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Occurrence, associated risk factors and antimicrobial resistance patterns of *Staphylococcus aureus* and methicillin resistant *S. aureus* from foods of bovine origin in Dessie and Kombolcha towns, Ethiopia

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Food-producing animals including bovine species are major reservoirs for different food-borne pathogens. *Staphylococcus aureus* is among the causes of food-borne diseases globally that can be transmitted mainly through consumption of contaminated foods of animal origin and emergence of multidrug resistant bacteria like methicillin resistant *S. aureus* (MRSA) become a significant public health concern. A cross-sectional study was conducted from October 2019 to July 2021 to estimate the prevalence, identify associated risk factors and determine antibiogram profiles of *S. aureus* and MRSA from foods of bovine origin in Dessie and Kombolcha towns. A total of 384 foods of bovine origin samples were collected using random sampling techniques. Isolation and characterization of *S. aureus* were done according to the standard bacteriological protocols. Agar disc diffusion method was employed to determine the *in vitro* antimicrobial resistance pattern of *S. aureus* and MRSA isolates. The collected data were analyzed using descriptive and inferential statistics. The overall prevalence of *S. aureus* and MRSA were found to be the equal (39.3%). The prevalence of *S. aureus* was 55.6, 44.0, 41.1, 36.4, 16.7, and 0.0% in yogurt, beef swab, udder milk, carcass swab, tank milk, and cheese samples, respectively. A statistically significant difference was observed in the prevalence of *S. aureus* among the different sample types ($P < 0.05$). The prevalence of *S. aureus* in milk samples from cows with and without treatment history was 47.1 and 26.0%, respectively. The difference in the prevalence of *S. aureus* among treatment history categories was statistically significant ($P < 0.05$). Higher prevalence of *S. aureus* was recorded in carcass swab samples collected from Dessie town (50.0%), municipal abattoirs (46.7%), slaughtering process with poor hygiene (57.1%); and carcasses slaughtered by butchers with poor hygiene (62.1%). 100.0, 97.4, 90.1, and 74.8% of *S. aureus* isolates were resistant to Cefoxitin, Penicillin G, Ampicillin, and Nalidixic acid, respectively. 97.3% of *S. aureus* isolates showed multidrug resistance to three and more than three drugs. To reduce the high magnitude of *S. aureus* contamination of foods of bovine origin, improvement of cattle health and good hygienic procedures along the production chain should be implemented in the study areas.

KEYWORDS

antimicrobial resistance, Dessie, Kombolcha, MRSA, prevalence, risk factors, *S. aureus*

1 Introduction

Food-borne diseases are globally important public health problems that can be transmitted to humans through consumption of food and water contaminated with microorganisms or chemicals (Mehrnaz et al., 2021). These diseases can occur in sporadic or outbreak forms and are frequently associated with morbidity, mortality, and economic losses around the world (Hemalata and Virupakshaiah, 2016; Khater et al., 2021; Borena et al., 2022; Pal et al., 2022).

Food-borne pathogens are responsible for a wide range of illnesses with serious consequences for both human health and the economy (Bintsis, 2017). Globally, food-borne disease outbreaks are becoming more frequent and more than 250 pathogens of food-borne illnesses have been identified (Liu et al., 2019; Al-Mohaithef, 2021). Due to the emergence of food-borne pathogens, microbial contamination of food is a significant public health concern (Al-Mohaithef, 2021). Food-producing animals including bovine species are the major reservoirs of different food-borne pathogens and foods of animal origin like meat, egg, milk and their products are the major sources and vehicles of food-borne infections (Oliver et al., 2005; Zakary et al., 2011; Heredia and Garcia, 2018; Kandil et al., 2018; Abebe et al., 2020).

Even though various microbial pathogens can contaminate food products of humans (Chao et al., 2007), both sporadic cases and outbreaks of food-borne illness are most commonly caused by bacteria and viruses (Truchado and Randazzo, 2022). Due to the high magnitude of occurrence and seriousness, some bacterial food-borne pathogens are significantly important (Loir et al., 2003). The leading bacterial causes of food-borne diseases include *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella enterica*, Shiga-toxin producing and other *Escherichia coli* strains, *Campylobacter jejuni*, *Vibrio species*, and *Bacillus cereus* (Zhao et al., 2014).

Among the major food-borne bacterial pathogens, *S. aureus* is the leading and most common cause of food-borne infections around the globe (Tsepo et al., 2016; Ayele et al., 2017; Fetsch and Johler, 2018; Hiko, 2018; Gebremedhin et al., 2022) and is responsible for food poisoning through production of enterotoxins (SEs; Denayer et al., 2017). *S. aureus* is a well-known, ubiquitous, commensal and opportunistic pathogen which can cause a wide spectrum of diseases in both humans and animals (Daka et al., 2012; Elemo et al., 2017; Adugna et al., 2018; Grace and Fetsch, 2018; Mphahlele et al., 2020; Mehrnaz et al., 2021; Alembo and Torka, 2023). Contaminated animal-source foodstuff mainly milk, dairy products and meat are common vehicles that are frequently implicated in Staphylococcal Food Poisoning (Abunna et al., 2017; Abebe et al., 2020; Feyissa et al., 2023).

In addition to the high prevalence and toxic effect of *S. aureus*, the emergence and increasing prevalence of antimicrobial resistant bacteria, in particular of methicillin resistant *S. aureus* (MRSA), due to the inappropriate and widespread use of antibiotics is a major concern to human and veterinary medicine worldwide (Chao et al., 2007; Tsepo et al., 2016; Abraha et al., 2018; Fetsch and Johler, 2018). The magnitude of MRSA in food producing animals is increasing and the emergence of livestock-associated

MRSA has become a growing concern for public health as it can be transmitted to humans via food chain (Lim et al., 2010; Mekuria et al., 2013; Ndahi et al., 2014; Rodríguez-Lázaro et al., 2017; Chai et al., 2020; Keyvan et al., 2020; Khater et al., 2021; Gajewska et al., 2022).

