Assessment of Hygienic Practices, Prevalence and Antimicrobial Susceptibility profile of S*taphylocoocus aureus* Isolated from raw cow's milk of Dairy farms and Its Public Health Importance in and around Banbasi Administrative town, Benishangul Gumuz Regional State, Western Ethiopia

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ABSTRACT: **Background**: *Staph.aureus* is pathogenic bacterium contaminating milk and milk products causing food poisoning primarily due to its enterotoxins. **Objectives**: Across -sectional study was conducted from November 2024 to May 2025 in Banbasi town, to estimate prevalence, risk factors, public health significance and antimicrobial susceptibility patterns of *S.aureus* from cow's milk sampled at different sampling points. **Methods**: A total of 384 samples; of which, 169 pooled milk samples from udder, 48 swab from milking buckets, 98 from milking container swab; and 69 from milkers' hand swabs were collected. Isolation and Identification of *S. aureus* were carried out following standard microbiological techniques. **Results**: From a total of 384 samples examined, the overall prevalence of *S.aureus* was 77/384(20.05%). There was statistically significant difference ($p<0.05$) in isolation of *S.aureus* among isolates from different sources and risk factors (parity, age, body conditions, pregnancy status, milking hygiene, udder shape, and management factors). The study has showed a higher contamination of *S.aureus* from milking containers, followed by milkers' hand swab, pooled milk samples and swab from milking buckets. 39 isolates were subjected to antimicrobial susceptibility tests for seven selected antibiotic discs. The isolates were susceptible to Ciprofloxacin (100%), followed by Chloramphenicol (89.74%). However, they were resistant to Penicillin (100%), Gentamycin (92.30% and Amoxicillin (84.61%), followed by Cefoxitin (64.10%) and Sulphonamide (56.41%). 53.84% of the isolates were developing multi-drug resistance. Lack of stringent regulation and monitoring in the dispensing and use of antimicrobials in the area might be contributed to the occurrence of high antimicrobial resistance to these drugs. **Conclusions and Recommendations**: An attempt was made to assess the milk handling practices and consumption behaviour of farmers and consumers but unsatisfactory result was recorded. Therefore, the study has revealed the possibility of public health risk posed by *S.aureus* in Banbasi town. Creation of public awareness about good milk handling practices, pasteurization or boiling of milk prior to consumption, rational use of drug, and periodic assessment of the antimicrobial sensitivity of drugs prior to use is recommended.

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1. INTRODUCTION

1.1. Back ground

Milk plays an important role in diets globally. Although, Milk is a complex mixture of macro and micro-nutrients and it is an important source of carbohydrates, fats, proteins, proteins with all ten aminoacids, immunoglobulins, essential fatty acids, and other micro nutrients, minerals and vitamins such as calcium, vitamin B12 and riboflavin (Nyokabi *et al*., 2021; Attah *et al*., 2021; Limbu *et al*., 2020).

Milk is considered as nature's complete food and is definitely one of the most valuable and regularly consumed foods (Regasa *et al.,* 2019). Milk is highly vulnerable to bacterial contamination, because it helps the growth and multiplication of pathogenic organisms leading to food spoilage, food borne infection and poisoning (Ayele *et al*., 2017). Milk-borne pathogenic bacteria pose a serious threat to human health, and constitute about 90% of all dairy-related diseases. *Staphylococcus aureus*, *Salmonella spp.*, *Listeria mono cytogenes*, *Escherichia coli* and *Campylobacter* are the main microbiological hazards associated with raw milk consumption (Berhe *et al*., 2020).

Milk is virtually a sterile fluid when secreted into alveoli of udder. However, beyond this stage of production, microbial contamination might generally occur from three main sources: within the udder, exterior to the udder and from the surface of milk handling and storage equipments, but the surrounding air, feed, soil, faeces and grass are also possible sources of contamination. The unhygienic and undesirable practices that decrease the quality of raw milk can be classified into three categories: Practices related to the animal: Animals are not healthy or suffer from mastitis; Animals are dirty, in particular the udder, the teats, the hind quarter and the tail. Practices related to the milker: Hands and clothes of the milker are not clean and he/she practices unhygienic personal habits. Practices related to the milking process: wrong milking procedures (like stripping) are used; the utensils and the milk-can are not cleaned properly (Melese *et al*., 2015).
The usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains (Shitandi and Sternesjo, 2004). These traits are coded for by particular genes that may be carried on the bacterial chromosome, plasmids, and transposons or on gene cassettes that are incorporated into integrons (Rychlik, 2006), thus are easily transferred among isolates. Multiple antibiotic resistant *S. aureus* strains have been isolated from milk obtained from cattle samples in many parts of the world (Pesavento *et al*., 2007).

1.2. Statement of the problem

In Africa, foodborne diseases are responsible for 33–90% of deaths in children and represent a serious problem for the continent. Nevertheless, in developing countries such as Ethiopia, milk is a significant source of foodborne diseases and other infectious diseases. This happens where milk production and various dairy products take place under unsanitary conditions and poor production practices (Aliyo *et al.*, 2022).

In Ethiopia, the fresh milk is sold unpasteurized to the public either directly from small producers, via informal markets or through dairy farmers cooperatives. This informal marketing system has been a challenge for milk quality control in urban and peri-urban areas at all levels (Tegegne and Tesfaye, 2017).

