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#### **Original Article**



# Risk Factors Associated with Sub-Clinical Mastitis and Antibacterial Resistance in Small-Holder Dairy Farms of Kajiado North Sub-County, Kenya

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#### ABSTRACT

**Introduction:** Worldwide, there is a paucity of literature on subclinical mastitis, and antimicrobial resistance patterns of bacteria isolated from dairy animals kept in peri-urban areas. This study aimed at determining the prevalence of sub-clinical mastitis (SCM) and the sensitivity of the isolated bacteria to selected antibiotics in dairy cows kept by small-scale farmers in Kajiado North Sub-County, Kenya. Simultaneously, a questionnaire was administered to determine and assess the risk factors associated with mastitis.

**Materials and methods:** Milk was obtained from all quarters of 101 lactating dairy cows, sampled from 50 farms, and screened for SCM using California Mastitis Test. The samples were cultured and bacteria identified using standard bacteriological methods. Antibacterial sensitivity of *Staphylococcus* spp. and *Escherichia coli* isolates were tested using the Kirby-Bauer disk diffusion method, against commonly used antibiotics.

Results: The prevalence of SCM at cow and udder quarter levels were 51.2% and 47.5%, respectively. The prevalence of the bacteria was *Staphylococcus* spp. (51.4%), Klebsiella spp. (40.5%), Pseudomonas spp. (34.6%) and E. coli (11.8%). The risk factors significantly associated with SCM were breed, parity, lactation stage, and milking hygiene. The highest prevalence of SCM was found in cows in late-stage lactation (78%) with the lowest in those in early-stage lactation (37.5%). A higher prevalence of SCM was found in cows housed in farm structures having poor hygiene (95%). The highest prevalence of SCM was in Friesian breeds (67.3%) and the least affected were the indigenous cows (27.3%). Cows in the fourth and higher parities were the most (64.7%) affected by SCM. Most of the Staphylococcus spp. isolates were found to exhibit resistance to oxytetracycline (73%) but had high sensitivity to gentamycin (69%). All E. coli isolates showed resistance to oxytetracycline while a 75% were sensitive to Chloramphenicol. In conclusion, the study showed that a large proportion of cows were affected by SCM, with the main causative agent being *Staphylococcus* spp. **Conclusion:** The study shows that antibiotic resistance was alarmingly high in the study animals. The predisposing factors should be further investigated with a view of developing necessary interventions.

#### 1. Introduction

The dairy industry in Kenya is one of the largest in the African region and is an important player in the economic and nutritional aspects of the local and growing population<sup>1</sup>. The sector contributes to about 8% of the gross domestic product with annual milk production of 3.43 billion liters<sup>1</sup>. Kenya's dairy cattle population is estimated at 4.3 million, mainly in the semi-

intensive and intensive production systems<sup>1</sup>. The main breeds for dairy production are Friesian, Guernsey, Ayrshire, Jersey, and their crosses<sup>2</sup>. In recent years, urban and peri-urban dairy production systems have gained prominence in Kenya and spread to nomadic livestock husbandry practices in counties such as Kajiado<sup>1</sup>. Dairy farming involves milk production in such

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areas, targeting major market outlets in urban centers, including Nairobi, Kenya<sup>3</sup>. Several studies have shown that urban and peri-urban dairy farming is mainly motivated by better prices for animal products, which also creates employment opportunities<sup>3-5</sup>. There are many challenges in peri-urban dairy farming, such as inadequate quantity and quality of feeds, poor access to breeding, and poor access to credit facilities and output markets, mainly diseases, such as mastitis<sup>5-7</sup>.