In many developing countries, the magnitude of food-borne diseases is relatively high. In order to reduce this high prevalence, the detection of food-borne pathogens in food is very important (Zhao et al., 2014). However, the true magnitude of food-borne diseases in these countries is not accurately estimated due to the poor surveillance systems (Savariraj et al., 2018). In Ethiopia, there is limited published information on the occurrence and distribution of *S. aureus* and its antibiotic resistance pattern in food animals and foods of animal origin (Mekuria et al., 2013; Abebe et al., 2020). Foods of animal origin are produced under unsanitary conditions and there is the habit of consumption of raw or under cooked cow milk and beef in most parts of Ethiopia (Demme and Abegaz, 2015). Moreover, there was no accessible published report on the magnitude and antimicrobial resistance pattern of *S. aureus* from foods of bovine origin in Dessie and Kombolcha towns. Detection of this bacterial pathogen in foods of bovine origin is crucial to implement different strategies which help to reduce bacterial contamination along the production chain to improve the safety of these food products. Hence, this study was conducted to estimate the prevalence, identify associated risk factors and determine antimicrobial resistance pattern of *S. aureus* and methicillin resistant *S. aureus* (MRSA) from foods of bovine origin in Dessie and Kombolcha towns.

2 Materials and methods

2.1 Study area

The study was conducted in Dessie and Kombolcha towns of South Wollo Zone, Eastern Amhara Region, Ethiopia. Kombolcha and Dessie towns are located at a distance of 376 km and 401 kilometers from Addis Ababa, respectively. Dessie is situated at 39°38'E-41°13/East longitude and 11°8'N-11°46' North latitude whereas Kombolcha town is located at 39°45/E longitude and 11°6' N latitude. The topography of Dessie is a highland type with an elevation between 2,470 and 2,550 meters above sea level. On the other hand, Kombolcha town is located at a range of altitudes between 1,500 and 1,840 meter above sea level with mean annual rainfall of 1,046 mm. The mean annual rainfall of Dessie town ranges from 1,100 to 1,200 mm and the annual minimum and maximum temperatures of the town are 9 and 23.7°C, respectively. With regard to Kombolcha town, the annual minimum and maximum temperatures are 12.9 and 28.1°C, respectively (Eskinder et al., 2010; Abebe et al., 2023).

2.2 Study population

Milking dairy cows from dairy farms, carcasses from municipal and ELFORA abattoirs, cheese and yogurt from milk product retail shops and beef from butcher shops and restaurants in the study

sites were considered as study population. In Kombolcha town, 164 registered dairy farms were found during the time of sample collection. The number of milking cows in these farms was 586 and daily average milk yield in the town was 6,261 liters (Kombolcha Town Animal Production Health Office, 2019). In Dessie town, the number of dairy farms and milking cows included in the study were 28 and 196, respectively. Around 30 to 35 oxen were slaughtered on Friday of each week at Dessie municipal abattoir and 5 oxen were slaughtered per day in average at the abattoir. The samples were collected during holidays at Kombolcha municipal abattoir since small numbers of oxen were slaughtered per day due to expansion of illegal field slaughtering. The number of oxen slaughtered at Kombolcha ELFORA abattoir ranged from 80 to 140 (120 in average).

2.3 Study design

A cross sectional study was conducted from October 2019 to July 2021 to estimate the prevalence, identify associated risk factors and determine antibiotic resistance pattern of *S. aureus* from foods of bovine origin in Dessie and Kombolcha towns.

2.4 Sample size determination

The sample size (n) was determined based on the formula recommended by Thrusfield (2005).

$$n = \frac{Z^2 P \exp(1 - P \exp)}{d^2} \quad n = \frac{1.96^2 P \exp(1 - P \exp)}{d^2}$$

Where, n = Sample size, Z = Statistic for a level of confidence, d = Required absolute precision, Pexp = Expected prevalence

Since there was no previous accessible and published report on the same title in the study sites, 50% of prevalence of *S. aureus* contamination was expected. Moreover, the sample size was calculated at 95% confidence interval with 5% desired absolute precision. Based on the above given formula and numbers, the sample size was calculated to be 384.

2.5 Sampling technique

Probability sampling methods were employed to select foods of bovine origin samples from different sources in the study sites. Milking cows were selected using simple random sampling technique from the sampling frame of total milking cows in dairy farms of the two study sites. Systematic random sampling method was employed to select carcass samples among cattle slaughtered at abattoirs of study sites and every 3rd carcasses were selected. Moreover, samples from tank milk and milk product shops, and beef swab samples from butcher shops and restaurants were selected using random sampling technique.

2.6 Sample collection

After the milkers prepared the cows for milking through usual practice, about 25 ml of milk sample was collected aseptically from all quarters of the selected individual cow's udder during the middle of milking procedure using sterile labeled screw cupped glass bottles. Using sterile screw cupped glass bottles, around 25 ml of sample from well-mixed tank milk and 25 ml/g of yogurt and cheese samples from milk product shops were collected aseptically. From the surface and deep part of the selected carcasses (neck, thorax, abdomen, breast, and crutch), carcass swab samples were collected at abattoirs using sterile cotton swabs and the beef swab samples were collected from different sites of individual beef at butcher shops and restaurants, and the swab samples were transferred into labeled test tubes containing 5 ml of sterile 0.85% NaCl solution. Moreover, different variables of each sample type were recorded on pre-designed data recording format. The collected samples were transported in ice box containing ice packs to Veterinary Microbiology Laboratory of School of Veterinary Medicine, Wollo University on the day of collection, stored aseptically in refrigerator and analyzed within 24 h.