In Ethiopia there are several studies reported with (51.56%) *S.aureus* prevalence by Shimelis (2014); in Selale /Fitche Area, in Holleta agricultural research centre (43.3%) by Duguma *et al*. (2013), in Hawassa (48.75%) by Daka *et al*. (2012); in Holeta town (47.1%) by Mekibib *et al. (*2010) and in Debre Ziet (39.5%) by Addis *et al.* (2011). Similarly, Bedada and Hiko, (2011), Workineh *et al*. (2002) and Tareke, (2003) who have reported as 39.1%, 39.2% and 40.3% *S. aureus* isolates at Assela, Addis Ababa and Southern Ethiopia, respectively.

Fresh milk drawn from a healthy cow normally contains a low microbial load of less than 10^3cfu/ml. However, the bacterial load may increase up to 100-fold or more if stored for sometimes at ambient (30 to 35^0C) temperature. Milk produced under hygienic conditions from healthy animals should not contain more than 1 x 10^5cfu/ml (Faisal and Ahmed, 2018).

Most research on the microbiological quality and safety of milk or milk products is from the central parts of the country representing urban or peri-urban cattle dairy production system (Amenu *et al.*, 2019). However, little has been done in agro-pastoral and pastoral communities where livestock production is the main livelihood of the people. There is limited data on hygienic practices throughout the dairy production system in Ethiopia and standard milking procedures do not exist. The microbial load of milk is a major factor in determining the quality of milk. The high bacteria count and the presence of pathogenic bacteria in milk not only degrades the milk quality and shelf-life of milk or milk related products but also causes serious health threats to consumers (Gunasena and Siriwardhana, 2021).

Awareness and resources aiding for hygienic milk production, storage, and transportation are very limited, especially smallholder production system is under developed when compared with the institutional and urban producers in the and around Banbasi Administrative Town. Microbiological status of raw milk is affected by several factors including a health status of the animal, farm management practices, environmental hygiene and poor temperature control (Berhe *et al*., 2020).

Therefore, the present study was initiated to generate base-line information on the isolation and identification of *Staphylocoocus aureus* in raw cow's milk and potential public health risks associated with the consumption of raw cow milk.

1.3. Objectives

1.3.1. General objective

- The objective of this work is to assess hygienic practices, prevalence and antimicrobial susceptibility profile of *Staphylocoocus aureu*s isolated from raw cow's milk along small scale dairy farm and it`s public health importance in and around Banbasi administrative town Benishangul Gumiuz Regional state

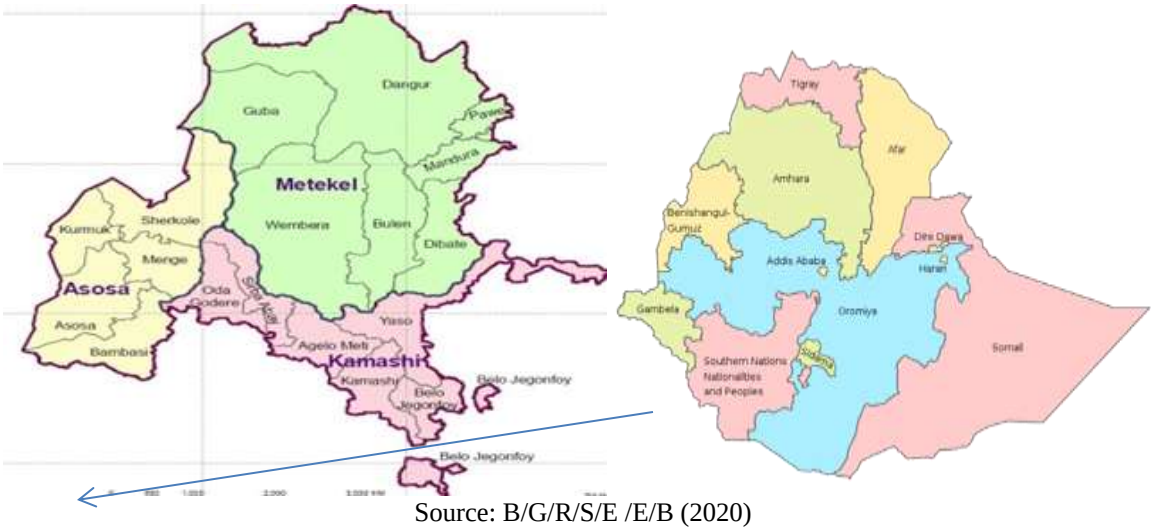
1.3.2. Specific objectives

- To estimate associated risk factors and prevalence of *S. aureus* in raw cow's milk in the study area.
- To assesses hygienic practices and its public health importance in farms.
- To determine the antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from raw cow milk in the study Area.

2. MATERIALS AND METHOD

2.1 Study Area

Bambasi is located in Assosa zone of Benishangul Gumuz Regional State at the distance of 676 km, west Addis Ababa,. The Town is located at 9.60^0 and 10.45^0 N and 34.20^0 and 34.58^0E longitude with altitude ranges from 580 to over 1544 m.a.s.l. The town is characterized by low land plane agro-ecology with average annual rain fall of 1316 mm with uni-modal type of rain fall that occurs between April and October. Its mean annual temperature ranges between 16.75^0C and 37.9^0C (National Meteorology Service Agency, 2024). The total human population of the Banbasi Adminstrative Town is 104,147. 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; Banbasi Adminstrative Town Agriculture and office and Animal resource development, 2017). The town is known for high milk production and supply to the town and surrounding areas.



