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Original Research

Prevalence and Antimicrobial Susceptibility Profile of Bacterial Isolates in Mastitis cases in Nekemte Town Dairy Farms, Western Oromia, Ethiopia

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Abstract

A study in Nekemte town screened 383 nursing cows to determine the frequency and antibiotic susceptibility of bovine mastitis, a common dairy cattle infection. The research aimed to identify factors contributing to economic losses and poor milk quality. The study found that 48.30% of lactating cattle tested positive for mastitis, with 132 being subclinical and 53 being clinical. Out of 1532 quarters examined, 42 were blind, and 149 had sub-clinical and 79 clinical mastitis. Mastitis was linked to factors like milking mastitic cows last, cleaning the udder, age, tick infestations, and using a towel to dry teat. Staphylococcus aureus was the most common bacterial species, accounting for 81 (35.53%). Of these, 81 (100%), 78 (96.3%), and 75 (92.59%) were very susceptible to gentamycin, penicillin, and chloramphenicol, whereas 4 (4.94%) and 5 (6.17%), respectively, showed strong resistance to ampicillin and streptomycin. The study found that E. coli bacteria were most effective against chloramphenicol (43.68%), streptomycin (43.89%), and gentamycin (44.67%). Streptococcus agalactiae bacteria were most resistant to vancomycin, tetracycline, and ampicillin. Of the 46 isolates, 20.18% were highly effective against gentamycin, erythromycin, chloramphenicol, penicillin, tetracycline, and streptomycin. Further molecular-based identification is needed due to high mastitis incidence in dairy cattle.

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INTRODUCTION

The dairy business is becoming highly structured in its long supply chain, employing 1.3 billion individuals in the world and directly supporting the livelihood of 600 million low-income peasant farmers in underdeveloped nations (Thornton, 2010). Farm animals, including cattle, poultry, sheep, goats, and swine, are a source of food and financial income around the world. By earning foreign currency from exploration of skin and hide, live animals, and meat, the livestock sectors contribute over 70% of the continent's income (Zeryehun *and Abera*., 2017).

Ethiopia's topography is incredibly varied, with a vast array of climatic conditions and an abundance of ecological zones for agriculture that are appropriate for supporting a vast number of animals (Amare *et al*., 2013). The livestock sub-sector produces 16.5% of total agricultural GDP (Anteneh *et al.,* 2009) and promises to rally around the country's economic prosperity. Seventy million cattle, forty-two million sheep, fifty-five million goats, eight million camels, fifty-six million chickens, two million horses, forty-four million mules, and eight million donkeys call this country home (CSA, 2021).

Cattle, in particular, are the backbone of the agricultural economy in Ethiopia and play an important role in producing revenue for farmers, guaranteeing food security, and making a contribution to wealth and social, cultural, and ecological values (Abera *et al*., 2021). However, the potential of livestock contributions to the country's economic development is less than expected due to frequent animal illness, a weak management system, low genetic performance, poor veterinary service, a lack of government attention, and a recurring drought (Tesfaye *et al.,* 2012).

Among the livestock diseases, bovine mastitis was the most common and costly disease, impacting all dairy farms (Lakew *et al.,* 2019). This infection was a mammary gland illness caused by pathogens invading the mammary gland via the teat canal, producing morphological alterations to the glandular tissues and causing chemical and physical changes in the milk (Radostits *et al.,* 2000). This infection is the result of a combination of three main factors: pathogens, the environment, and host defence factors (Gera and Guha, 2011). Mastitis is an infection of alveolar tissue and is clinically distinguished by milk visual abnormalities as well as pathological alterations of the mammary in the form of discomfort, mammary swelling, and fibrosis (Zeryehun and Abera, 2017). Infectious diseases are those in which diseased cows' udders act as the primary reservoir (Emana *et al.,* 2020). Infections are transmitted, especially during milking, and can cause chronic and subclinical illness (Girma *et al.,* 2012). The major infectious pathogens that cause bovine mastitis are *S. agalaciae, Mycoplsama, S. aureus*, and *Corrynebacteria* (Ashraf and Imran, 2018).

According to Cervinkova *et al*. (2013), environmental mastitis is a term used to describe intramammary illnesses caused by bacteria that primarily originate from the cow's environment. Environmental pathogens involve *Klebsiella* species and *Escherichia coli* (Panel, 2020). The most frequent pathogens are *Streptococcus uberis* and *dysgalactiae*, and most diseases formed by these bacteria are clinical and transient (Harmon, 1994). This infection is a worldwide issue since it impacts the health of animals, milk composition, and the milk supply economy in all countries, resulting in massive economic losses (Sharma *et al.,* 2007).

The financial effects of bovine mastitis were minimised milk supply, milk condemnation due to antimicrobial residues, the cost of treatment, the culling of persistently sick animals, milk waste due to poor quality, and unexpected mortality from serious conditions (Seegers *et al*., 2003). Milk from mastitis-affected cows cannot be consumed by humans due to changes in chemical components and taste qualities (Adkins and Middleton, 2018). Furthermore, milking from diseased animals has an impact on the future processing and lifespan of milk products (Seegers *et al*., 2003). Such a common illness in dairy cattle has been extensively reported as having a higher incidence in industrialized countries, but data for developing countries is scarce (FAO, 2014).