Bovine mastitis is a major concern as it affects milk production negatively<sup>3</sup>. It is the most costly disease for dairy animals, and losses mainly occur through rejected milk, reduction in milk vield, extra labor, cost of drugs, premature culling of animals, and replacement of breeding animals<sup>1</sup>. The disease can be managed by treating the clinical cases and designing appropriate mastitis control measures<sup>8</sup>. The clinical form of the disease can be detected easily by the farmers since it is associated with visible changes in milk composition and appearance, decreased milk production, and the presence of signs of inflammation<sup>8</sup>. However, sub-clinical mastitis is more difficult to detect because symptoms are not readily apparent<sup>8</sup>, so its diagnosis is challenging. Studies have shown that sub-clinical mastitis is up to 40 times more prevalent than the clinical form<sup>9</sup>. Further, the sub-clinical form is chronic and thus acts as a continuous source of infection on a farm<sup>10</sup>. The prevalence of sub-clinical mastitis in cows in high agricultural potential areas of Kenya ranges from 36% to 87% in different studies<sup>11-14</sup>.

In recent years, overuse and misuse of antibiotics in Kenya have encouraged the evolution of bacteria towards resistance, resulting in therapeutic failure <sup>14-16</sup>. In Kenya, most studies on mastitis in cattle have been done in high agricultural potential areas where intense dairy farming is practiced. There is a paucity of data on subclinical mastitis in dairy animals kept in urban and peri-urban areas. The present study aimed to evaluate the prevalence of subclinical mastitis, risk factors, and sensitivity of isolated bacteria to commonly used antibiotics. The study was undertaken in a peri-urban area of Kajiado North subcounty, Kenya.

# 2. Materials and Methods 2.1. Ethical approval

The approval to conduct this study was approved by the Animal Ethics Committee of Jomo Kenyatta University of Agriculture and Technology, Kenya (REF: JKU/2/4/896B). The study followed the protocols approved by the Committee. The participating farmers gave informed consent.

#### 2.2. Study area

The study was carried out between October and November 2019 in Kajiado North Sub-county, Kenya, located about 30 Km south of Nairobi City. It lies between latitude -1° 51' 8.57'S and longitude 36° 46' 36.59''E and covers approximately an area of 148Km<sup>2</sup>. The sub-county has three wards, Ngong, Olkeri, and Kiserian. The annual rainfall and temperature vary from 500-1500 mm and 7-35°C, respectively<sup>17</sup>. The sub-county has a humid tropical climate, with the two rainy seasons being April to May and October to December. Due to its proximity to Nairobi City, the county serves as a residential area for the city workers due to a reasonably cheaper option for residential purposes. Livestock farming is widespread, and according to the 2019 National Census, the sub-county was recorded to have a population of 304,404 people and livestock, including cattle (151,295 head), sheep (314,080), goats (236,790), chicken (257,367), and donkeys (2,113)<sup>17</sup>.

#### 2.3. Study design and sample size

The study utilized a cross-sectional study design. The sample size for the study was calculated based on the formula by Thrusfield<sup>18</sup>. The prevalence of mastitis in a peri-urban Nakuru County, Kenya, which has largely similar characteristics to the current study site, was 36%<sup>11</sup>. This study assumed a prevalence of 36% to calculate the sample size. On the basis of formula <sup>18</sup>, the sample size was to be 354 lactating cows. However, the population of lactating cows in the county at the time of the study was less than 10,000 (Kajiado Sub-County, Veterinary Officer, personal communication). Accordingly, 101 lactating cows were sampled from all milking quarters.

#### 2.4. Sampling strategy

The study used a multistage random sampling technique to select study wards, villages, households, and animals. Three villages were randomly selected from each ward (Ngong, Olkeri, and Kiserian administrative Wards). From these households, a sampling frame was prepared from the list provided by the local Veterinary Office. Thereafter, the lactating animals were randomly selected from each village. From each household, the lactating cows were randomly selected and sampled; a total of 101 milk samples from 50 farmers were obtained. Most of the farms had one or two lactating cows.

#### 2.5. Milk sample collection

The cows were physically examined, and thereafter milk was sampled. Milk samples were collected using a standard procedure <sup>19</sup> where the milking cows were restrained; the udder was scrubbed to remove dirt, bedding, and other physical matter. The udder and teats were washed using cotton wool soaked in 70% ethanol. The teat was then dried using disposable clean towels. The first three streams of milk from each teat were discarded. On the fourth stripping, California Mastitis Test (CMT) was carried out using commercial kits (California Mastitis Test Kit, Immucell Corporation, UK) as described by the manufacturer.