Source: B/G/R/S/E /E/B (2020)

Figure 1: Map of Banbasi Administrative town

2.1. Study Population

The study populations was lactating dairy cows (local breed and cross breed), randomly selected from in and around the administrative town. Individuals involved in dairy farm activities including lactating cow owners at households, milker`s men and /or women and cow milk seller at local milk markets were study participant.

2.2. Study Design

A cross-sectional study was conducted from November 2024 to May 2025 in and around Banbasi administrative town with the objective of assessment of hygienic practices, prevalence and antimicrobial susceptibility profile of *S. aureus* isolated from raw cow's milk along extensive dairy farms and its public health importance. Farmers involved in the study was smallholder dairy producers, milkers and retailers. The study unit was small-holder farmer with

lactating cows where a questionnaire was administered and raw cow's milk was collected. During, milk sample collection all the three dairy farms in the district were included purposively.

2.3. Sampling techniques and Sample Size Determination

The district was purposively selected based on cattle population and production potential of milk to the town and surrounding districts.. Random sampling technique was employed to select study households for milk sample collection and questionnaire survey. The sample size for this study was determined by using formula given by Thrusfield (2007). Therefore, the sample size "n" was calculated as $N = 1.962 * Pexp (1 - Pexp) d2$ (1) where n=required sample size, 1.96 = the value of Z at 95% confidence interval, Pexp=expected prevalence, and d =desired absolute precision. Therefore, the sample size was calculated taking into account 95% confidence interval, desired absolute precision of 5%, and an expected prevalence of 50% since there was no previous study. Accordingly, a total of 384 milk samples were collected. Therefore, 169 pooled milk samples from udder, 48 from milking buckets, 98 from milking container swab, 69 from milkers hand swab, were subjected for microbiological examination

For questionnaire survey: for assessment of community knowledge on milk hygiene practice; 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 required for the questionnaire survey was 100 respondents. The participants were interviewed using proportional sampling from the market and households.

2.4. Questionnaire survey

Semi-structured questionnaire was used to collect information from small-dairy holders; milker's and markets (traditional milk sellers). A total of 100 respondents were participated in the questionnaire survey, each from milk sellers and households were involved. 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 with regard to milk was done and noted.

2.5. Sample collection and Laboratory analysis

2.5.1. Sample collection and Transportation

Raw milk samples were collected from critical control points along the microbiological quality of raw cow milk in and around Banbasi Administrative Town. The presence of *Staphylococcus aureus* was assessed at critical control points along the bacteriological quality of raw cow milk; directly from the cows' udder at farm level, milkers hand, from the milking bucket at farm level, and from marketed milk containers up on arrival (from cafe, restaurants and home consumers). Among those households previously considered for questionnaire survey study. Accordingly, 169 pooled milk samples from udder, 48 from milking buckets, 98 from milking container swab, 69 from milkers hand swab, were subjected for microbiological examination. About 25ml of fresh whole milk samples was collected from sampling points (directly from the udder of lactating Cows, labeled and put in ice box). The samples were transported to the Assosa Regional Veterinary diagnostic laboratory and kept at +4 ^{0}C until microbiological analysis was done.

2.5.2. Isolation and Identification of S. aureus

The nutrient agar was prepared according to the manufacturer's recommendations and milk samples were subjected to bacterial culture and isolation according to the procedures described by Quinn *et al*. (2002). Briefly, a loop full of milk samples and swabs was inoculated on blood agar base enriched with 7% sheep blood and incubated aerobically at 37^{0}C for 24-48hrs. On blood agar and *S.aureus* colonies typically round, golden- yellow pigment, convex, opaque

colonies with a characteristic beta–hemolytic pattern. The presence of *Staphylococcus* was confirmed based on colony morphology; Gram's reaction; cellular morphology and organization; and catalase test. Suspected colonies were sub-cultured on mannitol salt agar and incubated aerobically at 37^0C for 24- 48hrs. The colonies of Staphylococci which produced a yellow pigment on the media was subjected to coagulase tests and cultured on purple base agar (with 1% maltose). Finally, *Staphylococcus aureus* was identified as coagulase-positive, catalase positive, gram positive cocci, non-motile, and rapidly ferment maltose and change in the medium and colonies appear to be yellow in color (Regasa *et al*., 2019).

2.6. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates were performed using disk diffusion method on Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (NCCLS, 2002). Pure colony was selected and emulsified in 5ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculums. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 35-37^0C for 24 hrs. An inhibition zone diameter of each antimicrobial was then measured and interpreted as 'Sensitive', 'Intermediate' and 'Resistant' by comparing with recorded diameters of a control organism, ATCC25923 (CLSI, 2007).

3.8 Quality Control Data Management and Analysis

Sample was collected consistently following strict aseptic conditions of sample collection procedure for microbial analysis to avoid cross contamination. Laboratory result was evaluated for their consistency with standard using manuals or positive/negative control isolates. Data validation was performed during data entry process for its consistency, accuracy, and missing value before analysis. Each steps of the research was conducted strictly following the data collection, analysis, and presentation procedures. The collected data was entered in to Microsoft excel spread sheet then transferred to STATA version 17 for analysis. Binary logistic regression model was used when appropriate to analyze the proportions of categorical data. Odd ratio and 95% CI was computed. Descriptive statistics were used to summarize questionnaire responses. Pearson's Chi-square test was used to assess the association of risk factors with the occurrence of *S.aureus* in samples. The prevalence was estimated with confidence level set as 95% and p-value less than 5% ($p<0.05$) was taken as significant to include into next analysis.

