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Research Article

Hygienic Practice, Isolation and Antibiogram Profiles of *S. aureus* from Goat Meat at Butcher House in Chelenko and Kulubi Towns, Eastern Ethiopia

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Abstract

Staphylococcus aureus contamination originating from meat represents a significant global public health concern, particularly in developing nations such as Ethiopia. A cross-sectional investigation was carried out between September 2022 and March 2023 to evaluate goat meat handling practices and determine the prevalence and antimicrobial susceptibility profile of *S. aureus* in the towns of Chelenko and Kulubi. A total of 206 swab specimens were collected from goat meat and environmental sources and tested for the presence of Staphylococcus aureus. All specimens underwent serial dilution to quantify the bacterial load in goat meat obtained from butcher shops. Additionally, 42 butcher shop workers with diverse demographic backgrounds were interviewed regarding their meat handling practices. Of the 206 total specimens (120 from goat meat and 86 from contact surfaces within butcher establishments), an overall prevalence of 24.8% of the organism was isolated. When categorized by specimen type, the highest prevalence was observed on cutting board swabs (35%), followed by worker hands (30.8%), while the lowest prevalence was recorded from meat samples (20.8%). Similarly, by sampling location, a higher prevalence was detected in Chelenko (27.2%) compared to Kulubi town (22.3%) for meat swab specimens. No statistically significant difference ($p > 0.05$) was observed in *S. aureus* prevalence between towns or among sample types. The mean bacterial counts were 5.56 ± 0.276 log_{10} CFU/cm^2 for Chelenko and 5.42 ± 0.309 log_{10} CFU/cm^2 for Kulubi. No significant difference was found in *S. aureus* load across sample sources in either town ($p > 0.05$). *S. aureus* isolates from goat meat demonstrated high resistance rates to amoxicillin (66.7%), penicillin-G (62.8%), and ampicillin (52.7%), whereas high susceptibility was observed to vancomycin (94%), gentamicin (88.2%), and kanamycin (86.3%). A questionnaire survey was also conducted to assess hygienic handling practices and potential risk factors associated with goat meat contamination in the study area. Poor meat handling practices and low community awareness regarding meat hygiene were observed in both towns, a finding consistent with the non-significant distribution of *S. aureus* across sampling locations and sites. Consequently, improving community awareness through education on hygienic meat handling is strongly recommended.

Keywords

Antimicrobial, Butcher House, Goat Meat, Hygiene, *S. aureus*

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1. Introduction

Food-borne diseases occur due to the consumption of contaminated food products, particularly foods of animal origin, and remain a major global public health problem. According to the World Health Organization [1], approximately 600 million food-borne illnesses and 420,000 deaths occur annually worldwide due to various pathogens and toxins present in food products. Although food-borne diseases affect both developed and developing countries, the burden is more severe in developing nations, including Ethiopia, because of poor food handling and sanitation practices, inadequate food safety regulations, weak regulatory systems, limited financial resources, and low public awareness regarding proper food hygiene. These conditions create a favorable environment for the spread of food-borne pathogens and food poisoning agents [2].

Meat is an important source of protein, vitamins, and essential nutrients for humans. However, due to its chemical composition and biological characteristics, meat is highly perishable and provides a favorable environment for the growth of various microorganisms that may cause infections in humans [3]. The occurrence of pathogenic and spoilage bacteria in goat meat and its by-products continues to be a significant public health concern. The level of bacterial contamination in goat carcasses depends on several factors, including the microbial load of the carcass, hygienic practices during handling and processing, storage temperature, and duration of storage [4]. Among food-borne pathogens, *Staphylococcus aureus* is recognized as one of the most important bacteria responsible for food contamination, food spoilage, reduced shelf life, and food poisoning through the production of heat-stable enterotoxins [5]. The enterotoxins produced by this bacterium are associated with food contamination resulting from poor personal hygiene of food handlers, inadequate packaging, improper sterilization, and contamination of surfaces, utensils, and equipment used during food handling and processing [6].

Staphylococcus aureus is one of the major causes of food poisoning and bacterial infections in both humans and animals. The organism is commonly found on the skin and mucous membranes of humans and animals and is recognized as an important invasive pathogen responsible for a wide range of clinical conditions, including skin and soft tissue infections, intravenous catheter-associated infections, food poisoning, toxic shock syndrome, osteomyelitis, pneumonia, and bloodstream infections [7]. It has frequently been isolated from animals and foods of animal origin [8]. Staphylococcal food poisoning occurs following the consumption of meat and meat products contaminated with staphylococcal enterotoxins [9]. *Staphylococcus aureus* can be transmitted from animals to humans through direct contact or consumption of contaminated meat products, while humans may also serve as reservoirs for transmission to animals. Therefore, the pathogen represents an important zoonotic agent capable of transmission between humans and animals [10].

Antimicrobial resistance among zoonotic bacteria has become a major global public health concern and is currently the focus of extensive research throughout the food production chain because of its impact on both veterinary and human medicine [11]. The emergence of multidrug-resistant strains of *S. aureus* has become an important global zoonotic issue. Resistant strains often fail to respond to commonly used antimicrobial agents, resulting in prolonged illness, increased treatment costs, higher mortality risks, and substantial economic losses to individuals and society [12]. Foods of animal origin, particularly meat, are among the major sources through which antimicrobial-resistant bacteria and drug residues can be transmitted to humans. Humans may acquire resistant infections through the consumption of contaminated meat products [13]. The spread of antimicrobial-resistant staphylococci is mainly associated with the indiscriminate use of antimicrobial agents by healthcare providers, untrained practitioners, and drug users in both veterinary and human medicine [6].

