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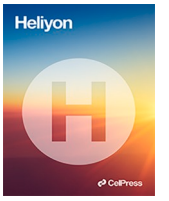

Isolation and identification of *Staphylococcus aureus* from bovine milk and community awareness on public health significance of mastitis in and around Jigjiga, Somali region, Ethiopia

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ABSTRACT

A cross-sectional study on lactating cattle was done from December 2019 to July 2020 to identify and isolate *Staphylococcus aureus* from bovine mastitic milk and assess community awareness of public health problems connected with mastitis in and around Jigjiga. Among 353 milk samples, 96 were determined to be mastitis positive. Clinical and subclinical mastitis were found in 79 and 17 respectively. Milk samples (n = 96) from clinical and subclinical animals were cultured to isolate *S. aureus*; *S. aureus* isolates were detected in 51.04 % (49/96) of the samples. The study was also conducted to investigate community awareness of the public health significance of mastitis and 89 % of people were aware of the disease's existence, particularly the clinical form of mastitis, due to its prominent clinical signs. In contrast, 80 % of them were unaware of the subclinical form of mastitis. About 78 % of those questioned said they had no idea mastitis could be passed from cow to cow. The majority of participants (65 %) stated that they consumed raw milk. The large percentages of respondents (92 %) were unaware that enterotoxigenic *S. aureus* can be found in mastitic milk. This study suggests that the most of the communities in the study site was unaware of the disease's effects. Therefore, community awareness about the public health importance of mastitis and hazardous of microbial in milk, particularly *S. aureus*, which causes food contamination and serves as a method of disease transmission to people through unpasteurized milk consumption, should be implemented.

1. Introduction

Mastitis is an infection of the mammary gland induced mostly by pathogenic microbes, with a range of both local and systemic manifestations. Because of modernization, an increased human population, and wealth expansion, the proportion of intensive and semi-intensive dairy operations in Ethiopia has indeed been expanding throughout time. Most dairy farms' management systems remained traditional [1]. However, the majority of Ethiopia's new dairy farms lack optimal management methods and are prone to mastitis [2].

Milk is an essential part of the global human diet, and it also serves as a favorable substrate for the development of numerous

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microorganisms, particularly hazardous bacteria. Raw milk consumption may cause health problems for consumers as a result of zoonotic infections and antibiotic residues [3]. A variety of factors, including additives, contamination during milking, and the existence of udder infections, can impair milk quality [4]. Microbes in milk may originate from the animal, humans' hands, or the environment [3]. *S. aureus* is a multifaceted bacterium that causes a wide range of diseases in humans and animals [5].

In the dairy food industry, *S. aureus* contamination of dairy cows and raw milk is still a problem. Multiple outbreaks of food-borne disease linked to tainted dairy products illustrate S. *aureus*' public health importance [6].

In order to prevent and control bovine mastitis, community awareness and perception of the disease must be raised. As a result, no previous study has been conducted on mastitis risk factors associated with farmer awareness, attitude, and practice in Ethiopia, as in other countries like Bangladesh [7]. Such information is essential for understanding farmers' perspectives on mastitis and developing appropriate methods to help minimize its prevalence and impact.

There is no recent study that reveals the present frequency of *S. aureus* generated from mastitic cattle' milk and community awareness about the public health relevance of mastitis in and around Jigjiga town. Furthermore, past studies undertaken in the area were few in number. Thus, the objectives of the current research were to isolate and identify *S. aureus* from bovine mastitis milk and assess community awareness about the public health significance of consuming raw mastitis milk in and around Jigjiga, Somali Regional State, Ethiopia.

2. Materials and methods

2.1. Study area

The research was carried out on several farming systems in and around Jigjiga. Jigjiga is the Ethiopian Somali Regional State's capital city. Jigjiga is situated about 625 km from Addis Ababa. The town's elevation spans from 1620 to 1720 m. Its climate is classified as semi-arid, with high temperatures and limited rainfall, resulting in considerable evaporation.

2.2. Study animals

The study animals was lactating dairy cows managed in different farming systems (intensive and extensive) and around Jigjiga.

2.3. Study design

Cross-sectional study methods were used to isolate and identify *S. aureus* in dairy herds and assess community awareness about the public health significance of consuming raw mastitis milk in and around Jigjiga.

