PREVALENCE, RISK FACTORS AND ANTIMICROBIAL RESISTANCE PROFILES OF MASTITIS-CAUSING BACTERIA ISOLATED FROM DAIRY GOATS IN MUKURWE-INI SUB COUNTY, NYERI COUNTY, KENYA

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Prevalence, Risk Factors and Antimicrobial Resistance Profiles of Mastitis-Causing Bacteria Isolated from Dairy Goats in Mukurwe-ini Sub County, Nyeri County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this research work to my parents, husband David, and children Lemuel, Jael and Joanna.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial Resistance
CMT	California Mastitis Test
CoNS	Coagulase negative Staphylococcus
DNA	Deoxyribonucleic Acid
EABL	Extended Spectrum Beta-Lactamases
Elisa	Enzyme – Linked Immuno Sorbent Assay
MALDI-ToF	Matrix -Assisted Laser Desorption Ionization -- Time of –Flight
MDR	Multiple Drug Resistance
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *S. aureus*
NGO	Non -Governmental Organization
PCR	Polymerase Chain Reaction
SCC	Somatic Cell Count
TMP	Trimethoprim
WHO	World Health Organization

ABSTRACT

Bovine mastitis continues to be a cause of economic losses in the dairy industry and remains a major public health hazard globally. Though dairy goat farming in the study area has realized tremendous growth in the recent years, there is limited region-specific data on the prevalence, associated risk factors, and antimicrobial resistance (AMR) profiles of mastitis-causing pathogens in the dairy goats. This knowledge gap hinders the development of targeted control strategies and prudent antimicrobial use policies tailored to the local context. Therefore, this study seeks to determine the prevalence, risk factors, and AMR patterns of mastitis-causing bacteria isolated from dairy goats in Mukurwe-ini Sub County, thereby contributing to informed interventions for mastitis control and antimicrobial stewardship. In this cross sectional study, farm level data on risk factors for mastitis was obtained from 56 farmers using semi-structured questionnaires. A total of 189 goat milk samples were collected from the farms. The goat's udder was observed for signs of clinical mastitis and the California Mastitis Test (CMT) used to test the milk for presence of sub-clinical mastitis. All milk samples were then cultured for morphological identification of bacteria using culture and standard identification methods. The bacteria species were further confirmed by MALDI-ToF technique. The isolated bacteria were tested for antibiotic sensitivity to eight commonly used antibiotics namely; Cefuroxime (30µg), Cefotaxime (30µg), Amoxicillin and Clavulanic acid (10µg), Oxacillin (10µg), Azithromycin (15µg, Meropenem (10µg), Ciprofloxacin (10µg) and Nitrofurantoin (300µg) using the Kirby- Bauer disc diffusion test. The presence of antibiotic resistance genes (*mecA*, and *blaTEM*) was determined using polymerase chain reaction (PCR) method. The prevalence of clinical mastitis was 1.1% (2/189) while that of sub-clinical mastitis was 84.7% (160/189). Higher ($p<0.05$) prevalence of mastitis was observed in goats whose houses were cleaned fortnightly and in cases where farmers used same towel to dry different does' udders during the milking process. Thirteen (13) different bacterial species were isolated from the milk samples and identified by MALDI-ToF, and these included *S. aureus* (21.16%), Coagulase- negative Staphylococci (19.58%), *E. coli* (17.46%), *Pseudomonas* spp. (13.76%), *Enterobacter* spp. (10.05%), *K. oxytoca* (5.82%), *E. vulneris* (1.59%), *Proteus vulgaris* (1.59%), *Raoutella ornithinolytica* (1.59%), *Stenotrophomonas maltophilia* (1.05%), *Pantoea agglomerans* (1.05%), *Serratia marcescens (1.05%)* and *Cedeceas* spp. (0.53%). Majority (97.5%) of *S. aureus* were resistant to Oxacillin and were 100% sensitive to Ciprofloxacin. The Coagulase-negative Staphylococcu*s* isolates were 100% resistant to Oxacillin and 100% sensitive to Ciprofloxacin. Most (93.9%) *E. coli* isolates were resistant to Oxacillin, 69.7% were sensitive to Ciprofloxacin, and 87.9% were sensitive to both Amoxicillin/Clavulanic acid and Meropenem. The antimicrobial resistant genes detected in *S. aureus* and *E. coli* were *mecA* (66.67%, 0%), and bla_{TEM} (20%, 78.26%), respectively. In conclusion, the study showed that most of the does were affected by subclinical mastitis with the main causative bacteria being *Staphylococci* spp. and coliforms. Farmers need to be trained on improved control of mastitis by adoption of good milking practices and use of CMT kit for early detection of mastitis. Occurrence of multidrug resistance by key mastitis - causing

pathogens was shown to be prevalent and therefore there is need for development of intervention strategies.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

