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Research Article

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Prevalence and Antimicrobial Susceptibility of Staphylococcus Aureus And Escherchia Coli Isolates of Bovine Mastitis And Associated Risk Actors in Shashemene Town, Ethiopia

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Abstract

The study was conducted from January 2021-July 2021 to assess the prevalence, antimicrobial susceptibility of S. aureus and E. coli isolates of bovine mastitis and associated risk actors in Shashemene town. In this study, following clinical examination and CMT screening test conducted on purposely selected 76 lactating cows. Milk samples from CMT-positive cases were aseptically collected and S. aureus and E .coli isolates were identified. Finally, an antibiotic sensitivity test was conducted on isolates. The overall prevalence of mastitis was 63.16% (n=48) of which 9 (11.84%) and 39 (51.32%) were clinical and subclinical mastitis. From a total of milk samples collected per cow, 22 (45.83%) S. aureus and 7 (14.58%) E. coli were isolated. S. aureus was resistant to Penicillin G (77.3%), Ampicillin (90%), Oxytetracycline (50%), and Erythromycin (54.5%) while E. coli isolates were found to be 100% resistant to penicillin G, Amoxicillin, Ampicillin, followed by Streptomycin (85.7%) and Cefotaxime (57.1%). The key factors associated with mastitis and drug resistance are parity, age, house hygiene, udder hygiene, and related management practices. In conclusion, the present study indicated that mastitis is a great problem in the study area and multi-drug resistant isolates are circulating. Hence, the factors contributing to the high prevalence and drug resistance developments need to be avoided in dairy farms. On top of this, alternative treatment options and drug preparations need to be considered for the treatment of bovine mastitis.

Key words: Bovine Mastitis, S. Aureus, E. Coli, Drug Resistance, Shashemene

Introduction

Ethiopia is one of the most populous countries in Africa that depends on agriculture. Livestock represents a main national resource and forms an essential part of the agricultural production. Ethiopia has the largest cattle population with an estimated 56.71 million cattle, of which 20.7% of them are milking cows [1]. However, milk production does not fulfill the demands due to different reasons which include the low genetic potential of local breeds on milk production, the extensive and low inputs farming practices and widespread livestock diseases [2].

Mastitis is among the costly diseases that cause loss of milk production, cost of treatment and unaffordable culling of dairy cattles. It is the major cattle health problem[3] and considered as a complex disease with multifactorial causation [4, 5]. It is an infection of the udder, caused usually by bacteria that enter through the teat end to the quarter and cause pathological changes in glandular tissues [6].

The predominant microorganisms that cause mastitis and milk spoilage are Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis, Mycoplasma species, Streptococcus uberis coliforms (E. coli, Klebsiella species and Enterobacter aerogenes), Serratia, Pseudomonas, Proteus species, environmental Streptococci, Enterobacter species [7, 8]. In addition to reducing milk quality and quantity many of them are accountable for zoonotic diseases in humans [5].

Among mastitis-causing bacteria, S. aureus represents the main causative agent that threatens the farmers not only due to the high incidence rate and zoonotic potential but also due to reports of multi-drug resistance. Whereas E. coli are is among the gram-negative bacteria that usually cause subclinical mastitis affecting the performance of dairy on top of its public health risks. The recurrent treatment failure due to antibiotic-resistance is also associated with sever human infection [9]. The studies in Ethiopia showed presence of multidrug resistant S. aureus (31.3-83%) and E. cli (26.1-72%) isolated from bovine mastitis and the recent report showed a very concerning increasing trend. As a result, mastitis remains to be main challenge impairing the development of the dairy industry in Ethiopia. In this regard, investigating the current status of the disease, antibiotic resistance profile and determining the risk factors associated with the spread of antibiotic resistance is very important. Therefore the objective assesses the prevalence, antimicrobial susceptibility of S. aureus and E. coli isolates of bovine mastitis and associated risk actors in Shashemene town [10-14].

Materials and Methods

Study Area

The study was conducted in Sheshemene town, West Arsi Zone of Oromia regional state, Ethiopia. Sheshemene town is found at 250km distance from Addis Ababa at 70 6' 40" to 70 23' 20" latitude and 380 28' 0" to 380 48'0" longitude and elevations ranging from 1500 to 2300m above sea level. Shashemene town is the administrative center of the West Arsi zone. There is mixed crop-livestock farming system. The livestock in the area: 3,352,768 Cattle, 1,302,776 Sheep and Goat, 497,509 Horse, Mule, Donkey and 1,174,292 poultry [15].

