Research Square

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Isolation of Methicillin-resistant Staphylococcus Aureus From Bovine Mastitis in Sebeta Town, Ethiopia

Dawit Alemu Lemma
HOSSANA
Sinidu Mengesha
sinidumengesha26@gmail.com

Lemo Woreda Agricultural Bureau

Research Article

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Abstract

Background

Bovine mastitis is a major production disease affecting dairy cattle worldwide, with Staphylococcus aureus being one of the most common etiological agents. The emergence of methicillin-resistant S. aureus (MRSA) poses significant public health and economic concerns. This study aimed to isolate methicillin-resistant Staphylococcus aureus from mastitic milk samples and determine the antimicrobial resistance patterns of S. aureus isolates in dairy farms around Sebeta town, Ethiopia.

Methods

A cross-sectional study was conducted from December 2017 to March 2018 in and around Sebeta town. A total of 200 lactating dairy cows were examined for mastitis using the California Mastitis Test (CMT). Milk samples were collected aseptically from CMT-positive quarters and cultured for bacterial isolation. S. aureus isolates were identified using standard microbiological techniques including Gram staining, catalase test, coagulase test, and the OMNILOG identification system. Antimicrobial susceptibility testing was performed using the disc diffusion method against ten antimicrobial agents.

Results

The overall prevalence of mastitis was 65% (130/200). S. aureus was isolated from 23 (17.69%) of the mastitic milk samples. Antimicrobial susceptibility testing revealed that the isolates were highly resistant to penicillin G (91.3%) and ampicillin (91.3%), with moderate resistance to oxytetracycline (39.1%). All isolates (100%) were susceptible to vancomycin, oxacillin, gentamycin, and cefoxitin. High susceptibility was observed for erythromycin (95.7%), sulphamethoxazole/trimethoprim (95.7%), and methicillin (95.7%). Multidrug resistance was observed in 91.3% of the isolates, with 47.82% showing resistance to both penicillin G and ampicillin, and 43.47% showing resistance to penicillin G, ampicillin, and oxytetracycline.

Conclusions

This study demonstrated a high prevalence of bovine mastitis in the study area, with a considerable proportion of S. aureus isolates showing resistance to commonly used antimicrobials. Although MRSA was not prevalent in this study population, the presence of multidrug-resistant S. aureus in milk samples is alarming and warrants continuous surveillance and prudent antimicrobial use in dairy farms.

Introduction

Ethiopia possesses the largest livestock population in Africa, with an estimated total cattle population of approximately 59.5 million, of which female cattle constitute about 55.5% (CSA, 2017). Dairying is a significant livestock production activity in Ethiopia, encompassing a wide range of small, medium, and large-scale operations (Guadu and Abebaw, 2016). However, the productivity of the dairy sector is severely constrained by various diseases, among which bovine mastitis is one of the most economically important (Mulshet et al., 2017).

Bovine mastitis is a complex inflammatory condition of the mammary gland that adversely affects milk quality and quantity, leading to substantial economic losses to dairy farmers and the national economy (Mulshet et al., 2017). The disease is caused by a diverse array of bacterial pathogens, with Staphylococcus species being the most frequently implicated agents in both clinical and subclinical mastitis cases (Dorgham et al., 2013). Staphylococci are Gram-positive, non-motile, non-spore-forming cocci that grow as facultative anaerobes and are typically arranged in irregular clusters resembling bunches of grapes (Quinn, 1999). Pathogenic staphylococci are distinguished by their ability to produce coagulase, with Staphylococcus aureus being the most significant coagulase-positive species (Harris et al., 2002). Staphylococcus aureus has emerged as a major pathogen of global concern due to its increasing antimicrobial resistance. The extensive therapeutic use of antimicrobials has been identified as the primary driver for the development of resistance, particularly the acquisition of the mecA gene, which encodes a modified penicillin-binding protein (PBP2a) with reduced affinity for β-lactam antibiotics (Tadesse et al., 2017). Methicillin-resistant S. aureus (MRSA) strains are resistant to essentially all β-lactam antibiotics, complicating treatment options and posing significant public health risks. Historically, MRSA was considered exclusively a human pathogen until its first isolation from a dairy cow with mastitis in 1972 (Devriese et al., 1972). Since then, MRSA has been increasingly reported in livestock populations worldwide, raising concerns about its zoonotic potential and the role of food animals as reservoirs for antimicrobial resistance genes. In Ethiopia, limited information exists on the prevalence of MRSA in bovine mastitis, particularly in the Sebeta area, which is a major dairy production hub. Therefore, the objectives of this study were: (1) to isolate methicillin-resistant Staphylococcus aureus from mastitic milk samples collected from dairy farms in and around Sebeta town, and (2) to determine the antimicrobial resistance patterns of S. aureus isolates to commonly used veterinary antimicrobial agents.

MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Sebeta town, located in the Oromia Special Zone surrounding Addis Ababa, Ethiopia. Sebeta is situated at approximately 8°55'N latitude and 38°37'E longitude, at an elevation of 2,356 meters above sea level, about 25 km southwest of Addis Ababa. The area covers a total of 102,758 km² and experiences an annual rainfall ranging from 860 to 1,200 mm, with average ambient temperatures reaching up to 28°C (CSA, 2013). The region is characterized by intensive and semi-intensive dairy production systems, making it an appropriate location for studying bovine mastitis.

2.2 Study Design and Study Population

A cross-sectional study was conducted from December 2017 to April 2018. The study population consisted of lactating dairy cows from selected dairy farms in and around Sebeta town. A total of 200 lactating cows were purposively selected based on the availability of dairy cows and the willingness of farm owners to participate in the study.

2.3 Sample Size Determination

A total of 200 dairy cows were conveniently sampled from available dairy farms in the study area. The selection was based on accessibility and owner cooperation, ensuring adequate representation of the dairy population in the region.

2.4 Study Methodology

2.4.1 Sample Collection

Udders of selected cows were thoroughly examined for clinical signs of mastitis, including the presence of lesions, pain, heat, and swelling. Milk from each quarter was grossly examined for changes in color and consistency. Subclinical mastitis was diagnosed using the California Mastitis Test (CMT) following standard procedures (Quinn, 1994). Briefly, a squirt of milk from each quarter was placed into the four shallow cups of the CMT paddle, and an equal volume of CMT reagent was added. The paddle was gently rotated in a horizontal plane, and the reaction was scored based on gel formation within a few seconds. Results were categorized as negative (score 0, no gel formation), trace (score 1, slight precipitation), weak positive (score 2, distinct precipitation without gel), distinct positive (score 3, gel formation with mixture thickness), or strong positive (score 4, strong cohesive gel with convex surface). A cow was considered positive if at least one quarter showed a positive CMT reaction (score ≥ 1) (Quinn, 1994).

Before sample collection, teat ends were thoroughly washed with water, and the first few streams of milk (foremilk) were discarded to minimize contamination from the teat canal. Approximately 10 mL of milk was aseptically collected from each CMT-positive quarter into sterile universal bottles labeled with permanent markers. To reduce cross-contamination, samples were collected from near teats first, followed by far teats (Quinn, 1999). Samples were transported to the National Animal Health Diagnostic and Investigation Center Microbiology Laboratory in an ice box for microbiological examination. Samples were stored at 4°C until processing, which was performed within 24 hours of collection (Tadesse et al., 2017).

2.4.2 Laboratory Procedures

2.4.2.1 Bacterial Isolation and Identification

A loopful of each milk sample was streaked onto Mannitol Salt Agar (MSA) plates and incubated aerobically at 37°C for 24 hours. Colonies that produced yellow zones (indicating mannitol fermentation) were subcultured onto nutrient agar for purification. Presumptive identification of *S. aureus* was performed using standard microbiological techniques including Gram staining, catalase test (positive), and coagulase test (positive) (Quinn, 1999). Additionally, isolates were tested for maltose fermentation using purple base agar with 1% maltose.

Confirmatory identification was performed using the OMNILOG fully automated bacterial identification system (BIOLOG, USA) according to the manufacturer's protocol. Briefly, a single colony grown on Biolog Universal Growth (BUG) agar was emulsified in inoculating fluid A (IF A), and cell density was adjusted to 90–98% transmittance using a turbidimeter. One hundred microliters of the cell suspension were inoculated into each well of the GEN III microplate and incubated aerobically at 33°C for 22 hours. The OMNILOG system automatically interpreted the results and provided species-level identification (OMNILOG, 2010).

