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Prevalence and Antibiotic Resistance of *Staphylococcus aureus* Mastitis in Holeta Area, Western Ethiopia

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Abstract: A cross-sectional study was carried out from November 2014 to April 2015 to determine the prevalence and antimicrobial resistant situation of *Staphylococcus aureus* with assessment of associated potential risk factors at selected dairy farms in Holeta area, western Ethiopia. Purposive sampling technique was employed to select the study sites, farms and animals. Physical examination, California Mastitis Test (CMT), Culture, Biochemical identification tests and Antimicrobial susceptibility tests were used in the study. A total of 384 lactating Holstein cross breed, jersey breed and zebu cows were included and of these, 216 (56.25%) were found to be reactive by CMT and only 21 cows (5.46%) were found clinically positive. A total of 33 (15.3%) isolates were identified via bacteriological analysis of the milk samples. The antimicrobial susceptibility test showed that the isolates were highly sensitive to Gentamycin (97.0%) and Amikacin (97.0%); moderately sensitive to Kanamycin (84.8%), Sulfamethoxazole (78.8%) and Tetracycline (66.7%). Whereas, highly resistant to Penicillin-G (100%). Statistically significant difference was observed ($P<0.05$) between isolates and antibiotic used. This study depicted that antimicrobial resistant *Staphylococcus aureus* was prevalent in the study farms. Thus, improved management options should be practiced in order to minimize the economic loss caused by *S. aureus* mastitis in dairy farms.

Key words: Antimicrobial susceptibility Test · Holeta · Lactating cows · Milk · *Staphylococcus aureus*

INTRODUCTION

In Ethiopia, the number of intensive and semi intensive dairy farms have been increasing from time to time due to urbanization, increased human population and income growth. However, the management practices of these dairy farms remained traditional. In such dairy farms, mastitis is the predominant disease. Mastitis is the inflammation of the mammary gland mainly due to a bacterial infection and it is characterized by a variety of local and systemic symptoms. Mastitis could be prevented by implementing proper animal health management systems. But, most of the emerging dairy farms in Ethiopia lack optimum management practices and are predisposed to mastitis [1].

Staphylococcus aureus (*S. aureus*) is an important cause of clinical mastitis in dairy cows causing a huge economic loss worldwide [2]. Reports from Ethiopia also indicate that, *S. aureus* is the most predominant cause of mastitis in dairy cows [3]. *S. aureus* can express a wide array of potential virulence factors, including surface proteins that promote adherence to damaged tissue and/or exotoxins and enzymes that can cause a variety of infections in skin and soft tissues, including intramammary mastitis [4]. Some evidence suggests that biofilm formation can be a virulence factor associated with *S. aureus* mastitis [5]. Furthermore, this organism can display resistance to several relevant antibiotics, making its eradication difficult [6].

The cure rate after antimicrobial treatment of clinical *S. aureus* mastitis is very variable due to both cow and bacterial factors such as parity of the cow, chronicity of the infection and bacterial genotype [7]. To approach the appropriate treatment and control measures for bovine mastitis, it is important to study the antimicrobial resistance mechanism and epidemiology of *S. aureus* infections.

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Currently, in Ethiopia especially in the central highlands where most of the dairy farms are found, the information on prevalence and distribution of S. *aureus* mastitis and the sensitivity of commonly used antimicrobials for treatment of *S. aureus* mastitis is scarce [8]. Thus, the study was designed to determine the prevalence and antimicrobial resistant of *S. aureus* and associated risk factors at selected dairy farms in western Shoa zone, Ethiopia.

MATERIALS AND METHODS

Sample Collection and Preparation: Using Purposive-sampling technique, 385 lactating cows kept under intensive, semi intensive and extensive management systems from western Shoa Zone, Ethiopia, were screened for mastitis using California mastitis test (CMT) [9] from November 2014 to April 2015. Milk samples from CMT positive cows were collected aseptically and transported immediately under cold chains to the Holetta Agricultural Research Center, animal biotechnology laboratory.

