

Antimicrobial Resistance of Staphylococci at Animal Human Interface in Smallholders and Dairy Farms in Central Oromia, Ethiopia

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Purpose: *Staphylococcus* species come from a variety of sources and can contaminate milk during milking, cause mastitis and other diseases in animals and humans. The enterotoxins they produce cause food poisoning. Our objectives were to isolate, biochemically characterize, and determine antimicrobial susceptibility profiles of *Staphylococcus* species from dairy farms in central Oromia, Ethiopia.

Methods: A total of 339 samples (n = 135 [raw milk], n = 135 [udders' swabs], n = 25 [milkers' hands swabs], n = 44 [pooled milking utensils' swabs]) were collected from smallholders and dairy farms. Bacteriological culture and biochemical tests were performed to isolate and identify *Staphylococcus* species, and the Kirby Bauer disk diffusion method was used for antimicrobial susceptibility testing.

Results: Across all sample types and dairy farms, 247 (72.9%) *Staphylococcus* isolates were obtained which comprised of 101 (74.8%) isolates from raw milk, 98 (72.6%) from udder swabs, 30 (68.2%) from pooled utensil swabs, and 18 (72%) from milkers' hand swabs. Fifty coagulase-positive *Staphylococcus* isolates (20 *S. aureus*, 20 *S. hyicus* and 10 *S. intermedius*) subjected to antimicrobial susceptibility tests have shown various degrees of resistance. All *S. aureus* isolates were 100% resistant to ampicillin and penicillin. Out of 20 *S. hyicus* isolates, 90% were resistant to ampicillin and 85% to penicillin. *S. intermedius* isolates (n=10) were 70% resistant to nalidixic acid and penicillin whilst remaining 100% resistant to ampicillin. Five *S. aureus*, three *S. intermedius* and two *S. hyicus* isolates from raw milk, milk utensil swabs and milkers' hand swabs were multidrug-resistant (resistance to at least three classes of antimicrobials).

Conclusion: This study revealed a high prevalence of staphylococci in the dairy cattle, milkers and milking utensils with multidrug-resistant coagulase-positive *Staphylococcus* species suggesting the significance of pasteurization. Further research is encouraged on the factors leading to antibiotic resistance in *Staphylococcus* species.

Keywords: antimicrobial susceptibility profile, cattle, dairy farms, smallholders, *Staphylococcus* species

Introduction

Staphylococcus species are Gram-positive bacteria that are commonly found on the skins and mucous membranes of humans and animals.¹ Staphylococci are grouped into coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS) based on their ability to coagulate blood plasma (coagulase reaction).² Coagulase-positive staphylococci such as *S. aureus*, *S. intermedius*, and most *S. hyicus* cause disease in their host throughout the world³ and intoxication that results from the consumption of foods containing sufficient amounts of one (or more) preformed enterotoxin results in staphylococcal food

poisoning (SFP).⁴ Staphylococcal enterotoxins are produced by enterotoxigenic strains of staphylococci, mainly CPS, with *S. aureus* being the most prominent, and only occasionally CNS.⁵ These enterotoxins can cause mild to life-threatening diseases.^{5,6}

Staphylococcal foodborne disease (SFD) is one of the most common foodborne diseases worldwide. Humans and animals have been known as the primary reservoirs of staphylococci.⁷ The pathogenic staphylococci are usually found on the mucous membranes of the upper respiratory tract, lower urogenital tract, digestive tract, and moist areas of the skin.⁸ Coagulase-negative staphylococci have emerged as important pathogens in human.⁹ One of the *S. aureus* routes of spread to humans is through ingestion of contaminated milk.¹⁰ *S. aureus* contaminates milk when there is mammary gland infection and bad hygienic habits such as coughing or sneezing and lack of hands washing when handling milk storage utensils during or after milking.¹¹ In Ethiopia, there is no standard hygienic practice followed by producers during milk production.¹²