In Ethiopia, the prevalence of *S. aureus*, including multidrug-resistant strains in foods of animal origin, has increased because of poor hygienic meat handling practices, inadequate sanitation, and indiscriminate use of antimicrobial agents for growth promotion and treatment of diseased animals, which contribute to the development of resistant bacterial strains [14-17]. These problems have significant public health and socioeconomic impacts because contaminated meat products can serve as important sources of food-borne infections. Previous studies have indicated that poor hand-washing practices before meat handling and after restroom use are major indicators of poor hygiene behavior among meat handlers [18]. Furthermore, factors such as age, sex, educational status, and work experience of food handlers are strongly associated with hygienic meat handling practices. Studies conducted in Bisoftu, Ethiopia, reported that educational level, sex, and work experience significantly influenced the implementation of good hygiene practices among food handlers [19].

Studies conducted in Ethiopia have also shown that only a few butcher shop workers use gloves during meat processing. Moreover, the widespread habit of consuming raw or undercooked meat increases the risk of food-borne infections, particularly under conditions of overcrowding, poor sanitation, poverty, and inadequate hygiene practices [20]. Raw meat is commonly sold in open-air retail shops without proper temperature control, and minced meat is often consumed raw or partially cooked in restaurants and households [21]. In addition, knives, wooden cutting boards, and weighing scales used in retail shops have been reported as important sources of bacterial contamination, particularly with *Staphylococcus aureus* [22]. Therefore, proper meat hygiene practices and adequate infrastructural facilities are essential to ensure meat safety and protect public health.

However, there is limited information regarding the prevalence, microbial load, antimicrobial susceptibility profile, and hygienic meat handling practices associated with *Staphylococcus aureus* contamination of goat meat in Chelenko and Kulubi towns, Eastern Ethiopia. Therefore, the present study was conducted to assess hygienic meat handling practices and determine the prevalence, microbial load, and antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from raw goat meat and meat contact surfaces in butcher shops located in Chelenko and Kulubi towns, Eastern Etehiopia.

General Objective

To assess hygienic handling practices and determine the occurrence and antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from raw goat meat in Chelenko and Kulubi towns.

Specific Objectives

To assess hygienic meat handling practices of butcher shop workers and identify factors associated with meat contamination in Chelenko and Kulubi towns.

To determine the prevalence and antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from raw goat meat and meat contact surfaces in the study area.

To enumerate the microbial load of *Staphylococcus aureus* contaminating raw goat meat in butcher shops in the study are.

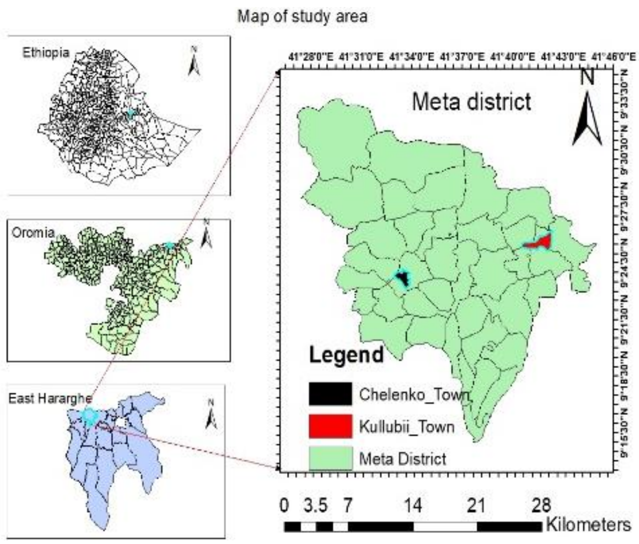
2. Materials and Methods

2.1. Description of the Study Area

This study took place in two towns Chelenko and Kulubi within the Meta district, situated in Eastern Hararghe, Oromiya, Ethiopia. The area lies 445 kilometers east of Addis Ababa, the nation's capital, and 80 kilometers west of Harar town. Geographically, its coordinates range from 9°7'55" to 9°28'45" north latitude and 41°31'40" to 41°52'30" east longitude. The district shares borders with Goro Muti to the south, Deder to the southwest, Goro Gutu to the northwest, Bedeno to the southeast, Kersa to the northeast, and the Somali Region to the north. Elevations in Meta district vary between 1,311 and 2,830 meters above sea level. Annual precipitation ranges from 600 to 900 mm, while temperatures fluctuate between 15°C and 37°C [23].

Two public slaughterhouses exist within Meta district, designed to cater to both Christian and Muslim communities. Each facility comprises two rooms. One room is designated for the actual slaughtering process, where all steps—from stunning to carcass splitting and all intermediate procedures—are performed directly on the floor. The other room serves as storage for slaughter equipment and animal skins, which are kept together temporarily until removal. Water for cleaning both the abattoir and carcasses is supplied via pipes from the municipal water system, though interruptions are frequent. The slaughterhouse lacks fencing, allowing scavengers such as carnivores and vultures unrestricted access to the premises.

Nevertheless, the slaughter of shoats (sheep and goats) has not yet commenced at the municipal abattoirs in either town. Instead, small ruminants are commonly slaughtered in warehouse or kitchen spaces, as well as behind restaurants, within the study area. Prior to slaughter, goats are transported to backyards around noon, where they are kept under stressful conditions at the butchers' homes. Without prior stunning, slaughter occurs entirely on the ground, followed by carcass splitting, cutting, and deboning—all conducted on the same contaminated backyard surfaces. Once slaughtering is complete, the carcass and edible offal are transported on foot (without a vehicle) to the butcher's shop by the slaughterer and other shop workers. Throughout the slaughter line, clean and unclean tasks are not physically separated, and individual workers perform both types of tasks, increasing the risk of contaminating the carcasses and edible by-products.