Isolation and Identification of Staphylococcus Species: For bacteriological analysis, milk samples were cultured according to the procedures described by Quinn *et al*. [9]. A loop full of milk sample collected from each infected quarters was inoculated into MacConkey agar and blood agar base enriched with 7% ovine blood. The inoculated plates were then incubated aerobically at 37°C for 24 to 48h. Identification of bacteria on primary culture were identified as *S. aureus* according to the following scheme: Gram-positive cocci, hemolytic on sheep blood agar, catalase-positive and coagulase-positive and oxidation and fermentation of mannitol [9] and OIE [10].

Antimicrobial Testing: The resistance profile of the isolated *S. aureus* was determined using Kirby-Baur disc diffusion method on Mueller-Hinton agar (Sigma-Aldrich Corp., St. Louis, MO, U.S.A.) following the procedures described by P. J. Quinn*, et al.* [9]. Then antibiotic impregnated paper disc (Oxoid, UK) were applied and pressed onto the plate with forceps. Plates were incubated at 37°C for 18 hrs. The diameters of zones of growth inhibition were measured in millimeter and interpreted as sensitive, intermediate and resistant to different antibiotics as per the procedure of P. J. Quinn*, et al.* [9]. The drugs used were Penicillin-G (10U), Tetracycline (30µg), Gentamycin (10µg), Erythromycin (15µg), Kanamycin (30µg), Sulfamethoxazole (300µg) and Amikacin (30µg).

Data Analysis and Interpretation: The collected data were analyzed using SPSS version 20. Descriptive statistics was used to determine the prevalence of antimicrobial resistant *Staphylococcus aureus* and Chi-square test (χ^2) was used to assess associated risk factors. In all the analyses, confidence level was held at 95% and $P<0.05$ was set for significance.

RESULTS

Prevalence of Mastitis: From 384 lactating cows of Holstein X Boran crosses, jersey and highland zebu cattle examined for mastitis using CMT reagent, 216 (56.3%) were positive for CMT (+1, +2, +3) and 21 (5.46%) were diagnosed for clinical mastitis (Table 1).

Prevalence of *Staphylococcus aureus:* Out of 216 CMT positive milk samples, *Staphylococcus aureus* was isolated only from 33 samples. Thus, the overall prevalence of *Staphylococcus aureus* was found to be 15.3% (33). The prevalence varied among farms; highest (13.5%) in farm 1 which have Jersey cows and lowest (5.55%) in farm 2 which have Holstein X Boran crosses (Table 1).

The 33 *S. aureus* isolates were further tested for drug sensitivity to different antibiotics. The antibiotics showed different sensitivity to the bacteria. Gentamycin and Amikacin showed high potency (100%) whereas Penicillin-G showed lowest potency (0%). The antibiotics used for *S. aureus* isolates showed different potency level

Table 1: Prevalence of *S. aureus* among Clinical and Subclinical Mastitis Lactating Cows at selected dairy farms of Holeta area from November 2014 to April 2015

Farms	No of Screened Animal	Clinical Mastitis	(%)	Subclinical Mastitis	(%)	*S. aureus* Isolates	(%)
1	126	5	3.96	67	53.17	17	13.49
2	144	6	4.16	85	59.02	8	5.55
3	42	3	7.14	15	35.71	3	7.14
4	30	2	6.66	19	63.33	2	6.66
5	42	5	11.9	19	45.23	3	7.14
Total	384	21	5.46	195	50.8	33	8.59

Table 2: Results of associated risk factors with the Occurrence of antibiotic resistant *S. aureus* mastitis at selected dairy farms in Holeta area