S. aureus is inherently resistant to nearly every antibiotic ever discovered. Antimicrobial resistance is frequently developed by horizontal gene transfer from outside sources, although chromosomal mutation and antibiotic selection are also crucial. *S. aureus* is unique in its capacity to develop drug resistance.¹³ Isolates of *S. aureus* identified from bovine mastitis milk in different parts of Ethiopia indicated that there was a high level of resistance to ampicillin, penicillin, polymyxin B, and streptomycin.^{14–19} Considering the large portion of the Ethiopian population that lives close to their livestock, there is potential for transmission of drug resistant *S. aureus* from livestock to humans through the consumption of raw milk and milk products.²⁰

Studies were conducted in some parts of Ethiopia on the isolation of *Staphylococcus* species from bovine mastitis milk^{16,19,21}; however, little information is available on the identification of *Staphylococcus* species from raw milk in smallholders and dairy farms in the country, particularly in the study area. Raw milk consumption is common in Ethiopia, and milk is usually handled under poor sanitary manner.²² Therefore, our objectives were to estimate the prevalence of *Staphylococcus* species infection, assess risk factors, and determine antimicrobial susceptibility profiles of *Staphylococcus* species isolated from raw milk, udder swabs, milkers' hands, and swabs of utensils.

Materials and Methods

Description of the Study Area and Study Design

This study was conducted in Ambo and Guder towns of West Shewa Zone, Oromia Region, Central Ethiopia. Ambo is located at 8°56'30" to 8°59'30" N latitude and 37°47'30" to 37° 55'15" E longitude at 114 km West of Addis Ababa. The altitude of the area ranges from 2000 to 2400 meters above sea level with annual temperature ranging from 15°C to 29°C (annual mean 22°C), annual mean rainfall ranges from 800 mm to 1000 mm (annual mean 900 mm), and the mean monthly relative humidity varies from 64.6% in August to 35.8% in December. Livestock is a major agricultural resource in this area. Ambo town has three Kebeles. The total number of cattle in and around Ambo and Guder towns is 144,243²³ and 174,799,²⁴ respectively. The total human population of Ambo and Guder towns is estimated to be 70, 900, and 21, 700, respectively. Guder is located 12 km West of Ambo, and it has an elevation of 2101 meters above sea level.²⁵

The study population includes all lactating dairy cows found in smallholders and dairy farms of selected three Kebeles of Ambo town and two Kebeles of Guder town, which are kept under an intensive, semi-intensive, and extensive management system. All dairy farms found in both Ambo and Guder towns were included, but lactating cows within farms were randomly selected. Smallholder had fewer than 10 animals per herd, but dairy farms had greater than or equal to 10 animals per herd. Smallholder farms were selected using purposive sampling. One hundred thirty-five lactating cows from 5 smallholders and 18 dairy farms were sampled. The sampling also included swabbing the milkers' hands of all personnel working in those farms or smallholders. A total of 339 samples were obtained from smallholders and dairy farms (135 raw milk, 135 udder swabs, 25 milkers' swabs, and 44 pooled milking utensil swabs).

Sample Collection, Holding, and Transport

A sterile wooden swab was moistened with buffered peptone water. The surface of milking equipment, milkers' hands, and udder skin were swabbed with the wet swab and placed separately in the labeled test tube that had buffered peptone water medium. Then, the udder and teats were washed with water and detergent. Using a clean towel, the surface of the udder was dried. Again, the teats were wiped vigorously with 70% alcohol. Two distant teats from the milker were wiped

first and then the two nearest teats. The first three squirts of milk were discarded to clean the teat canal. The first nearest teats were milked followed by the two furthest teats. Sterilized screw-capped test tubes were prepared and labeled to gather milk samples from each cow. A total of 10 mL of pooled milk sample (approximately equal in volume for each quarter) was collected from each cow. Samples were transported to Ambo University Veterinary Microbiology Laboratory under the cold chain.

Isolation and Identification of *Staphylococcus* Species

Swab samples in a buffered peptone water were incubated at 37 °C for 24 hrs. A loopful (10 µm) of cultured suspension was inoculated onto blood agar. Similarly, a loopful (10 µm) of milk was directly inoculated onto blood agar containing 7% sterile sheep blood and incubated for 24–48 hrs at 37°C. A colony typical of *Staphylococcus* was identified, and cell morphology, color, and shape characterization were done using Gram's staining. For each sample, one of presumptive colonies of *Staphylococcus* based on the Gram's staining was sub-cultured on nutrient agar plates for species-level identification using biochemical tests.