In Kenya, the dairy goat population is about 400,000 of which 80% are reared by small-scale farmers in the Mount Kenya region counties including Meru, Embu, Nyeri, Kirinyaga and Murang'a (Kikwatha and Kyalo, 2020; Mbindyo *et al*., 2018). Mukurwe-ini Sub-County in Nyeri County has a high population of dairy goats owing to the combined effort between government and Non-Governmental Organizations (NGO) to improve food and nutritional security in the region through dairy farming (Mburu *et al*., 2014). The social-economic studies done by Kinyanjui *et al*., (2008) showed that 57% of the dairy goats' milk produced was consumed in households while the surplus was sold to specific individuals mainly suffering from ailments such as diabetes, Acquired Immunodeficiency Syndrome (AIDS) and those allergic to cow milk (Mburu *et al*., 2014). The same study further noted that in Kenya, there is a high potential of dairy goat farming where each goat can yield up to 2.96 litres of milk daily. However, goat farming is affected by a number of challenges, the main one being infectious diseases such as mastitis (Mahlangu *et al*., 2018; Kagucia *et al*., 2020).

Mastitis is the most common disease that affects dairy goats (Gazzola *et al*., 2021), leading to heavy economic losses associated with poor milk quality, reduced milk yield, high cost of treatment, discarded milk while the animal is on treatment, early culling and sometimes death of the animal (Dahl, 1969; Kifaro *et al*., 2009). The losses that have been documented globally due to mastitis range from €61 to €97 per average cow per year (Hogevean *et al*., 2011) while that of goats range from 65 – 80 Euros (Sánchez *et al*., 1997; Oosterhuis., 2010). The disease results in the inflammation of the mammary glands as a result of infection by pathogenic agents such as bacteria, fungi or viruses, and spreads mainly through unhygienic conditions in dairy farms (Novac & Andrei, 2020). Contagious mastitis is caused by bacteria residing on the skin of the teat and inside the udder such as *Staphylococcus aureus* and *Streptococcus agalactiae*, and is transmissible from one goat to another. Environmental mastitis is caused by environmental pathogens such as *Escherichia coli*, *Streptococcus uberis* and

*Klebsiella s*pp., usually found on beddings, feed, manure and soil. Mastitis is, therefore, the outcome of interplay between three major factors: infectious agents, host resistance and environmental factors (Verma *et al*., 2018). Early detection and effective treatment of mastitis is of great importance to curb losses incurred by farmers while providing nutritional security. Knowledge on prevalence of mastitis in dairy goats, microbial diversity, risk factors associated with mastitis development, and antimicrobial resistance patterns would greatly improve prevention and guide treatment of the disease.

Mastitis is treated with antibiotics, mainly penicillins (Younan, 2002) which are administered through intramammary tubes and parenterally. While coliform mastitis may sometimes be treated using cephalosporins and fluoroquinolones (Aqib *et al*., 2019), their use should strictly be based on bacteriological diagnosis (Suojala *et al*., 2013). Early treatment with effective antibiotics significantly limits the severity of mastitis, economic loss and development of antimicrobial resistance (Verma *et al*., 2018). The overuse and misuse of veterinary antibiotics by farmers has been associated with infection resurgence , due to development of antimicrobial resistance (AMR) in mastitis-causing pathogens (Aqib *et al*., 2019). Cloxacillin, for example, an analog of Methicillin/oxacillin which is used in dry cow therapy, has been implicated in development of methicillin-resistant *S. aureus* (MRSA) isolates (Saini *et al*., 2012). *S. aureus* has been isolated from raw milk samples and is a leading cause of dairy mastitis. Worldwide, there has been reduced efficacy of drugs used to treat infections caused by MRSA due to its increased resistance to glycopeptides (Tarai *et al*., 2013). Further, extended spectrum beta-lactamases (ESBL) antibiotic-resistant *E. coli* and *Klebsiella* species have been isolated from dairy milk (Badri *et al*., 2017).