2.7 Standard organisms for quality control

A standard strain of *S. aureus*, which was obtained from Amhara Public Health Institute (APHI) Dessie branch, was used as quality control standard organisms.

2.8 Isolation and identification of *S. aureus* and methicillin-resistant *Staphylococcus aureus*

Standard microbiological protocols described by Quinn et al. (1999) and Quinn et al. (2002) were followed for the isolation and identification of *S. aureus* from different samples of foods of bovine origin. Briefly, from each well-mixed original sample, 1 ml was transferred into 9 ml of sterile peptone water (Micromaster, India) and incubated aerobically at 37°C for 24 h. A loopful of the enriched sample was streaked aseptically on blood agar base (HiMedia Laboratories Pvt. Ltd., India) enriched with 5% sheep blood plates and incubated aerobically at 37°C for 24–48 h. Then, the plates were examined for growth of bacterial colonies, colonial morphology (round, smooth, and white or yellow colonies) and presence or absence of hemolysis. Those isolates that were suspected as *Staphylococcus species* on the basis of their morphological aspects and β-hemolytic pattern on the surface of blood agar plates (Figure 1) were observed and recorded (Bannerman, 2003).

Pure presumed colonies were subcultured on nutrient agar plates (HiMedia Laboratories Pvt. Ltd., India) and subjected to Gram's stain and catalase test. Colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters (Figure 2) and positive for catalase test (forming bubbles) were presumptively identified as *Staphylococcus*.



FIGURE 1
β-hemolysis on blood agar.



FIGURE 3
Golden yellowish color colonies with yellow color of MSA.

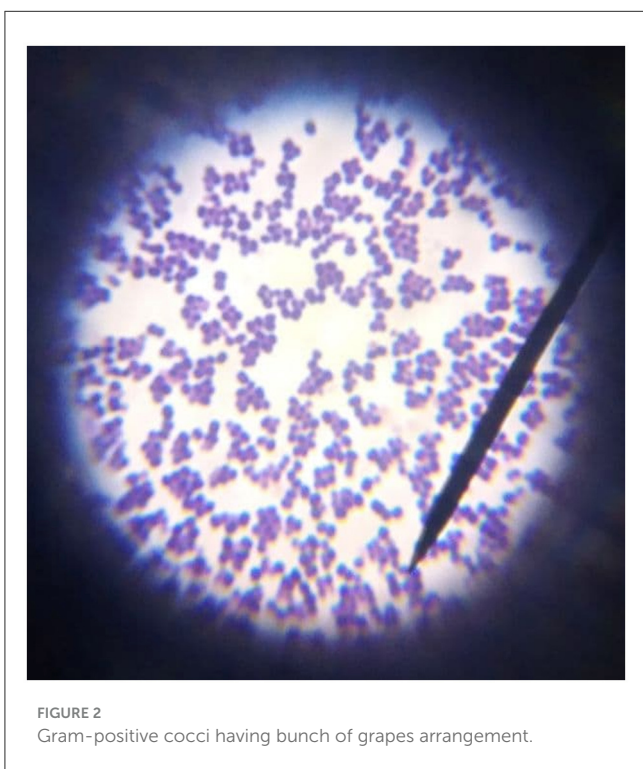


FIGURE 2
Gram-positive cocci having bunch of grapes arrangement.

The presumptive colonies were taken from nutrient agar plates and subcultured onto nutrient broth (Sisco Research Laboratories Pvt. Ltd., India) and incubated aerobically at 37°C for 24 h. A loopful of the subcultured isolates was streaked aseptically onto Mannitol Salt Agar (MSA; Microexpress, India; Figure 3) and incubated aerobically at 37°C for 24–48 h. Presumed colonies were then subcultured on nutrient agar plates and incubated aerobically at 37°C for 24 h for conducting different biochemical tests.

The purified *S. aureus* isolates were identified through different biochemical tests including Catalase test, Oxidation-Fermentation (OF) test, detection of hemolysis, DNase test, and both slide and tube Coagulase tests. Isolates which were hemolysis positive (complete hemolysis around the colonies; Figure 1), Gram-positive (blue color cocci with bunch of grapes arrangement; Figure 2), Catalase positive (forming bubbles), yellowish color colonies with yellow color of MSA (Figure 3), slide coagulase positive (clump formation), tube coagulase positive (clot formation), OF positive (both open and sealed media changed to yellow color), and DNase positive (distinct pink clear zone around the streak line) were considered as *S. aureus* (Quinn et al., 2002).

In this study, the Kirby-Bauer disc diffusion method using Cefoxitin (30 µg) and Oxacillin (1 µg) discs (Mast Group Ltd., Merseyside, U.K) was done for identifying MRSA strains from *S. aureus* isolates. However, *S. aureus* isolates resistant for Cefoxitin disc diffusion method were confirmed as MRSA since CLSI (2020) suggested that Oxacillin disc diffusion method is not reliable for *S. aureus* and recommended the use of the Cefoxitin for MRSA detection as it is a better inducer of PBP-2a encoding *mecA* gene. Moreover, Cefoxitin disc diffusion has 100% sensitivity and 100% specificity using PCR for *mecA* gene as gold standard comparison test (Ahmad et al., 2013; Fernandes et al., 2005; Pramodhini et al., 2011).