Catalase Test

Pure colonies from suspected isolates were picked up from the nutrient agar plate and mixed with a drop of 3% hydrogen peroxide on a clean glass slide. When the suspension formed a bubble, it was considered as catalase-positive bacteria.

Oxidase Test

Pure colonies were picked and smeared onto a Kovac's reagent dampened filter paper. The color of the paper was observed and waited until 30 seconds. The development of a deep blue to purple color in 10 to 30 seconds was considered a positive reaction.²⁶

Anaerobic Utilization of Glucose

Oxidation fermentation basal medium was prepared, and 0.5% glucose was added into the medium. Then, the media was distributed into individual test tubes. Suspected *Staphylococcus* colonies were inoculated into the medium using straight wire. Finally, the medium inside the tube was covered using sterile paraffin which was at least 25 mm thick. The inoculated tubes were incubated for 5–14 days at 37 °C. When acid was produced anaerobically, bromothymol blue changes to yellow throughout the tube indicating the presence of *Staphylococcus* species, which has been known to be fermentative. The development of acid in the medium indicated glucose fermentation.²⁷

The Growth of *Staphylococcus* on Mannitol Salt Agar Plate (MSA)

The colonies that were identified by Gram staining, oxidase test, and catalase test were sub-cultured on mannitol salt agar plates and incubated at 37 °C examined after 24–48 hrs for growth and change in the color of the medium. The presence of growth and pH change in the media (red to yellow color) was regarded as confirmative identification of the salt-tolerant staphylococci. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium.²⁸ Colonies that develop weak or delayed yellow color after 24 h of incubation were considered as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS.

Tube Coagulase Test

Colonies that were already grown on the MSA plate were transferred to Brain Heart Infusion medium (BHI) and incubated at 37 °C for 24 hr. The 0.5 mL of rabbit plasma and a drop of fresh colonies taken from BHI were mixed and incubated for 4–24 h at 37 °C. The clotting of the suspension was evaluated at 30 minutes intervals for the first 4 h of the test and then after 24 hrs of incubation. The reaction was considered positive if any degree of clotting was visible within the tube when tilted.²⁹

Fermentation of 1% Maltose Using Purple Agar Base

Purple agar base (PAB) with the addition of 1% maltose was used to differentiate the pathogenic staphylococci. The suspected colony was inoculated on PAB media with 1% maltose and incubated at 37 °C for 24–48 hrs. *S. aureus* rapidly

fermented maltose and acid metabolic products caused the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. intermedius* showed weak or delayed reaction and *S. hyicus* did not ferment maltose but attacked the peptone in the medium producing an alkaline reaction (a deeper purple) around the colonies.¹

Antimicrobial Susceptibility Test

Fifty isolates comprised 20 *S. aureus*, 20 *S. hyicus*, and 10 *S. intermedius* were randomly selected and subjected to 12 antimicrobial susceptibility disks. The lists of antimicrobial disks (Oxoid, Basing Stoke, UK) included erythromycin, penicillin, streptomycin, tetracycline, sulfamethoxazole-trimethoprim, vancomycin, ciprofloxacin, nitrofurantoin, amoxicillin, nalidixic acid, doxycycline, and ampicillin. The method applied for antimicrobial testing was the antibiotic disk diffusion method, using the Kirby Bauer technique. About 2–3 pure colonies of the isolates were taken from the nutrient agar and suspended in tryptone soy broth and then, incubated at 37 °C for 1–2 hrs. The suspension was then checked for the development of slight turbidity, against 0.5 MacFarland solutions. A sterile cotton swab was dipped into the suspension and the swab was then wiped on Muller Hinton agar, according to the standard procedure. Then, the antimicrobial disks were firmly placed on it and the plates were incubated at 37 °C for 24 hr. The zone of inhibition around each disk was measured using a caliper and the results were interpreted as sensitive, intermediate, and resistant using a standard zone of the interpretative chart.³⁰

Collection of Risk Factor Information and Data Analysis

During sampling on the farms, information about animal identifiers, the farm condition, and management was collected through interviews and personal observations. Information such as animal location, breed, type of farm, age, parity, lactation stage, presence of blind teat, teat lesion and udder tick infestation, floor type, previous udder treatment, usage of towels for cleaning and drying udder before milking and udder washing before milking were gathered.