Further, the milk sample was examined for consistency of the fluid as well as the amount of gel reaction<sup>20</sup>. The level

of mastitis was scored on a scale of 0-3 with 0 (negative),1 (trace/negative), and 2 and 3 (positive). A cow was considered to have mastitis if milk from at least one of the udder quarters was positive by California Mastitis Tests (CMT). Positive mastitis milk samples, approximately 10 ml of milk from each teat, were then collected and transported in a cold chain to the Microbiology Laboratory at the Jomo Kenyatta University of Agriculture and Technology (Kenya) for analysis.

#### 2.6. Culture and bacterial identification

Bacteriological cultures from the milk samples were conducted as described by the Laboratory Handbook on Bovine Mastitis<sup>21</sup>. From each milk sample, 0.01 ml was streaked on three agars plates, namely Mannitol Salt agar (MSA), Nutrient agar (NA), Eosin methylene blue agar (EMB), and M-PA agar (for detection and isolation of Pseudomonas aeruginosa). The plates were incubated at 37°C for 18-24 hours and examined for the growth of bacteria. Agar plates without growth were re-incubated for up to 4 days, indicating no bacterial growth. As soon as the bacterial growth occurred, the colonies were studied microscopically for both abundance and colonial morphology as described by Kenya National Bureau of Statistics (KNBS)<sup>17</sup>. The colony growth was stained for Gram reaction, and various biochemical tests (catalase, fermentation, indole tests) were used to determine the genus and species of the bacterial colonies. The catalase test was used to distinguish staphylococci from streptococci, while the indole test was employed to differentiate Escherichia coli (E. coli) from other Enterobacteriaceae.

#### 2.7. Antibiotic/Antimicrobial sensitivity tests

antimicrobial sensitivitv isolated The for the Staphylococcus spp. and E. coli was determined by the disk diffusion assay as recommended by the Clinical and Laboratory Standards Institute<sup>22</sup>. The two bacteria were chosen because they have been documented to cause more severe mastitis in dairy animals<sup>23</sup>. The isolated and identified bacteria were tested for antibiotic sensitivity through a panel of commercial paper-impregnated disks (Himedia Ltd, India) based on a panel of 5 locally available antibiotics that were used for the treatment of mastitis (Oxytetracycline (30µg), Gentamycin (10µg), Tetracycline (30µg), Streptomycin (20µg), and Chloramphenicol (50µg). The antibiotic-impregnated paper disks were applied onto the surface of Nutrient agar that was inoculated uniformly with the bacteria and then incubated overnight at 37°C. The sensitivity of the bacteria to the drug was determined by measuring the diameter (mm) of the zone of inhibition around the disk. The standard criteria were used to obtain and classify the antibiotic sensitivity of each antibiotic<sup>22</sup>.

# 2.8. Risk factors for the occurrence of sub-clinical mastitis

A structured questionnaire was administered to 50

farmers (Ngong (15), Olkeri (18), and Kiserian (17) wards). These farmers were from farms where the milk samples were obtained. The data collected included age, parity, stage of lactation, and management practice of dairy cattle farming. The cows' age was determined by the information from the owner and dentition characteristics. Parity was categorized using a scale of 1-4, with 1 being the least parity and 4 being the highest parity recorded. Lactation stage was classified as early (up to 90 days), mid (91 to 180 days), and late (181 days and above)<sup>8</sup>. Depending on the schedule of cleaning of the cow pens and the presence of manure, the level of hygiene was categorized as good (daily cleaning), fair (weekly cleaning), or poor (monthly cleaning)<sup>14</sup>.

#### 2.9. Data analysis

Data were entered into Microsoft Excel 2013 spreadsheet file (Microsoft, USA) and exported to a statistical package SPSS version 20 (Microsoft, USA) for further statistical analysis. Descriptive statistics were computed to establish the frequencies and percentages of cows diagnosed with mastitis. The prevalence of subclinical mastitis was based on the CMT results. A chi-square test was used to evaluate associations between risk factors and mastitis infection (p < 0.05).