2.9 Antimicrobial susceptibility testing of *S. aureus*

All the identified *S. aureus* isolates were subjected to *in vitro* antimicrobial susceptibility test using the standard Kirby-Bauer disc diffusion method on the Muller-Hinton

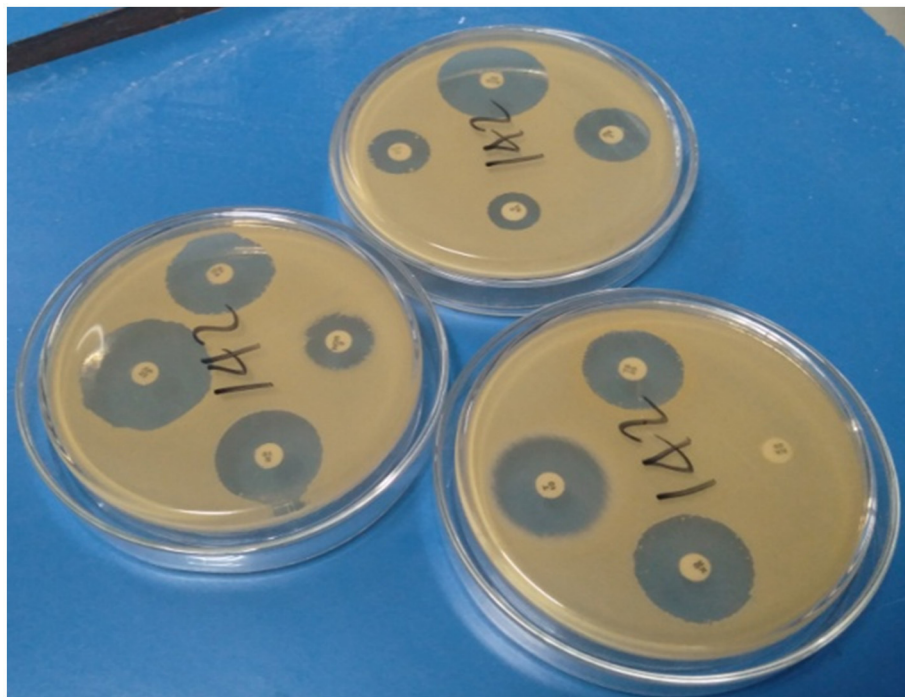


FIGURE 4
In vitro antimicrobial susceptibility pattern of *S. aureus* isolates.

TABLE 1 Prevalence of *S. aureus* among the sample types and study sites.

Sample sources	Variables	No. of examined	No. of positive (%)
Sample type			
Dairy farms	Udder milk	146	60 (41.1)
	Tank milk	6	1 (16.7)
Milk product shops	Yogurt	36	20 (55.6)
	Cheese	9	0 (0.0)
Butcher shops and restaurants	Beef swab	25	11 (44.0)
Abattoirs	Carcass swab	162	59 (36.4)
	χ^2		12.093
	P-value		0.034
Study site			
	Dessie	181	74 (40.9)
	Kombolcha	203	77 (37.9)
	χ^2		0.3497
	P-value		0.554
	Overall	384	151 (39.3)

Agar (HiMedia Laboratories Pvt. Ltd., India; Bauer et al., 1966). Along with Cefoxitin (30 mg) and Oxacillin (1 µg), other ten antibiotics discs (Mast Group Ltd., Merseyside, U.K) were used: Amoxicillin/clavulanic acid (30 mg), Ampicillin (25 mg), Kanamycin (30 mg), Nalidixic acid (30 mg),

TABLE 2 Bivariate logistic regression results of *S. aureus* among different sample types.

Sample type predictor	<i>S. aureus</i>	
	OR (95% CI)	P-value
Cheese	Reference	
Tank milk	0.349 (0.04–3.06)	0.342
Yogurt	2.18 (1.05–4.53)	0.036
Udder milk	1.22 (0.77–1.93)	0.400
Beef swab	1.37 (0.59–3.22)	0.467
Carcass swab	1	0.999

Norfloxacin (2 µg), Penicillin G (10 IU), Streptomycin (10 mg), Sulphamethoxazole-trimethoprim (25 µg), Tetracycline (10 mg), and Vancomycin (5 mg).

The subcultured colonies were transferred into test tubes containing 5 ml of sterile 0.85% saline solution and homogenized until the turbidity of the bacterial suspension is comparable to the 0.5 McFarland turbidity standards. The sterile cotton swab was immersed into the adjusted suspension and the inoculum was then spread uniformly over the entire surface of the Mueller-Hinton Agar plate (HiMedia Laboratories Pvt. Ltd., India). Using sterile thumb forceps, antibiotic discs were placed at a minimum distance of 24 mm on the agar surface after the inoculated plates dried for 3–5 min and gently pressed. The plates were inverted within 15 min of the antibiotic discs placement and incubated aerobically at 37°C for 24 h. Then, the diameters of growth inhibition zones of each antibiotic disc were measured using digital caliper and the

TABLE 3 Prevalence of *S. aureus* among the different variables of milk samples.

Variables	No. of examined	No. of positive (%)	χ^2	P-value
Study site				
Dessie	52	16 (30.8)	2.884	0.089
Kombolcha	100	45 (45.0)		
Sample type				
Udder milk	146	60 (41.1)	1.432	0.232
Bucket milk	6	1 (16.7)		
Farm system				
Intensive	131	49 (37.4)	2.935	0.087
Semi Intensive	21	12 (57.1)		
Treatment history				
No	50	13 (26.0)	6.193	0.013
Yes	102	48 (47.1)		
Milking practice				
Excellent	3	3 (100.0)		
Very good	42	20 (47.6)	8.025	0.045
Good	104	38 (36.5)		
Poor	3	0 (0.0)		
Farm hygiene				
Excellent	4	3 (75.0)	2.851	0.415
Very good	52	18 (34.6)		
Good	85	35 (41.2)		
Poor	11	5 (45.5)		
Total	152	61 (40.1)		

S. aureus isolates were grouped as Sensitive (S), Intermediate (I), and Resistant (R) based on the Clinical and Laboratory Standard Institute interpretation tables (CLSI, 2020; Figure 4). Bacterial isolates resistant to one or more antibiotic types in three or more antibiotic classes were considered multidrug-resistant (Magiorakos et al., 2012).