Microsoft Excel spreadsheet program was used for raw data storage, and the analysis was carried out by STATA Version 11.³¹ Descriptive statistics were conducted to summarize the raw data. Univariate logistic regression analysis was used to check the presence of an association between potential risk factors and isolation of *Staphylococcus* species. The significance level was set at $\alpha = 0.05$, and P-value less than 0.05 was taken as statistically significant.

Results

Isolated *Staphylococcus* Species

Out of 339 samples collected from the smallholders and dairy farms, 247 (72.9%) *Staphylococcus* isolates were identified (Table 1). Most isolates were CNS species (41%) followed by *S. aureus* (13%) and *S. hyicus* (11.7%). *S. aureus*, *S. hyicus*, and CNS were identified in samples from all sources, while *S. intermedius* was not isolated from the milker's hand swabs (Table 1).

Univariate logistic regression analysis showed that none of the study variables in cows was found to be significantly associated with the isolation of *Staphylococcus* as indicated in Table 2.

Table 1 Prevalence of *Staphylococcus* Species Isolates from Smallholders and Dairy Cows in Central Ethiopia

Sample Type	Positive/Total Sample (%)	CNS n (%)	<i>S. aureus</i> n (%)	<i>S. intermedius</i> n (%)	<i>S. hyicus</i> n (%)
Raw milk	101/135 (74.8)	54 (40)	17 (12.6)	8 (5.9)	22 (16.2)
Udder swab	98/135 (72.6)	59 (43.7)	21 (15.5)	9 (6.6)	9 (6.6)
Utensil swab	30/44 (68.2)	16 (36.6)	4 (9)	4 (9)	6 (13.6)
Milker's hand swab	18/25 (72)	10 (40)	5 (20)	0	3 (12)
Total	247/339 (72.9)	139 (41)	47 (13)	21 (6.2)	40 (11.7)

Abbreviation: CNS, coagulase-negative *Staphylococcus* species.

Table 2 Univariate Analysis of the Risk Factors for Isolation of *Staphylococcus* Species from Raw Milk of Cows in the Study Area

Risk Factors		Total Sample	Positive N (%)	Regression Coefficient	P-value
Site	Ambo Kebele 01	29	21 (72.4)		
	Ambo Kebele 02	40	33 (82.5)	0.5855	0.319
	Ambo Kebele 03	29	20 (69.0)	-0.16657	0.773
	Guder	37	27 (73.0)	0.02817	0.960
Town	Ambo	98	74 (75.5)	-0.02537	0.764
	Guder	37	27 (73.0)		
Breed	Local	46	38 (82.6)	-0.6731	0.138
	Cross	89	63 (70.8)		
Farm type	Smallholders	11	9 (81.8)	-0.4480	0.579
	Dairy farms	124	92 (74.2)		
Age (Years)	<3	71	53 (74.6)	0.01869	0.962
	3–6	64	48 (75.0)		
Parity	1–2	86	63 (73.3)	0.2321	0.581
	≥3	49	38 (77.6)		
Lactation Stage (months)	<3	42	35 (83.3)	-0.4021	0.140
	3–6	61	44 (72.1)		
	>6	32	22 (68.8)		
Blind Teat	No	115	88 (76.5)	-0.5625	0.277
	Yes	20	13 (65.0)		
Teat Lesion	No	118	91 (77.1)	-0.8583	0.111
	Yes	17	10 (58.8)		
Tick Infestation	No	71	53 (74.6)	0.01869	0.962
	Yes	64	48 (75.0)		
Floor Type	Muddy	41	31 (75.6)	-0.06096	0.888
	Concrete	94	70 (74.5)		
Usage of Towels	No	83	63 (75.9)	-0.1489	0.713
	Yes	52	38 (73.1)		