Source: Arc GIS version

Figure 1. Map of the study site.

2.2. Study Design

A cross-sectional study was conducted from September 2022 to March 2023 in Chelenko and Kulubi towns to assess hygienic handling practices and to determine the occurrence and antimicrobial susceptibility of *S. aureus* isolated from goat meat at butchers.

2.3. Sample Source

The sample source were the goat meat swab sample, meat cutting boards, knives, workers' hands, meat hanging hooks in butcher shop. The source for survey data was the goat meat workers in study area.

2.4. Sample Size and Sampling Technique

The sample size was determined using the formula described by Thrusfield [24] by assuming 5% precision, 95%

level of confidence interval and 16% expected prevalence of *S. aureus* in raw goat meat reported by [17] from Addis Ababa, Ethiopia. Accordingly;

$$n = \frac{\left(1.96^2 \times \ Pexp \ \times \ (1 \ - \ Pexp)\right)}{d^2}$$

Where; n= required sample size; P_{exp} = expected prevalence and a desired absolute precision (d) of 0.05. Accordingly, the number of sample needed to determine the prevalence of *S. aures* from goat meat is determined to be 206. From the total of 206 samples out of which, 120 meat swab samples from butcher house and 86 for environmental swab samples was purposively allocated (Table 1). Due to limited number of butcher shop in the district, the sample size for questionnaire survey was taken purposively i.e. all the butcher houses in the study were recruited.

Table 1. *Number of collected sample from different source.*

Sample source	Number of examined
Goat meat	120
Worker hands	26
Cutting boards	20
Knives	20
Hanging hooks	20
Total	206

2.5. Questionnaire Survey and Observation

Data was collected by administering butcher shop workers with structured questionnaires. Main items on the questionnaire paper included the general conditions of the butcher shops, butcher shops facilities and general hygienic conditions, processing practices, personnel, equipment, demographic information's of the participants and etc. Those personnel who were responsible in the processing of goat meat were interviewed and required samples were taken after the oral consent of the participants. Before start of the data collection, the questionnaires were translated to *Afaan Oromoo* and then interview was conducted using local language.

2.6. Sample Collection and Transportation

Prior to the sample collection, all butcher shops were visited to facilitate research collaboration. Subsequently, cooperation letter was written and sent to towns in the study site. In general, all samples were sampled aseptically for the purpose of *Staphylococcus aureus* isolation. The swab samples were collected twice peer week for three months at Chelenko and Kulubi Towns. For carcass swab sampling, four different sites of the carcass (viz., ribs, neck, flank and hind leg) was swabbed using the method described in ISO 6888-2 [25], one site covering 100 cm^2 by placing sterile template (10cm x 10cm) on carcass. For each sampling area, sterile cotton wool swabs rolled on wooden sticks were moistened in 10 ml buffered peptone water, and the swabs were rubbed over the completely selected area with pressure continuously for 30 seconds. On completion of the rubbing process, the wooden sticks were broken by pressing it against the inner wall of the test tube and disposed leaving the cotton wool swabs were transferred to screw-capped test tubes containing the buffered peptone water. Subsequently, the same pressure was repeated to sample each location with dry swabs. The swab samples were placed in to the same test tube filled with 10 ml buffered peptone water. The test tube was shaken vigorously for 2 minutes before transportation. The samples were properly labeled and immediately transported used icebox to Laboratory within 24 hours.

The environmental samples (meat contact surfaces) were collected from butcher house worker's hand worker's swab and equipment (knife, cutting board swab, and hook swab) samples (n=86) were collected. For knives, combined samples were collected from the blade and handle of the knives. In the butcher house, the samples were collected from knife swab, cutting board swab and hook swab. The swabs were then returned to a test tube containing 10 ml sterile Buffered Peptone Water (BPW). A second sterile cotton swab of the same type was used as before over the entire sampled area as above and this swab was placed into the same container. All samples were properly labeled and transport to Haramaya University Veterinary Microbiology Laboratory using an icebox on ice packs and analyzed upon arrival or within 24 hours of sampling.

2.7. Laboratory Analysis

2.7.1. Isolation and Identification of *S. aureus*

Isolation of *S. aureus* was performed using standard bacteriological methods, plate culture and biochemical tests [26, 27] Samples was processed and analyzed separately using the following procedure.

The swab samples which has been dipped into 10 ml buffered Peptone water during collection were directly incubated for 24 h at 37°C. A loopful of the culture suspension was streaked on blood agar plate (BAP) enriched with 7% heparinized sheep blood (HiMedia, Pvt., India) and incubated for an additional 24hr at 37°C aerobically. The next day, those zones of light to golden yellow pigment-colored presumptive *S. aureus* colonies were subculture onto mannitol salt agar from blood agar (HiMedia Pvt. India). Colonies with yellow zones on mannitol salt agar were later streaked on nutrient agar and pure colonies cultured on nutrient agar were taken for further identification for gram staining as well as appropriate biochemical tests [27].