The diseases have been identified as one of the leading causes of financial losses for Ethiopian dairy farms and milk producers. In each year, more than 140 to 200 USD per farm was costed due to bovine mastitis; in approximation, 8% was discarded milk, 14% was for treatment costs, 6% was due to death in acute cases, and 70% was due to reduced milk product (Dereje *et al.,* 2018; Hundera *et al.,* 2005). Everyone, including veterinarians, producers, and dairy sector experts, wants to understand the pathogens that cause clinical and subclinical illness in dairy cattle, as well as how to manage and avoid them (Dinka *et al.*, 2018).

Klebsiella species, streptococcus species, staphylococcus species, mycoplasma, corrynebacteria, and *Escherichia coli* are some of the major pathogens causing bovine mastitis in Ethiopia (Adane *et al*., 2017). Certain studies on bovine mastitis showed a prevalence of 62.6% in southern Ethiopia (Abebe *et al.,* 2016), 74.5% in Holota (Abebe and Bakala, 2022), and 68.0% at the Addis Ababa commercial dairy farm (Tilahun and Aylate, 2015), but very little attention has been paid to this problem in Nekemte town dairy farms.

The global spread of new and emerging resistance mechanisms has led to an increase in the threat posed by antimicrobial resistance (AMR). It is getting harder and sometimes impossible to treat some infections as antibiotics lose their effectiveness. Thus, the growing challenge that AMR poses is also raising concerns about public health. It is deemed a food safety concern when pathogenic bacteria are present in milk and raw milk is consumed. (Naranjo-Lucena *et al.,* 2023). Antimicrobial resistance to bovine mastitis is a great problem in Nekemte town. The aim of this study is to estimate the prevalence, predisposing factor, and antimicrobial susceptibility profile of bacterial isolates of mastitis in dairy cattle in Nekemte town, western Oromia, Ethiopia.

MATERIAL AND METHODS

Study Area

This study was carried out in Nekemte town, east Wollega, Oromia regional state, western Ethiopia. The settlement of Nekemte town lies 335 km west of Addis Abeba, located at 9^{o} 03 to 9^{o} 07 north latitude and 36^{o} 29 to 36^{o} 38 east longitude, with a distance of 1960 m to 2170 m above sea level (Emana *et al*., 2020). The rainy season is mainly at the end of May to early September; additionally, April, March, and February are seasons of "belg rain," with yearly rainfall ranging from 1500 to 2200 mm. The annual maximum temperatures range from 14 to 26 °C. Nekemte was the capital of the former Wollega Province and is home to a museum of Wollega Oromo culture (Dinka *et al.*, 2018).

Nekemte town has an estimated 78,178 cattle, 9,894 sheep, 6,477 goats, 3,287 donkeys, 1,598 horses, 665 mules, 4572 dogs, and 2,428 cats (Emana *et al*., 2020). Figure 1 below indicates the map of the study area.

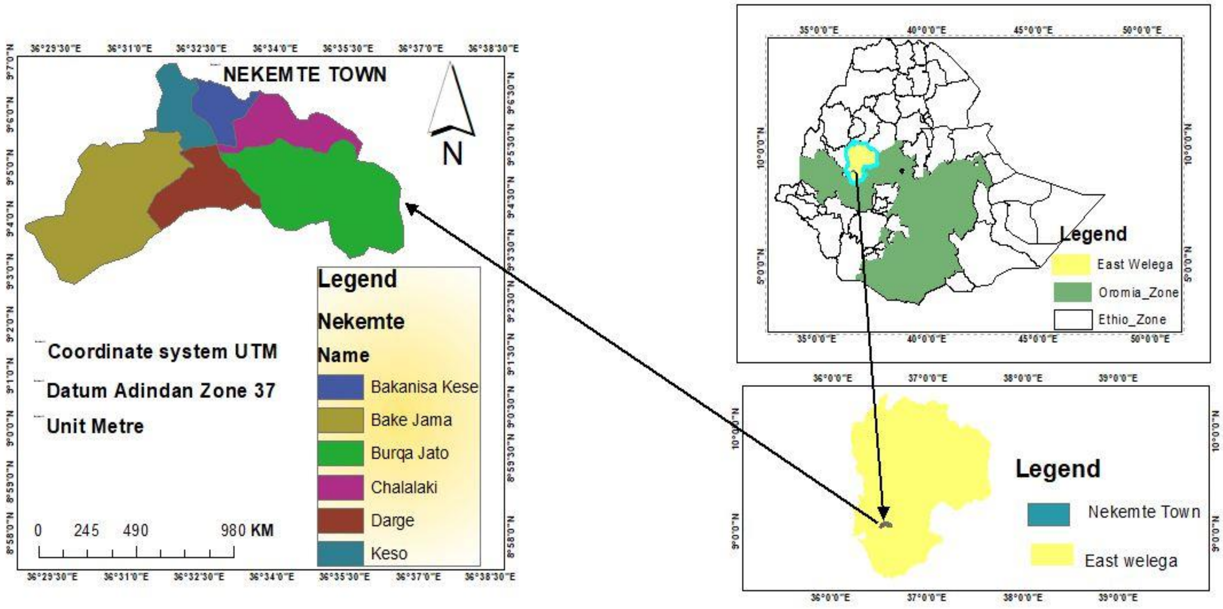

Figure 1 *Maps of the study area*

Study Animals

The study animals include exogenous and cross-breed lactating cows from the Nekemte town dairy farms, which are managed intensively and semi-intensively. According to Kidane *et al.* (2019), there are no large-scale dairy farms (greater than 30 dairy animals) in the study area; therefore, the farms were categorised as small (<5) and medium (6–30) dairy farms. Out of 42 dairy farms found in Nekemte town, the current study included 34 farms.