Antimicrobial Susceptibility Profiles of *Staphylococcus* Species

From 50 isolates of *Staphylococcus* species subjected to antimicrobial susceptibility tests, most isolates (90% to 100%) were resistant to the beta-lactam antibiotics, namely ampicillin, penicillin, and amoxicillin (Table 3). From 10 *S. intermedius* isolates subjected to antimicrobial susceptibility testing, all isolates were resistant to ampicillin, 7 (70%) to nalidixic acid and penicillin, 5 (50%) to vancomycin. *S. hyicus* isolates exhibited 90% (18/20) resistance to ampicillin, 85% (17/20) to penicillin, 60% (12/20) to amoxicillin. All 50 isolates of *Staphylococcus* species subjected to antimicrobial susceptibility testing were 100% susceptible to ciprofloxacin. In addition, all 20 tested isolates of *S. aureus* were susceptible to ciprofloxacin, streptomycin, and sulphamethoxazole–trimethoprim. A total of the 20 tested isolates of *S. hyicus* were susceptible to doxycycline and ciprofloxacin. All tested *S. intermedius* isolates were susceptible to ciprofloxacin and sulphamethoxazole–trimethoprim, but all the 10 tested isolates were resistant to ampicillin (Table 3).

Antimicrobial Resistance Pattern of *S. aureus*, *S. intermedius*, and *S. hyicus*

Seven isolates of *S. aureus* were resistant to three beta-lactam antibiotics (ampicillin, penicillin, and amoxicillin) (Table 4). Likewise, eight isolates of *S. hyicus* were resistant to these beta-lactam antibiotics.

Multidrug Resistance

In this study, five *S. aureus* isolates (two from raw milk, two from utensil swabs and one from milker's hand swabs), three *S. intermedius* (two from utensil swab and one from raw milk), and two *S. hyicus* isolates from raw milk were found to be resistant to three antimicrobial classes (Table 5).

Table 3 Percentage of Antimicrobial Susceptibility Profiles of *S. aureus* (n=20), *S. intermedius* (n=10), and *S. hyicus* (n=20)

Antimicrobials Class	Antimicrobial Disks (Concentration)	Species	% (No. of Susceptible)	% (No. of Intermediate)	% (No. of Resistance)
Aminoglycosides	Streptomycin (10 µg)	<i>S. aureus</i>	100 (20)	0 (0)	0 (0)
		<i>S. intermedius</i>	70 (7)	30 (0)	0 (0)
		<i>S. hyicus</i>	85 (17)	0 (0)	15 (3)
Nitrofurantoin	Nitrofurantoin (300µg)	<i>S. aureus</i>	65 (13)	35 (7)	0 (0)
		<i>S. intermedius</i>	70 (7)	30 (3)	0 (0)
		<i>S. hyicus</i>	80 (16)	10 (2)	10 (2)
Macrolide	Erythromycin (15µg)	<i>S. aureus</i>	80 (16)	20 (4)	0 (0)
		<i>S. intermedius</i>	70 (7)	30 (3)	0 (0)
		<i>S. hyicus</i>	75 (15)	25 (5)	0 (0)
Tetracycline	Tetracycline (30µg)	<i>S. aureus</i>	60 (12)	0 (0)	40 (8)
		<i>S. intermedius</i>	100 (10)	0 (0)	0 (0)
		<i>S. hyicus</i>	85 (17)	0 (0)	15 (3)
	Doxycycline (30 µg)	<i>S. aureus</i>	65 (13)	15 (3)	20 (4)
		<i>S. intermedius</i>	80 (8)	20 (2)	0 (0)
		<i>S. hyicus</i>	100 (20)	0 (0)	0 (0)
Fluoroquinolones	Ciprofloxacin (5 µg)	<i>S. aureus</i>	100 (20)	0 (0)	0 (0)
		<i>S. intermedius</i>	100 (10)	0 (0)	0 (0)
		<i>S. hyicus</i>	100 (20)	0 (0)	0 (0)
Sulfonamide	Sulfamethoxazole–trimethoprim (23.75µg)	<i>S. aureus</i>	100 (20)	0 (0)	0 (0)
		<i>S. intermedius</i>	100 (10)	0 (0)	0 (0)
		<i>S. hyicus</i>	90 (18)	10 (2)	0 (0)
Quinolones	Nalidixic acid (30µg)	<i>S. aureus</i>	35 (7)	20 (4)	45 (9)
		<i>S. intermedius</i>	30 (3)	0 (0)	70 (7)
		<i>S. hyicus</i>	30 (6)	65 (13)	5 (1)
Glycopeptide	Vancomycin (30 µg)	<i>S. aureus</i>	100 (20)	0 (0)	0 (0)
		<i>S. intermedius</i>	10 (1)	40 (4)	50 (5)
		<i>S. hyicus</i>	85 (17)	15 (3)	0 (0)
β-lactams	Ampicillin (10µg)	<i>S. aureus</i>	0 (0)	0 (0)	100 (20)
		<i>S. intermedius</i>	0 (0)	0 (0)	100 (10)
		<i>S. hyicus</i>	10 (2)	0 (0)	90 (18)
	Amoxicillin (25µg)	<i>S. aureus</i>	0 (0)	10 (2)	90 (18)
		<i>S. intermedius</i>	90 (9)	0 (0)	10 (1)
		<i>S. hyicus</i>	25 (5)	15 (3)	60 (12)
	Penicillin G (10 unit)	<i>S. aureus</i>	0 (0)	0 (0)	100 (20)
		<i>S. intermedius</i>	30 (3)	0 (0)	70 (7)
		<i>S. hyicus</i>	15 (3)	0 (0)	85 (17)