2.10 Data management and analysis

All collected raw data were compiled, entered, and coded in Microsoft Excel 2007 spread sheet and transferred to STATA Version 12 software for statistical analysis. Descriptive and inferential statistical techniques were used to analyze the collected data. Descriptive statistics such as mean, frequency, and/or percentage were calculated. Besides to proportion, chi-square test (χ^2), P-value, and logistic regression were computed to see the association of risk factors with that of the occurrence of the isolates and the degree of association was determined using Odds ratio

TABLE 4 Prevalence of *S. aureus* among the variables of milk product samples.

Variables	No. of examined	No. of positive (%)	χ^2	P-value
Study site				
Dessie	20	5 (25.0)	5.513	0.019
Kombolcha	25	15 (60.0)		
Sample type				
Yogurt	36	20 (55.6)	9.000	0.003
Cheese	9	0 (0.0)		
Equipment type				
Aluminum can	2	0 (0.0)	1.674	0.196
Plastic	43	20 (46.5)		
Hygiene				
Excellent	6	2 (33.3)	0.592	0.744
V. good	20	10 (50.0)		
Good	19	8 (42.1)		
Total	45	20 (44.4)		

(OR) with 95% confidence interval (CI). P-values < 0.05 were considered as statistically significant.

3 Results

3.1 Overall prevalence

Out of the total of 384 examined samples of foods of bovine origin, 151 (39.3%) were found to be contaminated with *S. aureus*. The site-based prevalence of *S. aureus* was 40.9 and 37.9% in Dessie and Kombolcha towns, respectively. The difference in the prevalence of *S. aureus* among the study sites was not statistically significant ($P > 0.05$). The prevalence of *S. aureus* was 55.6, 44.0, 41.1, 36.4, 16.7, and 0.0% in yogurt, beef swab, udder milk, carcass swab, tank milk, and cheese samples, respectively. A statistically significant difference in the *S. aureus* prevalence ($P < 0.05$) was observed among the different sample types of foods of bovine origin (Table 1).

The contamination of yogurt with *S. aureus* was 2.18 times more likely to occur compared to cheese and it was statistically significant ($P < 0.05$; Table 2).

3.2 Prevalence of *S. aureus* among variables of different sample types

The recorded prevalence of *S. aureus* in milk samples from cows with treatment history was 47.1%. The difference in the prevalence of *S. aureus* among treatment history categories was statistically significant ($P < 0.05$; Table 3).

Though *S. aureus* was not detected from cheese, it was isolated from 55.6% of yogurt samples and the difference was statistically

TABLE 5 Prevalence of *S. aureus* among variables of carcass swab samples.

Variables	No. of examined	No. of positive (%)	χ^2	P-value
Study site				
Dessie	96	48 (50.0)	18.767	0.000
Kombolcha	66	11 (16.7)		
Source				
Municipal Abattoir	105	49 (46.7)	13.532	0.000
ELFORA	57	10 (17.5)		
Hygiene of slaughtering process				
V. good	78	17 (21.8)	15.217	0.000
Good	49	22 (44.9)		
Poor	35	20 (57.1)		
Hygiene of butchers				
V. good	78	17 (21.8)	16.681	0.000
Good	55	24 (43.6)		
Poor	29	18 (62.1)		
Hygiene of slaughtering materials				
Excellent	57	10 (17.5)	14.808	0.001
Good	83	41 (49.4)		
Poor	22	8 (36.4)		
Total	162	59 (36.4)		

significant ($P < 0.05$). The proportion of *S. aureus* from milk products was higher in Kombolcha town (60.0%) than Dessie town (25.0%). Thus, there was a statistically significant difference in the prevalence of *S. aureus* from milk products among the two study sites ($P < 0.05$; Table 4).

A statistically significant difference in the *S. aureus* prevalence ($P < 0.05$) was observed among all hypothesized variables of carcass swab samples as shown in Table 5. Higher prevalence rates of *S. aureus* were recorded in carcass swab samples collected from Dessie town (50.0%), municipal abattoirs (46.7%), slaughtering process with poor hygiene (57.1%); and carcasses slaughtered by butchers with poor hygiene (62.1%) as presented in Table 5.

The difference in the prevalence of *S. aureus* among all hypothesized variables of beef swab samples was not statistically significant ($P > 0.05$) as shown in Table 6.

3.3 In vitro antimicrobial susceptibility pattern of *S. aureus* isolates

Phenotypical antibiotic sensitivity test of 151 *S. aureus* isolates against 12 antimicrobial agents revealed that 100.0, 97.4, 90.1, and 74.8% of the isolates were resistant to Cefoxitin, Penicillin G, Ampicillin, and Nalidixic acid, respectively. Most of the isolates (93.4%) were susceptible to Norfloxacin as illustrated in Table 7.

TABLE 6 Prevalence of *S. aureus* among the variables of beef swab samples.