#### 3. Results

#### 3.1. Characteristics of the dairy farm and herd structure

The characteristics of the dairy farms and the sampled cattle are shown in Table 1. The majority (44%) of the sampled farmers had attained a tertiary level of education. The animal husbandry system indicated that most (54%) farmers practiced an intensive production system with the dairy cows under zero-grazing. Additionally, 66% of the farmers kept other types of livestock besides cattle. Exotic cattle breeds were predominant, accounting for 62% and only 10% was for indigenous breeds. It was noted that 46% of the zero-grazing cattle sheds and milking parlor were cleaned once a week. The study indicated that a large

**Table 1.** Characteristics of the farms and dairy cattle sampled in Kajiado

 North Sub-county, Kenya

Characteristics		Frequency	Percentage
	Non-formal	1	2
Education level	Primary	11	22
Education level	Secondary	16	32
	Tertiary	22	44
	Intensive	27	54
Production system	Semi intensive	13	26
	Extensive	10	20
Frequency of	Daily	16	32
cleaning	Once a week	23	46
cleaning	Once a month	11	22
Other forms of	Yes	33	66
livestock	No	17	34
	Exotic	31	62
Breed of dairy cattle	Indigenous	6	12
	Crosses	13	26
Previous history of	Yes	32	64
mastitis in cows	No	18	36

number (64%) of the farmers had previous cases of persistent mastitis.

# 3.2. Prevalence of sub-clinical mastitis and identification of bacteria

The CMT results showed that 52 (51.2%) of the 101 samples tested were mastitis positive. The prevalence of subclinical mastitis (SCM) at the quarter (number of a quarter of udder of udders infected) level was found to be 47.5%. The results among the cows from the three administrative Wards in the study area included Olkeri (37.5%), Kiserian ward (48.4%), and Ngong ward (15.4%).

Out of the 101 samples collected, 71.3% had a positive bacterial culture, and a total of 140 bacterial isolates were obtained. In descending order, the prevalence of the bacterial infections was *Staphylococcus* spp. 51.4%, *Klebsiella* spp. 40.5%, *Pseudomonas* spp. 34.6% and *E. coli* 11.8% (Table 2).

**Table 2.** Prevalence and distribution of bacteria in milk samples of dairycows from Kajiado North Sub-county, Kenya (Number of sampled cows:Olkeri Ward: 37, Kiserian Ward: 34, Ngong Ward: 30)

Isolated Bacteria	Distribution inwards			Total
	Olkeri (n, %)	Kiserian (n, %)	Ngong (n, %)	(n, %)
Staphylococcus spp.	21 (56.8)	23 (67.7)	8 (23.6)	52 (51.4)
Escherichia coli	3 (8.1)	9 (26.5)	0 (0)	12 (11.8)
Pseudomonas spp.	12 (32.4)	17 (50)	6 (17.6)	35 (34.6)
Klebsiella spp.	12 (32.4)	19 (55.9)	10 (29.4)	41 (40.5)

n = number of cows positive for a given bacteria

# 3.3. Relationship between prevalence of sub-clinical mastitis and identified risk factors

The CMT results were used to evaluate the relationship between prevalence and risk factors (Table 3).

**Table 3.** Association of risk factors and prevalence of subclinical mastitisin dairy cattle as identified by California Mastitis Test in Kajiado NorthSub-County, Kenya

Breed	Number	Frequency of positive cows	Proportion
Friesian	55	37	67.3
Ayrshire	20	7	35
Simmental	2	0	0
Cross-breeds	13	5	39
Indigenous	11	3	27.3
Lactation stage			
Early	32	12	37.5
Mid	30	12	40
Late	39	28	72
Parity of the cow			
1	28	11	39.3
2	31	14	45.2
3	25	16	64
4	17	11	64.7
Level of hygiene			
Good	18	3	16.7
Fair	63	30	47.6
Poor	20	19	95

The highest prevalence of sub-clinical mastitis was observed in Friesian (67.3%), followed by crosses, Ayrshire, and the indigenous cows were the least affected. There was a significant (p<0.05) difference in the prevalence of sub-clinical mastitis in the different breeds. The highest (72%) prevalence of sub-clinical mastitis was found in the cattle at the late lactation stage, while the lowest prevalence (37.5%) was found in the early lactation stage. The lactation stage was found to be significantly (p < 0.05) associated with the prevalence of mastitis.