Study Design

A cross-sectional study was carried out from November 2022 to October 2023 to estimate the prevalence and antibacterial susceptibility profiles of bacterial isolates of bovine mastitis.

Questionnaire survey

Information was acquired during farm visits through a semi-structured questionnaire administered on all farms by personal conversation. Management system (intensive or semi-intensive), size of farm (small or medium), breed (Holstein Friesians or cross), age, parity, house with roof (yes or no), surface concreted (yes or no), bedding (yes or no), washing time (weekly or daily), appropriate milking methods (yes or no), udder washing before milking (yes or no), milking mastitis positive cow (yes or no) udder drying after washing (yes or no), lactation stage, using separate towels (yes or no), feeding system, and educational levels tick infestations, regular checks for udder health (yes or no), applying teat dips (yes or no), using dry cow therapy (yes or no), and culling positive cows with mastitis (yes or no). All are recorded during study time.

Sample size calculation

The sample size for this study was calculated by the Thrusfield (2018) formula with a 95% confidence interval and a significant level of 5%. The sample size was determined using a prevalence of 46.75% isolated from mastitic dairy cows in Horro Guduru Wollega by Beyene and Tolosa (2017).

$$n = \frac{Z^2 P_{exp}(1 - P_{exp})}{d^2} = 383$$

Whereas n = sample size required, z = reliability coefficient (1.96), d= desired absolute precision (0.5), Pexp = expected prevalence (46.75).

Sampling Technique and Sample Collection

The peasant association and study farm were purposefully selected based on the availability of lactation cows and the farm owners' interest in participating in the research. Simple random sampling techniques were used to select the study animals. The sample size per kebele (peasant association) and per farm was calculated based on the available proportion of lactating dairy cows. A stand milk collection procedure was used to collect the milk sample; the teat was sterilized with alcohol prior to sampling, and the initial 3 drops of milk were discarded. The nearest teats were collected first, followed by the farthest teat. Again, for the existence of clotting, flaking, blood, and watery discharges, the consistency and color of the sample from all teats were investigated (Radostits *et al*., 2007). Five millimeters of CMT-positive milk were collected and put in a test tube rack for quality management, and then the samples were delivered to the microbiology laboratory of Wollega University's School of Veterinary Medicine for microbiological examination.

Study Methodology

Field-level examination

A Californian mastitis test was used for examination of affected udders at field level (dairy farms). Approximately 5 ml of sample were taken from separate teats into each of the containers on the Californian mastitis test paddle; the same amounts of three percentages of CMT solution were dropped into all cups and stirred well for 20–30 seconds. After swirling the solution in a rotary motion, the outcomes were documented as negative, trace, weak positive, distinct positive, and strong positive, depending on the degree of gel formation (Dinka *et al.*, 2018).

Bacteriological examination

The sample was tested for bacteria using the methods outlined by Quin *et al*. *(2011). A*ccordingly, on the blood agar medium (Oxoid England), which consists of 7% defibrinated sheep blood and macConkey agar (HIMIDIA, India), a loop-full of milk samples were inoculated and thereafter cultured aerobically at 37 oC for 24 hours. The cultured colony was examined for the growth of microorganisms producing bovine mastitis.

All microorganisms were identified upon primary culture using colony characterization, Gramme stain reactivity, morphology and arrangement of the bacterium, catalase, oxidase, growth or failure to grow on MacConkey agar, and the motility test (Moges *et al*., 2011; Sarba and Tola, 2017). The catalase test, mannitol **s**alt agar growth conditions, and motility testing were used to identify *Staphylococcus* species. The catalase test distinguishes catalase-producing pathogens (*Staphylococcus* species) from non-catalase-producing pathogens (*Streptococcus* species) (Abebe *et al.*, 2016). The catalase enzyme catalyzes the release of H_2O_2 into both oxygen and water, resulting in the release or formation of bubbles and a positive screening response (Hanson, 2006).

Staphylococcus aureus was identified from other *staphylococci by a* coagulase test. *Streptococcus agalactiae* (Group B *hemolytic streptococcus*) were identified because they produce a chemical called Christie-Atkins-

Munch-Peterson (CAMP) factors that increase the region of hemolysis formed by *Staphylococcus aureus* (Beyene and Tolosa, 2017; Amin *et al*., 2017). Growth parameters on MacConkey agar were used to detect gramme-negative bacteria, the oxidase response, the catalase result, the TSI (triple sugar iron agar) test, and the IMViC test (Quin *et al.*, 2011). *Staphylococcus epidermidis* forms greyish-white, elevated, round, smooth, glistening, translucent to slightly opaque, non-heamolytic on blood agar, and 1 to 2 mm in diameter of 24-hour cultured bacteria (Lasseter *et al.*, 2010).

Antimicrobial Sensitivity Test

According to the National Mastitis Council's guidelines (1990), the Kirby-Bauer disc diffusion methods were used to assess the antibiotic resistance profiles of bacterial isolates. In a sterile test tube, isolated organisms were chosen and mixed in 3 ml of normal saline that was sterilized. To standardise the size of the inoculum, the average density of bacterial cells in the suspension was determined to be 0.50 using a McFarland densitometer (Wikler, 2006; Amin *et al.*, 2017).