Variables	No. of examined	No. of positive (%)	χ^2	P-value
Study site				
Dessie	13	5 (38.5)	0.337	0.561
Kombolcha	12	6 (50.0)		
Where get slaughtered				
Abattoir	21	8 (38.1)	1.857	0.173
Field	4	3 (75.0)		
Hygiene of butchers				
V. good	18	8 (44.4)	0.875	0.646
Good	6	3 (50.0)		
Poor	1	0 (0.0)		
Hygiene of cutting utensils				
V. good	5	3 (60.0)	0.649	0.420
Good	20	8 (40.0)		
Hygiene of butcher shops				
Excellent	2	2 (100.0)	4.279	0.233
V. good	13	6 (46.2)		
Good	8	3 (37.5)		
Poor	2	0 (0.0)		
Total	25	11 (44.0)		

Moreover, 100.0% of resistant to Cefoxitin indicated that all of the *S. aureus* isolates were classified as MRSA. Hence, the overall prevalence of MRSA in the present study was 39.3%.

The result shown in Figure 5 indicated that 3 (2.0%), 1 (0.7%), 14 (9.3%), 21 (13.9%), 42 (27.8%), 47 (31.1%), 11 (7.3%), 9 (6.0%), and 3 (2.0%) of *S. aureus* isolates showed resistance to one, two, three, four, five, six, seven, eight, and nine antibiotics, respectively. In general, 147 isolates of *S. aureus* (97.3%) showed multidrug resistance to three and more than three antimicrobial drugs.

4 Discussion

In the present study, the overall prevalence of *S. aureus* was 39.3%, which was nearly comparable with the findings of Weldeselassie et al. (2020) (39.1%), Addis et al. (2011) (39.5%), Sudhanthiramani et al. (2015) (39.09%), Enquebahe et al. (2015) (38.7%), Zerabruk et al. (2019) (37.5%), Derib et al. (2017) (37.14%), Awad et al. (2017) (42.0%), and Abo-Shama (2014) (36.7%) in Mekelle town, Debre-Zeit town, Tirupathi (India), Tigray region, Addis Ababa, Wolaita Sodo, Dakahlia and Damietta Governorates (Egypt), and Sohag Governorate (Egypt), respectively. The high proportion of *S. aureus* in foods of bovine origin is indicative of poor hygienic measures during production, handling, storage, and distribution (Jahan et al., 2015). *S. aureus* is

TABLE 7 *In vitro* antimicrobial sensitivity pattern of *S. aureus* isolated from different sample types of foods of bovine origin.

Antimicrobial agents	Interpretation categories		
	Sensitive [n (%)]	Intermediate [n (%)]	Resistant [n (%)]
Cefoxitin	0 (0.0)	0 (0.0)	151 (100)
Vancomycin	1 (0.7)	137 (90.7)	13 (8.6)
Penicillin G	4 (2.6)	0 (0.0)	147 (97.4)
Tetracycline	72 (47.7)	0 (0.0)	79 (52.3)
Streptomycin	49 (32.5)	62 (41.1)	40 (26.5)
Sulfamethoxazole-trimethoprim	83 (55.0)	30 (19.9)	38 (25.2)
Amoxicillin/Clavulanic acid	93 (61.6)	0 (0.0)	58 (38.4)
Ampicillin	15 (9.9)	0 (0.0)	136 (90.1)
Nalidixic acid	6 (4.0)	32 (21.2)	113 (74.8)
Oxacillin	123 (81.5)	18 (11.9)	10 (6.6)
Kanamycin	63 (41.7)	81 (53.6)	7 (4.6)
Norfloxacin	141 (93.4)	2 (1.3)	8 (5.3)

an obvious contributor to milk contamination since it is the most common cause of bovine mastitis (Matallah et al., 2019).

However, the current prevalence of *S. aureus* was higher than the previous reports of -, Hiwot et al. (2016) in Arsi and East Shewa Zones (3.2%), Arafa et al. (2016) in Great Cairo zone (Egypt) (4.67%), Argaw et al. (2018) in Selected Districts of Jimma Zone (5.0%), Riva et al. (2015) in Northern Italy (9.1%), Kalayu et al. (2020) in Mekelle town (12.5%), Basanisi et al. (2017) in South Italy (12.9%), Beyene et al. (2017) in Addis Ababa (14.67%), Ektik et al. (2017) in Balikesir (Turkey) (14.85%), Massawe et al. (2019) in Mbeya Region (Tanzania; 15.0%), Shrestha et al. (2021) in Chitwan (Nepal; 15.2%), Mekuria et al. (2013) in Addis Ababa (16.2%), Abunna et al. (2017) in and around Asella town (16.24%), Asiimwe et al. (2017) in South-West Uganda (17.15%), Lemma et al. (2021) in Addis Ababa (20.5%), Hamid et al. (2017) in Jammu (India; 22.5%), Ayele et al. (2017) in Sebeta town (23.4%), Reta et al. (2016) in Jigjiga City (24.2%), Jahan et al. (2015) in Bangladesh (25.53%), Tsepo et al. (2016) in North West Province (South Africa; 26.5%), Tessema and Tsegaye (2017) in Alage (Ziway; 28.2%), Girmay et al. (2020) in Shire (Tigray; 29.09%), Tesfaye et al. (2021) in and around Adama town (30.6%), Olatoye et al. (2018) in Oyo State (Nigeria; 31.5%), Matallah et al. (2019) in Algeria (31.56%), and Hassan et al. (2018) in Asella (34.3%).