Livestock bacteria can be reservoirs of antibiotic resistance genes such as those associated with ESBL in *Enterobacteriaceae* which could be transferred to human beings through multiple routes (Ljungquist *et al*., 2016). In addition to expanded-spectrum cephalosporins (ceftriaxone, ceftiofur, cefotaxime and ceftazidime), ESBL producers often carry determinants that confer resistance to fluoroquinolones, aminoglycosides and trimethoprim (TMP) – sulfamethoxazole combination (Saini *et al*., 2012).

Antimicrobial resistance is a public health threat and can potentially cause human mortalities approximated to 10 million per annum by 2050 if new effective antimicrobials are not developed (O'Neill, 2016). In 2019, over 1.05 million were associated with antimicrobial resistance and 250000 deaths were attributed to AMR in the World Health Organization (WHO) African regions, posing unprecedented health threat (Sartorius *et al*., 2019). The mortality rates of infections associated with multidrug-resistance (MDR) microorganisms have consistently increased over the last two decades across different populations (Ali *et al*., 2019). Over time the bacteria that cause mastitis have developed resistance to the antibiotics administered (Kromker et al., 2017).

1.2 Problem Statement

In Kenya, the major challenges to goat production are diseases, sub-optimal husbandry, inadequate supply of breeding stock, lack or poor supply of inputs including drugs, feed, water (Kinyua, 2011), unavailability of appropriate markets, poor market organization, poor infrastructure and lack of efficient information networks, poor public policy on environment, poor administration of animal health policies, decreasing farm size and insecurity (Mbindyo *et al*., 2020). Mastitis is a significant health challenge affecting dairy goats, with substantial economic, productivity, and animal welfare implications. The disease is the most widespread and costly disease in dairy goats occurring throughout the world. Costs due to mastitis include reduced milk production, condemnation of milk due to antibiotic residues, veterinary costs, culling of chronically infected goats and occasional deaths. In Kenya, particularly in smallholder systems like those in Mukurwe-ini Sub County, Nyeri County, dairy goat farming plays a crucial role in improving rural livelihoods through milk production and income generation. However, the burden of mastitis remains underreported and inadequately addressed in goats compared to cattle. Furthermore, the misuse and overuse of antibiotics in treating mastitis have contributed to the emergence of antimicrobial-resistant bacteria (Abebe *et al*., 2016*)* , complicating treatment outcomes and posing a public health threat through potential transmission of resistant pathogens via the food chain. Previously, studies have documented the major pathogens of mastitis such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and

Coliforms. However, recent studies have reported that coagulase-negative *Staphylococcus* and other *bacilli* could be potential mastitis - causing p

athogens. These reports have shown that these minor pathogens may play a significant role in the pathogenesis of mastitis and vary from herd to herd (Mbindyo *et al*., 2020). The challenge has been compounded by the fact that some of the pathogens responsible for causing mastitis in dairy goats have developed multi drug resistance which could be through amongst other mechanisms, reduced intracellular drug accumulation and biofilm formation (Yalew, 2020).Despite the growing concern, there is limited region-specific data on the prevalence, associated risk factors, and antimicrobial resistance (AMR) profiles of mastitis-causing pathogens in dairy goats in this area. This knowledge gap hinders the development of targeted control strategies and prudent antimicrobial use policies tailored to the local context.

1.3 Justification

Mastitis causes a sharp decrease in milk production and farm revenue. The milk yield in dairy goats can decrease by as much as 25% and at times up to 100% (Kifaro *et al*., 2009). The disease is also a public health concern as the bacteria are potentially zoonotic and can cause sicknesses associated with food toxins (Cobirka *et al*., 2020). Only a few studies have been done in Kenya on mastitis and the antimicrobial resistance in bacteria causing mastitis in dairy goats. This study is important in order to profile all the microbes responsible for mastitis including the emerging microbes.

Antimicrobial resistance is a growing problem in Kenya and this puts the country in danger if no interventions are made to address the challenge. In 2019, there were 8,500 deaths attributed to AMR and 37,300 deaths associated to AMR in Kenya (Gitaka *et al*., 2020). Globally, AMR is projected to cause 10 million deaths by 2050 if inadequately mitigated. There are already fears of running out of antibiotics in the near future due to AMR (Gitaka *et al.*, 2020).

