from Mekele[36] which no resistant to drug like vancomycin. The finding was contrast with report from Central Ethiopia [35] which 93.6%, and 61.3% of *S. aureus* isolates was susceptible to gentamycin and ciprofloxacin but line with reports from Northern Portugal [37], 83.9% of *S.aureus* isolates was sensitive to ciprofloxacin and also 90.3% of *S.aureus* isolates was sensitive to gentamycin.

The present study showed that higher proportion of *S. aureus* isolates showed resistance to ampicillin (84.1%), and tetracycline (69.6%).The result of ampicillin resistance of isolate was lower than finding from Holeta, central Ethiopia [31] which 95% of *S. aureus* isolate was resistant to ampicillin.The result was higher than result high resistant to ampicillin occur from Egypt [28] which was 70.8%. Also this finding line with finding from Central Ethiopia [35] which 66% of*S. aureus* isolated was resistant to tetracycline. The finding was lower than result from Addis Ababa [34] which 94% of *S.aureus* isolate was resistant to ampicillin. This report was higher resistant to tetracycline when compared to report from Mekelle [36] which 35.4% resistant to tetracycline, report from Addis Ababa [34] which 46.2% of *S. aureus* was resistant to tetracycline.

The high percentage (60.00%) and frequencies of isolates was MDR. This result was higher than result from [29], which only 10.42% was MDR.This finding was lower than finding from Central Ethiopia [35] which 98.4% of *S. aureus* isolated was MDR respectively. The finding line with finding from Holeta, Central Ethiopia [31] which 65%.This finding of resistance could be due to unrestricted accessibility of antimicrobial agents on the hand of sellers, not caring for professions, which leads to abuse, lack/weak of rule and regulation of drugs, heightened selection pressure for resistant strains of bacteria, and weak or absence of antimicrobial resistance observation programs, the bacteria was exposed to various types of drugs, or carry resistant genes [38].

The present study on the risk factors for the contamination and occurrence of *S. aureus* in the raw cow milk showed that the odds ratio of *S. aureus* in raw cow milk with late lactationstage was (AOR=3.17 and P=0.044) higher than milk of cows with middle and early lactation stage. This could be due to the fact that late lactation stage are more susceptible to infection than medium and early lactation stage cows because of their long period of time during milking the teat was exposed to bacteria through milkers' hands, teat cup liners and drying clothes [39].

As the current finding reveal 77% of farmers/ farm workers have knowledge of milk borne disease. This result was lower than finding from North Shoa Zone, Ethiopia [40] which 95.1% of the responded that drinking raw milk could potentially cause a serious health problem. This finding was line with finding from DebreBirhan town [41], Jinka, Ethiopia [42] which the 61.3%, and 56.41% of the respondents are knowledgeable about the milk borne zoonotic diseases respectively. Also, this result was better than finding from and from Sebeta[13] which 54% respondents did not know any things about food borne diseases.

The result revealed that all of the farmers (100%) did not use any antiseptic before and after milking, this result was line finding from central Ethiopia [43] which all (100%) of respondents did not use antiseptic before or after milking. Most of the farmers (90%) of them not use refrigerator for cooling purpose until consuming or selling. This result was line with finding from Sebeta, central Oromia [13] which 90% of respondents do not use refrigerator for cooling purpose. Also 98% of farmer uses plastic containers as milking equipment or milking transportation services. This result was comparable with result finding by interview from selected sites of southern Ethiopia [44] results 95% of them uses plastic equipment.

Knowledge on milk borne diseases, storage of milk in refrigerator, milk consumption habit, and washing hands and milking containers with soup and detergents significantly differ with educational standard of respondents. This was line with finding from Southern Ethiopia, Jinka [42] the illiterate did not know prevention methods of milk borne zoonosis.

In the current study 77% of them know side effect like vomiting, abdominal pain, nausea on you after consuming raw milk was sighing of milk contamination. This result was line with results finding from Fiche town [45] which 44.8% of the food handlers know the vomiting and 65.6% of respondents know diarrhoea was side effect of food borne disease. Only 49% of respondents know consuming raw milk has risk. This finding was lower than finding from Jinka, Southern Ethiopia [42] which 64.30% knows the raw milk has serious health effect. 88% of respondents were knowledgeable on using antimicrobial for minor and unidentified cases was not important.The finding from Turkey [46] was better which only 41% of respondents uses drugs for minor problems has side effect.