Abbreviation: n, the number of isolates.

Discussion

Cow milk may be contaminated with *Staphylococcus* and other bacterial species at any stage of the process. At the point of production, milk may be positive if the cow is mastitic and is shedding pathogens, or can be contaminated from unclean udders or teats, from the dust, from the unclean milking machines or milkers' hands, from dirty milking containers. Milk can also be contaminated at post-harvest stages. In this study, the contamination of milk with *Staphylococcus* species was investigated at the smallholders and dairy farms from various sources such as the skin of udder, hands of a milking personnel, and surface of milk containers. A total of 247 (72.9%) isolates of staphylococci were found from 339 total samples, which consists of raw milk samples, swabs of milking utensils, udder swabs, and swabs of milkers' hands. The overall prevalence of *Staphylococcus* species identified in this study was slightly higher than the report of Gizaw,³² who reported 55.1% of *Staphylococcus* species isolates from milk in Bishoftu, Ethiopia. The

Table 4 Antimicrobial Resistance Patterns of *S. aureus* (n=20), *S. intermedius* (n=10), and *S. hyicus* (n=20)

Antimicrobial Resistance Pattern of <i>S. aureus</i>	No. of Resistant Isolates	Percentage
NAL-AMP-PEN	1	5
AMP-PEN-AMX	7	35
NAL-AMP-PEN-AMX	3	15
DOX-AMP-PEN-AMX	1	5
TTC-AMP-PEN-AMX	3	15
TTC-NAL-AMP-PEN-AMX	2	10
TTC-DOX-NAL-AMP-PEN-AMX	2	10
Antimicrobial Resistance Pattern of <i>S. intermedius</i>		
NAL-AMP	1	10
VAN-NAL-AMP	1	10
VAN-AMP-PCN	2	20
NAL-AMP-PCN	2	20
NAL-AMP-AMX-PCN	1	10
VAN-NAL-AMP-PCN	2	20
Antimicrobial Resistance Pattern of <i>S. hyicus</i>		
NIT-STM	1	5
AMP-PCN	4	20
AMP-AMX-PCN	8	40
NIT-AMP-AMX-PCN	2	10
TTC-AMP-AMX-PCN	1	5
TTC-STM-AMP-PCN	1	5
TTC-STM-AMP-AMX-PCN	1	5

Abbreviations: AMP, ampicillin; AMX, amoxicillin; DOX, doxycycline; NAL, nalidixic acid; NIT, nitrofurantoin; STM, streptomycin; PCN, penicillin G; TET, tetracycline; VAN, vancomycin.