The lowest prevalence of sub-clinical mastitis was found in dairy cattle in the first parity (39.3%). An increase in prevalence was noted as the parity increased, with the fourth parity recording the highest prevalence (64.7%). In this case, parity had a significant effect on the occurrence of mastitis (p < 0.05). The highest prevalence of sub-clinical mastitis (95%) was found among dairy cattle whose structures had poor hygiene, and a decrease in prevalence was observed as the level of hygiene improved. Hygiene was a significant predictor of mastitis in cows (p < 0.05; Table 3).

As can be seen in Table 4, the highest occurrence of *Staphylococcus* spp. was found in the Friesian breed (65.5%), cows in late lactation stage (72%), those with high parity (64.7%), and those with a poor level of hygiene (95%). The highest number of *Klebsiella* spp. isolates was found among the Friesian breed (54.5%), in the early lactation stage dairy cattle (87.5%), in dairy cattle with high parity (94.1%), and in dairy cattle with a poor level of hygiene (90%, Table 4).

The occurrence of *Pseudomonas* spp. was found to be highest in the Friesian breed (45.4%). The highest prevalence of *Pseudomonas* spp. was also observed in dairy cattle in the late lactation stage (51.2%), those with higher parity (88.2%), and those with poor hygienic level (100%, Table 4). The highest number of *E. coli* isolates was found among the Friesian breed (14.5%), in the late lactation stage dairy cattle (17.9%), in dairy cattle with high parity (41.1%), and in dairy cattle with a poor level of hygiene (50%, Table 4).

#### 3.4. Antibiotic sensitivity

Antibiotic sensitivity was only tested against Staphylococcus spp. and E. coli, as they are major causes of mastitis in Kenya<sup>23</sup>. These bacteria are also considered the most predominant mastitis-causing microorganisms coupled with their significance as zoonotic pathogens<sup>23</sup>. These results are summarized in Table 5. It was observed that the *Staphylococcus* spp. isolates were most sensitive to gentamycin (69%) and least sensitive to oxytetracycline (15%). The *Staphylococcus* spp. isolates were found to have high resistance to oxytetracycline (73%), tetracycline (35%), and streptomycin (25%). The obtained results indicated that 75% of E. coli isolates were most sensitive to Chloramphenicol. However, 100% of the E. coli isolates were resistant to oxytetracycline, and 92% were resistant to tetracycline.

Parameter	N	Distribution of bacterial species (n, %)			
Breed	N -	Staphylococcus spp.	Klebsiella spp.	Pseudomonas spp.	E. coli
Friesian	55	36 (65.5)	30 (54.5)	25 (45.4)	8 (14.5)
Ayrshire	20	7 (35)	7 (35)	5 (25)	2 (10)
Simmental	2	0	0	0	0
Crosses	13	6 (46.1)	3 (23)	3 (23.1)	1 (7.6)
Indigenous	11	3 (27.2)	1 (9)	2 (18.1)	1 (9.1
Lactation stage					
Early	32	12 (37.5)	28 (87.5)	7 (21.8)	2 (6.2)
Mid	30	12 (40)	3 (10)	8 (26.6)	3 (10)
Late	39	28 (71.8)	10 (25.6)	20 (51.2)	7 (17.9)
Parity of the cow					
1	28	8 (28.6)	2 (7.1)	1 (3.5)	1 (3.5)
2	31	11 (35.4)	9 (29)	4 (12.9)	2 (6.4)
3	25	17 (68)	10 (40)	15 (60)	2 (8)
4	17	16 (94.1)	16 (94.1)	15 (88.2)	7 (41.1)
Level of hygiene					
Good	18	4 (22.2)	1 (5.5)	3 (16.6)	0
Fair	63	28 (44.4)	23 (36.5)	12 (19)	2 (3.1)
Poor	20	20 (100)	18(90)	20 (100)	10 (50)