2.4. Sampling method and sample size

The total sample was established using a cluster of 68 small-holder dairy farms situated in and around Jigjiga. According to the sample frame from the study area, the farm was a small-holder dairy farm with an average of three to four lactating cows. As a result, 353 lactating cows from the 68 dairy farms were included in the research.

The sample size for the questionnaire survey was estimated using formula $N = 0.25/SE2$ [8]. Where N is the sample size and SE is the standard deviation (5 %). In accordance with the aforementioned formula, the sample size necessary for a questionnaire survey about community awareness of the public health relevance of mastitis was 100 respondents.

2.5. Study methodology

2.5.1. Physical examination

The udders, or teats, were physically checked visually and palpated to determine the existence of gross lesions. During the inspection, the cardinal symptoms of inflammation, uniformity, size, and consistency of the udder quarters were all examined. Clinical mastitis was determined by the presence of obvious indications of inflammation and contaminated milk [9]. The breed of the cow, age of the cow, health state of the mammary glands, and farm names were all documented at the moment of each examination.

2.5.2. Milk sample collection and handling

Samples were taken using the aseptic technique outlined in Ref. [10]. They were taken before milking. After washing the teats, the first drops of milk were removed, and the teat tips were disinfected with cotton swabs wet with 75 % alcohol. The samples were collected in sterile glass bottles and sealed with screw closures. The bottles were labeled with a permanent marker so that they could be easily read when placed on racks. After the initial drops of milk were discarded (stripping), 10 ml of milk were collected into a horizontally held vial. Following collection, the samples were placed in an icebox and transported to the Jigjiga Regional Laboratory for processing, where they were maintained at 4 °C until inoculation for *S. aureus* isolation and identification began.

2.5.3. California Mastitis Test

According to the National Mastitis Council guidelines [11], milk samples were collected in order to identify sub-clinical mastitis. The teats were cleaned with tap water and dried when there was a substantial quantity of dirt to be eliminated. The teat ends were

disinfected with cotton soaked in ethyl alcohol (70 %). An equivalent amount of California Mastitis Test (CMT) reagent was combined with an appropriate amount of sample milk in each compartment of the CMT paddles and mixed with a gentle circular motion for 15 s. The thicknesses of the gels created by the CMT reagent and milk combination were read as 0 (the mixture staying unaltered) to 3 (an almost-solid gel developing), with a score of 2 or 3 deemed a favorable outcome. Cows were classified as positive for CMT if at least one of the quarters tested positive [9].

2.6. Isolation and identification of Staphylococcus aureus

Isolation and identification of *S. aureus* from milk and milk products were performed by following the ISO [12] procedures. The tests performed to identify the *S. aureus* isolates included growth characteristics on blood agar, Gram staining, catalase tests, growth on Mannitol salt agar base, slide and tube coagulase tests, and growth on purple agar base.

2.7. Questionnaire survey

A questionnaire was used to collect data on assessing community awareness about the public Health significance of mastitis. The questionnaire was based on if people consumed pasteurized milk or not, transmission of disease, knowledge of clinical and subclinical mastitis, impact of mastitis in milk composition, and enterotoxin of *S. aureus,* etc. The questions were originally translated from English into the local language (Somali) when administered, and the answers were then translated to English and entered into the original form.

2.8. Data management and analysis

The data obtained was loaded into the Microsoft Excel program and analyzed by applying SPSS statistical software version 21. Descriptive statistics were applied to analyze the data and reported in terms of frequency, percentage, tables, and charts. The odds ratio using logistic regression was utilized to assess the existence of an association between the probable risk factors and the incidence of mastitis. Tables were used to represent the community's knowledge regarding sickness. All statistical tests were determined to be significant at $p < 0.05$.

3. Results

3.1. Isolation of Staphylococcus aureus

During the investigation, out of a total of 353 lactating cows examined for mastitis, 96 samples of cow level were determined to be positive for mastitis. There were 17 cases of clinical mastitis and 79 cases of subclinical mastitis among them. The samples were cultured on blood agar media for isolation and identification of *Staphylococcus aureus*. According to Table 1, *Staphylococcus aureus* was found in 51.04 % (49/96) of the milk samples. Of these, 35.3 % (6/17) were clinical mastitis, and 53.2 % (43/79) were subclinical mastitis.