Table 5 Multidrug Resistance of *Staphylococcus* Species Isolates

Types of Samples	Multidrug-Resistant Isolates	No. of Isolates	Class of Antimicrobial
Raw milk	<i>S. aureus</i>	2	Tetracycline, quinolones, and β -lactams
Utensil swab	<i>S. aureus</i>	2	Tetracycline, quinolones, and β -lactams
Milker's hand swab	<i>S. aureus</i>	1	Tetracycline, quinolones, and β -lactams
Raw milk	<i>S. intermedius</i>	1	Glycopeptide, quinolones, and β -lactams
Utensil swab	<i>S. intermedius</i>	2	Glycopeptide, quinolones, and β -lactams
Raw milk	<i>S. hyicus</i>	2	Tetracycline, aminoglycosides, and β -lactams

variation in the prevalence of *Staphylococcus* species reported may be due to the usage of cleaning agents and the sample size.

The prevalence of *S. aureus* in raw milk in the current study (13.9%) was comparable with the reports of Asrat et al³³ who reported 15.5% *S. aureus* from milk samples of dairy cows and nasal swabs of farmworkers in selected dairy farms around Addis Ababa, Ethiopia. Likewise, Gizaw³² reported 17.2% of *S. aureus* from cattle, food chains, and humans in Bishoftu, Ethiopia. However, our finding was lower than that of Bendahou et al,³⁴ who reported 40% *S. aureus* isolates from milk and milk products in North Morocco. The low prevalence of *S. aureus* in the current study compared to the study in the north of Morocco could be attributed to the collection of the milk samples directly from the cows' udders before contacting milking utensils in this study, which might have reduced the prevalence of *S. aureus*.

The prevalence of *S. intermedius* (6.2%) and *S. hyicus* (11.7%) recorded in this study agrees with the study conducted by Gizaw,³² who reported 7.4% of *S. intermedius* and 8.2% of *S. hyicus* from milk and samples of human origin (udder milk, tank milk, bucket swab, tank swab farm hand swab, nasal swab) and is similar to the study by Addis et al,³⁵ who reported the prevalence of 4% of *S. hyicus* from raw bovine milk in Bishoftu, Ethiopia. Our findings were also similar to the study of Bendahou et al,³⁴ who reported 2% *S. intermedius* and 4% *S. hyicus* in dairy cows in North Morocco. Similarly, the prevalence of CNS (41%) recovered from milk, swab samples from the udder, milking utensils, and milkers' hands in the current study was closely comparable with the findings of Bendahou et al,³⁴ who reported a 54% CNS from milk and milk products in North Morocco.

Out of 44 swab samples collected from milking utensils in farms in the study area, 4 (9%) were found to be *S. aureus*. Parmar et al³⁶ reported a high prevalence of 18.8% of *S. aureus* from swabs of milking equipment of a dairy farm in Anand, Gujarat, India. The dissimilarity in the prevalence may be due to the difference in the hygienic status of the equipment sampled. The prevalence of *S. aureus* in swab samples collected from milkers' hands was 20% (5/25). This finding was higher when compared with a study conducted by Parmar et al,³⁶ who reported a 2.55% prevalence of *S. aureus* from swabs of milkers' hands. The variation might be due to the small number of samples in this study.

The results of the questionnaire survey to find out potential risk factors associated with the prevalence of *Staphylococcal* species indicated that none of the variables studied were significantly associated with the prevalence of *Staphylococcal* species.

The prevalence and degree of antimicrobial resistance in veterinary medicine are increasing worldwide. The dissemination of antimicrobial-resistant staphylococci are presenting a challenge to both human and animal health professionals.³⁷ The antimicrobial susceptibility tests carried out in the current study indicated that *S. aureus* was 100% resistant to ampicillin and penicillin, and 90% resistant to amoxicillin. This finding was in agreement with a study conducted by Addis et al,³⁵ who reported 100% resistance of *S. aureus* to both penicillin G and amoxicillin. This finding is also comparable with the result of Sori et al,³⁸ who reported 98% resistance of *S. aureus* isolates to penicillin G and 67% to ampicillin. A study conducted in Bangladesh by Begum et al³⁹ showed a slightly lower antimicrobial resistance profile, where *S. aureus* isolates were found to be 82.86% resistant to penicillin G and 37.14% to amoxicillin.