2.4.2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on all confirmed *S. aureus* isolates using the standard disc diffusion method on Müller-Hinton Agar (MHA) plates (Quinn, 1999). The following antimicrobial discs were used: penicillin G (10 µg), ampicillin (10 µg), oxytetracycline (30 µg), vancomycin (30 µg), gentamycin (10 µg), cefoxitin (30 µg), erythromycin (15 µg), sulphamethoxazole/trimethoprim (25 µg), oxacillin (1 µg), and methicillin (5 µg).

Inoculum was prepared by suspending isolated colonies from an 18–24 hour culture on non-selective medium (blood agar) in sterile saline. The suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard. Within 15 minutes, a sterile cotton swab was dipped into the suspension and streaked evenly over the entire surface of the MHA plate. Antimicrobial discs were applied, and plates were incubated at 37°C for 24 hours. Inhibition zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Intermediate results were recorded when applicable. Multidrug resistance was defined as resistance to two or more antimicrobial agents.

2.5 Data Analysis

Data were entered into Microsoft Excel and analyzed using descriptive statistics. The prevalence of mastitis and isolation rates of *S. aureus* were calculated as proportions. Antimicrobial susceptibility patterns were expressed as percentages of resistant, intermediate, and susceptible isolates.

Results

3.1 Prevalence of Mastitis and Isolation of *S. aureus*

Of the 200 lactating dairy cows examined, 130 (65%) tested positive for mastitis based on the California Mastitis Test (CMT). From the 130 CMT-positive milk samples cultured for bacterial isolation, *Staphylococcus aureus* was identified in 23 (17.69%) samples (Table 1).

Table 1
Prevalence of mastitis and isolation of *S. aureus* from CMT-positive milk samples

Sample Source	Total Examined	CMT Positive n (%)	Samples Cultured	*S. aureus* Positive n (%)
Dairy cows	200	130 (65.0%)	130	23 (17.69%)

3.2 Antimicrobial Susceptibility Patterns

Antimicrobial susceptibility testing was conducted on all 23 *S. aureus* isolates against ten antimicrobial agents (Table 2). The highest resistance rates were observed for penicillin G (91.3%) and ampicillin (91.3%), followed by oxytetracycline (39.1%). All isolates (100%) were susceptible to vancomycin, oxacillin, gentamycin, and cefoxitin. High susceptibility rates were observed for erythromycin (95.7%), sulphamethoxazole/trimethoprim (95.7%), and methicillin (95.7%).

Table 2
Antimicrobial susceptibility patterns of *S. aureus* isolates (n = 23)

Antimicrobial Agent	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin G	21 (91.3%)	0 (0.0%)	2 (8.7%)
Ampicillin	21 (91.3%)	0 (0.0%)	2 (8.7%)
Oxytetracycline	9 (39.1%)	0 (0.0%)	14 (60.9%)
Vancomycin	0 (0.0%)	0 (0.0%)	23 (100.0%)
Gentamycin	0 (0.0%)	0 (0.0%)	23 (100.0%)
Cefoxitin	0 (0.0%)	0 (0.0%)	23 (100.0%)
Erythromycin	0 (0.0%)	1 (4.3%)	22 (95.7%)
Sulphamethoxazole/Trimethoprim	0 (0.0%)	1 (4.3%)	22 (95.7%)
Oxacillin	0 (0.0%)	0 (0.0%)	23 (100.0%)
Methicillin	0 (0.0%)	1 (4.3%)	22 (95.7%)

3.3 Multidrug Resistance Patterns

Multidrug resistance (resistance to two or more antimicrobial agents) was observed in 21 (91.3%) of the *S. aureus* isolates (Table 3). The most common resistance pattern was resistance to both penicillin G and ampicillin, observed in 11 isolates (47.82%). Resistance to penicillin G, ampicillin, and oxytetracycline was observed in 10 isolates (43.47%).