Pure fresh colony from nutrient agar was inoculated in a test tube containing Mueller hinton agar (Fluka Biochemal, Germany) and cultured for 24 hours at 37 oC (Ashraf and Imran, 2018). The bacterial solution was prepared to the density of the McFarland standard of 0.5 ml with sterile saline (0.85% w/v NaCl: Scharlay, Brazil). After modifying the turbidity, Mueller-Hinton agar was inoculated with the modified solutions, and the inoculates were permitted to dry for 5 minutes (Emana *et al.*, 2020).

Multo discs (England) that included ampicillin (10µg), tetracycline (10µg), gentamicin (10µg), penicillin (10µg), streptomycin (10µg), chloramphenicol (30µg), erythromycin (15µg), and vancomycin (10µg) have been placed on the Mueller-Hinton agar surfaces and incubated for overnight at 37°C. Finally, the inhibition zone was measured using digital calibre, and the result was recorded in millimeters. Pathogens were categorised as resistant, susceptible, or intermediate based on the standard interpretive table (Eby Bassiri, 2013) (Table 1).

Table 1

Zone diameter inhibitor standard interpretation

Antibiotic disc	Disc content	Resistance	Intermediate	Susceptible
Streptomycin	10µg	≤11	12-14	≥15
Ampicillin	10µg	≤11	12-13	≥14
Chloramphenicol	30µg	≤12	13-17	≥18
Erythromycin	15µg	≤13	14-17	≥18
Penicillin	10 unit	<20	21-28	≥29
Vancomycin	30µg	≤9	10-11	≥12
Gentamycin	10µg	≤12	13-14	≥15
Tetracycline	30µg	≤14	15-18	≥19

Data Management and Analysis

Before statistical analysis, the recorded data was entered into a Microsoft Excel spread sheet and coded. Stata 14.0 statistical software was used to carry out all data analysis. The prevalence of mastitis was calculated by dividing the total positive animals by the total examined cows (Ashraf and Imran, 2018). Study animals were categorised as positive if at least one teat of examined cows was positive for clinical or subclinical mastitis. The relationship between the dependent variable and the categorical response variable was analysed by the chi-square test and logistic regression. The variables with the highest P-values were removed one by one, until only significant variables ($P < 0.05$) remained. The likelihood ratio test ($P<0.05$) was used to determine significant variables.

RESULTS AND DISCUSSION

Results

Cow-level prevalence

Out of the 383 breastfeeding cattle that were tested, 185 (or 48.30%) had mastitis. Of these, 132 (34.46% of the total) had preclinical mastitis and 53 (13.84% of the total) had clinical mastitis. Out of the 1532 quarters that were analyzed, 42 (2.74%) were determined to be blind, while 149 (9.73%) had subclinical and 79 (5.15%), respectively, clinical. The prevalence of mastitis at the quarter level is seen in Table 2 below.

Table 2

Quarter level prevalence

Quarters	No. of examined	Clinical No. Positive (%)	Sub clinical No. Positive (%)	Total
RFQ	383	12(3.13)	35(9.14)	47(12.27)
RHQ	383	28(7.31)	41(10.70)	69(18.01)
LFQ	383	14(3.66)	36(9.40)	50(13.06)
LHQ	383	25(6.53)	37(9.66)	62(16.19)
Total	1532	79(5.15)	149(9.73)	228(14.88)

Note: *RFQ, Rightfore quarter; RHQ, Righthind quarter; LFQ, Leftfore quarter; LHQ, Left-hind quarter.*

Bacteriological result

The most common microorganisms recorded in the current study was *Staphylococcus aureus* 81 (35.53%), *Escherichia coli* 48 (21.05%), *Streptococus agalactiae* 46 (20.18%), *Klebsiella pneumonie* 20 (8.77%), Staphylococcus *chromogen* 18 (7.89%), and *Staphylococcus epidermidis* 15 (6.58%). Table 3 below indicates the bacterial isolated during study period.

Table 3

Frequencies of bacterial isolated (total isolated was 228)

Bacterial species	Frequency	percent
Staphylococcus aureus	81	35.53
Streptococcus agalactiae	46	20.18
Escherichia coli	48	21.05
Staphylococcus chromogen	18	7.89
staphylococcus epidermidis	15	6.58
Klebsiella pneumonie	20	8.77
Total	228	14.88

Prevalence in relation to risk factors

Mastitis occurrence is associated with age, management system, washing the udder before and after milking, farm size, milking a mastitic cow last, tick infestation, breed, and cleaning the room (P<0.05), while parity has no significant effect (P > 0.05) on the prevalence of the diseases. The current investigation revealed a significantly higher prevalence (*P* = 0.037) of mastitis 102 (53.68%) recorded in Holstein Friesian compared to cross-breeding 83 (43.11%). A significantly higher prevalence (*P*<0.002) was seen in ≥8-year-old cows (83,60.14%) than in ≤4-year-old cows (62,50.40%), and a lower prevalence of 40 (32.78%) was recorded in 5-7-year-old cows. The current result indicated that a higher prevalence of 105 (53.85%) was seen in cows intensively managed than 80 (42.55%) in cows semi-intensively managed (*P*<0.027). The univariable and multivariable logistic regression analyses on the prevalence of mastitis are explained in Tables 4 and 5, respectively.