Study Design

A cross-sectional study conducted between January 2021 to July 2021 to assess the prevalence, antimicrobial susceptibility of S. aureus and E. coli isolates of bovine mastitis and associated risk actors. The dairy cattle kept under the small-scale dairy farm in Shashemene town were identified purposively and farm owners were interviewed about the farm management as well as other mastitis-related issues. The animal age, parity, breed, management, milking practice, house hygiene and herd size were recorded for each case. 76 individual lactating cows were randomly selected and clinically examined. Following general clinical examination, primary screening was conducted using the CMT test and CMT positive milk samples were collected and transported to the microbiology laboratory at Hawassa University Faculty of Veterinary Medicine for further microbiological analysis. The antibiotics sensitivity tests were conducted on the S. aureus and E. coli isolated from bovine mastitis.

California Mastitis Test (CMT)

CMT were conducted to determine the prevalence of sub-clinical mastitis and to screen the selection of samples to be cultured. Briefly, 2ml milk sample from each quarter of 76 cows was collected into a four plastic paddle marked A, B, C and D and mixed with equal amount of CMT reagent. CMT positive cows were screened according to standard procedure [8].

Sample Collection, Bacteria Isolation and Identification

Milk samples (5 ml) from CMT-positive cows were collected into sterile universal bottles and transported using an ice-box to Hawassa University, Faculty of Veterinary Medicine, Microbiology Laboratory and cultured on standard bacteriological media [16]. Isolation and identification were done according to the [8] to identify S. aureus and E. coli: the two common bacterial that has been reported to develop drug resistance. Briefly, a loop full of milk samples were streaked onto sterile blood agar plates and MacConkey agar plates using the quadrant streaking method. Then the plates were incubated aerobically at 37°C for 24-48 hours and they were examined for growth, morphology, pigmentation and hemolytic characteristics of the colonies.

For identification S. aureus colonies characterized in primary culture were subcultured onto selective and differential media (Mannitol Salt Agar (MSA) plates) and incubated at 370C aerobically for 24 - 48 hours. The presence of growth and color change from red to yellow within below 24hours were regarded as confirmative identification of the salt-tolerant Staphylococci which ferment mannitol. Those colonies of pathogenic Staphylococci which grew on Mannitol Salt Agar (MSA) and fast fermenter of mannitol which develop yellow discoloration of the medium were suspected as S. aureu s [8].

Isolation of E. coli was done by characterizing their hemolytic pattern on blood agar plates and lactose fermentation on MacConkey agar plates. Primary identification of E. coli was done using biochemical tests which include catalase, oxidase, and motility test using growth on Eosin methylene blue (EMB) agar plates. Further identification of pathogenic E. coli was done using indole test, methyl red test, Voges-Proskeur test and citrate utilization test (I/MR/VP/C) according to the protocol [16].

Antimicrobial Sensitivity Test

Antimicrobial sensitivity test was conducted using the agar disc diffusion method. Briefly S. aureus and E. coli isolates from bovine mastitis were inoculated into the nutrient broth and suspension was made in sterile saline. Turbidity of the bacterial suspension was adjusted by comparing with 0.05 McFarland turbidity standards and plated on Mueller- Hinton agar. In this study, Oxytetracycline, Penicillin G, Streptomycin, Chloramphenicol, Kanamycine, Amoxicillin, Erythromycin, Ampicillin and Cefoxatime were used. These antimicrobials were selected based on the frequent use for the treatment of clinical bovine mastitis and other diseases in dairy farms. After incubation at 370C for 24hrs, the diameter of the zone of inhibition (in millimeter) was measured using a caliper for each antibiotic discs and results were recorded as susceptible, intermediates or resistant by comparing with standard values for each antibiotic disc [17].

Data Collection

During farm visits, a structured questionnaire was used to collect data about various herd and animal level factors supposed to influence the occurrence of mastitis in the dairy herds and cow levels. The questionnaire was administered to farm owners/attendants through a face-to-face interview. Information on the management system, udder washing practices, house cleaning, disinfection of teat, cleaning udder, isolation of infected cow, more affected breed, were recorded.

Data Management and Analysis

The data obtained from questioners and laboratory results were stored in an Excel spreadsheet. Agents isolated, cows with mastitis and its associated risk factors were analyzed by descriptive statistics. STATA 14 was used for multiple comparisons. Chi-square tests followed by Logistic regression models were used to test the association between mastitis, S. aureus, E. coli, and risk factors analysis.

Results

Mastitis and Associated Risk Factors

From a total of 76 lactating cows, 48 (63.16%) cows were found to be affected with mastitis of which 39 (51.32%) and 9 (11.84%) were subclinical mastitis and clinical mastitis respectively (Table 1). The result at the teat quarter level also showed that compared to the others, the left rear quarters were affected with the highest infection rate (44.74%) followed by the right rear quarters (30.26%) (Table 2).