Table 3
Multidrug resistance patterns of *S. aureus* isolates

Resistance Pattern	Number of Isolates	Percentage (%)
Penicillin G + Ampicillin	11	47.82%
Penicillin G + Ampicillin + Oxytetracycline	10	43.47%
Total	**21**	**91.29%**

Discussion

This study investigated the prevalence of mastitis, isolation of *S. aureus*, and antimicrobial resistance patterns in dairy farms in and around Sebeta town, Ethiopia. The overall prevalence of mastitis (65%) observed in this study is comparable to previous reports from Asella (66.6%) (Abera et al., 2013) and Holleta (71%) (Mekbib et al., 2010). However, the prevalence in this study was higher than those reported by Sori et al. (2005) (52.7% in Sebeta), Mitiku et al. (2017) (56.5% in Sebeta), Tadesse et al. (2017) (40.9% in Wolayta), Yilma et al. (2017) (49.23% in Addis Ababa), Tekle and Berhe (2015) (43.78% in Sidama), Tassew et al. (2016) (56% in Kombolcha), Tassew et al. (2017) (39.32% in Asossa), Faris (2016) (11.4% in Afar), and Belayneh et al. (2013) (39.5% in Adama). The prevalence was lower than the 83.1% reported by Tesfaye (2014) in Selalle. These variations in mastitis prevalence among different study areas may be attributed to differences in management practices, hygienic conditions during milking, milking techniques, improper antibiotic use, and other farm-specific risk factors (Mekbib et al., 2010). The relatively high prevalence observed in the current study suggests that mastitis remains a significant constraint to dairy production in the Sebeta area, necessitating improved control measures. The isolation rate of *S. aureus* (17.69%) from mastitic milk samples in this study is consistent with the 15.1% reported by Tadesse et al. (2017) in Wolayta. However, it is lower than the rates reported in Holleta (47.1%) (Mekbib et al., 2010), Sebeta (44.03%) (Sori et al., 2005), Sidama (21.1%) (Tekle and Berhe, 2015), Asella (37.71%) (Abera et al., 2013), Asossa (22.14%) (Tassew et al., 2017), Selalle (40.69%) (Tesfaye, 2014), and Adama (26.8%) (Belayneh et al., 2013). The isolation rate was higher than the 11.4% reported by Faris (2016) in Afar. These geographical variations in *S. aureus* isolation rates indicate that other bacterial and fungal pathogens also contribute significantly to mastitis in the study area, and there is likely spatial heterogeneity in the distribution of mastitis-causing pathogens.

The high resistance to penicillin G (91.3%) observed in this study is consistent with previous reports from Asossa (95.55%) (Tassew et al., 2017), Bahir Dar (95.8%) (Bitewa, 2015), and Jimma (87.2%) (Sori et al., 2011). However, it is higher than the 67.9% reported by Tafa et al. (2011) and 48.9% by Belayneh et al. (2013). The 100% penicillin resistance reported by Tadesse et al. (2017), Faris (2016), and Tassew et al. (2016) in different parts of Ethiopia indicates widespread penicillin resistance in *S. aureus* isolates across the country. This extensive resistance is likely attributable to the widespread and often indiscriminate use of penicillin for mastitis treatment in dairy farms, which has exerted strong selective pressure for the development and dissemination of penicillin resistance genes.

Resistance to oxytetracycline (39.1%) in this study was lower than the rates reported in Asossa (63.41%) (Tassew et al., 2017), Wolayta (69.2%) (Tadesse et al., 2017), Haramaya (54.3%) (Tafa et al., 2011), Bahir Dar (72.2%) (Bitewa, 2015), and Kombolcha (77.4%) (Tassew et al., 2016). However, it was higher than the 0% resistance reported by Sori et al. (2011) in Jimma, where all isolates were susceptible to tetracycline. This variability may reflect differences in antimicrobial usage practices among dairy farms in different regions.

Notably, all isolates in this study were susceptible to vancomycin, oxacillin, gentamycin, and cefoxitin. This finding contrasts with several previous reports that documented resistance to these agents. For instance, Tassew et al. (2017), Tadesse et al. (2017), Belayneh et al. (2013), Tesfaye (2014), and Bitewa (2015) reported vancomycin resistance rates of 56.75%, 56.4%, 17.6%, 70.1%, and 52.4%, respectively. The 100% vancomycin susceptibility observed in our study is similar to the findings of Sori et al. (2011) and Tassew et al. (2016), suggesting regional variations in vancomycin resistance. The complete susceptibility to oxacillin and cefoxitin indicates the absence of methicillin resistance in our study population, as these agents are reliable markers for methicillin resistance (CLSI guidelines).