3. RESULTS

3.1. Overall Prevalence of *Staphylococus aureus*

In the present study, out of the total lactating cows examined (N=384), 77/384 (20.05%) *Staphylococcus aureus* prevalence was identified using bacteriological methods. Higher *S.aureus* (23.46%) contaminants were recorded in swab samples taken from milking container followed by samples from milkers' hand swab (21.74%); (19.52%) from pooled milk from udder and 12.50% of *S.aureus* isolated from milking bucket (Table 1).

Table 1*: Prevalence of* Staphyloccocus aureus *with sample types*

Sample Type	**N=384**	**No. positive**	**Prevalence**	**CHI2**	**OR**	**95%CI**	**P-value**
Pooled milk from udder	169	33	19.52	2.37	1.006	0.81,1.24	0.49
Milk sample from bucket	48	6	12.5				
Milking container(swab)	98	23	23.46				
Milkers' hand swab	69	15	21.74				
Total	**384**	**77**	**20.05**				

3.2. Risk Factors Associated with *S.aureus* Prevalence

Prevalence of *S.aureus* related to the specific risk factors were determined as the proportion of affected cows out of the total examined. The questionnaire survey and observation data result shows association of age factors, parity, body conditions, milking hygiene, udder shape, pregnancy status, and management factors, are amongst the potential

risk factors, which was associated with *S.aureus* in dairy cows farmstead (Table 2). Accordingly, *S.aureus* prevalence showed statistically significant variation ($p<0.05$)with respect to number of parity age groups; body conditions scores, and pregnancy status, milking hygiene; udder shape; management system. However, breed, lactation stage, floor system, blind teat, teat lesion, and previous mastitis treatment history have no significant difference with prevalence of *S.aureus* ($p>0.05$) (Table 2).

Table 2: Result of multivariate logistic regression of attribute risk factors with Staph aureus

Factor	Categories	N=169	No (%)positives	ChI2	P-value
Age(years)	≤3 (year)	10	7 (70%)	15.74	0.000*
	4-7 years	115	20 (17.39%)		
	> 7 years	44	8(18.2%)		
Breed	Cross	46	9(19.56%)	0.05	0.82
	Zebu	123	26(21.13%)		
Parity	1-3	105	29(27.62%)	8.28	0.02*
	4-6	60	6(10%)		
	>6	4	0(0%)		
Lactation Stage (m)	Early (≤3)	58	10(17.24%)	2.43	0.48
	Mid (4-6)	39	9(23.07%)		
	Late (7-9)	22	7(31.81%)		
	Dry (>9)	50	9(18%)		
Pregnancy Status	Pregnant	27	0(0%)	8.39	0.004*
	Non- Pregnant	142	35(24.64%)		
Previous mastitis History	Infected	155	33(21.29%)	0.38	0.53
	Non- infected	14	2(14.28%)		
Barn floor type	Concrete	70	12(17.14%)	0.92	0.33
	Muddy (soil)	99	23(23.23%)		
Milking hygiene	Good	101	7(6.93%)	29.02	0.000*
	Poor	68	28(41.17%)		
Blind teat	No	144	31(21.52%)	0.39	0.52
	Yes	25	4(16%)		
Teat lesion	No	161	32(19.87%)	1.44	0.23
	Yes	8	3(3.75%)		
BCS	Good	93	13 (13.97%	5.70	0.02*
	Poor	76	22 (28.94%)		
Udder shape	Pendiculous	44	18(40.90)	14.78	0.000*
	High up	125	17(13.60%)		
Udder treatment history	Yes	15	2 (13.33)	0.54	0.46
	No	154	33(21.42)		

**BCS= body conditions*

3.3. Antimicrobial Susceptibility Test

Out of 77 *S. aureus*, only 39 isolates were subjected to antimicrobial susceptibility tests because of antibiotic disc availability constraints. All 39 isolates were susceptible to Ciprofloxacillin (100%) and Chloramphenicol (89.74%). However, higher antimicrobial resistance were recorded in penicillin G (100%) followed by Gentamycin (92.30%), Amoxicillin (84.61%), Cefoxitin (64.10%) and Sulphonamides (56.41%) (Table 3)

Table 3: Antimicrobial susceptibility patterns of *S. aureus* isolates to antimicrobials

Antimicrobial agents	Disc content (µg)	No. of Isolates	Resistance No-(%)	Intermediate No- (%)	Susceptible No-(%)
Cefoxitin	CK-30	39	25(64.10)	0(0)	14(35.89)
Amoxicillin	Amx-10	39	33(84.61)	0(0)	6(15.38)
Sulphonamide	S-300	39	22(56.41)	12(30.76)	5(12.82)
Gentamycin	Gen-10	39	36(92.30)	0(0)	3(7.69)
Penicillin G	P-10	39	39(100)	0(0)	0(0)
Chloramphenicol	C-30	39	3(7.69)	1(2.56)	35(89.74)
Ciprofloxacin	Cip-5	39	0(0)	0(0)	39(100)

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

4.3.1 Multi drug resistance of Staphylococcus aureus

In the present study, 21 (53.84%) of the isolates were developed multidrug resistance. Of these, The maximum multiple drug resistance registered for two isolates were resistance to four classes of antimicrobials (penicillin, sulphonamides, ciprofloxacin, and gentamycin) (Table 4).