The FAO action plan on antimicrobial resistance 2016–2020 recommended the need for situation analysis and surveillance of AMR in animals and human beings so that effective strategies can be implemented (World Health Organization, 2017). As a result, the Kenyan Ministry of Health (MOH) developed its National Action Plan (NAP) on prevention and containment of AMR in 2017, using the Global Action Plan as the blueprint. The plan is to improve awareness and understanding of antimicrobial resistance through effective communication, education and training, reduce the incidence of infection through effective sanitation, hygiene and Infection Prevention and Control (IPC) measures. The information obtained from this study will therefore be vital not only in identifying bacteria associated with mastitis in dairy goats and their associated antimicrobial resistance profiles, but also in creating awareness and inform the need for proper hygiene in preventing spread of AMR in the study region.

The study results will have an impact on Kenya AMR policies as it can be used to improve awareness and understanding AMR, strengthen the knowledge and evidence base, reduce the incidence of infections through effective sanitation, hygiene and infection prevention and control measures. The study will also help in the achievement of sustainable development goals number 3 on Good health and wellbeing, Kenya's Vision 2030 through control of AMR and therefore improvement of good health and wellbeing of both humans and animals. Therefore, this study seeks to determine the prevalence, risk factors, and AMR patterns of mastitis-causing bacteria isolated from dairy goats in Mukurwe-ini Sub County, thereby contributing to informed interventions for mastitis control and antimicrobial stewardship

1.4 Research Questions

1. What is the prevalence and risk factors of mastitis in dairy goats in Mukurwe-ini Sub County, Nyeri County?
2. Which bacterial strain(s) cause mastitis in dairy goats in Mukurwe-ini Sub County, Nyeri County?
3. What are the antimicrobial susceptibility patterns of mastitis-causing bacteria isolated from dairy goats in Mukurwe-ini Sub County, Nyeri County?
4. Which genes confer antimicrobial resistance in mastitis-causing bacteria isolated from dairy goats in Mukurwe-ini Sub County Nyeri County?

1.5 Hypothesis

1. There is a significant association between specific dairy management practices and the incidence of mastitis in dairy goats from the study region.

2. Mastitis-causing bacteria isolated from goat milk in Mukurwe-ini Sub County exhibit distinct antimicrobial resistance profiles.

1.6 Objectives

1.6.1 General Objective

To determine the prevalence and risk factors of mastitis, and the antimicrobial resistance profiles of bacteria causing mastitis, in dairy goats kept by farmers in Mukurwe-ini Sub County, Nyeri County, Kenya.

1.6.2 Specific Objectives

1. To determine the prevalence and risk factors of mastitis in dairy goats kept by farmers in Mukurwe-ini Sub County, Nyeri County.
2. To determine the spectrum of bacteria causing mastitis in dairy goats in the study area.
3. To evaluate the antimicrobial susceptibility patterns in mastitis-causing bacteria isolated from dairy goats in the study area.
4. To determine the antimicrobial resistance genes in key bacteria causing mastitis in dairy goats from the study area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Goat Production

The report of Food and Agriculture Organization indicates that globally goat population continues to grow and stands at approximately 1.2 billion animals. Africa had over 506 million heads of goats as of the year 2022 with Kenya having a population of 34.53 million goats (FAOSTAT, 2022). The lessons gained globally in the sector of the dairy goats reveals successful building blocks in industries related to dairy goats but Asia leads in production and consumption of dairy goats, (Miller & Lu, 2019). Some of the dairy goat breeds includes; Sannen, Toggernburg, Alpine and Anglo-Nubian. The main goat breed kept for meat is Boer. Some goat breeds kept for fibre include Angora and Cashmere (Ryder, 2001).