3.2. Sociodemographic characteristics of respondents

The study was also carried out to assess community awareness of the public health significance of mastitis. About 65 % of the participants in the study were female, whereas 35 % were male. According to respondents' ages, 49 %, 33 %, and 18 % were between the ages of 15 and 30, 30–50, and over 50, respectively. During the survey, respondents were asked about their marital status, and around 45 % were married, 33 % were single, and 22 % were divorced. Approximately 55 % of respondents were illiterate; 22 % could only read and write; and 14 % and 9 % were in secondary or primary school, respectively. Respondents' occupations were as follows: 60 % were livestock owners, 20 % were milk sellers, and 20 % were consumers as stated in Table 2.

3.3. Community awareness of public health problems of mastitis

Approximately 89 % of respondents were aware of the existence of mastitis, while 11 % were not. In the study, the majority of respondents (76 %) had knowledge of clinical mastitis, whereas 24 % did not. Of those respondents, 80 % were unaware of subclinical mastitis, while 20 % were. When asked about the major causative agents of the disease, 58 % of the community said they didn't even know. However, 27 % and 15 % stated that it was caused by injuries and microorganisms, respectively. As shown in Table 3, the

Table 1
Isolation and identification of *Staphylococcus aureus*

Clinical mastitis		Subclinical mastitis		
Sample cultured	Positive (%)	Sample cultured	Positive (%)	Total
17	6 (35.3 %)	79	43 (53.2 %)	49 (51.04 %)

Table 2
Sociodemographics of respondents.

Questions (n = 100)	Categories	Frequency and Percentage
1. Gender	Male	35 (35 %)
	Female	65 (65 %)
2. Age	15–30	49 (49 %)
	30–50	33 (33 %)
	Above 50	18 (18 %)
3. Marital Status	Single	33 (33 %)
	Married	45 (45 %)
	Divorced	22 (22 %)
4. Respondent's education level	Illiterate	55 (55 %)
	Read and write	22 (22 %)
	Primary level	14 (14 %)
	Secondary level	9 (9 %)
5. Occupation	Livestock owners	60 (60 %)
	Milk sellers	20 (20 %)
	Consumers	20 (20 %)

majority of respondents (42 %) said that the main source of the disease was an unhygienic floor, while roughly 15 % believed it was due to unwashed hands, 13 % believed it was related to suckling, and 30 % were unsure. About 78 % of the community was unaware that mastitis might be transmitted from cow to cow, although 22 % were aware that it could. The study also assessed milk consumption, and 35 % of respondents said they drank pasteurized milk, but the majority (65 %) said they drank raw milk. During the study, 62 % of the community was unaware that milk transmission of several zoonotic diseases can occur. According to the study, 63 % of the community in the study area had no knowledge of the transmission of some zoonotic diseases, but others mentioned the most common zoonotic diseases such as tuberculosis, diphtheria, Q fever, and others. Mastitis can alter the composition of milk, but the community in the study area is unaware of this, as 88 % of them said no, while only 12 % were aware that mastitis can change the composition of milk. Approximately 92 % of the participants were unaware that mastitis milk could be the primary source of enterotoxigenic *S. aureus*, while 8 % were aware. Hence, subclinical mastitis is more common than clinical mastitis, and the California mastitis test is required to monitor the disease. This kit was unknown to 88 % of respondents, while it was known to 12 %.

Table 3
Community awareness about public health significance of mastitis.

Questions (n = 100)	Categories	Frequency & Percentages
1. Do you know about mastitis?	Yes	89 (89 %)
	No	11 (11 %)
2. What causes mastitis?	Micro-organism	15 (15 %)
	Injury	27 (27 %)
	Unknown	58 (58 %)
3. What is the Source of the infection?	Unhygienic floor	41 (41 %)
	Unhygienic hands	15 (15 %)
	Sucking	13 (13 %)
	Unknown	31 (31 %)
4. Do you know about clinical mastitis?	Yes	76 (76 %)
	No	24 (24 %)
5. Are you aware of subclinical mastitis?	Yes	20 (20 %)
	No	80 (80 %)
6. Cow to cow transmission of mastitis	Known	22 (22 %)
	Unknown	78 (78 %)
7. Which kind of milk do you drink?	Pasteurized milk	35 (35 %)
	Raw milk	65 (65 %)
8. Raw milk transmission of zoonotic diseases	Yes	38 (38 %)
	No	62 (62 %)
9. kind of zoonotic disease	TB and Brucella	5 (5 %)
	Diphtheria and Q fever	12 (12 %)
	All above	20 (20 %)
	Unknown	63 (63 %)
10. Changing of milk composition.	Yes	12 (12 %)
	No	88 (88 %)
11. Source of enterotoxins from *S. aureus*.	Yes	8 (8 %)
	No	92 (92 %)
12. California mastitis test kit.	Known	12 (12 %)
	Unknown	88 (88 %)