2.7.2. Biochemical Confirmation of *S. aureus* Isolates

All suspected cultures of *S. aureus* were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, and shape and cell arrangements. The Grams stained smears from typical colonies that showed gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive *S. aureus*. After gram stain the pure culture of the isolates were picked up by bacteriological loop from the agar plate and mixed with a drop of 3% H_2O_2 on a clean slide for catalase test. When the organism is positive, bubbles of oxygen is liberated within a few seconds. Those catalase positive cocci were presumptively considered as *S. aureus*. The colonies that were identified by gram's staining reaction and catalase test were streaked on Mannitol salt agar plate and incubated at 37 ℃ and examined after 24 hrs for growth and color change of the medium. The golden yellow discoloration due to highly mannitol fermentation within 24 of incubation shows the *S. aureus*. The coagulase test was used to confirm *S. aureus.* The identified *S. aureus* by MSA fermentation were sub-cultured to nutrient agar plate and after 24 hours culture colonies of *S. aureus* were picked by bacteriological loop and placed on clean slide with a small drop of distilled water and emulsified. The test suspension was treated with a drop of freeze-dried rabbit plasma (Santa Fe Drive, Lenexa, USA) and mixed well with a needle for 5-10 seconds. Those forming Clumping of cocci were taken as coagulase positive and confirmed as *S. aureus* [26].

2.7.3. Enumeration of *Staphylococcus aureus*

For enumeration of the *Staphylococcus aureus*, the meat swab and environmental swab samples mixed with 10 ml of buffered peptone water (BPW) and incubated overnight at 37 ℃ taken for original samples. 10 ml of swab sample was diluted in 90ml of 0.1% peptone water to make 10^{-1} dilution from which a serial dilution up to 1: 10^{-6} prepared. From each critical dilution 0.1 ml of diluted sample was transferred to Baird Parker Agar (BPA) (HiMedia, India) supplemented with 20% egg yolk and 3.5% potassium tellurite (Oxoid Ltd, Basingstoke, England) and spread by a bent glass rod. The plates were incubated at 37°C for 24 hours. The plate containing colonies with typical appearance of circular, smooth, convex, moist, and gray to jet-black, frequently with light-colored margin, surrounded by opaque zone and frequently with an outer clear zone in the medium was taken as *Staphylococcus aureus.* Two consecutive Petri dishes that contained 30 -300 colonies were selected for *S. aureus* count and total *S. aureus* colonies from two consecutive plates of each sample were converted into colony forming units per ml (cfu/ml) using a formula given in British Standard Institute [28].

$$N = \frac{\sum C}{V\ (n1+\ 0.1\ n2)\ d}$$

Where; N= number of bacterial colonies counted,

C= sum of colonies identified on two consecutive dilution steps.

n1= is the number of plates counted at the first dilution n2= is the number of plates counted at the second dilution V= volume of inoculums on each dish/plate, in milliliter and d= dilution rate corresponding to the first dilution selected (the initial suspension is a dilution). Finally, the results were converted to $\log_{10}$ cfu/cm^2, and mean values of total viable plate counts were determined. The results were classified as below average and above average comparing with the standards described by [29]. Maximum limit of bacterial load that is acceptable with plate count of 10^5cfu/cm^2 from raw meat [30].

2.7.4. Antimicrobial Susceptibility Tests

Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method on Mueller-Hinton agar, From each biochemically confirmed isolate, loop full of three to five well grown colonies on nutrient agar were transferred with sterile loop into sterile tubes containing 5ml of normal saline solution. The broth culture was incubated at 37°C for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swabs were dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate. The plate was held at room temperature for 15min to allow drying. Antimicrobial discs were placed on the agar surface with disk dispenser and gently pressed down to ensure contact. The plates were kept at room temperature for at least 30 minutes for diffusion of active substance of the agent and incubated at 37°C for 24 hours and diameter of the inhibition zone formed by each antibiotics; amoxicillin, penicillin, vancomycin, kanamycin, Ampicillin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin, and tetracycline were measured using a digital caliper and the results were interpreted according to clinical laboratory standard protocols [31].

2.8. Data Processing and Analysis

The data collected was entered, recorded and stored in Microsoft excel spread sheets program version 2010. Then it was transferred to SPSS software version 20. Pearson's chi square test was used to analyze the proportion of categorical data. Bacterial count data were normalized by $\log_{10}$ transformation. Maximum, minimum and Average values of microbial count was determined using transformed data. A p-value <0.05 at 95% confidence level was considered indicative of a statistical significant difference.

3. Results

3.1. Questionnaire Survey

A total of 42 workers were interviewed for their socio demographic characteristics in Chelenko and Kulubi Towns. All participants were males with age up to 40 years. Majority (61.9%) of Chelenko and (66.7%) of Kulubi respondents had an experience of less than five years. None of respondents had attended any training on meat hygiene and food handling (Table 2).

Table 2. *Socio demographic characteristics of respondents (n=42).*

Factors	Values	Chelenko (n=21) No. (%)	Kulubi (n=21) No. (%)
sex	Male	16(76.2)	18(85.7)
	Female	5(23.8)	3(14.2)
Age	18-30 years	12(57)	14(66.7)
	Above 30 years	9(43)	7(33.3)
Educational status	Primary education	10(47.6)	9(43)
	Secondary education	8(30.1)	7(33.3)
	College	3(14.3)	5(28.7)
Experience	Less than 5 years	13(61.9)	14(66.7)
	Above 5 years	8(38.1)	7(33.3)

In the same manner among respondents interviewed on their hygienic handling practices 39(70.9%) of Chelenko and 31(56.4%) of Kulubi town were not proper hand washing at butchery. Similarly, 16(76.2%) and 15(71.4%) were washed their hands rinsing with water only. In addition to this, 13(61.9%) and 12(57.1%) of butcher shop of Chelenko and Kulubi Town had no a refrigerator for meat preservation respectively as shown in Table 3 below.