Table 4: Multi-drug resistance (MDR) pattern of S. auerus isolates

No. AMR	AMR patterns	No. isolates	No.of isolates (%)	Total isolate(%)
TWO	S3+PG GEN+ CIP FOX+S3	2 5 4	2 (5.12) 6 (15.38) 3(7.69)	11(28.20)
THREE	GEN+ PG+ S3 AMX+ CHLO+S3	3 5	3(7.69) 5(12.82)	8(20.51)
FOUR	PG+ S3+ CIP+ GEN	2	2(5.12)	2(5.12)
		MDR pattern of S. auerus isolates		=21 (53.84)

Key: No. = number; %=percent; AMX= amoxicillin; PG=penicillin, S3= sulfonamides; CHL= chloramphenicol; FOX= Cefoxitin, GEN= gentamycin

3.4. Questionnaire Survey related to Public Health

The issues of public health significance arising from *S. aureus* and possible sources of milk, general hygiene, hand wash, use of towel, disinfectant, udder hygiene, time of cleaning, milkers' hand and milk container contamination with *S. aureus* were assessed using semi- structured questionaire survey in Banbasi district.

In this study in addition to risk factors for prevalence of *S. aureus* were assessed by questionnaire for public health significance for milk consumption, form of milk consumption acquiring illness due to consuming milk, clinical signs of illness showed for milk born disease, awareness on milk born disease and staphylococcal food poisoning frequency of milk consumption and other question raised. Among the total of 50 interviewed dairy farmers, 78% of them consume milk while the rest not. From those consume milk, 10%, 14%, 28% and 48% of them consume in the form of Ergo, raw milk, Ayib, and boiled milk respectively.

The consumption of raw milk is relatively higher (70%) among people who can read and write than un-educated dairy farmers (20%). Only (31%) of the dairy farmers were aware of the occurrence of food borne diseases due to raw milk consumption and (34%) of them have aware of staphylococcal food poisoning associated with consumption of raw milk and milk products. Among farmers consuming milk, 52% acquiring illness and 48% were no acquiring illness. Of the total respondent farmers, 54% of them practiced cleaning of dairy house daily, 50% of them practiced hand washing before milking; however, 64% of respondents' cow udder hygiene was slightly dirty (Table 5 and 6).

Table 5: Socio- demographc data of livestock owners related to public health

Factors	Categories	Freq.	Percentage(N=50)	Chi2 (p-value)
Sex	Male	19	38	8.78 (0.03)
	Female	31	62	
Age	15-30 yrs	24	48	0.8(0.64)
	30-50 yrs	15	30	
	>50 yrs	11	22	
Education level	Illiterate	10	20	0.52 (0.91)
	Read and write	35	70	
	Primary	4	8	
	Secondary	1	2	
Occupation	Livestock owner	31	62	1.88 (0.38)
	Milk seller	17	34	
	Consumers	2	4	

Table 6: Knoweldge and practices of Dairy farm owners with related to public health

Factors	**Categories**	**Freq.**	**Percentage(n=50)**	**Chi2 (p-value)**
Housing system	Separated pen	12	24	0.82(0.36)
	Group (shared)	38	76	
Bedding material	Yes	14	28	9..62(0.14)
	No	36	72	
Types of floor	Good concrete	6	12	12.92 (0.16)
	Bad concrete	11	22	
	Muddy	33	66	
Time length of	Daily	27	54	5.24 (0.01)

cleaning -bedding and manure of the farm	Weekly	21	42	
	Monthly	3	6	
Udder hygiene	Slightly dirty	32	64	9.84(0.007)
	Moderate	18	36	
	Very dirty	0	0	
General hygiene	Yes	12	24	1.41(0.23)
	No	38	76	
Milk yield	Low	37	74	1.67(0.79)
	Medium	11	22	
	High	2	4	
Inflammatory signs	No	39	78	30.90 (0.000)
	Yes	11	22	
Teat shape	Pointed	42	84	16.30 (0.000)
	Round	7	14	
	Flat	1	2	
Blindness	Yes	2	4	0.79(0.67)
	No	48	96	
Tick infestation	Yes	5	10	19.56(0.000)
	No	45	90	
Gross milk quality	Normal	32	64	38.32 (0.000)
	Watery	1	2	
	Clots/flakes	2	4	
	Blood tinged/pus	15	30	
Hand washing practices	Before milking	24	48	8.35(0.21)
	After milking	0	0	
	Before and after milking	25	50	
	Between milking process	1	2	
	Not at all	0	0	
Use of dry towel	Yes	4	8	0.27 (0.60)
	No	46	92	
Use of antiseptics	Yes	3	6	19.73(0.000)
	No	47	94	