European countries more so the developed ones like France have sectors that are well aligned to dairy goat rearing. The specialty in the goat sector in Europe is production of milk, skewed to making of cheese and manufacturing that is traditional on the farms, (Miller & Lu, 2019). The government is visible in the regulation of the sanitary, extension, research, organizations involved in production support in marketing as well as quality and safety assurances. However, producers still experience market fluctuations (Miller & Lu, 2019). The ownership of herds in Europe stands at 2.51% of goats herds worldwide, producing 18.1% of the milk produced globally, (Escareño *et al*., 2012). There is continued variation in the milk production on worldwide scale as avenues for selling of milk product grows in many states and countries. The strides experienced rely on the farming concepts that specify practices that are well organized and are top notch in the industry, superior management of animals and consumers acceptance of quality products. These dimensions in marketing are expected to give competition to cow milk (Kinyua, 2011).

In the rural economies the stake of goat contribution is underestimated due to its informality and not well enshrined in the economic system, selling and marketing of goat products and the nature of goats reared, (Kinyua, 2011). There are widespread bottlenecks that curtail the Africa rearing of goats mainly the resources hindrance, bias that is cultural and political, limited stock of breeding, diminished understanding on its importance, support in marketing, information, untrained personnel and infrastructural deficiency, (Mahlangu *et al*., 2018).

On a global dimension the production of goat milk amounts to 18.67million tons, with 6.17 tons emanating from India, with Bangladesh, Sudan, Pakistan, France, Greece, turkey and Spain following respectively, (FAO, 2018). This has led to a gain in the stakes that are interested in goat milk production and associated products over the recent years, (Escareño *et al*., 2012).

On a comparison mode the milk produced by goats against milk produced by cows, there is a buffering capacity that is higher as well as digestibility, therapeutic use in human nutrition and alkalinity that is diverse. More so goat milk fat globules are smaller, and protein's polymorphism that is distinct with significantly lower as1- and higher as 2-case in which makes it to have a higher tolerability than cow milk (Sepe *et al*; 2019). Moreover, fatty acids are short and medium chain and there by being vital for boosting growing children ability. The composition of milk products of goats milk are ice cream that is blended out of the mixture of diverse milk (cows or sheep) or from the pure milk, cheese, yoghurt, curd and fermented milk, (Sepe *et al*; 2019).

2.1.1 Goat Production in Kenya

In totality the Kenyan goat's population stands at 34.53 million according to an article published by Statista (2024). Most of these goats were not bred for milk production but for meat. In Kenya, dairy goats' population is about 400,000 with a majority of 80% being reared in Mount Kenya region (Kikwatha & Kyalo, 2021). The goats are reared as a remedy for the increased search for small household's milk demands especially in the lower populations that are poor. Due to the density of population and small parcels of land this rearing becomes beneficial and economical, (Mbindyo *et al*., 2018). In Mukurwe-ini Sub County, the breeds of goats kept include; Alpine, Toggernburg, Saanen, Cross-breeds and Local breeds (Mbacho, 2011).

The representation of goat milk in the Kenyan gross domestic product remains unknown (Mbindyo *et al*., 2020). According to FAO (2006), total goat milk yield in Kenya was estimated at 0.129 million tons, most of which is produced and consumed by Kenya's pastoralists who are settled in arid regions with limited potential for agriculture. Hitherto, the determinant aspects remains visible in those goats in East Africa are small and their production is principally meant to raise their offspring, (Mbindyo *et al*., 2020).

In the mountain region of Kenya, the introduction of goats of exotic nature by organizations that were not affiliated to the government like FARM Africa, was seen in 1996, putting emphasis on development of rural agriculture in Africa (Mbindyo *et al*., 2018). FARM Africa collaborated with the agricultural docket in Kenya in order to steer the low farmers to adopt better breed from their local breeds. Thus Alphine and Toggenburg introduction led to crossbreeds of goats of local nature to breeds that were pure through community enablement (Ahuya *et al*., 2001). Currently, Kenya common breeds of dairy goats include; Toggenburg and crossbreeds (Kinyua, 2011; Ndegwa *et al*., 2001, Mbindyo, 2014).

In Kenya the farming of dairy goats has turned to be a profitable venture for the farmers in low scale, since through selling the animals they gain extra income as well from their produce like milk, skin and meat. In addition, goats are able to provide manure which is used by farmers (Ogola and Kosgey, 2010). Goats have the capacity to produce leaner, nutritious meats and milk because they possess a capacity of feeds conversion. They are highly adaptable and can use forage that is marginalized and can withstand conditions that are harsh. Subsistence farmers rely on these characteristics as the most valuable asset, (Ogola and Kosgey, 2010).