The high susceptibility to erythromycin (95.7%), sulphamethoxazole/trimethoprim (95.7%), and methicillin (95.7%) observed in our study suggests that these drugs could potentially be effective for treating bovine mastitis caused by *S. aureus* in the study area. However, the presence of one intermediate isolate for each of these antibiotics warrants caution, as continued antimicrobial use may lead to the development of full resistance.

Multidrug resistance was observed in 91.3% of the isolates, which is a concerning finding. The most common resistance patterns were penicillin G + ampicillin (47.82%) and penicillin G + ampicillin + oxytetracycline (43.47%). This indicates that the majority of *S. aureus* isolates in the study area harbor resistance to multiple antimicrobials, which significantly limits treatment options and may lead to therapeutic failures. The development of multidrug resistance is almost certainly a consequence of repeated and indiscriminate antimicrobial use in dairy farms, highlighting the critical need for antimicrobial stewardship programs and the implementation of rational antimicrobial use guidelines.

The absence of methicillin-resistant *S. aureus* (MRSA) in this study population is reassuring; however, the presence of an intermediate isolate for methicillin and the widespread antimicrobial resistance patterns observed should serve as an early warning signal. The emergence of MRSA in livestock populations has been documented in many countries (Vishnupriya et al., 2014), and Ethiopia is not immune to this threat. Continued surveillance is essential to monitor the emergence of MRSA and to inform evidence-based antimicrobial use policies.

The high prevalence of mastitis, the substantial isolation rate of *S. aureus*, and the concerning antimicrobial resistance patterns identified in this study have significant implications for both animal health and public health. The presence of drug-resistant *S. aureus* in milk samples may increase the risk of transmission to humans through consumption of unpasteurized milk or direct contact with infected animals, posing a potential zoonotic threat (Normanno et al., 2007). Additionally, these resistant strains

can serve as reservoirs for antimicrobial resistance genes, contributing to the global challenge of antimicrobial resistance.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated a high prevalence (65%) of bovine mastitis among dairy cows in and around Sebeta town, Ethiopia, with *Staphylococcus aureus* isolated from 17.69% of mastitic milk samples. The antimicrobial susceptibility testing revealed considerable resistance to penicillin G (91.3%), ampicillin (91.3%), and oxytetracycline (39.1%), while all isolates were susceptible to vancomycin, oxacillin, gentamycin, and cefoxitin. Multidrug resistance was observed in the majority (91.3%) of *S. aureus* isolates. Importantly, MRSA was not prevalent in this study population.

The widespread antimicrobial resistance observed, particularly to commonly used antibiotics, is alarming and likely reflects the indiscriminate and therapeutic use of antimicrobials in dairy farms. This situation has significant economic and public health consequences, as it may lead to treatment failures, increased production costs, and potential zoonotic transmission of drug-resistant pathogens.

Based on the findings of this study, the following recommendations are made:

1. **Strengthen surveillance programs**: Implement continuous monitoring and surveillance of mastitis pathogens and their antimicrobial resistance patterns in dairy farms to guide evidence-based treatment decisions and detect emerging resistance.
2. **Promote rational antimicrobial use**: Develop and implement antimicrobial stewardship programs in dairy farms, including guidelines for judicious antimicrobial use, to reduce the selective pressure driving the emergence and spread of antimicrobial resistance.
3. **Improve mastitis prevention and control**: Enhance farm-level biosecurity measures, improve milking hygiene, implement proper milking techniques, and cull chronically infected animals to reduce the prevalence of mastitis.
4. **Raise awareness**: Educate dairy farmers, veterinarians, and other stakeholders about the risks of antimicrobial resistance and the importance of responsible antimicrobial use.
5. **Further research**: Conduct molecular characterization studies to identify the genetic determinants of antimicrobial resistance, including the *mecA* gene, and to investigate the genetic relatedness of *S. aureus* isolates between animal and human populations to assess zoonotic transmission risks.
6. **Strengthen laboratory capacity**: Enhance the capacity of veterinary diagnostic laboratories to perform routine antimicrobial susceptibility testing to guide treatment decisions and monitor resistance trends.

Declarations

Animal Ethics Statement: The study protocol was reviewed and approved by the College of Veterinary Medicine, Haramaya University, Ethiopia, through its Animal Research Ethics Committee. All animal handling and milk sample collection procedures were conducted in accordance with institutional

guidelines for the ethical use and care of animals. Prior permission was obtained from the owners of the dairy farms before sample collection.

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