On the contrary, prevalence rates of *S. aureus* higher than the present study were reported by Salauddin et al. (2020) in Rangpur division (Bangladesh; 100.0%), Ateba et al. (2010) in North West province (South Africa; 100.0%), De-Oliveira et al. (2011) in Bahia (Brazil; 68.0%), Lingathurai and Vellathurai (2011) in Madurai (South India; 61.7%), Gundogan and Avcı (2014) in Turkey (56.0%), Megersa et al. (2012) in Hawassa town (53.5%), Wu et al. (2018) in China (50.4%), Daka et al. (2012) in Hawassa town (48.75%), Mohanta and Mazumder (2015) in Southern Assam (India; 47.86%), Limbu et al. (2020) in Dharan (Nepal; 45.0%),

and Elemo et al. (2017) in Asella town (44.62%). This variation in the prevalence of *S. aureus* between the present study and previous reports could arise from differences in dairy and beef cattle production and health, milking and slaughtering procedures and hygiene, cleanliness of equipments, and hygienic conditions during handling, transportation, storage, and distribution up to consumption. Additionally, differences in the research methods used by the investigators, such as study design, sample source, sampling method, sample size, sample type, and methods of detection in laboratories could also contribute to the variations of the results in the different reports.

In the current study, the occurrence of *S. aureus* was highest in yogurt (55.6%), followed by beef swab (44.0%), udder milk (41.1%), carcass swab (36.4%), tank milk (16.7%), and cheese (0.0%). A statistically significant difference in the *S. aureus* prevalence ($P < 0.05$) was observed among different sample types of foods of bovine origin. The contamination of yogurt with *S. aureus* was 2.18 times more likely to occur compared to cheese and it was statistically significant ($P < 0.05$). The higher prevalence of *S. aureus* in yogurt might be associated with the initial contamination of the milk due to unsatisfactory hygienic practices during milking and poor dairy cow health status or further contamination of the milk during collection and transportation as well as storage for long time under ambient temperature.

The prevalence of *S. aureus* was higher in milk samples from cows with teat treatment history (47.1%) than cows without treatment history (26.0%) and the difference was statistically significant ($P < 0.05$). In dairy cattle, *S. aureus* is the most common mastitis-causing bacterium (Kotzamanidis et al., 2021). Thus, the higher occurrence of *S. aureus* in milk samples from cows with teat treatment history might be due to mastitis since cows with previous mastitis history are more prone to re-infection.

Though *S. aureus* was not detected from cheese, it was isolated from 55.6% of yogurt samples and the difference was statistically significant ($P < 0.05$). According to Pazakova et al. (1997), the antibacterial action of yogurt is insufficient to prevent food poisoning in the case of high *S. aureus* contamination of milk. Moreover, Soliman and Ahmed (2019) stated that *S. aureus* could contaminate yogurt and survived fermentation process for 8–10 days.

The proportion of *S. aureus* from milk products was higher in Kombolcha town (60.0%) than Dessie town (25.0%). Thus, there was a statistically significant difference in the prevalence of *S. aureus* from milk products among the two study sites ($P < 0.05$). The difference might be associated with the variation in hygienic practices at dairy environment, herd health status of dairy farms and variation in sanitation during collection, transportation and storage of milk.

A statistically significant difference in *S. aureus* prevalence ($P < 0.05$) was observed among all hypothesized variables of carcass swab samples. The carcass swab samples collected from Dessie town were more contaminated by *S. aureus* (50.0%) than those collected from Kombolcha town (16.7%). This could be associated with the difference in slaughtering operations and hygienic practices at abattoirs. Higher prevalence rate of *S. aureus* was recorded in carcass swab samples collected from municipal abattoirs (46.7%) than ELFORA (17.5%). The higher prevalence of *S. aureus* in carcass swab samples collected from municipal abattoirs could arise from the slaughtering operation.

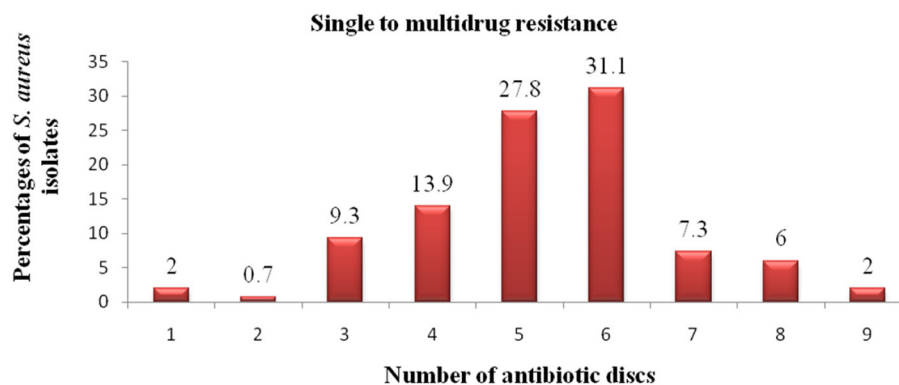


FIGURE 5

Antibiotic resistance pattern of *S. aureus* isolates. The numbers (1–9) in the x-axis indicate the total number of antibiotics to which the *S. aureus* isolates showed resistance. The exact percentage of resistant *S. aureus* isolates to each number of antibiotics is indicated above each column.

In municipal abattoirs, slaughtering process was conducted on the floor which could increase the risk of contamination from the ground, outer integument, slaughter men and other sources. The higher prevalence of *S. aureus* from carcasses slaughtered under poor hygienic operation (57.1%) and carcasses slaughtered by butchers with poor hygiene (62.1%) were not astonishing since *S. aureus* are commonly associated with poor hygiene and sanitation (Bagumire and Karumuna, 2017) and contamination of beef at the slaughterhouse in particular occurs because of inadequate hygienic conditions and poor handling practice (Fasanmi et al., 2018). The difference in the prevalence of *S. aureus* among different hypothesized variables of beef swab samples was not statistically significant ($P > 0.05$).