2.1.2 Challenges of Goat Production in Kenya

The underlying challenges in Kenya as concerns the production in the goat farming are market related concerns after maturity, susceptibility to diseases, high cost of concentrates, feeds that are not sustainable, unreliability of programs of buck rotation, and loss due to insecurity, (Mbindyo *et al*., 2018). The existence of diseases at a large scale makes the health of the goats susceptible and this diminishes their milk productions that affects directly the goats or alternatively influences their fertility levels on a large scale thereby leading to delays in the process of reproduction and reduced lactations in the long run. Reports that have been established on the diseases that are infectious in dairy goats includes mastitis, disease of the mouth and foot, tuberculosis; pox, caseous lymphadenitis; mycoplasma, brucellosis, pneumonia; Johne's disease; enterotoxaemia; and other diarrhoeal diseases such as colibacillosis (Kagucia *et al*., 2023;Mbindyo *et al*, 2018).

2.2 Mastitis in Dairy Goats

Mastitis is an infection or inflammation of the mammary glands or udder of dairy animals (Contreras *et al*., 2007) (Figure 2.1). The infectious agents implicated to cause caprine mastitis include bacteria, fungi and algae (Bhatt *et al.*, 2012).The disease is visible through the milk or udder characteristics that are bacteriological, chemical, physical, and pathological, (Amin *et al*., 2013). The economic losses that emerges with mastitis include treatment cost escalations, labour demands also rises, culling costs, yield in cheese diminishes, and decrease in quality as well as produced milk, (Gomes *et al*., 2016).

Figure 2.1: Inflammation of the Udder

Source: (Camacho *et al*., 2005).

2.2.1 Forms of Mastitis

In dissecting the forms, it can gangrenous sometimes but mostly it is clinical or sub clinical. The presence of these two forms on the herds is limited in clinical mastitis whereas sub clinical mastitis may go unnoticed. Sub-clinical mastitis has the highest cost implication due to lowered production in milk and a predominant factor of mastitis of clinical nature, (Ali *et al*., 2016).

2.2.1.1 Clinical Mastitis in Goats

Mastitis that is clinical in nature is an inflammatory response that causes visibly abnormal milk color change and fibrin clots. The acute systemic form of mastitis occurs within 4-6 hours after infection and is associated with an increase in fever over 39.2^{o}C and a trajectory pulse rate (Siivonen *et al*., 2011). There is appetite loss in doe's feeding behavior, depression experience and slow movement may be seen. There is a water secretion of milk that is yellow in color, mammary glands that are swollen, hard and color red, hot and sensitive to touch. The milk of the doe may clot and be flake mostly in cases that are severe, fatality may occur due to mastitis, (Kateete *et al.*, 2013; Ceniti *et al.*, 2017).

2.2.1.2 Sub-clinical Mastitis (SCM) in Goats

Subclinical mastitis occurs in the udder gland as an infection that has no clinical visible signs. This makes mastitis of sub clinical nature to go unseen not unless subjected to tools of diagnostic nature, Koop *et al.*, 2012). The suffering from subclinical mastitis is a constant threat to the entire herd as it poses a threat of mastitis transmission. Subclinical mastitis may turn out to be acute and eventually chronic clinical mastitis. Subclinical mastitis leads to substantial somatic cell count (SCC), alterations in composition of milk and yield in milk lowered, (Martins *et al.*, 2020).

2.2.1.3 Chronic Mastitis

This form of mastitis that is chronic is an infection that is incurable and persistent. It can manifest itself as clinical or sub clinical through udders inflammation characteristics that extends for a prolonged time period, (Jan & Jaśkowski, 2014). Animals suffering from chronic mastitis are normally culled (Merz *et al.*, 2016).

2.3 Prevalence of Mastitis

Determining the exact prevalence of mastitis in dairy goat worldwide could be challenging due to variation in reporting, data collection methods, management practices and geographical factors. However, mastitis is recognized as a significant issue affecting dairy goat herds globally, with prevalence rates varying among regions and farms. Various studies and surveys have reported mastitis prevalence rates ranging from 5% to over 50% in different dairy goat populations (Sánchez-Macías., *et al.*, 2017).