Table 1: Clinical and Subclinical Mastitis at Cows and Quarter Level

Type of mastitis	Total examined cows	Total affected cows	Total examined quarter	Total affected quarter
Clinical	76	9 (11.84%)	304	18 (6%)
Subclinical	76	39 (51.32%)	304	66 (21.7%)
Total	76	48 (63.12%)	304	84 (27.6%)

Table 2: Quarter Level Mastitis

Quarters	Examined quarters	Affected quarters
Right front	76	12 (15.79%)
Right rear	76	23 (30.26%)
Left front	76	15 (19.74%)
Left rear	76	34 (44.74%)
Total	304	84(27.6)

In this study, various possible risk factors at cow and farm levels were evaluated for their effect on the prevalence of mastitis. The association of mastitis with parity, age, house hygiene, udder washing, and management level and herd size was statistically significant. The study also revealed a significantly highest prevalence of mastitis in cows having more than five calves (Table 3).

There was also a significant association between house hygiene and mastitis. There was a lower infection of mastitis in cows kept in good house hygiene (26 (51%)) while having more in cows kept in poor house hygiene (22 (88%)). Washing of the cow's udder before milking has also significantly reduced the prevalence of mastitis. On the contrary, the prevalence of mastitis was not significantly influenced by the breed of cows; however, there was a higher prevalence in exotic breeds than crossbreeds. It has been also noted that there are no farmers that disinfect the cow's teat before and after milking (Table 3).

Table 3: Association of Different Risk Factors With Cow's Mastitis In The Study Area

Risk factor	Category	Number of animal examined	Number of positives (%)	Univariable analysis		Multi-variable analysis	
				COR(95% CI)	p value	AOR(95% CI)	p value
Age	≤4year	19	9(47.4)	Ref	0.02	Ref	0.4
	5-6year	35	20(57.1)	0.25(0.05-0.8)		0.51(0.08-2.9)	
	≥7year	22	19(86.4)	1.5(0.5-4.55)	0.028	1.4(0.31-5.9)	0.6
Management system	Semi-intensive	14	5(35.7)	Ref		Ref	
	Intensive	62	43(69.35)	4.07(1.2-13.8)	0.018	4.8(1.07-21.7)	0.04
Parity	1-2	23	9(39.1)	Ref			
	3-4	42	29(69)	0.20(0.08-0.7)	0.022	0.3(0.03-3.18)	0.048

	≥5	11	10(90.9)	3.4(1.2-10.04)	0.007	2.1(0.67-6.73)	0.03
Breed	Exotic	39	27(69.23)	1.71(0.67-4.4)	0.260	1.9(0.57-6.6)	0.29
	Cross	37	21(56.76)	Ref		Ref	
Herd size	>10	50	36(72)	3(1.12-8.05)	0.027	3.7(0.95-14.4)	0.05
	≤10	26	12(46.15)	Ref		Ref	
Teat disinfection	Yes	0	-				
	No	76	48(63.16)				
House hygiene	poor	25	22(88)	7(1.87-26.5)	0.002	1.9(0.12-28.8)	0.6
	Good	51	26(51)	Ref			
Udder washing	No	24	22(91.7)	11(2.34-51.6)	0.000	6.9(1.4-34.79)	0.019
	Yes	52	26(50)	Ref			
	Ref						
Isolation of mastitis cow	No	48	33(68.75)	0.5(0.20-1.37)	0.186	0.53(.12-2.4)	0.4
	Yes	28	15(53.6)	Ref		Ref	

COR= Crude odd ratio, CI= Confidence interval, Ref= Reference

Co-linearity was also checked between variables and the results of the multi-co-linearity matrix revealed that all independent variables were not collinear with each other (r < 0.5) except house hygiene versus udder washing (gamma = 0.85). The independent variables entered into the multivariable model were parity, age, management system, udder washing and herd size of mastitis. Thus, considering p-value, non-collinearity, and biological importance of variable, the following variables were selected for entry into the multivariable model: parity, udder washing, and management system. The final multivariable logistic regression model of risk factors analysis revealed that management (OR = 4.82, CI= 1.07- 21.7, p < 0.05 for cows managed intensively), parity (OR=2.1, CI=0.67-6.73, p < 0.051for cow having more than five calves) and udder washing (OR= 6.9, CI=1.44-34.79, P < 0.05 for cows their teat were didn't wash very well during milking), had a significant association with mastitis and hence are independent predictors (Table 3).

Isolation and Identification of *S. aureus* and *E. coli*

From a total of 48 cow milk samples collected, 22 (45.83%) and 7 (14.58%) were found to be positive for S. aureus and E. coli, respectively while 19 out of 48 (39.6%) milk samples were cultures negative. Out of 9 clinical mastitis cows and 39 CMT-positive subclinical mastitis cows 5(55.6%) and 17 (43.6%) S. aureus was observed while 1 (11.1%) and 6 (15.4%) of E. coli were isolated respectively. 8.3% of mastitis milk samples were having mixed infections of S. aureus and E. coli (Table 4).