Table 4

Analysis of bovine mastitis by univariable logistic regression

Predisposing factor	Category	Number examined	Number positive	Prevalence (%)	*P-value*	95% CI (OR)
Breed	Cross breed	193	83	43.005	0.037	1.026-2.29 (1.536145)
	H. F	190	102	53.68		
Management system	Intensive	195	105	53.84	0.027	1.052-2.357 (1.575)
	S. intensive	188	80	42.55		
Washing udder before and after milking	Yes	192	80	41.67	0.009	0.390- 0.876 (0.585034)
	No	191	105	54.97		

Table 4 continues…

Age	≤4 year	123	62	50.40	0.003	0.677-1.509 (1.01087)
	5-7 year	122	40	32.78		
	≥8 year	138	83	60.14		
Farm size	Small	141	52	36.88	0.002	1.147-1.883 (1.470418)
	Medium	242	133	54.96		
Feed	Hay	189	105	51.98	0.001	1.364-3.196 (2.088391)
	Mixed feed	194	80	44.20		
Tick infestation	Absent	193	84	43.52	0.029	0.6993-1.046 (0.8554124)
	Present	190	101	53.16		
Milking mastitic cow last	No	191	107	56.02	0.050	0.984-2.202 (1.472579)
	Yes	192	78	40.63		
Cleaning room	Daily	196	92	46.94	0.003	0.358- 0.805 (0.5371372)
	Weekly	187	93	49.73		
Parity	<4	191	92	51.83	0.584	0.7488-1.670 (1.118409)
	≥4	192	93	51.56		
Educational background	Elementary school	175	102	58.29	0.007	0.5104-0.9015 (6784001)
	University	149	56	37.58		
	Sec. school	59	27	45.76		
Use towel to dry teat	Separate	301	132	43.85	0.003	0.3814- .8179 (0.5586101)
	Common	22	14	63.64		
	No use	60	39	65.00		
Teat and hand disinfectant	No	250	132	52.80	0.016	0.3864-.9075 (0.5922348)
	Yes	133	53	39.85		

Based on information obtained from the 34 farm owners, 20 (58.82%) of the farmers attended elementary school, while 9 (26.47%) completed high school. The remaining 5 (14.7%) were educated up to college or university level. To dry teats before or after milking, 7 (20.58%) used individual towels, 10 (29.41%) used common towels, and 17 (50%) did not use a towel. From the total farms interviewed, 4 (11.76%) milkers decontaminated their hands before milking the next cow, whereas 11 (33.25%) milkers decontaminated their hands only at the beginning of milking, and the remaining 19 (55.88%) did not use disinfectant.

Table 5

Multi variable logistic regression

Predisposing factor	Category	Odds	S. Error	Z	*P*	95% CI
Breed	Cross breed					
	H. F	1.537099	0.351278	1.88	0.040	0.9821-2.4056
Washing udder before and after milking	Yes	Ref				
	No	0.619359	0.150227	-1.98	0.048	0.3851-0.9963
Age	≤4 year					
	5-7 year	1.601748	0.232013	3.25	0.001	1.2058-2.127
	≥8 year					
Tick infestation	Absent					
	Present	1.651696	.381535	2.17	0.030	1.0502-2.597
Milking mastitic cow last	No	Ref				
	Yes	0.563983	0.131851	-2.45	0.014	0.3566-89179
Use towel to dry teat	Separate					
	Common	0.470459	0.104243	-3.40	0.001	0.30473-7263
	No use					

Note : *H. F= Holstein Friesian*

Antimicrobial Sensitivity Test

The disc diffusion method was used to assess the antibiotic resistance profiles (N.M.C., 1990). Muller-Hinton agar was used as the plating medium. An antibiotic susceptibility test was applied to all isolated bacterial species, and the findings are reported in Table 6. Out of the total 81 bacteria tested for antibiotic profiles, Gentamycin 81 (100%), penicillin 78 (96.30%), chloramphenicol 75 (92.59%), erythromycin 71 (87.65%), and tetracycline 57 (70.37%) were all effective against *S. aureus*, whereas they were resistant to streptomycin 5 (6.17%) and vancomycin 5 (6.17%). Of the total 46 isolates, *Streptococcus agalactiae* were susceptible to Erythromycin 44 (95.65%), Gentamycin 44 (95.65%), chloramphenicol 43 (93.48%), penicillin 42 (91.30%), tetracycline 42 (91.30%), and ampicillin 40 (86.96%), and resistant to Streptomycin 2 (4.35%) and vancomycin 8 (17.39%).

Out of the 48 *Escherichia coli* tested for antibiotic susceptibility profiles, 44 were sensitive to gentamycin (91.67%), chloramphenicol (89.58%), streptomycin (89.58%), erythromycin 40 (83.33%), and penicillin (77.08%), but vancomycin (4.33%) and 5 (10.42%) were resistant. Out of the total 18 bacteria tested for antibiotic susceptibility profiles, *Staphylococcus chromogen* were susceptible to gentamycin 18 (100%), chloramphenicol 18 (100%), ampicillin 1 (5.56%), streptomycin 2 (11.11%), and tetracycline 7 (38.89%). Ampicillin 14 (93.33%), gentamycin 14 (93.33%), streptomycin 14 (93.33%), tetracycline 13 (86.67%), penicillin 12 (80%), and chloramphenicol 11 (73.33%) are all susceptible to the total of 15 isolated *Staphylococcus epidermidis* and resistant to vancomycin 17 (26.67%). *Klebsiella pneumonie* was sensitive to all antimicrobial discs except ampicillin 2 (10%), which was highly resistant.