The current study shows only 26% of respondents are knowledgeable on frequent use of the same drug have negative impact on curing of sick animals. This result was not better than finding from

Amhara region, north western Ethiopia [47] which only 42.9% of respondent's aware improper use of drug can cause AMR.57% of respondents have good attitudes on treating animals with same antimicrobial frequently may cause AMR.This result was line with finding from Amhara region, NorthWestern Ethiopia [48] 59.3% of farmers believes antimicrobial can cure all types of diseases.

The study result finds 47% of respondents have positive attitudes, while53% have negative attitudes on factors causing milk contamination and antimicrobial resistivity. This finding line with finding from Vadodara District, Gujarat [49] which all livestock keepers interviewed, about 52% showed a positive attitude toward hygiene with regard to personal as well as animal hygiene. As the current find illiterates respondents had less knowledge about factors contributing for the milk contamination and AMR than those elementary and above education levels. This finding was line with finding from southern Ethiopia [44] which formal education on milk handling can have a great impact on knowledge, attitude and practices of proper milk handling.

Generally, the currents study revealed that *S. aureus* isolated and identified from raw cow milk, milkers hand swab, and milking buckets and they were resistance to many different types of antimicrobials which could be result in great public health and economic impacts. This prevalence of *S. aureus* also indicated there was a deficiency of proper milk handling practice which have direct effect on milk hygiene, lack of advanced management in the farm and which may lead to the occurrence Staphylococcal food poisoning. There was also high numbers of the farmers didn't aware about the contributing factors of milk contamination and AMR due to lack of awareness and low educational standards. This study strongly clarifies the improper use of antimicrobial and the development of antimicrobial resistance in the community.

Limitation of this study:This study failed to use molecular diagnostic methods, identify types toxin produced by bacteria, to estimate bacterial load, to perform molecular studies and detect genes responsible for antimicrobial resistancedue to lack of materials and shortage of time.

5. CONCLUSION AND RECOMMENDATIONS

The current studyconducted in Fiche and Hambiso towns of North Shewa, Ethiopiaconfirmed that there is contamination of raw milk, milkers' hand, and milking buckets. A high prevalence of *S. aureus*was obtained in milkers hand swabs, which was 21.05% followed by raw cow milk, which was 17.33% and milking bucket swabs which was 17.31%. A large percentage of *S. aureus* isolates were susceptible to ciprofloxacin 82.61%, gentamycin81.16% and vancomycin81.16%.But there were also high resistant of *S. aureus* isolates to various antimicrobial agents, particularlyampicillin84.1% and tetracycline 69.6%.

However, the majority 60% of the *S. aureus* isolates tested were resistant to three or more antimicrobial classes, asserting the presence of MDR. More parity, late lactation stage of the cows from animal's factor and poor draining condition were crucial predictors for the occurrence of *S. aureus* in cow milk. Most of the farmers have good knowledge and practice on factors causing milk contamination and antimicrobial resistance. But most of them have more negative attitudes towards the factors causing milk contamination and antimicrobial resistance. There is huge gaps milking storage condition, using antiseptics during milking and check-up of milkers at hospital every six month.

Thus, based on the above finding, regular trainings on milk hygiene, and milk handling practices should be given to all actors in the milk value chain by the concerned bodies. Update antimicrobial usage policyfor dairy cows, and the proper use of veterinary and human drugs to ensure their safety, efficacy and quality. Antimicrobial sensitivity testing should be done at regular interval in order to select effective drugs for treatment or prophylaxis and to avoid drug resistance problem. Creating awareness on applications of antimicrobials as the last line of defence by avoiding their use for miner and undefined cases and by prohibition of left over drugs for future use.

Acknowledgements

Authors are very grateful to Prof.Dr. R.K.Narayan for going through the manuscript and to the dairy farmers for their kind cooperation. This paper is dedicated to the scientists who made pioneer contribution in the field of Staphylococcus aureus.

Author's contribution

All the authors contributed equally. They read the final version, and approved it for the publication.