In Tanzania, a study showed a prevalence of 76.7% of mastitis of sub clinical nature in dairy goats (Mbilu, 2007). In Uganda, research in the District of Wakiso showed that 80.5% positivity to mastitis of dairy cows out of which 76.0% had subclinical mastitis (Kakooza *et al.*, 2023). A study done in Haramaya district in the Eastern of Ethiopia between 2018 and 2019 showed that 45.05% of the lactating dairy goats suffered from mastitis out of which 40.62% suffered from subclinical mastitis (Belina & To-fiq, 2023).

In totality the prevalence of mastitis in India dairy goats stood at 69.9% with 63.0% of the cases being subclinical (Mohanty *et al*., 2022). A Malaysian study done established that 40.93% lactating does suffered from mastitis (Ghazali *et al*., 2022) while in China, 45.82% dairy goats were found to have subclinical mastitis (Zhao *et al*., 2015).

The prevalence of mastitis in Kenya has risen over the recent years. Mbidyo *et al*, (2014), in a study revealed that prevalence of sub clinical mastitis stood at 59% in goats from County of Meru, 58.1% in county of Embu, and 54.2% in those from county of Nyeri. This was a rise from the study done by Ndegwa *et al* (2000) that established the prevalence of sub clinical mastitis to be 28.7%. Another study done by Mahlangu *et al* (2018) in Thika East Sub County found that prevalence of sub clinical mastitis to be 50.9%. Okoko *et al* (2020) study resulted to a prevalence of 72.5% of sub clinical mastitis in dairy goats. Sub clinical mastitis of such a high magnitude emanates from hygiene that is poor, procedures of milk that are unstandardized, non-usage of tat dips and nonexistence of proper washing both pre and post udder.

2.4 Causative Agents of Mastitis

The mastitis causing bacteria has been projected to emanate from the milk handling process as well as environment of the farm, (Marimuthu *et al.*, 2014). The association of mastitis with fungi and toxins related to it, as well as bacteria and virus has been cited, (Mahlangu *et al*., 2018). Out of the many bacteria's that are associated with mastitis cause in goats are *S. aureus* and Coagulase-negative staphylococci bacteria (Fox *et al*., 1993).Other causative agents of mastitis include; *K. pneumoniae, dysgalactia, K. oxytoca, Mycoplasma capricolum, S. uberi,* S. *S. caprae*; *E. coli, Pseudomonas aeruginosa, Streptococcus agalactiae,* and *Clostridium* spp.(Fahim *et al.*, 2019, Siivonen *et al*., 2011).

2.5 Diagnosis of Mastitis

Clinically, the mastitis diagnosis of goats are linked on the history and signs exhibited by the herd. The use of diagnostic methods that can recognize indirectly the mastitis of sub clinical nature include microbiological culture, Enzyme – Linked Immunosorbent Assay (ELISA), California Mastitis Test (CMT), milk electrical conductivity tests, Somatic Cell Count (SCC), pH and catalase test. The most common methods are CMT and SCC.

2.5.1 Microbiological Culture

A reliable diagnostic tool of mastitis in goats has been the microbiologic culture. Due to the economic and simplicity in performance of the bacterial culture techniques, the usage of repeated methods that are standardized needs to be performed. Whilst majority mastitis pathogens are readily grown under aerobic conditions on blood-based agar medium, some pathogens require specific growth media and growth conditions, for example *Mycoplasma* spp. (Adkins, 2018).

After primary isolation of a bacterial colony or colonies, additional tests must be applied to determine the identity of the organism. Some of the common phenotypic tests used to preliminary differentiate organisms isolated from milk include visual evaluation of colony morphology, examination of the culture medium for hemolysis, and Gram staining and KOH gelation testing. Bacteria are majorly classified into two; gram positive and gram negative bacteria. To differentiate between contagious and non-contagious gram-positive bacteria, catalase test, coagulase test and CAMP/esculin test are performed. Some examples of contagious gram-positive bacteria include; *S. aureus* and *S. agalactiae*. Noncontagious gram-positive bacteria include; non-aureus *Staphylococci*, non-agalactiae *Streptococci* and Streptococci-like organisms. Gram-negative bacterial isolates are identified by growth on selective medium like lactose fermentation on MacConkey agar, triple iron reaction, Simmons citrate agar and oxidase test. Motility testing may be used to differentiate the various environmental gram-negative pathogens (Patel, 2001).