Table 6

Antibiotic sensitivity of microbial pathogens to different antibiotics

Antibiotics	Isolated bacterial species and percentage of their antibiotic sensitivity					
	S.Aureus (%) (N=81)	*Strep. Agalactiae*(%) (N=46)	*E.coli* (%) (N=48)	*S.Chromogen* (%) (N=18)	*S.Epidermidis* (%) (N=15)	*K.pneumonie* (%) (N=20)
Am10µg	4(4.94)	40(86.96)	7(14.58)	1(5.56)	14(93.33)	2(10.00)
C30µg	75(92.59)	43(93.48)	43(89.58)	18(100.00)	11(73.33)	19(95.00)
E15µg	71(87.65)	44(95.65)	40(83.33)	15(83.33)	11(73.33)	20(100.00)
GN10µg	81(100.0)	44(95.65)	44(91.67)	18(100%)	14(93.33)	20(100.00)
p10µg	78(96.30)	42(91.30)	37(77.08)	16(88.89)	12(80.00)	20(100.00)
St10µg	5(6.17)	2(4.35)	43(89.58)	2(11.11)	14(93.33)	19(95.00)
T10µg	57(70.37)	42(91.30)	5(10.42)	7(38.89)	13(86.67)	19(95.00)
V10µg	5(6.17)	8(17.39)	4(8.33)	6(5.56)	17(26.67)	20(100.00)

Note: *ST, Streptomycin; AM, Ampicillin; C, Chloramphenicol; E, Erythromycin; P, Penicillin; V, Vancomycin; GN, Gentamycin; T, Tetracycline; k, Klebsiella; S, staphylococcus;* N, *Number of bacterial examined.*

DISCUSSION

The current investigation indicated that the prevalence of mastitis in the study area was 48.30% at the cow level and 14.88% at the quarter level. Clinical and subclinical mastitis were found in 53 (13.84%) and 132 (34.46%) at cow level, and 79 (5.15%) and 149 (9.73%) at quarter level, respectively. The current total cow level prevalence was similar to research reported by Amin *et al.* (2017) in Haramaya town, Hundera *et al.* (2005) in Sebeta, Beyene, and Tolosa (2017) in Horo Guduru Wollega, which reported a prevalence of 49.2%, 52.78%, and 46.75%, respectively.

The current prevalence of mastitis was lower when compared to the previous studies of 71% and 63.02% by Mekibeb *et al. (*2010) in Holota town, central Ethiopia, and Lakew *et al.* (2019) in Haramaya District, eastern Ethiopia, respectively. This finding was higher than the research of Elemo *et al. (*2018) in Bale, Tassew *et al. (*2017) in and around Assosa Town, and Hundera *et al. (*2005) in Sebata Town, who reported 36.72%, 39.32%, and 34.6%, respectively. Different breeds of cattle, management systems, and animal risk factors might be the variables related to the diversity of mastitis occurrences among the studies.

The current study was also compared with the findings elsewhere in the world, and the result was higher when compared to the results of Ebrahimi *et al.* (2007) and Nam *et al. (*2008), who indicated 29.03% in Iran and 35.5% in South Korea, respectively. Insufficient milking procedures and a lack of mastitis prevention and control strategies were some predisposing factors to the higher prevalence of the diseases. The cow level occurrence observed in this investigation is relatively lower than the 68.3% and 72.6% reported by *Ngwa et al. (*2018) in the Adamawa region of Cameroon and Ranjan *et al. (*2010 in India, respectively. Management systems, cattle breeds, agro-climatic areas, and animal risk factors are the factors that contribute to the variation in the occurrence of diseases across countries.

The present cow-level clinical mastitis (13.84%) was closely similar to the results of Mekibib *et al*. (2010), Bedane *et al.* (2012), Biffa *et al. (2005), and* Mbindyo *et al.* (2020), who reported the prevalence of 22.4%, 21.1%, 19.6%, 16.11%, and 12% in Holeta Town, Yabello District, Southern Ethiopia, and Kenya, respectively. The current result was higher than those of Moges *et al. (*2011), Elemo *et al. (2018),* and Sarba and Tola (2017), who reported prevalences of 4.9%, 4.95%, and 9.9% in Gondar, in and around Sinana district, and Ambo district, respectively. The current prevalence was higher than the result of Mekonnen *et al.* (2019) in Bahir Dar and Gondar, who reported a 5.6%. The different prevalence in the study area may be due to the management system of the farm and quality handling during milking.

The current study of cow-level sub-clinical mastitis found 132 (34.46%) cases, which is consistent with the prevalence of 31.8%, 38%, and 28.6% found in previous studies. The current result was fewer compared to the studies of Mekibib *(*2010), Amer *et al. (*2018), and Moges *et al. (*2011), which reported cow-level subclinical mastitis at 48.6%, 54.4%, and 56%, respectively. The present cow-level subclinical mastitis was high compared to the findings of Biffa *et al. (*2005) and Adane *et al. (*2017), which recorded 23% and 7.3%, respectively, in different study sites in Ethiopia. The difference among farms was due to less attention from farmers to the prevention of subclicical mastitis. Subclinical mastitis (SM) is a non-symptomatic form of intramammary inflammation that affects cows in given herds.