Table 4. Relationship between risk factors and prevalence of specific bacteria isolated from milk of dairy cattle from Kajiado North sub-county, Kenya

N= number of animals sampled in each category

Table 5. Antibiotic susceptibility pattern of bacteria isolated from milk of dairy cows with subclinical mastitis in Kajiado North Sub-county, Kenya

Isolate	Antibiotic disc	Ν	Sensitive	Slightly sensitive	Resistant
Staphylococcus spp.	Tetracycline	52	24 (46%)	10 (19%)	18 (35%)
	Chloramphenicol	52	26 (50%)	20 (38%)	6 (11%)
	Oxytetracycline	52	8 (15%)	6 (11%)	38 (73%)
	Streptomycin	52	19 (36%)	20 (38%)	13 (25%)
	Gentamycin	52	36 (69%)	12 (23%)	4 (8%)
Escherichia coli	Tetracycline	12	0 (0%)	1 (8%)	11 (92%)
	Chloramphenicol	12	9 (75%)	2(17%)	1 (8%)
	Oxytetracycline	12	0 (0%)	0 (0%)	12(100%)
	Streptomycin	12	0 (0%)	1 (8%)	11 (92%)
	Gentamycin	12	4 (33%)	5 (42%)	3 (25%)

#### 4. Discussion

The obtained results of the current study indicated a high prevalence of subclinical mastitis in small-holder dairy farms within the peri-urban setting in Kajiado County. However, this is lower than the result of the study reported in Thika Sub-County (64%), Juja Sub-County (67%), and Kajiado (73%) counties<sup>12-14</sup>. The reported prevalence was higher than the national prevalence levels of 34-36% in Kenya<sup>11</sup>. The results of this study show prevalence levels are also higher than the rates reported in the sub-Saharan regions, such as 50.4% in Rwanda, 38.9% in Sudan, 21.1% in Zimbabwe, and 42.9% in Egypt<sup>24-26</sup>, respectively. The higher burden of mastitis in peri-urban areas could be due to poor management strategies for mastitis.

In the present study, there was a significantly higher prevalence of sub-clinical mastitis in cows whose houses had poor hygiene compared to those which had good hygiene, which is similar to other studies by<sup>12, 14, 27</sup>. Several studies have shown that failure to implement routine prevention practices, such as good hygiene, is one of the main causes of sub-clinical mastitis in dairy animals in developing countries<sup>13, 16</sup>. The current study revealed the Friesian breed as most affected by mastitis which agrees with previous studies <sup>14,28</sup>. In contrast, local breeds were more resistant. Udders of high-yielding cows, such as Friesian, are more susceptible to infection<sup>8</sup>. Studies have shown that in Friesian cows, selective pressure for increased milk production has led to a higher propensity for the occurrence of mastitis<sup>29</sup>. Although farmers are encouraged to keep high-yielding exotic animals, this is not accompanied by the awareness of proper prevention and control of mastitis. Another strategy might include cross-breeding exotic and indigenous breeds in order to increase the disease resistance of the latter while maintaining high milk production traits.

Cows in the late lactation stage had a higher prevalence of subclinical mastitis compared to those in mid and early lactation. These results agree with the findings of another study indicating that there are more cases in late-lactation stage cows because the udder could be more susceptible to infections<sup>30</sup>. In some cows, there is no keratin plug formation in the teat canal during the dry period, which can increase susceptibility to infections<sup>31</sup>.

In the current study, a higher prevalence of sub-clinical mastitis was mostly associated with cows with higher parity. This scenario has also been documented in some studies<sup>12</sup>. This might be partly associated with the position of the udder in older cows which are often pendulous and more susceptible to infections<sup>32</sup>.