Conflict of interest

The authors declare that they do not have conflict of interest.

Source of financial grant

There was no financial support for this manuscript.

REFERENCES

1. Grace, D., Wu, F. and Havelaar, H. [2020]. MILK Symposium review: Food borne diseases from milk and milk products in developing countries—Review of causes and health and economic implications. Journal of Dairy Science, 130, 9715-9729.

2. Pal,M., Shuramo, M.Y.,Tewari, A.,Srivastava,J.P. and Steinmetz., C.H.D. [2023]. Staphylococcus Aureus from a Commensal to Zoonotic Pathogen: A Critical Appraisal. International Journal of Clinical and Experimental Medicine Research, 7, 220-228.

3. CDC.[2020]. Food borne germs and illness. (https://www.cdc.gov/ assessed online on 27 September, 2020).

4. Pal, M., Ketchakmadze D., Durglishvili N. and Ketchakmadze I. (2022). *Staphylococcus aureus*: A Major Pathogen of Food Poisoning: A Rare Research Report. *Nutrition and Food Processing*, 5, 2637-8914/074.

5. Pal, M., Gutama, P. K., and Koliopoulos, T. (2021). Staphylococcus aureus, an important pathogen of public health and economic importance: A comprehensive review.*Journal Emerging Environmental Technologies and Health Protection*, 4, 17-32.

6. Arumugam, G., Periasamy, H. and Maneesh, P. [2017]. *Staphylococcus aureus*: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. *Frontiers in Staphylococcus aureus,*InTech. doi: 10.5772/67338.

7. Li, Y., Li, X., Huang, T. Xu, K. and Li, C. [2019]. Molecular characteristics and virulence gene profiles of *Staphylococcus aureus* isolates in Hainan, China. *BMC Infectious Diseases*, 19, 873.

8. Hachemi, A., Zenia, S., Denia, M., Guessoum, M., Hachemi, M. and Ait-Oudhia, K. [2019]. Epidemiological study of sausage in Algeria: Prevalence, quality assessment, and antibiotic resistance of *Staphylococcus aureus* isolates and the risk factors associated with consumer habits affecting food borne poisoning, *Veterinary World*, 12, 1240-1250.

9. Li, G., Wu, S., Luo, W., Su, Y., Luan, Y. and Wang, X. [2015]. *Staphylococcus aureus* ST6-t701 isolates from Food-Poisoning Outbreaks (2006–2013) in Xi'an, China. *Food borne Pathogen Diseases*,12, 203-206.

10. Pulia, M., Anderson, J., Ye, Z., Elsayed, N., Le, T., Patitucci, J., Ganta, K., Hall, M., Singh, V. and Shukla, S. [2022]. Expression of Staphylococcal Virulence Genes In Situ in Human Skin and Soft Tissue Infections. *Antibiotics*, 11, 527.

11. Oniciuc, E., Nicolau, A., Hernández, M. and Rodríguez, D. [2017]. Presence of Methicillin-Resistant *Staphylococcus aureus* in the Food Chain. *Trends in Food Science and Technology*, 61, 49–59.

12. Santy, J. [2018]. Antimicrobial resistance stewardship: it's up to us all. *International Journal of Orthopedic Trauma Nursing*, 28, 1–3.

13. Ayele, y., Desissa, F., Mamo, B., Girma, R., Beyene, T., Jibat, T., Tadase, F., Geloye, M., Feyisa, A. [2017]. Assessment of *Staphylococcus aureus* Along Milk Value Chain and Its Public Health Importance InSebeta, Central Oromia, Ethiopia.*BMC Microbiology,* 17, 141.

14. Adugna F, Pal M and Girmay G. [2018]. Prevalence and Antibiogram Assessment of Staphylococcus aureus in Beef at Municipal Abattoir and Butcher Shops in Addis Ababa, Ethiopia. Biomed Res Int, 2018:5017685.

15. Beyene, T., Hayishe, H. and Gizaw, F. [2017]. Prevalence and antimicrobial resistance profile of Staphylococcus in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. *BMC Research Notes*,10, 171.