The current quarter-level prevalence of 14.88% is in close agreement with Biffa *et al. (*2005) in south Ethiopia and Lakew *et al.* (2019) in Haromaya, Ethiopia, who report prevalences of 28.2% and 29.04%, respectively. Nevertheless, the total quarter-level prevalence recorded in the current investigation is lower compared to the results of Mbindyo *et al. (*2020) in Kenya, Amer *et al. (*2018) in Japan, and Ngwa *et al*. (2018) in the Adamawa region of Cameroon, who reported 56.9%, 57.7%, and 76.6%, respectively. The disparity in this finding might be attributed to differences in veterinary service, inadequate disease control strategies, and poor cleanliness standards at the research sites.

In this study, mastitis was found to be more prevalent in the right hind quarter 69 (18.01%) as well as the left hind quarter 62 (16.19%), whereas a lower prevalence was recorded in the left fore 50 (13.06%) and 47 (12.27%) in the right fore quarters. The finding was consistent with the finding by Hundera *et al. (2005),* who stated that infection was more common in the hind quarter. The increased prevalence of right quarters may be attributed to the convenience of holding the right hind quarter while milking as well as the greater production capabilities of the hind quarter (Radostits *et al.,* 2000). Faecal and environmental contaminants are also higher in the hind quarter.

Subclinical mastitis was more common than clinical mastitis in both cows and quarters. These considerable variations may be attributable to the fact that clinical mastitis is easily detected and managed. Sub-clinical mastitis has no physical abnormalities; therefore, it is rarely detected by producers and veterinarians and has continued to be the leading cause of illness in farms. Sub-clinical mastitis was much more common than clinical mastitis due to the udder's defence system, which lowers the disease incidence (Abebe *et al.,* 2016).

The significant mastitis occurrence observed in the present investigation is a big issue that decreases milk production and negatively impacts milk hygiene, causing financial damage and a risk to public health. In contrast, the hazards that contribute to mastitis have been breed, absence of milking an infected cow, lack of mixed feed, lack of control over tick infestation, lack of daily manure removal and cleaning room, farm size, management system, and age.

According to the current study, few dairy farms practice regular mastitis prevention, like pre- and post-milking quarter disinfection with different disinfectants in 32.35% of farm owners (11/34) and separate towels to dry teats for each cow in 17.65% of farm owners (6/34). Furthermore, all of the farm owners do not perform the CMT or even other diagnostics on a regular basis to check their cows for subclinical mastitis.

According to the findings of this investigation, farms that failed to milk mastitis cows last are more affected by mastitis. Abebe *et al. (*2016) and Nielsen *et al.* (2013) also reported similar results at Hawassa and in Sweden, respectively. Researchers observed that failing to milk a mastitis-positive cow last increases the transmission of mastitis in the herd. There was a statistically considerable variation in occurrence between cows of various age groups (P<0.000). The higher prevalence (41.30%) has been observed in lactating dairy cows of age greater than or equal to 8 years, followed by cows aged 5-7 years (21.31%), and cows aged less than or equal to four years had the lowest prevalence (17.89%). This outcome was consistent with reports by Tilahun and Aylate (2015) in Adis Abeba and Amer *et al. (2018),* who revealed that older age increases the prevalence of mastitis. Older animals were more affected by different diseases as their age increased (Abebe *et al.*, 2016).

Mastitis was far more common among farmers who did not use udder drying towels for every cow (73.53%) than among farmers who did (17.65%). This finding was almost the same as the findings of Mekonnen *et al.* (2019) and Mbindyo *et al. (*2020)' who found that employing the same drying towels for the herds was contributing to the spread of mastitis infections. The medium-sized farm had a higher prevalence of 31.82%, while the small farm had a lower prevalence of 19.86%. The result had statistical significance (*P*<0.011). It was discovered that almost all farms with more than 10 cows were intensively managed, causing the higher prevalence of disease. Mastitis may be caused by cows being overexposed to microorganisms in their environment as a result of high stocking density, an unclean floor, contaminated equipment, poor ventilation, and high humidity. The current outcome agrees with the reports by Sarba and Tola (2017).

The influence of feed on the present study was investigated and evaluated, and the study found that feed had a significant impact on the occurrence of the disease (P<0.05). A greater frequency (33.17% of the disease) was detected in farms with feeding systems that only used harvested hay, and a lower prevalence was found in 20.99% of farms with mixed feeding (concentrate). The influence of breed on the occurrence of mastitis was studied, and the results proved that breed had a significant impact (*P*<0.012). The result of the analysis of the breed indicated that Holstein-Friesian had the largest mastitis prevalence (33.16%), while cross-breeds had the smallest (21.76%). This demonstrates that hybrid cows are more resistant to infectious diseases than Holstein-Friesian cows. The breed variation in sensitivity to mastitis was consistent with the studies of Mekibib *et al.* (2010) and Lakew *et al.* (2019).