Table 4: Number and percentage of S. aureus and E. Coli Isolated From Clinical Mastitis Cases And CMT Positive Subclinical Mastitis Cows

Types of mastitis	Examined cows	Number of *S.aureus* isolated	Number of *E.coli* isolated	Number of mixed isolates
Clinical	9	5(55.6%)	1(11.1%)	1(11.1%)
Subclinical	39	17(43.4%)	6(15.4%)	3(7.7%)
Total	48	22(45.8%)	7(14.6%)	4(8.3%)

Risk Factors Associated with the Occurrence of *E. coli* and *S. aureus*

The effect of specific risk factors on the prevalence of S. aureus and E. coli was investigated using the Chi-square test and logistic regression analysis. The factors significantly associated with the occurrence of S. aureus are age, parity, breed, herd size, house hygiene and udder washing. While management, parity, house cleaning and udder washing were significantly associated with the occurrence of E. coli (Table 5).

Table 5: Association of cows and farm risk factor with isolation of S. aureus

Risk factor	Category	Number of animal examined	Number of S.Aureus isolated (%)	COR(95% CI)	X^2	p value
Age	≤4year	9	1(11.1)	Ref	12.7	0.002
	5-6year	20	7(35)	0.13(0.03-0.56)		
	≥7year	19	14(73.7)	3.27(0.34 -31.9)		
Management	Intensive	43	18(41.86)	Ref	2.62	0.105
	Semi intensive	5	4(80)	5.55(0.57-53.9)		
Parity	1-2	9	1(11.1)	Ref	9.55	0.008
	3-4	29	12(41.4)	0.16(0.03-0.86)		
	≥5	10	9(90)	4.94(0.54-45.6)		
Breed	Exotic	27	17(63)	5.44(1.52-19.4)	7.29	0.007
	Cross	21	5(23.8)	Ref		
Herd size	≤10	12	3(25)	Ref	7.1	0.008
	>10	36	19(52.7)	6.3(1.5-26.73)		
Teat disinfection	Yes	0				
	No	48	22(45.8)			
House hygiene	Good	26	7(26.9)	Ref	8.7	0.004
	Poor	22	15(68.2)	5.82(1.67-20.2)		
Udder washing	Yes	26	8(30.8)	Ref	5.2	0.023
	No	22	14(63.6)	3.94(1.18-13.1)		
Isolation of mastitis cow	Yes	15	7(46.7)	Ref	2.1	0.09
	No	33	15(45.45)	1.05(0.30-3.57)		

COR= Crude odd ratio, X^2 = chi square, CI= Confidence interval, Ref= Reference

Multivariate logistic regression analysis between the isolation of S. aureus and associated risk factors were shown after removing a variable which has an insignificant effect from univariable logistic analysis (Management system, Isolation of mastitis infected cow) and Co-linearity was checked between variables. Accordingly, age was dropped from further analysis due to co-linearity with parity (gamma = 1) while udder washing was dropped due to co-linearity with house hygiene and herd size (gamma = 0.9, 0.71 respectively). Thus, the variables subjected to multivariable logistic regression analysis were herd size, breed, and house hygiene. Accordingly, the final logistic regression model revealed that all the variables entered remained significant predictors of isolation of S. aureus from mastitis cows ($p < 0.05$). The final model showed that larger herd size (OR = 9.67, CI = 1.6-58.2, $p < 0.013$), exotic breed (OR = 11.4, CI= 1.89-68.6, $p < 0.008$) and poor house hygiene (OR = 14.9, CI= 2.36- 94.5, $p < 0.004$) were significant factors associated with isolation of S .aureus from mastitis cow (Table 6).

Table 6: Multivariate Logistic Regression Analysis of Associated Risk Factors with S. aureus Isolates

Risk factor	Category	Number of animal examined	Number of S. *Aureus* isolated (%)	COR(95% CI)	AOR(95% CI)	p value
Breed	Exotic	27	17(63)	5.4(1.52-19.4)	11.6(1.9-68.6)	0.008
	Cross	21	5(23.8)	Ref	Ref	
Herd size	≤10	12	3(25)	Ref	Ref	0.013
	>10	36	19(52.7)	6.3(1.5-26.73)	9.66(1.6-58.2)	

house hygiene	good	26	7(26.9)	Ref	Ref	0.004
	Poor	22	15(68.2)	5.82(1.7-20.2)	14.9(2.4-94.5)	
constant					0.05(0.00-0.3)	0.002

AOR= Adjusted odd ratio, COR= Crude odd ratio, CI= Confidence interval, Ref= Reference