16. GWAO. [2022]. Annual reports of Girar Woreda Agriculture Office.

17. DWAO. [2022]. Annual reports of Dagam woredaAgriculture Office.

18. Thrusfield, M. [2007].Veterinary Epidemiology. Third Edition, Blackwell Science, 233.

19. Sema, R., Shimelis, M. and Asheber, A. [2019]. Milk Safety Assessment, Isolation, and Antimicrobial Susceptibility Profile of *Staphylococcus aureus* in Selected Dairy Farms of Mukaturi and Sululta Town, Oromia Region, Ethiopia.*Veterinary Medicine International*,2019, 3063185.

20. NSZAO. [2022].Annual Reports of North Shewa Agriculture Office.

21. Miller, M., Binnicker, M., Campbell, S., Carroll, K., Chapin, K. and Gil, P. [2018]. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology.*Clinical Infectious Disease*, 67, e1-e94.

22. Youssif, N., Hafiz, N., Halawa, M. and Saad, M. [2020]. Influence of some hygienic measures on the prevalence of subclinical mastitis in a dairy farm. *International Journal of Dairy Science*, 15, 38-47.

23. ISO (6888-3).[2003]. Microbiology of food and animal feeding stuffs: - Horizontal method for the detection and identification of Staphylococci, Part 3. Geneva, Switzerland.

24. Quinn, P., Markey, B., Carter, ME., Donnelly, WJC., Leonard, F. and Maguire, D. [2002].Veterinary Microbiology and Microbial Disease. 1st ed. Blackwell Science Ltd UK.

25. CLSI.[2021]. Performance Standards for Antimicrobial Susceptibility Testing, 31st edition; Supplement M100S; Clinical and Laboratory Standards Institute: Wayne, PA, USA.

26. Belay, N., Mohammed, N. and Seyoum, W. [2022]. Bovine Mastitis: Prevalence, Risk Factors, and Bacterial Pathogens Isolated in Lactating Cows in Gamo Zone, Southern Ethiopia.*Veterinary Medicine: Research and Reports*, **13**, 9-19.

27. Girmay, W., Gugsa, G., Taddele, H., Tsegaye, Y., Awol, N., Ahmed, M. and Feleke, A. [2020]. Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Milk in Shire Dairy Farms, Tigray, Ethiopia. *Veterinary Medicine International*, 4, 1-7.

28. Asmaa, S., Radwa, R., Alshimaa, M., Farag, M., Hazem, R, Adel, A., Mohamed, M., Mohamed, E., Amira, A. and Amal, A. [2022]. Prevalence and Characterization of PVL-Positive *Staphylococcus aureu*s Isolated from Raw Cow's Milk. *Toxins*, 14, 2.

29. Lakech, T., Yersega, B., Eyasu, T. and Wondewossen, T. [2021]. Prevalence of *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* and Potential Risk Factors in Selected Dairy Farms at the Interface of Animal and Human in Bishoftu, Ethiopia.*Veterinary Medicine: Research and Reports,*12, 241-251.

30. Megersa, L. [2015]. Identification and antimicrobial susceptibility profiles of *Staphylococcus* species isolated from raw milk, swabs of udders, milking utensils and milkers hands in small holder and dairy farms in Ambo and Guder town, Addis Ababa University, MScThesis.

31. Endrias, Z., Addisu, B., Bizunesh, M., Kebede, A., Nega, D., Lencho, M. and Edilu, J. [2022]. Isolation and Identification of *Staphylococcus aureus* from Milk and Milk Products, Associated Factors for Contamination, and Their Antibiogram in Holeta, Central Ethiopia. *Veterinary Medicine International*, 2022, 13.

32. Abdi, K., Laura, R., Aynadis, T., Kindinew, D., Jessie, V., Jasna, K. and Ashagrie, Z. [2020]. Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. *International Dairy Journal*, 109, 104762.

33. Tadesse, B. [2014]. Isolation and identification of methicilin resistant *Staphylococcus aureus* From bovine mastitic milk in and around wolaitasodo, Southern Ethiopia. Addis Ababa University, MSc Thesis, 1-5.

34. Fikirte, L., Haile, A., Andrew, S. and Tadesse, E. [2021]. Prevalence and Antimicrobial Susceptibility Profile of *Staphylococcus aureus* in Milk and Traditionally Processed Dairy Products in Addis Ababa, Ethiopia. *BioMedical Research International*, 2021, 5576873.