Microbiological investigation of the milk samples confirmed that *Staphylococcus aureus* (*S. aureus*) was the most common bacterial identified in subclinical and clinical mastitis, representing 35.53% of the total isolates. The predominance of *Staphylococcus aureus* in the current study was also reported by another researcher, Ngwa *et al.* (2018), in the Adamawa region of Cameroon. The greater prevalence of *S. aureus* observed could be attributed to inefficient drying, washing, postmilking teat dipping, and cleaning of the milker's hands. On the other hand, *S. aureus* was resistant to several types of antibiotics, particularly beta-lactam antimicrobials (David, 2010). Next to *S. aureus, Escherichia coli (E. coli)* was the next most prevalent, accounting for 21.5% of the total isolate. The current finding was similar to the finding of Ngwa *et al. (2018),* who indicated a prevalence of 22.0% in the Adamawa region of Cameroon; this finding was higher than the reports of Belayneh *et al. (*2013) in Adama, Ethiopia.

The greater occurrence of *Escherichia coli* might be attributed to the management of the dairy farm. The high percentage of environmental *Escherichia coli* isolated in this investigation could be linked to poor farm hygiene and a weak management system. *Klebsiella pneumonie* (8.77%), *Streptococcus agalactiae* (20.18%), *Staphylococcus chromogen* (7.89%), and *Staphylococcus epidmidis* (6.58%) were the major pathogens isolated. The prevalence of 20.18% of *Streptococcus agalactiae* in the current finding was high compared to the reports of Belayneh *et al.* (2013), who indicated a prevalence of 10%. The higher frequency of *Streptococcus agalactiae* in the study area was attributed to a lack of good management, the absence of milking mastitic cows, a shortage of mixed feeding systems, and a lack of daily manure removal.

Gentamicin was found to be the most effective antibacterial in the present study, followed by chloramphenicol. This result was the same as that of Dereje *et al. (2018),* other researchers who stated that gentamycin was the most effective antibiotic against the total isolate pathogen. Since these antibiotics were the least commonly used in veterinary services in the research area, drug resistance had not developed.

The antimicrobial investigation of the present study indicated that *S. aureus* was susceptible to gentamycin at 100%, penicillin at 96.30%, erythromycin at 87.6%, tetracycline at 71.60%, and chloramphenicol at 92.59%, whereas the resistance to ampicillin, vancomycin, and streptomycin was 4.94%, 6.17%, and 6,17%, respectively. Dabele *et al.* (2021) and Dereje *et al. (*2018) also reported similar results. According to the current finding, *Streptococcus agalactiae* was susceptible to Erythromycin (95.6%), Gentamycin (93.48%), Chloramphenicol (93.48%), Ampicillin (86.96%), Penicillin (60.875%), and Tetracycline (60.875%), but was highly resistant to Streptomycin (4.35%) and Vancomycin (17.39%). Dereje *et al. (*2018) also reported a similar result. The high resistance of streptomycin and vancomycin in the current study was due to the frequent use of the drugs.

The antibiotic sensitivity tests indicated that *Escherichia coli* was susceptible to gentamycin (91%), chloramphenicol (89%), and erythromycin (81.25%), but resistant to vancomycin (8.33%) and tetracycline (10.42%). The current results were similar to those of Tassew *et al. (2017),* who stated that *Escherichia coli was* susceptible to gentamycin, chloramphenicol, and erythromycin but highly resistant to vancomycin and tetracycline. Gentamycine (100%), chloramphenicol (94.44%), erythromycin (89%), and penicillin (83.33%) were the most effective against *Staphylococcus chromogen* in this study, but it was resistant to vancomycine, streptomycin, and ampicillin, with resistance parentage of 5.56%, 11.11%), and 5.56%, respectively. The frequent resistance observed in the current study was due to frequently treating positive animals with similar drugs. The current antibiotic sensitivity tests indicated that *Staphylococcus epidermidis* were highly susceptible to gentamycin (93.33%), ampicillin (93.33%), penicillin (80%), and tetracycline (86.67%), whereas they were highly resistant to vancomycin (26.67%). The current study is in disagreement with the findings of Belayneh *et al.* (2013), who indicated that S. *epidermidis* was highly susceptible to vancomycin. The high resistance of the current study may be due to long-term diseased animals, and vancomycin was the first choice of treatment in the study area. *Klebsiella pneumonia is susceptible to* all antimicrobial discs used for this study. Shilpa *et al.* (2016) also reported a similar result.

CONCLUSIONS

The current studies indicate bovine mastitis was the prevalent illness in dairy cows in Nekemte, a town in western Ethiopia. Lack of washing the udder before and after milking, age, failure to milk a mastitic cow last, tick infestation, educational background, and use of disinfectant teats are the key predisposing factors associated with the prevalence of mastitis in dairy cattle. The antibiotic sensitivity profile of the isolated pathogen to antibiotics indicates that a high number of isolated pathogens are very sensitive to Gentamycin and Chloramphenicol but resistant to *Ampicillin and Vancomycin*; therefore, a permanent resistance surveillance programme should be established in the study area. Moreover, washing the udder before and after milking, milking mastitic cows last, controlling tick infestations, and using towels to dry teats should be implemented for the prevention of mastitis at Nekemte town dairy farm. Further molecular-based studies are needed to identify drug resistance genes in the study area.

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DECLARATION

The authors state that they do not have any conflicting interests.

DATA AVAILABILITY

Data used for this study are available from the corresponding author for reasonable issues.

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