Table 7: Association of Cows and Farm Risk Factor With Isolation of E. coli

Risk factors	Category	Number of animal examined	Number of E.coli isolated (%)	COR(95% CI)	X^2	p value
Age	≤4year	9	1(11.1)	Ref	0.11	
	5-6year	20	3(15)	1.11(0.1- 12.5)		0.95
	≥7year	19	3(15.8)	1.4(0.12-15.9)		0.786
Management	Intensive	43	43	Ref	9.2	0.002
	Semi intensive	5	5	14.6(1.9-115.2)		
Parity	1-2	9	1 (11.1)	Ref	11.2	0.034
	3-4	29	1(3.4)	0.25(0.013-4.5)		0.004
	≥5	10	5(50)	5.8(0.52-64.8)		
Breed	Exotic	27	5 (18.5)	0.46(0.08-2.67)	0.8	0.381
	Cross	21	2(9.5)	Ref		
Herd size	≤10	12	1(8.3)	Ref	4.1	0.488
	>10	36	6(16.7)	2.2(0.24-20.4)		
Teat disinfection	Yes	0				
	No	48	7(14.6)			
House hygiene	Good	26	1(3.8)	Ref	5.3	0.047
	Poor	22	6(27.3)	9.38(1.03-85.3)		
udder hygiene	Yes	26	1(3.8)	Ref	5.3	0.022
	No	22	6(27.3)	9.37(1.03-85.3)		
Isolation of mastit cow	Yes	15	2(13.3)	Ref	2.7	0.08
	No	33	5(15.15)	1.16(0.198-6.8)		

COR=Crude odd ratio, X^2 = chi square, CI= Confidence interval, Ref= Reference

After removing a variable that has an insignificant effect from univariable logistic analysis and Co-linearity was checked between variables, Multivariate logistic regression analysis between the isolation of E. coli and associated risk factors were recorded. Accordingly, age, breed, herd size, and isolation of mastitis diseased cow were excluded due to insignificant effect from univariable logistic analysis while udder washing was removed due to co-linearity with house hygiene and parity (gamma = 0.9, 0.75 respectively). Therefore the final multivariable logistic regression model of risk factors analysis revealed that management and house hygiene were significantly associated with isolation of E. coli from mastitis cow (Table 8).

Table 8: Multivariate Logistic Regression Analysis of Associated Risk Factors with E. coli isolates

Risk factor	Category	Number of animal examined	Number of S.Aureus isolated (%)	COR(95% CI)	AOR(95% CI)	p value
Management	Intensive	43	4(9.3)	Ref	Ref	
	Semi intensive	5	3(60)	14.6(1.9-115.2)	19.5(1.5- 247)	0.022
House hygiene	Good	26	1(3.8)	Ref	Ref	
	Poor	22	6(27.3)	9.38(1.03-85.3)	11.9(0.9-151)	0.049

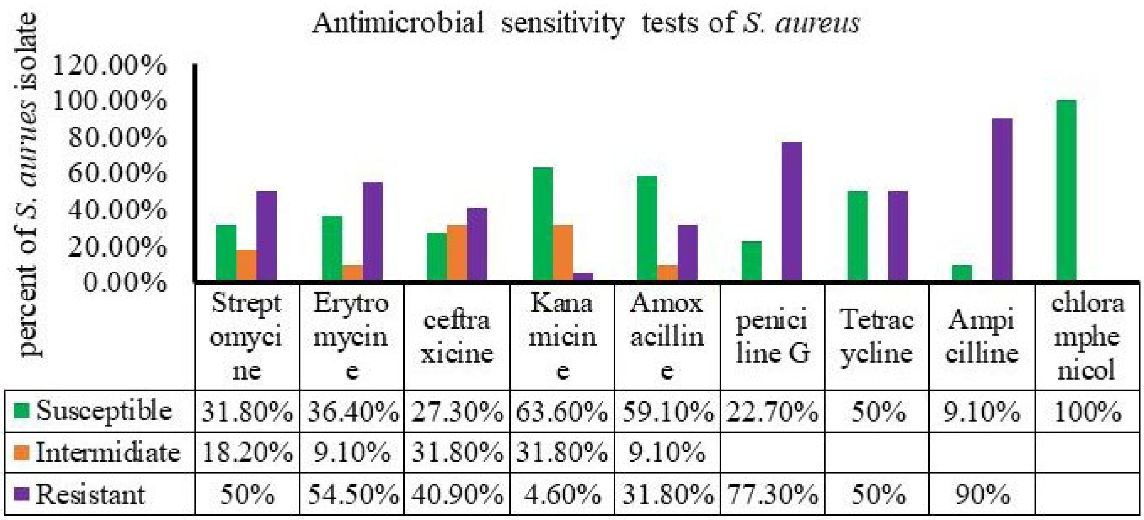
Constant					0.02(0.00-0.2)	0.002

AOR= Adjusted odd ratio, COR=Crude odd ratio, CI= Confidence interval, Ref= Reference

Antimicrobial Susceptibility Tests Result

A total of 22 isolates of S. aureus and 7 isolates of E. coli were tested against 9 commonly used antimicrobial agents following CLSI guidelines. From 22 isolates of S. aureus, all showed 100% susceptibility for Chloramphenicol, similarly susceptible to Kanamycin (63.6%) and Amoxicillin (59.1%). However, 90% of the isolate were resistant to Ampicillin, 77.3% for Penicillin G, 54.5% for Erythromycin, 50% for Oxytetracycline, 50% for Streptomycin and 40.9% for Cefotaxime (Figure 1).