Risk Factors	No of examined Lactating cows	No of Positive	X^2	P-value
Age Group (Year)				
2-4 years	16	4	0.429	0.333
5-8 years	62	20		
>8 years	36	9		
Parity in number				
1-2	37	10	0.791	0.421
2-4	43	14		
>4	34	9		
Body Condition Score				
Good	53	17	3.215	0.482
Medium	41	8		
Poor	20	8		
Production Type				
Intensive	43	17	4.1	0.079
Semi-intensive	61	13		
Extensive	10	3		
Average Production Potential				
1-3Lt	24	11	4.358	0.44
3-6 Lt	54	14		
>6 Lt	36	8		
Type of Vet. Services				
Advanced	82	25	0.429	0.333
Medium	7	2		
Poor	25	6		
Breed				
Jersey	43	17	4.1	0.079
HF cross	61	13		
Local	10	3		
Types of Mastitis				
Clinical	19	6	0.077	.205
Subclinical	95	27		

Table 3: Sensitivity of different antibiotics to *S. aureus* isolates

Drugs Used		No	X^2	P-value
Penicillin(10u)	S	0	120.43	0.00
	M	4		
	R	29		
Tetracycline (30µg)	S	22	125.48	0.00
	M	8		
	R	3		
Gentamycin(10µg)	S	32	128.54	0.00
	M	1		
	R	0		
Erythromycin(15µg)	S	30	127.66	0.00
	M	2		
	R	1		
Sulfamethoxazole (300µg)	S	26	126.71	0.00
	M	6		
	R	1		
Kanamycin (30µg)	S	28	127.03	0.00
	M	4		
	R	1		
Amikacin (30µg)	S	31	127.88	0.00
	M	2		
	R	0		

Where S=Sensitive, M = Intermediate, R= Resistant

and a statistical significant difference was observed ($P<0.05$) between the isolates and antibiotics used (Table 3).

DISCUSSION

Bovine mastitis is considered as the most common and economically significant disease in dairy animals worldwide [11]. Losses occur from decreased milk production, treatment and labor costs, risk of culling or death of the cow and reduced milk quality and milk price [12]. Furthermore, milk from mastitis cow can contain pathogens and their toxins, which may have hazardous effects for human health. *S. aureus* is a major cause of mastitis in dairy cows causing a huge economic loss worldwide [2]. The organism is resistant to most antibiotics like penicillin and has become a challenge to the dairy industry [13]. Thus, continuous surveillance and monitoring of its prevalence in dairy animals and its antibiotic resistance patterns have a paramount importance for control and prevention of mastitis.

The present study showed an overall mastitis prevalence of 56.3% as determined by the CMT and clinical examinations of the udder at selected dairy farms in and around Holeta. This finding is comparable with that of H. Sori, *et al.* [14] who reported (52.8%) in Sebata area and lower than that of finding of B. Mekibib, *et al.* [15] who reported (71.0%) in Holeta. However, it is higher than the finding of M. Bitew, *et al.* [16] who reported (8.20%) and (5.20%) in Bahir Dar town dairy farms and Gondar town dairy farms, respectively. These differences could be attributed to difference in milking practices, management systems, breeds and location. The prevalence of the disease in the present study in different farms is quite different among farms ranging from 63.33 to 35.7%. This could be due to difference in hygienic status, breed, milking system and house of the farm. In the same manner, the prevalence rate (5.46%) for clinical mastitis obtained in this study area was comparable with the finding reported across different parts of Ethiopia like, G.A. Enyew [17] (3.9%) from Bahir Dar. However, the present finding is lower than the report made by G.D. Delelesse [18] (10.3%) around Holeta area; S. Workineh, *et al.* [19] (25.1%) in Addis Ababa and M. Alemnew [20] (21%) in Modjo district. As mastitis is a complex disease involving interactions of various factors such as animal management and husbandry, environmental conditions, animal risk factors and causative agents, its prevalence will vary [21].

In the present study, higher proportion of *S. aureus* was isolated from CMT positive cows kept in poor housing (Muddy) conditions compared to cows kept in clean and dry environments. This could be because *S. aureus* is environmentally very robust, surviving wide extremes of temperature and moisture [22]. The organism also readily colonizes teat orifices, damaging roughened epithelium. The main source of the infection is the udder of infected cows transferred via milker's hands, utensils, towels and the environment (Floor) in which the cows are kept [13]. Thus, the prevalence of *S. aureus* in dairy farms who use machine milking was significantly ($p < 0.05$) lower than in farms that use hand milking.