Do you know the d/nt type of mastitis	Yes	12	24	0.11(0.73)
	No	38	76	
Clinical mastitis cases	Yes	11	22	21.48(0.000)
	No	39	78	
Sub clinical mastitis cases	Yes	6	12	41.77(0.000)
	No	44	88	
Previous exposure of mastitis problem in the farm	Yes	13	26	26.68(0.000)
	No	37	74	
When do you milk the cows with mastitis	First	3	6	9.23(0.026)
	Last	34	68	
	Any time	13	26	
Do you treat mastitis case as they occur	Yes	35	70	11.72 (0.000)
	No	15	30	
Do you know the name of drugs used for treatments	Yes	12	24	12.20(0.000)
	No	38	76	
Practices of culling chronically infected cows	Yes	24	48	0.02(0.89)
	No	26	52	
Practice of dry cow therapy	Yes	36	72	13.09(0.000)
	No	14	28	
Time length of cleaning milking cows udder	Not cleaning	17	34	115.96(0.000)
	Only before milking	5	10	
	Only after milking	6	12	
	Before and after milking	22	44	
Equipments used for milking	Aluminum cans	2	4	102.65(0.000)
	Plastic can	33	66	
	Clay pot	5	10	
	Others	10	20	
Use of soap, detergent to clean milk container	Yes	26	52	12.90(0.005)
	No	24	48	
Awareness on milk borne illness	Yes	15	30	4.59(0.20)
	No	35	70	
Awareness about	Yes	17	34	18.3790.000)

staphylococcal food poisoning	No	33	66	
Acquiring illness	Yes	26	52	35.39(0.00)
	No	24	48	
Signs of illness showed	Diarrhea	5	10	9.739(0.000)
	Vomiting	8	16	
	Diarrhea , vomiting	27	54	
	Abdominal pain, cramp	10	20	
Form of milk consumption	Raw	7	14	24.106(0.000)
	Ergo	5	10	
	Ayib	14	28	
	Boiled	24	48	
Milk consumption	Yes	39	78	22.72(0.000)
	No	11	22	

Table 7: Questionaire survey for milk Consumers'

Issues raised for Milk consumers	**Categories**	**Response rate(N=50)**	**Percentage%**
Form of milk consumption	Boiled milk	27	54%
	Yoghurt/Ergo	11	22%
	Cheese/Ayib	8	16%
	Raw milk	4	8%
Awareness about milk born disease	Yes	21	42%
	No	29	58%
Awareness bout staphyloccocus food born disease	Yes	4	8%
	No	46	92%
Acquiring illness after consuming milk and milk product	Yes	18	36%
	No	33	66%
Where do you purchase milk	Farm	27	54%
	Milk selling center	15	30%
	Hotel/ café	15	30%
Type of container do you use to collect milk	Plastic	38	76%
	Metallic	11	22%
Duration of milk stay at home prior consumption	<1hr	9	18%
	1-2hr	14	28%
	>2hr	0	0
Temperature of milk storage	$<4^0$c / refrigerator	34	68%
	Room temperature	16	32%

4. DISCUSSION

The present study revealed that, *S.aureus* was detected in each critical points of the milk supply chain. The overall prevalence of *S. auerus* was 20.05% with higher *S.aureus* contamination rate (23.46%) recorded in milking containers swab, followed by milkers hand swab (21.74%), pooled milk samples (19.52%) and milking bucket swab (12.50%), with statistically non-significantly associated (p>0.05).

This finding was compared with the previous findings of Fissaha *et al*. (2024) in Assosa town of dairy cows, which were 13.4%, 24.63%, 23.08%, and 15.63% of *S. aureus* prevalence in milk from udder, milking bucket, milkers' hand swab and milking container swab respectively. Besides, as compared to the present findings, lower findings were reported by Tibebu *et al*. (2021) in udder swab (10%) from Bishoftu town, central high lands of Ethiopia.

The present study showed 20.05% overall prevalence of *S. aureus* in the lactating dairy cows in the study area which is comparable with the reports of Lema *et al*. (2021) (24.6%) in Addis Ababa and of Tibebu *et al*., (2021) (21.46%) in Bishoftu, Ethiopia. This finding is consistent with previous findings of Mesfin (2015) in Kombolcha, Abinet (2015), in Batu, Abebe *et al*. (2013), in Addis Ababa, Seedy *et al* .(2010) in Egypt, Biniam (2014) in Wolayita Sodo, Alemayehu (2015) in Bahir Dar, indicated that, 26.7%, 17.13%, 16.0%, 17.2%, 18.39%, and 15.02% respectively. It is also comparable with 15.6% prevalence reported from Ambo and Bako towns in the west Shoa zone of the Oromia regional state, Ethiopia (Bizunesh *et al*., 2023).

However, this finding is lower as compared to the earlier findings of (51.56%) by Shimelis (2014) in Selale/Fitche Area, (44.03%) by Sori *et al*. (2005) around Sebeta; (43.3%) by Duguma *et al*. (2013) in Holleta agricultural research centre; (48.75%) by Daka *et al*. (2012) in Hawassa area; (47.1%) by Mekibib *et al*. (2010) in Holeta town and (39.5%) by Addis *et al*. (2011) in Debre Ziet area.

Higher findings were reported by Bitew *et al*. (2010) at Bahir Dar, and Mulugeta and Wassie (2013), around Wolaita Sodo, with 28.8%, 29.5% prevalence in cows respectively. Moreover, this finding is lower when compared with the previous findings of Shimelis (2014) in Selale/Fitche area, Alemayehu (2015) in Bahir Dar and its surroundings, Mesfin (2015) in and around Kombolcha, (83.1% v 65.42%), (62.06% v 42.44%), (56% v 33.7%) in cows and quarters respectively. it also disagrees with the previous findings of Lakew *et al*. (2009) in Asella; Abaineh (1997) in Fiche, Abera *et al*. (2013) in Adama, Zerihun (1996) in Addis Ababa and Nesru (1986) in Dire-Dawa, who reported 64.4%, 65%, 66.6%, 68.1%, 85.6% prevalence in cows respectively. The variable prevalence of *S. aureus* in lactating dairy cows across different reports may be attributed to differences in farm management practices, environmental conditions, and awareness of disease transmissions. *S. aureus* is a contagious pathogen that spreads from infected cows to healthy ones during unhygienic milking practices and contact with animals. It may be also associated with its frequent colonization of teats, its ability to exist intracellular and localize within micro abscesses in the udder and hence resistant to antibiotic treatment (MacDonald, 1997). The Bacteria usually establish chronic, sub clinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. The possible explanation for the variation might be that *S. aureus* is a contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures (Rowe, 1999).