S. aureus is a notorious bacterium which rapidly develops resistance to many antibiotics of different classes (Parvin et al., 2021) and the acquisition of resistance to several antibiotics worsen the risk and severity of its infection. Moreover, the emergence and spread of livestock-associated MRSA is a great concern for public health (Tamendjari et al., 2021). In the present study, the *in vitro* antibiotic sensitivity testing revealed that 97.3% of the *S. aureus* isolates showed multidrug resistance to three and more than three drugs which was consistent with previous reports of Girmay et al. (2020) in Shire town, Beyene et al. (2017) in Addis Ababa, Weldeselassie et al. (2020) in Mekelle town who reported 100.0, 100.0, and 93.75% of multidrug resistant isolates, respectively.

In the current study, the large proportions of the *S. aureus* isolates were phenotypically resistant to Cefoxitin (100.0%), Penicillin G (97.4%), Ampicillin (90.1%), and Nalidixic acid (74.8%). Thus, all isolates were classified as MRSA and the overall prevalence of MRSA in the present study was 39.3%. The 100% resistance of *S. aureus* to Cefoxitin was consistent with the previous findings of Ayele et al. (2017) in Sebeta town, Aliyu et al. (2019) in Nasarawa State (Nigeria) and Yakubu et al. (2020) in Nasarawa State (Nigeria) who reported similar 100.0% resistance to Cefoxitin. However, Mekonnen et al. (2018) reported 100% sensitive *S. aureus* isolates to Cefoxitin in North-Western Ethiopia and Sudhantiramani et al. (2015) reported low magnitude of Cefoxitin resistance (4.65%) in Tirupathi, India.

The high resistance pattern of the isolates to Penicillin G (97.4%) was in agreement with the previous studies reported by Ayele et al. (2017) (98.85%) in Sebeta town, Pati and Mukherjee (2016) (96.0%) in Northern Plains of India, Daka et al. (2012) (100.0%) in Hawassa area, Girmay et al. (2020) (100.0%) in Shire town, Derib et al. (2017) (100%) in Wolaita Sodo town, Ayana et al. (2017) (100%) in Bishoftu town, and Mathenge et al. (2017) (99.6%) in Nairobi, Kenya. The higher resistance to Ampicillin (90.1%) was consistent with the reports of Pati and Mukherjee (2016) (93.0%), Mathenge et al. (2017) (93.1%), and Lemma et al. (2021) (94.2%) in Northern Plains of India, Nairobi (Kenya), and Addis Ababa, respectively. However, 100% Ampicillin susceptible isolates were reported by Adugna et al. (2018) in Addis Ababa. According to Girmay et al. (2020), the production of β -lactamase enzyme by *S. aureus* could be responsible for its resistance to penicillin and other related antibiotics. The proportion resistance to Nalidixic acid obtained in the present study was consistent with the report of Anueyiagu and Isiyaku (2015) who reported 71.4% resistance to Nalidixic acid in Nigeria.

The antimicrobial sensitivity testing result indicated that 52.3% of the isolates showed resistance to Tetracycline which was consistent with Mekonnen et al. (2018) who reported 54.0% resistance to Tetracycline in North-Western Ethiopia. However, 100.0% susceptibility to Tetracycline was reported by Sori et al. (2011) in Jimma town. In the current study, 38.4% of the isolates were also resistant to Amoxicillin/Clavulanic acid which was consistent with the report of Pati and Mukherjee (2016) (37.0%) in Northern Plains of India. Furthermore, high proportions of *S. aureus* isolates (90.7%) showed intermediate susceptibility to Vancomycin. However, Massawe et al. (2019) and Pati and Mukherjee (2016) reported *S. aureus* isolates which were 100.0% susceptible to Vancomycin in Mbeya Region (Tanzania) and Northern Plains of India, respectively. Most of the *S. aureus* isolates (93.4%) were susceptible to Norfloxacin which was in accordance with the finding of Sori et al. (2011) who reported 100.0% sensitivity to Norfloxacin in Jimma town.

In human and veterinary medicine, the extensive, indiscriminate, and injudicious use of antibiotics may lead to

development of drug resistance and the magnitude of drug resistance among pathogens is increasing (Mokgophi et al., 2021; Qamar et al., 2020). Thus, the high resistance pattern of *S. aureus* to readily available and relatively inexpensive antibiotics might be associated with these factors.

5 Conclusion and recommendations

The present study revealed that the prevalence of *S. aureus* from foods of bovine origin was very high. The treatment history of milking cows was the risk factor for the high prevalence of milk contamination with *S. aureus*. The sources of secondary contamination including municipal abattoirs with slaughtering operation on the floor, poor hygiene of the slaughtering process and butchers with poor hygiene were the determinants of high magnitude of detection of *S. aureus* in carcass samples. Nearly all of the *S. aureus* isolates showed multidrug resistance to readily and commonly used antimicrobial agents. The gold standard techniques for identifying MRSA, specifically detection of *mecA* gene and identifying PBP2a through latex agglutination were not done in this study due to limitations in budget and their unavailability in nearby laboratories. Hence, improvement of cattle health and good hygienic procedures in farms, abattoirs, milk product and butcher shops, and restaurants should be implemented to reduce primary and secondary bacterial contamination of foods of bovine origin. Moreover, rational use of antibiotics should be practiced in animal and public health and regular assessment of antibiotic resistance among pathogenic bacteria in food animals and their products should be done. Further studies on molecular characterization of *S. aureus* and their resistant genes should be carried out in the study areas.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by Research and Publication Director, Wollo University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

GG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. NA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YT: Conceptualization, Funding acquisition, Writing – review & editing. SA: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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