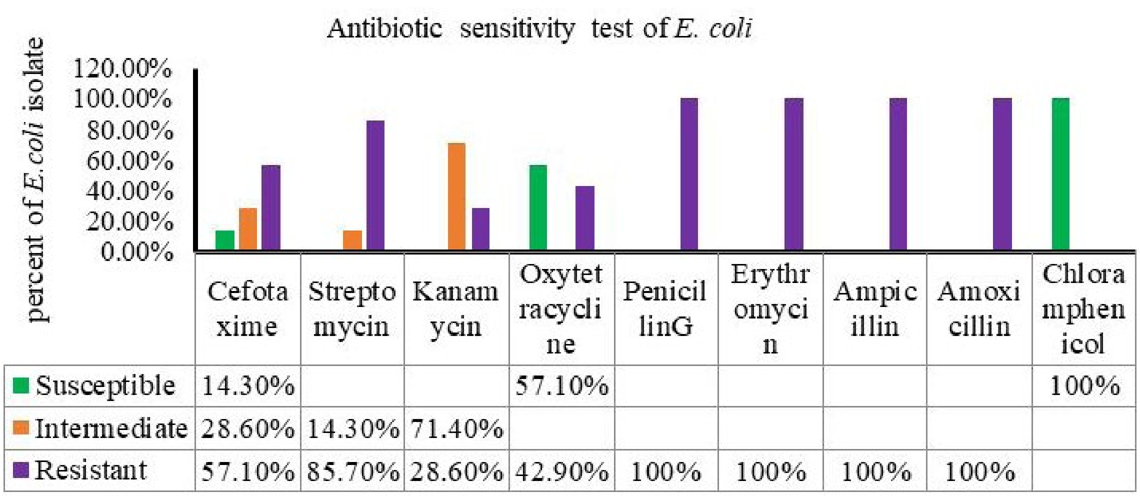
Based on analysis of multidrug resistance patterns of S. aureus isolates (data not shown), 4.45% exhibited resistance to Ampicillin, Cefoxamine, Penicillin, Erythromycin, Streptomycin, Amoxicillin and Oxytetracycline whereas 14.3% isolates of E. coli were shown resistance to Streptomycin, Ampicillin, Kanamycin, Amoxicillin, Erythromycin, Penicillin, Cefoxamine. The most frequent multi-drug-resistant isolates were those exhibiting resistance to Ampicillin, penicillin, Cefoxime at a frequency of 13.64% and Penicillin, Ampicillin, Oxytetracycline, Streptomycin at a similar frequency of 13.64% for S. aureus while in the case of E. coli Streptomycin, Ampicillin, Amoxicillin, Oxytetracycline, Penicillin, Erythromycin were the most frequent of 42.86% multidrug-resistant isolates.



	Streptomycine	Erytromycine	ceftraxicine	Kanamicine	Amoxacilline	penicline G	Tetracycline	Ampicilline	chloramphenicol
Susceptible	31.80%	36.40%	27.30%	63.60%	59.10%	22.70%	50%	9.10%	100%
Intermidiate	18.20%	9.10%	31.80%	31.80%	9.10%				
Resistant	50%	54.50%	40.90%	4.60%	31.80%	77.30%	50%	90%	

Figure 1: Antimicrobial Susceptibility Pattern of S. aureus Isolated from Mastitic Cow's Milk

Out of 7 isolates of E. coli all were highly susceptible to Chloramphenicol (100%) and there was lower susceptibility to Tetracycline (57.1%). However, a total resistance was observed to Ampicillin (100%), Amoxicillin (100%), Erythromycin (100) and penicillin (100), furthermore, different proportion resistance was observed to Streptomycin (85.7%), Cefotaxime (57.1%) and Kanamycin (28.6%) (Figure 2).



	Cefotaxime	Streptomycin	Kanamycin	Oxytetracycline	PenicillinG	Erythromycin	Ampicillin	Amoxicillin	Chloramphenicol
Susceptible	14.30%			57.10%					100%
Intermediate	28.60%	14.30%	71.40%						
Resistant	57.10%	85.70%	28.60%	42.90%	100%	100%	100%	100%	

Figure 2: Antimicrobial Susceptibility Pattern of E. coli Isolated from Mastitis Cow

Discussion

The 63.16% overall prevalence of dairy mastitis in this study is in agreement with the previous 62.6% report from from Hawassa, 62.6% from Eastern Hararghe Zone and 64.3% from Wolaita Sodo respectively [18-20]. However higher than the 37.1% prevalence report before nine year from Shashemene indicates an increasing trend of the disease [21]. The 88.9% highest prevalence has been reported from the North showa zone 71% from Holeta town and 74.7% around Addis Ababa where the dairy industries are blooming [23, 24].