The prevalence of *S. aureus* in local zebu (19.56%) and cross breeds (21.13%) were non-significantly associated with the occurrence of *S. aureus* (p>0.05). This finding is inconsistent with result reported by Almaw *et al*. (2009) in Gondar town and its surroundings, Sori *et al*. (2005) in and around Sebeta showed that breed significantly influenced the occurrence of mastitis. In addition, it is not inline with Bitew *et al*. (2010) who reported in Bahir Dar, between Cross and Fogera breed, Lakew *et al*. (2009) in cross and local Arsi breed and Biffa *et al*. (2005) found significant difference between local Zebu, Holstein-Frisian and Jersey breeds in Ethiopia, the difference is due sample size difference, management system/hygienic practice and genetic variability of the breeds that increase possibility of *S. auerus* occurrence (Schutz, 1994).

In this study; higher occurrence of *S. aureus* was detected in cows with late lactation (31.81%) followed by mid lactation (23.07%) and early lactation stage 17.24% and dry periods (18%) with no scientifically significant difference among lactation stages (p>0.05). This finding is in agreement with Mulugeta and Wassie, (2013); Biffa *et al*. (2005) and Tamirat, (2007) who indicated higher *S. aureus* infection occurs in cows at late lactation stage

followed by dry, mid and early lactation stages. Radostits *et al*. (2000; 2007) also support; most new infections occur during late and early dry, mid periods of lactation stages of dairy cows. This may be due to an absence of dry period therapy, low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply and absence of udder washing and teat dipping (Aylate *et al*., 2013).

Binary logistic regression analysis revealed that the prevalence of *S. aureus* isolates were significantly different among parity groups. Early birth of cows with 1-3 parity has 27.62% (higher) of infection followed by cow with 4-6 parity or frequency of birth (10) with statistically significant difference among parity ($p<0.02$). This finding is comparable with the previous reports of Mulugeta and Wassie (2013) in Wolaita Sodo town; Mekibib *et al*. (2010) in Holota town and Haftu *et al*. (2012) in northern Ethiopia who reported higher prevalence of *S. auerus* low parity numbers. A similar result of a significant association of parity with the prevalence of *S.aureu*s isolates. This might be due to the increased opportunity and contamination of udder and the prolonged duration of infection (Radostits *et a*l., 2007; Markos *et al*., 2023).

In this study, higher occurrence of *S. auerus* infection was detected in muddy floor system (23.23%) followed by concrete floor (17.14%) with non- significant difference in diseases occurrence on bedding types ($p>0.05$). This finding is agreement with Abera *et al*. (2013) in Adama town and Fekadu *et al*. (2005) in southern Ethiopia, Lakew *et al*. (2009) and Sori *et al*. (2005) who reported high prevalence of mastitis (due to *S. auerus*) in farms with muddy (soil) floors (48.36%) when compared with concrete floor types (35.22%). 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 mastitis pathogens. The main sources of infection are udder of infected cows transferred via milker's hand, towels and environment (Radostitis *et al*., 2007). This study revealed that dairy cow's house with poor drainage was 4.93 times more likely to be harbor *S. aureus* than well drainage housing systems. This supported by finding of Bizunesh *et al*.(2022) who indicated 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.

Occurrence of *S. aureus* in this study; in cows with respect to milking hygienic practice at farms revealed higher (41.2%) occurrence with poor milking hygiene practices than those with good milking hygiene practices (6.93%) with significant difference in occurrence of *S. auerus* ($p=0.000$). This finding is comparable with earlier reports of Mulugeta and Wassie, (2013); Lakew *et al*. (2009) and Sori *et al*. (2005). It is also supported by Radostitis *et al*. (2007) who suggested contagious mastitis occurrence higher in farms due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths.

In this finding, the prevalence of *S.aureus* was significantly influenced by age categories ($P < 0.000$). Age with less than or equal to 3 years was 70% prevalent with *S. aureus*, than 4-7 years (17.39%) and greater than 7 years age (18.2%) of dairy cows in the study areas. Similar findings were reported by Shimelis (2014) in Selale /Fitche.

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, 21% had a habit of drinking raw milk and 79% 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, who reported 21.7% of the raw milk consumption and 62% of respondents 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 greater risk of contracting food born intoxication infection than those who do not consume raw milk. Consumers are last group of food chain and therefore they are risk of any mal-practice occurring in the chain. Also 31% 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).