From 216 milk samples subjected to bacteriological examination, 33 (15.3%) were *S. aureus* isolates. This finding is in agreement with other studies [3,23,24] reported in Ethiopia, in which *S. aureus* was the predominant isolate from clinical and subclinical mastitis. Besides, mastitis caused by *S. aureus* has been reported from different countries with prevalence ranging from 5% up to 70% of cows and 90% of herds [13]. It does appear that these levels are decreasing due to the improvement of health management of dairy cows, but eradication is far to be achieved [25]. The high prevalence of S. *aureus* might be attributed to the wide distribution of the organism inside mammary glands and on the skin of teats and udders. *S. aureus* has adapted to survive in the udder and establish chronic, gangrenous and subclinical infections. From there, it is shed into the milk, which serves as a source of infection for healthy cows during the milking process [21].

Besides its effects in diary production, *S. aureus* is also regarded as the third most important factor causing food-borne diseases [26]. However, the treatment, control and prevention of this infection often fail due to the development of antibiotic resistance mechanisms by different *S. aureus* strains. The development of drug resistance is a significant feature of this organism. The present study reveals that the susceptibility of *S. aureus* to penicillin was 0%, Tetracycline (66.7%), Sulfamethoxazole (78.8%), Kanamycin (84.8%), Erythromycin (90.9%), Gentamycin and Amikacin (97.0%) and the average susceptibility of *S. aureus* was 73.6%. The current finding disagrees with H. Mekonnen, *et al.* [8] who reported 62.7% as an average susceptibility of *S. aureus* in Ethiopian dairy farms.

In this study, *S. aureus* showed 100% resistance to Penicillin-G. This is in parallel with the previous findings conducted in Holeta by A. Tesfaye, *et al.* [27] who indicated the *S. aureus* isolates were resistant to penicillin-G. Some reports from India also indicated the presence of high rate of antibiotic resistance patterns for Penicillin-G. This might be explained by the expression of inducible â-lactamase enzyme that is found encoded by the *BlaZ* gene in *S. aureus*, which causes hydrolysis of the â-lactam ring of penicillin [28]. Impaired treatment response has been associated with penicillin resistance of the infectious *S. aureus* strain [14,29]. However, the connection is not straightforward, which may indicate that some other bacterial factors could be involved in the phenomenon [30]. In accordance with this finding, higher sensitivity for Gentamicin and Amikacin has also been reported by A. Tesfaye, *et al.* [27] in Holeta. This might be due to high sensitivity of these drugs to Gram positive bacteria but factors like treatment factor, animal factor and storage of drug might decrease their sensitivity.

In general, for the selection of effective therapeutic agent against bovine mastitis, antibiotic sensitivity test has been widely used in many countries. However, it has been difficult to judge the clinical efficacy of an antimicrobial agent solely based on *in vitro* test. This is because, the presence of variations in response among

individual animals and among herds, due to the type of strain of an organism involved, location of infected sites, degree of udder indurations, physico-chemical properties and kinetic behavior of antibiotics in udder and milk and site of injection.

CONCLUSIONS

Generally, *S. aureus* mastitis was prevalent in both clinical and subclinical Bovine mastitis at the selected dairy farms in and around Holeta. Consequently, *in vitro* antimicrobial sensitivity test indicated both resistivity and sensitivity to some drugs. Higher prevalence of antimicrobial resistant *S. aureus* was isolated in the dairy farms of highly condensed cows with poor milking hygiene and poor environmental hygiene. Thus, routine improved management system should be practiced in order to design effective prevention and control methods. Moreover, further studies should be conducted to isolate the antimicrobial resistant strains and genes of *S. aureus* in study area.

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