Table 3. *Hygienic handling practices in butcher shops.*

Factors	Values	Chelenko No. (%)	Kulubi No. (%)
Take medical examination before	Yes	6(28.6)	13(61.9)
	No	15(71.4)	8(38.1)
Proper hand washing	Yes	13(61.9)	12(57.1)
	No	8(38.1)	9(42.9)
Regularly clean and wear clean, protective clothing	Yes	7(33.3)	14(66.7)
	No	14(66.7)	7(33.3)
Proper cleaning equipment's and butcher house	Yes	6(28.6)	12(57.1)
	No	15(71.4)	9(42.9)
Wearing Jewelry materials during meat han-dling	Yes	13(61.9)	11(52.4)
	No	8(38.1)	10(47.6)
Manner of Washing hands	Water only	16(76.2)	15(71.4)
	Detergents and Water	5(23.8)	6(28.6)
Money collection from buyers by person handling the meat	Yes	12(57.1)	14(66.7)

Factors	Values	Chelenko No. (%)	Kulubi No. (%)
	No	9(42.9)	7(33.3)
Meat cutting equipment sterilization	With hot water	3(14.3)	6(28.6)
	No	18(85.7)	15(71.4)
Presence of refrigerator for meat preserva-tion	Present	8(38.1)	9(42.9)
	Absent	13(61.9)	12(57.1)
Smoking/chewing while working	Yes	11(52.4)	8(38.1)
	No	10(47.6)	
Presence of a sink for hand washing at the display	Present	12(57.1)	13(61.9)
	Absent	9(42.9)	8(38.1)
Use disinfectant	Yes	7(33.3)	9(42.9)
	no	14(66.66)	12(57.1)
Knife and cutting board used for carcass and offal's	Single	17(81)	15(71.4)
	Separate	4(19)	6(28.6)

3.2. Prevalence of *Staphylococcus aureus*

The present study revealed that 24.8% an overall prevalence of *Staphylococcus aureus* from goat meat, contacting materials and workers from study area. Depending on the types of sample the high prevalence were observed on cutting boards (35%), while the lowest isolation was observed from meat swab sample (20.8%). Similarly, depending on the sample site the highest prevalence was recorded in Chelenko (27.2%) than Kulubi (22.3% Towns. Nevertheless, the study results showed sample type and the site of sample were not significantly associated risk factors for occurrence of *S. aureus* at the study area (Table 4).

Table 4. *Prevalence of S. aureus from sample type and study area.*

Variable Sample type	No. Examined	No. Positive	Prevalence	$\chi 2$	P-Value
Goat meat	120	25	20.8		
Worker hands	26	8	30.8		
Cutting boards	20	7	35	2.918	0.572
Hanging hooks	20	5	25		
Knives	20	6	30		
Sample site					
Chelenko	103	28	27.2	0.651	0.259
Kulubi	103	23	22.3		
Total	206	51	24.8		

3.3. Enumeration of *Staphylococcus aureus*

The present study revealed that, the minimum and maximum *S. aureus* counts in goat meat samples were 5.21 and 6.01(log CFU/cm^2) at the Chelenko and 5.02 and 5.99 (log CFU/cm^2 at the Kulubi town respectively and the mean counts of *S. aureus* were 5.56±0.276 (log_{10} / CFU/cm^2 and 5.42±0.309 (log_{10} / CFU/cm$^{2)}$ Chelenko and Kulubi Towns respectively. There was no significant difference was observed in the load of *s. aureus* within source of sample in both Towns (P> 0.05) (Table 5).

Table 5. The mean S. aureus (log_{10} cfu/ml load in goat meat and environmental samples.

Study Area	Sample Type	No. +Ve	Min	Max	Mean ±SD	t-test	P- value
Chelenko	Meat	13	5.21	6.01	5.56±0.276	2.541	0.150
	Worker hands	5	5.29	5.98	5.58±0.314		
	Cutting boards	4	5.34	5.94	5.54±0.249		
	Hanging hooks	3	5.11	5.86	5.51±0.319		
	Knives	3	5.09	5.82	5.49±0.316		
Kulubi	Meat	12	5.02	5.99	5.42±0.309	1.607	0.123
	Worker hands	3	5.25	5.98	5.53±0.352		
	Cutting boards	3	5.07	5.96	5.52±0.629		
	Hanging hooks	2	5.15	5.64	5.39±0.346		
	Knives	3	5.32	5.36	3.34±0.028		

3.4. Antimicrobial Susceptibility Profile of *Staphylococcus aureus*

All the isolates of *S. aureus* from goat meat (n=51) were tested for antimicrobial susceptibility profile by nine selected antibiotics, amoxicillin, penicillin, vancomycin, kanamycin, ampicillin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin, and tetracycline. The current study showed that the isolates were highly (90.2%) susceptible to vancomycin, and followed by gentamicin (88.2%), and kanamycin (86.3%). However, the isolates were highly resistant to penicillin-G (70.6%), amoxicillin (66.7%) and ampicillin (59.6%) (Table 6). *Staphylococcus aureus* isolates were developed resistance to various antimicrobial agents. From the 51 isolated *S. aureus*, 12 (23.5%), 10 (19.6%) and 8 (15.7%) isolates were resistant to multidrug of three drugs, four drugs and five drugs respectively. The most frequent multidrug resistance isolates were those exhibiting resistance to penicillin G and amoxicillin, ampicillin (Table 7).

Table 6. Antimicrobial susceptibility pattern of S. aureus isolates (n=51).