The present study showed that the resistance of *S. aureus* to penicillin G (100%), (92.30%) gentamycin, amoxicillin (84.61%), Sulphonamide (56.41%), and (64.10%) cefoxitin, and 7.69% in chloramphenicol observed in milk

samples. Comparable research works were reported in various parts of Ethiopia by Biniam T (2014) revealed resistance of *S. aureus* to Penicillin G (100%) in and around Wolaita Sodo, southern and Alemayehu (2015) indicated resistance of *S. aureus* to Penicillin G (95.8%), Cefoxitin (75.7%), and Tetracyline (72.2%) from Bovine mastitic milk in Dairy farms of Bahir Dar. Comparable resistance was reported by Tibebu *et al*. (2021) reported 94% resistance to penicillin in bishoftu; Abebe *et al.* (2013) who reported 96.7% resistance of *S. aureus* to penicillin G around Addis Ababa and Abera *et al*. (2010) who indicated 94.4% resistance to penicillin G in Adama. In addition, this results is in consistent with reports from earlier studies in the other countries (Edward *et al*., 2002; Gentilini *et al*., 2002 and Jakee *et al*., 2008) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials.

The resistance of *S. aureus* isolates to beta-lactam antibiotic was evident in current study. High percentage of *S.aureus* was resistant to the most frequent drugs. This finding is in agreement with the finding of Derese *et al*. (2012) who showed cefoxitin resistant isolates from the milk. Similarly, *S. aureus* isolates, were also resistant to amoxicillin (84.61%) and Penicillin G (100%) as reported by Daka *et al*. (2012). This is due to the fact that resistance of *S. aureus* to these drugs may be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antimicrobials (Wubishet *et al*., 2012; Sharma *et al*., 2011; Green and Bradely, 2004). In addition, the current investigation was agreed with the reports of (Tsige, 2018) who reported the resistance of *S.aureus* to penicillin 100%. Moreover, the present report was compared with the results of Markos *et al*. (2023) in Shishicho town recording 100% resistance for both penicillin and amoxicillin. This is supported by the findings of Endries *et al*. (2022) from Holeta who reported *S. aureus* resistance to Amoxicillin 95% and Oxacillin 87.50%.

In the present study, 21(53.84%) of the isolates developed multi-drug resistance. The maximum multiple drug resistance registered for two isolates were resistance to four classes of antimicrobials (penicillin, sulphonamides, cefoxitin, and gentamycin). Antibiotic resistance *S. aureus* isolates had been a challenge to both animal and public health. Comparably, Alemayehu (2015) reported multidrug resistance, to penicillin G, Cefoxitin and tetracycline. Similar finding by Mekuria *et al*. (2013) also reported MRSA isolate with resistant to more than two of non-β-lactam antimicrobials. The resistance of S. aureus isolates to penicillin G may be attributted to the production of B-lactamase enzyme that inactivates penicillin and closely related antibiotics. Resistance to penicillin G is used as a marker to assess the susceptibility of *S. aureus* isolates against other beta lactam antibiotics (Markos *et al*., 2023).

This result was higher than the finding of Lemma *et a*l. (2021) who reported 13.5% of multiple drug resistance *S. aureus* isolated from cow milk in Addis Ababa. This might be due to the variation in the type and frequency of use of these antibiotics for the treatment and prevention of prevailing bacterial diseases. However, this finding is lower than Shimelis (2014), who reported 86.46% of the isolates were resistant to different combinations of two or above tested antibiotics and the most frequent multidrug resistance pattern consisting of three drugs' is exhibited for, Gentamicin, Ceftazidime and Streptomycin. This multi-drug resistance occurred might be due to administration of multiple antibiotics for prophylaxis or infection, lack of drug sensitivity tests in the dairy farms, uncontrolled or discriminate use of antibiotics in the farms and another possibility is that cattle are being treated with antibiotics for other conditions, thereby selecting for resistant populations of *S. aureus* (Shitandi and Sternesjo, 2004).

Antimicrobial susceptibility tests of *S. aureus* isolates revealed that the highest rate of susceptibility among the isolates was recorded against Ciprofloxacin, Gentamycin and chloramphenicol. The results agree with the findings of Tibebu *et al*. (2021) who reported susceptibility to *S. aureu*s to Ciprofloxacin and Gentamycin. The variability in susceptibility results could partly arise from how frequently a drug was in use for dairy cows treatment in the study area.

5. CONCLUSION AND RECOMMENDATIONS

The present study has shown that *Staphyloccus auerus* contaminates were highly prevalent in milking supply chains. Higher *S.aureus* were recorded in milking containers swab, followed by milkers' hand swab, pooled milk samples and swab from milking buckets. In addition, hands of milkers' and milk containers were found to be the potential sources of milk contamination with the pathogen. The prevalence of *S. aureu*s indicates the higher public health risk due to the widespread consumption of raw milk and its products in study area. The study also revealed inadequate knowledge of milk borne disease. In addition, majority of *S.aureus* isolates developed multi-drug resistance (MDR) where the highest rate of resistance among the isolates was against penicillin followed by gentamycin, amoxicillin

and cefoxitin. However, it was observed that *S. aureus* isolates were highly sensitive to Ciprofloxacin and Chloramphenicol. In general, the study has revealed the possibility of the public health risk posed due to high prevalence of *S.aureus*, multidrug resistance, low level of knoweldege and awareness of farm workers and poor hygienic practices. Therefore; based on this conclusion the following recommendations are forwarded:

- Awareness should be created to improve on public health importance of the *S.aureus* and good hygienic practices.
- Hygienic practices in the area should be improved
- Monitoring, rational use of drugs and periodic assessment of the antimicrobial sensitivity of drugs prior to use should be practiced.
- Further study should be done molecularly to identify public health important and resistance gene of *S. auerus*.

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