The bacteria isolated during this study included

*Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *E. coli. Staphylococcus* spp. was the predominant pathogen in the area, comparable to what has been found in such studies in dairy animals <sup>11, 13, 14, 30</sup>. Coliforms that cause environmental mastitis thrive in dirty environments encountered on some farms during the study; hence, good hygiene is recommended.

The high predominance of *Staphylococcus* spp. may be partly attributed to poor milking hygiene associated with milkers' hands and udder drying towels, which have been known to enhance the spread of infections from teat to teat and between cows<sup>13</sup>. Additionally, low success following treatment of active clinical cases could act as reservoirs of S. aureus <sup>8</sup>. The present results agreed with a previous one, where the Friesian breed was the most common breed affected by each isolated bacteria<sup>33</sup>. Further, higher infection by Staphylococcus spp., Klebsiella spp., and Pseudomonas spp. was mostly associated with cows with higher parity, a scenario that has been well documented<sup>34</sup>. The current study showed that a higher number of bacteria isolates was found in cows in late-stage lactation, probably due to a lower immune status<sup>35</sup>. The higher number of Staphylococcus spp., Klebsiella spp., and Pseudomonas spp. in a poor hygienic environment supported the findings of another study <sup>28</sup>. The study reported a high prevalence of bacterial isolates in cows in early-stage lactation and attributed this to higher milk production, which is positively correlated with the spread of mastitis. The poor hygiene environment conditions at some of the farms along with other risk factors, were attributed to these results in the current study.

The results of the present study showed that the two selected bacterial isolates Staphylococcus spp. and E. coli were highly resistant to commonly used antibiotics for mastitis treatment. The isolates were least sensitive to oxytetracycline and more sensitive to gentamycin and Chloramphenicol. It has been shown that misuse and overuse of these antibiotics are a leading cause of bacterial resistance, also known as antimicrobial resistance. The overuse of oxytetracycline in treating various diseases could lead to heavy selection pressure and consequently the emergence of resistance. Previous studies in Kenya have shown that high prevalence of bacteria resistant to oxytetracycline<sup>13,14</sup>. Treatment of mastitis is often instituted by farmers who rarely complete the course of treatment<sup>13</sup>. Most of the farmers reported that they obtain the drugs at local drug stores often without a prescription which could lead to widespread misuse of the antibiotics. Oxytetracycline is the most common drug available with vendors in the area of study, which would tie with the observations of this study. According to the local Veterinary Office reports, gentamycin and Chloramphenicol have only recently been introduced in the area where this study took place (Kajiado North sub-county, unpublished data). However, it should be noted that Chloramphenicol is contraindicated in milk-producing cows due to long milk and meat withdrawal time<sup>36</sup>. The reason for the high prevalence of resistant strains in the study area warrants

further scientific investigation, considering that dairy farming is relatively new and shifts from traditional pastoralism to animal husbandry.

#### **5.** Conclusion

This study has documented the extent of antibacterial resistance associated with sub-clinical mastitis in dairy cattle in a peri-urban setting in Kajiado County, Kenya. The high antimicrobial resistance levels require urgent attention from relevant authorities. This study recommends the regular use of CMT as a screening test to test and treat cattle with mastitis at all stages of lactation. Further, some of the bacteria reported in this study are of zoonotic significance, and thus they could be spread to human beings through various means, such as consumption of raw milk and its by-products, thereby imparting antimicrobial resistance in humans.

#### Declarations

#### Competing interests

The authors declare that they haven't competing interests.

#### Authors' contribution

All authors conceived and designed the study. Maina Ngotho carried out the risk factor and other data analyses. John Kagira and Naomi Maina undertook the literature review and microbial analyses. Janet Njoroge and Daniel Nkoiboni participated in the collection of data, culturing and identification of the bacteria. All authors analyzed data and wrote the manuscript. All authors read and approved the analyzed data and the final revised article.

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#### Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

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