Class	Antimicrobial agents (dose)	Susceptibility Profiles		
		S No (%)	I No (%)	R No (%)
β-lactams	Penicillin G (10 µg)	8(15.7)	7(13.7)	36(70.6)
	Ampicillin (10 µg)	13 (25.5)	9(17.6)	29(56.9)
	Amoxicillin (30 µg)	9(17.6)	8(15.7)	34(66.7)
Aminoglycosides	Gentamicin (10 µg)	45(88.2)	1(1.96)	5(9.8)
	Kanamaycin (30 µg)	44(86.3)	5(9.8)	2(3.9)
Macrolides	Erythromycin (15 µg)	24(47)	11(21.6)	16(31.4)
Tetracycline	Tetracycline (30 µg)	20(39.2)	17(33.3)	14(27.5)

Class	Antimicrobial agents (dose)	Susceptibility Profiles		
		S No (%)	I No (%)	R No (%)
Sulfonamides	Trimethoprim-Sulfamethazole (1.25/23.75 μg)	32(62.7)	9(17.7)	10(19.6)
Glycopeptides	Vancomycine (30 μg)	46(90.2)	1(2)	4(7.8)

Table 7. Antimicrobial resistance of S. aureus isolates in Chelenko and Kulubi Towns.

		Total isolates n=51	Chelenko n=28	Kulubi n=23
1	Penicillin G	36(70.6)	21(75.0)	15(65.2)
2	Ampicillin	29(56.9)	16(57.1)	13(56.5)
3	Amoxicillin	34(66.4)	20(71.4)	14(60.9)
4	Gentamicin	5(9.4)	3(10.71)	2(8.69)
5	Kanamaycin	2(3.9)	2(7.14)	None
6	Erythromycin	16(31.4)	9(32.1)	7(30.43)
7	Tetracycline	14(27.5)	10(35.7)	4(17.39)
8	Trime-thoprim-Sulfamethazole	10(19.6)	6(21.4)	4(17.39)
9	Vancomycine	4(7.8)	2(7.14)	2(8.69)

Table 8. Multidrug resistance pattern of isolated S. aureus.

Number of drug resistant	Resistant drug patter	Number (%)
One drug	AX	3(5.88)
	PG	4(7.84)
	AMP	2(3.92)
Two drugs	AX, PG	4(7.84)
	AX, ERY	2(3.92)
	AX, AMP	1(1.96)
	TTC, SXT	3(5.88)
	PG, VAN	2(3.92)
Three drugs	AX, AMP, PG	1(1.96)
	AMP, PG, ERY	1(1.96)
	AMP, AX, TTC	4(7.84)
	AMP, PG, K	2(3.92)
	PG, ERY, SXT	2(3.92)
	AMP, AX, PG	1(1.96)
	PG, AX, SXT	1(1.96)
Four drug	AX, AMP, PG, VAN	2(3.92)
	AX, AMP, PG, TTC	1(1.96)

Number of drug resistant	Resistant drug patter	Number (%)
	AX, AMP, PG, ERY	2(3.92)
	AX, AMP, PG, GN	1(1.96)
	ERY, AX, PG, SXT	2(3.92)
	AMP, AX, GN, PG	1(1.96)
	AMP, PG, AX, TTC	1(1.96)
	AMP, PG, SXT, GN, ERY	1(1.96)
	AX, AMP, PG, GN, ERY	2(3.92)
Five drugs	AX, PG, SXT, TTC, ERY	1(1.96)
	AX, AMP, PG, ERY, TTC	1(1.96)
	AX, AMP, PG, TTC, ERY	3(5.88)
Total		51(100)

Keys: AMP=Ampicillin, AX=Amoxicillin, ERY=Erythromycin, GN=Gentamycin; K=Kanamycin, PG= Penicillin G; TTC -Tetracycline, SXT = Trimethoprim-Sulfamethazole, VAN= Vancomycin

4. Discussion

Proper meat handling practices play a significant role in ensuring meat quality and safety. Knowledge of meat hygienic handling practices during goat meat production, processing and distribution is essential to formulate preventive measures to mitigate the contribution of meat to foodborne diseases [32]. In current study males most likely involved in goat meat processing than females. Out of total respondent where 76.2% and 85.7% of male worker was involved from Chelenko and Kulubi butcher shops respectively. The result agree with report of Eguale [33] who reported males more participated than females in meat handling in central Ethiopia.

Regarding age of participants, a majority (57% in Chelenko and 66.7% in Kulubi) of workers age was in range of 18-30 years old. This is comparative with the study conducted by Mbonabucha and Fweja [34] who reported that the majority of participants (about 50%) were aged 20-30 years, about one third were between 31-40 years, and the least involved age group was that above 40 years (1.6%) in Rwunge district, Tanzania. None of the study participants took training regarding sanitation and hygienic food handling practices. However, training of food handlers concerning basic concepts and requirements of personal hygiene plays a key role for ensuring safe food. Zerabruk et al. unlike of the study finding report this result [19] where 65.5% of butchers were took training concerning food safety and hygiene food handling practices in Addis Ababa. Among interviewed respondents 47.6% and 43% were graduates of Primary School and 30.1% and 33.3% of participants were secondary school graduates respectively in Chelenko and Kulubi towns. This finding is almost similar with study conducted by Michael *et al*. [35] who reported that, 50% and 35% of participants included in his study were secondary and primary school graduates respectively in Accra, Ghana. Similarly, about 61.9% and 66.7% of butcher shop workers had an experience of less than 5 years, which comparable with report of Birhan *et al*, [14] reported 68.3% of the food handlers' had experience less than 5 years.

Routine medical examinations for butcher shop employees are crucial, as they aid in preventing and controlling the spread of foodborne illnesses to consumers. Nevertheless, this study found that 71.4% of workers in Chelenko and 38.1% in Kulubi had never undergone any health checkup. Health officials may attribute this high proportion to factors such as limited awareness, low economic status, and infrequent supervisory visits. This finding contrasts with that of Gutema et al, [36], who reported that 98% of surveyed retail shop employees had received medical checkups. Regular periodic health assessments can partially reduce the transmission of pathogens from sick or potentially carrier staff [37].