S. aureus isolates found in this study have also shown 45% resistance to nalidixic acid, 40% to tetracycline, and 20% to doxycycline. This is similar to the findings of Sori et al,³⁸ where *S. aureus* was reported to have 46% resistance to amoxicillin–clavulanic acid, 87.2% to penicillin, 92% to nalidixic acid, and 46% to amoxicillin. However, these isolates were found to be susceptible to tetracycline. The probable explanation for this could be that *S. aureus* strains can change their resistance behavior to the exposed antimicrobials.¹³

Al-Thani and Al-Ali³⁷ reported that 78% of *S. aureus* isolates showed resistance to tetracycline, which is comparable with the report in this study. This is not surprising because penicillin G and tetracycline are the most used antimicrobials for the treatment of infection or mastitis in veterinary practice in Ethiopia.⁴⁰ Moreover, penicillin resistance is plasmid based and, it spread out very quickly to several other strains.^{33,41} Similarly, Daka et al⁴² reported a 67.9% resistance pattern of strains of *S. aureus* isolates from milk to penicillin G, 70.9% to ampicillin, 30.9% to amoxicillin–clavulanic acid, and 0% to ciprofloxacin. Daka et al⁴² also reported that *S. aureus* isolates were resistant to erythromycin (32.1%), trimethoprim–sulfamethoxazole (7.7%) and vancomycin (38.5%). On the contrary, this study indicated that *S. aureus* isolates were susceptible to erythromycin (80%), sulfamethoxazole–trimethoprim (100%) and vancomycin (100%).

Among the *S. intermedius* isolates identified in this study, 100% were resistant to ampicillin, 70% to nalidixic acid and penicillin, and 100% susceptible to ciprofloxacin, tetracycline, and sulfamethoxazole–trimethoprim. These findings are correlated with the report of Intorre et al⁴³ where 100% *S. intermedius* isolates were susceptible to ciprofloxacin.

In this study, multidrug-resistant *S. aureus*, *S. intermedius* and *S. hyicus*, which were resistant to three classes of antimicrobials were found. Five *S. aureus* isolates were recovered from raw milk, milk utensil swabs and milkers' hand swabs suggesting that this pathogen is circulating at human animal interface. This signifies a public health risk as *S. aureus* is known to be one of the most common foodborne pathogens worldwide.⁴⁴ In addition, the emergence of multidrug-resistant *S. intermedius* and *S. hyicus* complicates treatment and poses a serious threat to infection control and public health.

Conclusion

The present study showed a high prevalence of *Staphylococcus* species in dairy cows in Guder and Ambo towns. The result of swab samples from the udder of the cow, milkers' hands, and storage containers also showed prevalence of *Staphylococcus* species. This shows the possibility of milk contamination with *Staphylococcus* species from milkers' hands, milking utensils, and udder of the cow after milking. The presence of pathogenic *S. aureus* poses a public health hazard and concerns about the safety of milk and milk products. The results obtained in this study also showed that the *Staphylococcus* species isolated from raw milk, swab samples from milking utensils, and milker's hands were resistant to common antibiotics that could be a threat to the control of mastitis and public health. Therefore, raw milk should be pasteurized and hygienically handled. Further research on the investigation of factors that help the increase of antimicrobial-resistant *Staphylococcus* species and characterization of CNS at species level are suggested.

Abbreviations

BHI, brain heart infusion; CNS, coagulase-negative *Staphylococcus*; CPS, coagulase-positive *Staphylococcus*; MSA, mannitol salt agar; PAB, purple agar base; SFD, staphylococcal foodborne disease.

Data Sharing Statement

The corresponding author and first author will provide datasets that support the results of this article upon reasonable request.

Ethics Approval and Consent to Participate

We confirm that the animals were handled with the best practice of veterinary care. We also confirm that verbal informed consent was acceptable and approved by the College of Veterinary Medicine and Agriculture, Addis Ababa University. The study's aims and methods were explained to all participating farm owners. The information utilized in this study was acquired from the participants and coded to maintain confidentiality after getting a verbal agreement. Ethical clearance for the study was obtained from the College of Veterinary Medicine and Agriculture, Addis Ababa University (AAU, CVMA), minutes of the Animal Research Ethics and Review Committee (Min NO: VM/ERC/04/10/14, Review Date: 23/10/2014).

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Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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