The 51.32% prevalence of subclinical mastitis revealed in this study is comparable with the 51.3% from Akaki kality 46.6% from Holota 54% from Asella and 49.2% from Addis Ababa[25-27]. The prevalence of clinical mastitis (11.84%)) was comparable with the previous 10% prevalence reports [10, 26]. The higher proportion of subclinical mastitis than that of clinical mastitis observed in the present study agrees with numerous previous reports from different parts of Ethiopia [23, 28-30] and Kenya [31]. This happens dues to low attention given under detected infection of sublicinical mastitis and associated milk production loss [32].

The overall 27.6% quarter level prevalence recorded in the present study was comparable with the 29% finding in Adama and 29.4% in southern Ethiopia[18, 28]. Compared to the other quarter, higher infection rate (44.74%) were on the left rear quarters. The right rear quarters were the second with an infection rate of 30.26% followed by left front quarters (19.74%) and right front quarters (15.79%). The rear quarters were affected more than the front quarters. This might be due to the pendulous nature of hind quarters with higher production volume and relaxed teat sphincters[33] and the high unintended fecal and environmental contamination [34].

The prevalence of mastitis was significantly associated with age and parity. The higher prevalence in older cows can be related to muscles of teats with relaxed sphincter that may allow the infection [6, 35]. Kocak also stated that multiparous cows has less protective immunity than primiparous cows [33].

The prevalence of mastitis significantly varies with the management system, house hygiene and udder/teat hygiene. In this study, since washing of udder reduces the load of bacteria that able to enter through the opened teat canal during milking for farmers who do not practice teat washing before and after milking. Lack of dairy house hygiene and and poor practice of cleaning dairy udder are among the key factors that increase prevalence of dairy mastitis [6, 36]. The dairy house drainage system for urine and water, if not properly cleaned, it creates hostile condition for bacteria like E. coli that cause environmental mastitis [37].

Bacterial Isolation

Based on the bacteriological examination, S. aureus was isolated in 45.83% mastitic milk samples [13]. This is comparable with the 44.62% previous findings in and around Asella town 42.1% in Adama town and 47% from dairy farms of Holeta town in Ethiopia [23, 28]. The present finding is higher than the 15.5% reports at Akaki kaliti and 18.5% reported in and around Sululta sub-city [25, 26]. Relatively higher prevalence than the current result were recorded from other parts of Ethiopia [38, 39]. The variation in prevalecnce of S. aureus could be asociated with difference in the dairy production practive and dairy health intervention.

The current study revealed that the prevalence of S. aureus increases as the parity number increases ($P \leq 0.05$). Accordingly, the high prevalence was recorded in cows having greater than five calves (9/10(90%)). This study indicates that the risk of S. aureus associated mastitis increases significantly with advancing parity of the cow. Statistically significant association was detected among age categories ($p \leq 0.05$) with high prevalence recorded in cows greater than seven years old age (14 (73.4%)). This could be due to lower immune systems and relaxed teats sphincter of high milk producing cows [13, 40].

The study also showed poor udder hygiene are more infected S. aureus (63.6%) and this finding is in line with the previous report [13, 41]. Hygienic milking practices are key to avoid the transmission of S. aureus bacteria, washing of teat before milking reduces loads of S. aureus contaminate the udder and reduce the chance of entrance of this bacteria through teat canal [6].

The study also showed that a higher percent of S. aureus was isolated from the exotic and crossbreed. Compelling result was also reported in and around Asella town and at Ambo and Guder town [40, 41]. This can be explained by the breed characterstics, anatomy of the udder for genetically selected cow for high milk yeald in the expence of predisposing to genetic vulnerability to mastitis [6].

E. coli was isolated from 14.58% of the milk samples. The present finding was closely related to the 12% report in Bishoftu [42], 12.91% in Borona pastoral community in Ethiopia and the 11.2% report from Ghana [43, 44]. In contirary, the present report is relatively higher than the 8.20 % report in Hawassa and 3.80 % in Sidama Zone [45, 46]. The higher isolation rate of E. coli from raw milk is apparently because of environmental contaminantion of E. coli due to poor hygiene [6].

According to the current study, the occurrence of E. coli increases with parity number, cow's management system, udder/teat hygiene, housing hygiene. This finding was in aline with the result of Ashenafi et al., [14] who reported high prevalence of E. coli from poor farm hygiene than good farm hygiene. Poor housing hygiene may attribute to the conducive environment as reserviour for E. coli that potentially cause mastitis in dairy cows [47].