The study also revealed that 61.9% of workers in Chelenko and 52.4% in Kulubi wore jewelry during meat processing. This result aligns with Birhan et al, [14], who reported that 59.41% of meat handlers wore items such as watches, earrings, and rings—contradicting the principle that jewelry should not be worn in food handling areas, as bacteria and food debris can accumulate on and under these items, where warmth further promotes bacterial growth and spread [38]. The majority of butchers 57.1% in Chelenko and 66.7% in Kulubi handled money with bare hands while processing meat. This rate is lower than that reported by Fereda et al, [39], who found that 93.3% of butchers in Dire Dawa city handled money during meat processing. Additionally, handling food with bare hands can lead to cross-contamination and the transfer of microorganisms to otherwise safe food. Since meat handlers are potential sources of microbial contamination, all feasible measures should be taken to limit or eliminate this risk [40].

Among the interviewed participants, 76.2% in Chelenko and 71.4% in Kulubi used only water without detergents to clean various work equipment and working surfaces. Regarding meat preservation methods, 38.1% in Chelenko and 42.9% in Kulubi reported using refrigerators. This finding is comparable to Tegegne and Tesfaye [41], who found that 45.1% of respondents knew refrigeration could reduce meat spoilage and extend shelf life.

The current study reported that 43.6% of respondents in Chelenko practiced proper handwashing before, during, and after handling meat—slightly agreeing with Tegegne and Tesfaye, [41], who reported 40.7% in Jigjiga town. However, this contradicts the finding for Kulubi (34.5%). Such discrepancies may be due to insufficient awareness of the importance of handwashing in reducing spoilage and disease transmission, as well as poor hygienic safety practices in the study area. Furthermore, both findings from Chelenko and Kulubi are inconsistent with Sani and Siow [42], who reported that about 98.2% of respondents were aware of the need to wash hands before, during, and after handling meat and equipment. Understanding proper meat handling, handwashing, and other essential hygiene procedures is critical, as meat handlers can act as vehicles for cross-contamination and the spread of foodborne pathogens. Proper handwashing among meat handlers significantly reduces the risk of transmitting gastrointestinal disturbances [43]. It has also been suggested that *Staphylococcus aureus* and other pathogens are transmitted from livestock to humans through direct contact or via contaminated meat or meat products [44].

In this investigation, the overall prevalence of *S. aureus* in goat meat and environmental swab samples from butcher shops in Chelenko and Kulubi was 24.8%. This finding aligns with reports by [45-48], who reported prevalence rates of 9.1%, 6.3%, and 8.87% from meat swab samples in Ambo Town, Bishoftu City, and Addis Ababa, respectively. The result falls within the range reported in other regions, possibly due to similar sample sizes, sampling techniques, and laboratory methods. No significant difference was observed in bacterial isolates between goat meat and equipment swabs, likely because uniform hygiene practices were applied across them.

However, the current prevalence was lower than findings by [49, 46, 15, 14], who reported rates of 36.5%, 51.56%, and 54.45% from meat swabs in Assela Town, Dangla Town, and Central Ethiopia, respectively. Lower rates were also reported from other countries, including 16% in Addis Ababa [17] and 15% in Addis Ababa [50]. A study by Kim et al. [51] in Korea isolated *S. aureus* from 33.2% of meat samples at retail shops higher than the present finding. Variations in *S. aureus* prevalence across studies may stem from differences in sample size, sample type, isolation techniques, handling practices, worker awareness and skill, animal health delivery systems, and geographic location. The isolation rate observed here reflects poor hygiene and work practices among meat handlers during processing, as well as inadequate sterilization of equipment and work surfaces. Retail shops represent one segment of the food industry that contributes to foodborne disease risks and potential health hazards unless food hygiene principles are implemented [52].

The environmental prevalence of *S. aureus* varied by sample type: 35% on cutting boards, 30.8% on worker hands, 30% on knives, and 25% on hooks. The high prevalence on cutting board swabs suggests significant contamination, likely because more than half of the butcher shops used a single cutting board for both carcasses and offal. Additionally, contamination levels were elevated due to improper meat handling in butcher shops, personnel hygiene issues, possible contamination during handling procedures, and poor washing practices for cutting boards. Likewise, the 30.8% prevalence on worker hand swabs may be explained by the fact that half of the butchers in both towns were unaware of the need to wash hands before, during, and after handling meat and equipment. Recognizing proper meat handling, handwashing, and other hygiene procedures is essential, as meat handlers can transmit foodborne pathogens. Proper handwashing significantly reduces gastrointestinal disease transmission [53].

In the current study, the mean *S. aureus* loads on goat meat were 5.56±0.276 $\log_{10}$ CFU/cm² in Chelenko and 5.42±0.309 $\log_{10}$ CFU/cm² in Kulubi. This finding is consistent with reports from Badele town (6.22±0.63 $\log_{10}$ CFU/g; Amanu et al., [54] and Dangila town (5.7 $\log_{10}$ CFU/cm²; Dessayew, [15]. In contrast, the present results are higher than those reported in Bahir Dar city (3.40±0.63 $\log_{10}$ CFU/g; Bzuneh, [55] and by Kibrom [56] (3.28 $\log_{10}$ CFU/cm^2). This variation may be due to differences in *S. aureus* contamination levels along the meat value chain, where strict hygiene practices were not implemented in the study areas. Another possible cause is the timing of sample collection contamination probability increases over time due to cross-contact.

Currently, antibiotic-resistant strains of *S. aureus* pose one of the most challenging problems worldwide, representing a significant risk in food products. Meat of animal origin is among the most common sites where drug residues can accumulate for extended periods. Humans can acquire infections by consuming contaminated meat [13]. In this study, the antimicrobial resistance of *S. aureus* isolates was tested against nine antimicrobials, and results were graded according to CLSI [31].