4. Discussion

The study also investigated the abundance of *S. aureus* in bovine mastitis in and around Jigjiga, and the overall prevalence of *S. aureus* found in mastitic milk samples was 51.04 %, which was comparable with a previous report done by Ref. [13], who indicated a prevalence of 5.1 % in Hawassa, South Ethiopia, and was also comparable with [14], who reported 47 % in central Ethiopia. The current study was higher than previous findings by Refs. [15,16], in northern Ethiopia and Kenya, which stated 36.0 % and 35.5 %, respectively, but it was lower than the finding by Ref. [17], in Kenya, which indicated 72.9 %. Other investigations have revealed that *Staphylococcus aureus* is one of the most frequent sources of bovine mastitis. Other studies have likewise revealed *S. aureus* isolate dominance and key functions in bovine mastitis [14,18]. The high prevalence of *S. aureus* in the study area was related to the lack of culling of chronically sick animals, dry cow treatment and post-milking teat cleaning, hand washing before milking, and inadequate house hygiene. This bacterium is contagious and can be found on the udder, infected teats, and even the quarters, which allows it to spread from infected cows to uninfected ones during milking. In this sense, a favorable chance for the organism's introduction into dairy cows has developed.

The study was also conducted to assess the level of community awareness about the public health significance of mastitis. The majority of the participants in the study (89 %) were aware of the existence of mastitis. Because there have been no previous studies on community awareness in Ethiopia, the study is not compared in other countries' reports. It is lower than the previous study in Bangladesh, which indicated 98.5 % of the communities were aware of the disease [7]. Most respondents (58 %) in the study area were unaware of the causative agents of mastitis in lactating cows. The majority of the community was aware of the source of the disease because 41 % responded that it originates from poor hygiene on farm floors. This figure was lower than a study conducted by Ref. [7], which reported that the source of infection was unhygienic floors in 87.7 % of cases. This suggests that the occurrence of mastitis in lactating cows may be caused by inadequate farm hygiene. Since clinical mastitis changes the appearance of milk and causes inflammation of the mammary gland, farmers are more aware of it, and the majority of participants (76 %) are aware of it. On the contrary, most respondents (80 %) in the study area were unaware of subclinical mastitis. This could be due to a lack of indications and the normal appearance of milk from cows, as well as a lack of screening tests for subclinica mastitis. The findings were comparable [19].

According to the findings, the majority of the community in the study area used raw milk, which increased the risk of infection with enterotoxins from *S. aureus*, which was the most causative agent of mastitis in the bacteriological investigation (51.04 %) in the study area. The majority of respondents (63 %) were unaware of the possibility of contracting other zoonotic diseases through raw milk. The farmers in the area of study did not utilize the California mastitis test to detect mastitis since 88 % of them did not know it. This study is comparable to a prior study by Ref. [7]. This could be due to a lack of knowledge, the availability of the kit, and national veterinary authority awareness of the CMT kit and other diagnosing tools in the area.

5. Conclusion and recommendations

Staphylococcus aureus was isolated during the study because of its high pathogenicity and public health concern. Both clinical and subclinical mastitis milk samples contained *Staphylococcus aureus*. The communities' social demographics were surveyed, including gender, age, marriage status, educational level, sex, and occupational role. On the other hand, community awareness about the public health significance of mastitis was assessed. According to current findings, the communities were unaware of mastitis and its importance to public health. As a result, the community should be made aware of the public health significance of mastitis, and methods of prevention by the hazardous microbials in milk should be implemented, specifically *S. aureus*, which causes food poisoning and offers a mechanism for disease transfer to humans via raw milk consumption.

Ethical approval

This research was approved by the Research Ethics Review Committee of the School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University. The study was conducted in compliance with the ARRIVE 2.0 guidelines. All methods were carried out in accordance with relevant guidelines and regulations. Before conducting the study, the objectives, expected results, and benefits of the study were explained to the farm owners, and the required permission was obtained from all the farm and cattle owners for the use of their animals for the study.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

Samatar Abshir Mahamed: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology,

Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Asma Ibrahim Omer:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **Nesra Yusuf Osman:** Investigation, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Mustafe Abdi Ahmed:** Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20981.

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