Antimicrobial Susceptibility Profiles of Isolates

In vitro antimicrobial susceptibility patterns in this study revealed that S. aureus was found to be resistant to Penicillin G (77.3%), Ampicillin (90%), Oxytetracycline (50%), Erythromycin (54.5%), Cefotaxime (40.9%) and Streptomycin (50%). This finding agrees with the previous report of 76.5% resistance to Penicillin G, 100% resistance to Ampicillin and Tetracycline and 70.6% to Erythromycin from akaki kaliti [25]. Similar resistance profiles were also reported in other parts of the country [13]. The highest rate of susceptibility among the isolates was reported only against chloramphenicol (100%) followed by Kanamycin (63.4%) and Amoxicillin (59.1%). The variation in the susceptibility results could depends on the frequency of usage of a given drug in that area. The resistance for Oxytetracycline, Penicillin G, and Ampicillin maybe these drugs that have been in use for many years in Ethiopia. Lack of strict regulation in the distribution and use of antimicrobials could be the main reason for the increase in the antimicrobial resistance to those drugs [48]. The better and optional drugs like quinolone and cephalosporin are relatively expensive and not avialable particularly in developing country like Ethiopia [49].

Multidrug resistance pattern of S. aureus exhibited resistance to (Erythromycin, penicillin), (Ampicillin, penicillin), and (Amoxicillin, Ampicillin and penicillin) while 13.6% isolates showed resistance to the three and four drugs (Ampicillin, Cefotaxime and Penicillin) and (Ampicillin, Penicillin, Oxytetracycline and Streptomycin). On the other hand, 9.09% of S. aureus isolates showed resistance to seven drugs. Antimicrobial resistance rate for S. aureus from bovine mastitis is in an increasing trend [50]. The emergence of drug resistant strains poses public health hazard as in the case of multidrug resistance of S. aureus in the food of animal origin [49].

The E. coli isolates were also found to be 100% resistant to penicillin G, Amoxicillin, Ampicillin, Erythromycin, followed by Streptomycin (85.7%) and Cefotaxime (57.1%). Relatively similar findings have been reported from Bushoftu [42]. The isolates were found to moderate susceptibility to Chloramphenicol followed by Oxytetracycline and Kanamycin. Similar to this finding, Reuben and Owuna, were also reported all E. coli isolates were 100% resistant to Penicillin and 84.2% to Amoxicillin. Salehi and Bonab, lso reported E. coli isolates were 53% resistant to Amoxicillin, 77% resistant to Kanamycin, from Iran. A recent report in Ethiopia also showed E. coli is resistant to Tetracycline [12, 51]. This could be misuse of antibiotics for disease prevention and treatment. In addition to these, wrong dosage, wrong routes of administration, arbitrary drug combinations, unwise use of drugs, and poor awareness on antimicrobial residues in animal products and drug withdrawal period might lead antimicrobial resistance development. Evidence has also been indicated that resistant strains of pathogens can be transmitted to humans through food [53]. Therefore, raw milk apart from the potential source of food borne bacterial pathogens, it can also cause severe health risks to consumers due to antimicrobial residues [54].

Conclusion

The study indicated a relatively high prevalence of clinical and subclinical dairy mastitis. This may hamper the expansion of dairy development in the country. Implementation of proper milking and dairy hygiene practices could reduce the prevalence. The finding of bacterial isolates from bovine mastitis indicated the presence of drug resistance. In the present study resistance of S. aureus isolates to Penicillin G (77.3%), Ampicillin (90%), Erythromycin (54.5% and Oxytetracycline (50%) and E. coli isolate to Penicillin G (100%), Ampicillin(100%), Streptomycin (85.7%) and Cefotaxime (57.1%) were identified. Thus, prior to the treatment of clinical cases, the infectious bacteria need to be identified and its drug response need to be tested. As continuous use of these conventional drugs for any clinical cases predispose to the development of drug resistence, there should be strict regulation and monitoring proper drug usage. On top of this awareness should be created for dairy farm owners to ensure early diagnosis and regular screening of cows for subclinical mastitis together with the treatment of clinical cases. Further in-depth and wider antimicrobial susceptibility profiles of other mastitis-causing bacteria and molecular characteristics of drug-resistant isolate should be carried out. Alternative therapeutic medication needs to be used against multidrug-resistant strains.

Abbreviations

AMR: Antimicrobial resistance, AOR: Adjusted odds ratio, COR: Crude odds ratio, CI: Confidence interval CMT: California mastitis test, MRSA: Methicillin-resistant Staphylococcus aureus, MR-VP: Methyl red Vogous-Proskauer test: NMC: National Mastitis Council's, SCC: Somatic cell count, Spp: Species, TSI: Triple sugar iron.

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Availability of Data and Materials

The data supporting the findings are presented in the manuscript. The corresponding author can also be reached for any data inquiry.

Ethics Approval and Consent

The study was approved by the research review committee of the faculty of veterinary medicine, Hawassa University. Informed consent was obtained for questionnaires interview and sample collection to keep the confidentiality of dairy farms at the time of sample collection. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for Publication

Not applicable.

Authorship Contribution Statement

SAN: Conceptualizing the study, writing, review & editing the manuscript. FBB: Data curation and laboratory analysis. MS: Supervision, review and edit the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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