The *S. aureus* isolates showed the highest resistance to β-lactam antibiotics (penicillin-G, ampicillin, and amoxicillin). This is attributed to the production of the enzyme β-lactamase. Penicillin has traditionally been used to treat *S. aureus* infections, particularly mastitis, in many countries including Ethiopia. However, many *S. aureus* infections are now resistant to the penicillin group due to β-lactamase production, which destroys the drug's antibacterial activity by hydrolyzing the critical β-lactam bond [57].

The high resistance rates observed—penicillin-G (70.6%), amoxicillin (66.7%), and ampicillin (56.9%) agree with findings by Tefera et al. [17], who reported resistance to chloramphenicol (80%), amoxicillin (85.5%), ampicillin (72.7%), vancomycin (56.4%), kanamycin (38.2%), and gentamicin (36.4%). This finding is also consistent with Zeyni et al. [16], who recorded resistance to ampicillin (72.7%) and amoxicillin (85.5%), and noted that 95% and 97.6% of *S. aureus* isolates were resistant to penicillin-G. This high percentage of penicillin-resistant *S. aureus* may result from widespread antibiotic use to control and treat infections on dairy farms, including mastitis [57].

In this study, isolated *S. aureus* strains were highly susceptible to vancomycin (94.2%), followed by gentamicin (88.2%) and kanamycin (86.3%). This finding is consistent with Tefera et al. [17], who reported susceptibility to chloramphenicol (90.2%), gentamicin (96.7%), kanamycin (91.8%), and vancomycin (100%). It is, however, inconsistent with Million et al. [58], who detected susceptibility to tetracycline (53.1%), gentamicin and kanamycin (50%), and vancomycin (40.9%).

The *S. aureus* isolates in this study were susceptible to erythromycin (74.5%) and tetracycline (52.7%). A comparable result was obtained by Adugna [50], who reported susceptibility to erythromycin (72.9%) and tetracycline (50.5%). Our results showing susceptibility to tetracycline (52.7%), gentamicin and kanamycin (50.9%), and vancomycin (43.6%) are inconsistent with Million et al. [58], who reported susceptibility to tetracycline (53.1%), gentamicin and kanamycin (50%), and vancomycin (40.9%).

The overall prevalence of multidrug resistance (resistance to at least one agent in three or more antimicrobial categories) among *S. aureus* isolates from goat meat was 63.4% (51 out of 206? Note: The original says "206/51"—likely a typo; I have preserved the original expression but note it should be verified). The most common multidrug-resistant isolates exhibited resistance to penicillin G, amoxicillin, and ampicillin. This finding aligns with a study conducted in Jigjiga city, Somali Regional State (69.6%; Befekadu et al., [59]). It also agrees with Daka et al. [60], who reported 62.8% multidrug resistance among *S. aureus* isolates from beef in Hawassa town. The presence of multidrug-resistant *S. aureus* represents an alarming situation requiring special attention. The increasing number of MDR isolates may be due to extensive misuse of antibiotic treatments.

5. Conclusions

This investigation was carried out in the towns of Chelenko and Kulubi, located in eastern Ethiopia, with the aim of assessing the prevalence, bacterial load, and antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from raw goat meat sold at butcher shops. Additionally, a questionnaire-based survey was employed to evaluate goat meat handling practices. The findings revealed a high prevalence and elevated load of *S. aureus*. Strong antimicrobial resistance was observed among penicillin-group antibiotics (penicillin-G, amoxicillin, and ampicillin). Nevertheless, the majority of *S. aureus* isolates remained susceptible to vancomycin, followed by gentamicin and kanamycin. Improper handling and inadequate storage conditions, which favor the growth of this pathogen, contribute to meat contamination, bacterial proliferation, and the production of enterotoxins by *S. aureus*. Most butcher shops did not store meat at appropriate low temperatures and failed to use sanitizers or detergents in their establishments. Furthermore, the majority of butcher shop workers did not practice proper handwashing during meat handling, and none had attended any course or training related to hygienic food handling practices.

Based on the above conclusion the following recommendation were forwarded:

1) It is essential to raise awareness regarding sanitation and good hygiene practices in butcher shops, as well as the importance of thoroughly cooking meat before consumption.
2) Appropriate hygienic measures should be implemented to the greatest extent feasible, including the application of Good Hygiene Practices (GHP), the adoption of HACCP principles, and the establishment of standard operating procedures for meat handling in butcher shops.
3) Veterinarians and medical professionals should work together for the possibility of awareness creation to the public about severe health consequences associated with raw meat consumption.
4) Rational use of antimicrobial drugs and regular surveillance of antimicrobial resistance should be made to combat drug resistance.
5) Further studies should have to be conducted on genetic determinant and virulence factor of pathogen for prompt detection and control of *S aureus* in the community.

Abbreviations

AMR	Antimicrobial Resistance
BAP	Blood Agar Plates
BPW	Buffered Peptone Water
CDC	Center for Disease Control and Prevention
CFU	Colony Form Unity
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistical Agency
ELISA	Enzyme-linked Immune Sorbent Assay
FBD	Foodborne Diseases
HACCP	Hazard Analysis Critical Control Point
ISO	International Organization for Standardizations
MRSA	Methicillin-Resistant *S. aureus*
MSA	Mannitol Salt Agar
PCR	Polymerase Chain Reaction
SE	Staphylococcus Enterotoxin
SFD	Staphylococcus Food Born Disease
SFP	Staphylococcus Food Poisoning
WHO	World Health Organization

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Author Contributions

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

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