35. Umer, S., Munera, A. and Abdulmuen, M. [2022]. Isolation, Identification, and Susceptibility Profile of *E. coli*, *Salmonella*, and *S. aureus* in Dairy Farm and Their Public Health Implication in Central Ethiopia. *Veterinary Medicine International*, 2022, 13.

36. Alem A., Daniel, A., Yimtubezinash, W., Shu-Hua, W., Wondwossen, A. and and Tadesse, T. [2020]. Burden and antimicrobial resistance of *S.aureus* in dairy farms in Mekelle, Northern Ethiopia. *BMC Veterinary Research*, 16, 20.

37. Oliveira, R., Pinho, E., Almeida, G., Azevedo, N. and Almeida, C. [2022]. Prevalence and Diversity of *Staphylococcus aureus* and

Staphylococcal Enterotoxins in Raw Milk From Northern Portugal. *Frontiers in Microbiology*, 13, 846653.

38. Guanghua, L., Zihao, W. Junfan, L., Qiang, R. and Wei, C. [2022]. Investigation of clonal diversity, virulence genes, and antibiotic resistance of *Staphylococcus aureus* recovered from raw cow milk in southern Xinjiang, China. *Folia Microbiologica*, **67**, 245-252.

39. Abebe, R., Hatiya, H., Abera, M., Megersa, B. and Asmare, K. [2016]. Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12, 270.

40. Seyoum, E.,Mekonen, T., Kebede, N., Gezahegn, H.,Mehirete, T. and Mengesha, Z. [2016]. Knowledge, Attitude and Practice Among Small Scale Dairy Farmers on MilkBorne Zoonotic Diseases, North Showa Zone, Ethiopia. *Journal of Food borne and Zoonotic Diseases*, 4, 19-28.

41. Mandefero, D. and Yeshibelay, G. [2017]. Assessment of community knowledge, attitude and practice on milk borne zoonoses disease in DebreBirhan town, north Shewa, Ethiopia. *Journal of Public Health Epidemiology*, 10, 123-131.

42. Abebe, F., Getachew, S., Hailu, T. and Fesseha, H. [2020]. Assessment of Community Knowledge, Attitude, and Practice on Milk Borne Zoonotic Diseases in Jinka, Southern Ethiopia. *Annals of Public Health and Research*, 7, 1096.

43. Tigabu, E., Asrat, D., Mihrete, S. and Gebreyes, A. [2015]. Assessment of risk factors in milk contamination with *S. aureus* in urban and peri-urban small-holder dairy farming in Central Ethiopia. *Zoonoses and Public Health*, 62, 637-643.

44. Senay, G., Shemlash, E. and Arega, A. [2020]. Assessment of knowledge, attitudes and practices of people about milk quality and common zoonotic diseases in small holder dairy production chain in selected sites of southern Ethiopia. *International Journal of Advanced Research and Biological Science*, 7, 25-36.

45. Samuel, C., Israel, S. and Birhanu, A. [2021].Food Safety Practices and Associated Factors among Food Handlers of Fiche Town, North Shewa Zone, Ethiopia. *Journal of Environmental and Public Health*, 2021, 1-7.

46. Yasin, O., Senol, C., Emre, S., Mehmet, N. and Burhan, C. [2019]. Assessment of Farmers' Knowledge, Attitudes and Practices on Antibiotics and Antimicrobial Resistance. *Animals*, 9, 653.

47. Kindu, G. and Mulugeta, K. [2021]. Knowledge, attitudes and practices of animal farm owners/workers on antibiotic use and resistance in Amhara region, north western Ethiopia.*Scientifc Reports*, 11, 21211.

48. Geta, K. and Kibret, M. [2021]. Knowledge, attitudes and practices of animal farm owners/workers on antibiotic use and resistance in Amhara region, north western Ethiopia. *Scientific reports*, 11, 21211.

49. Vaibhavi, K. and Divya, A. [2020]. Knowledge, Attitude, and Practice about Hygiene among Livestock Keepers in Peri-Urban Area of Vadodara District, Gujarat. *Indian Journal of Community Medicine*, 45, S16–S20.