2.5.2 Somatic Cell Count (SCC)

Somatic cell count (SCC) or logarithmic transformation of SCC, the somatic cell scores (SCS), is the most common diagnostic test used for detection of subclinical mastitis. In a laboratory set up, SCC can be measured using microscopy, referred to as direct microscopy or by using automated electronic cell counters. The direct microscopy SCC is performed by spreading a specific volume of milk within a calibrated area of a microscope slide. After the milk dries, the slide is stained and visible cells are counted within a defined area. This method is labour intensive, requires a high quality microscope, and requires highly trained personnel. Automated electronic cell counters, which are commonly based on flow cytometric methods, allows for rapid and easy determination of SCC (Adkins, 2018).

The SCC and the California Mastitis Test (CMT) are the most common test used to diagnose mastitis in dairy goats. The somatic cell count is a main indicator of milk quality. The SCC is defined as the number of cells per ml of milk (Forster *et al*., 2024). In healthy goat, there should be less than 1000 SCC/ml while in infection with weak pathogen it is 500,000-200,000 SCC/ml and over 1,500,000 SCC/ml in severe infections. SCC of milking does can therefore be used to define subclinical mastitis and a threshold of 200,000 to 400,000 cells/ml and above will accurately identify most infected ewes (Ali *et al.*, 2016).

2.5.3 California Mastitis Test (CMT)

California Mastitis Test is used to detect subclinical mastitis. The interpretation of the CMT is based on the visual inspection of the milk after mixing with the CMT reagent. The reaction takes place between the CMT reagent and the genetic material from the somatic cells found in the milk, forming a gel whose concentration is proportional to the number of somatic cells (Pradieé *et al*., 2012). A higher concentration in somatic cells leads to a higher CMT score (Ali *et al.*, 2016).

2.5.4 Enzyme – Linked Immuno Sorbent Assay (ELISA)

ELISA test involves detection of antibodies in the milk sample with a view of establishing existence or nonexistence of mastitis-causing pathogens. This method can only detect one pathogen at a time. The disadvantage of ELISA test is that antibodies detected in the milk may not be indicative of intramammary infection as they may have crossed over from other parts of the body that may have been infected with the said pathogen. Therefore, these crossovers may cause false negatives (Hicks *et al*., 1994).

2.6 Risk Factors of Mastitis

Microbial exposure is a major contribution in causing mastitis. In dairy animals bacteria are the main cause of mastitis.

2.6.1 Source of Bacteria

The infections of bacterial origin are categorized depending on the source of bacteria. Contagious pathogens like *S. aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis* are evident on teat skin and udder of cows, growing and colonizing the teat canal, (Kibebew, 2017). Infections of subclinical capacity can be established. The contact of animals and the reservoirs can be controlled in order to reduce the infections that are contagious, (Cheng and Han, 2020). Exposure to environmental pathogens such as *E. coli, Streptococcus uberis* and *S. aureus* increases the risk of infection. Contaminated bedding, dirty udders and unsanitary milking equipment can introduce bacteria to the udder (Aliyi, 2023).

2.6.2 Breeding and Genetic Factor

Mastitis vulnerability has been on breeding and genetic factor where pure breeds and cross breeds which have yields that are more genetically connected. Some breeds have weaker immune responses and anatomical traits that make them more prone to mastitis (Waller *et al*., 2014). Infections are also affected by age. The susceptibility to infections is more seen in older animal because of the wider or permanently partially - open teat canal due to frequent milking (Kibebew, 2017).

2.6.3 Lactation Stage

Another factor is the lactation stage where mastitis risk varies throughout the lactation cycle, as lactation in early days has high risk occurrence as well as in dry periods. Hormonal changes and alterations in milk composition during these stages can predispose cows to mastitis. Transition period which is the period between 3 weeks before and after parturition, being a period where the dairy animals are at a high risk of infection as a result of suppression of immunity which is associated with increased oxidative stress and low oxidative defense (Abebe *et al*., 2016). Due to the higher demand of energy for synthesis of colostrum and milk by the dairy animal, some animals suffer from nutritional imbalances where they do not meet the animal's lactation demands. The animal suffers from deficiency of trace elements (i.e. selenium, iron, copper, zinc, cobalt, and chromium), amino acids and vitamins. This weakens the animal's immunity and making her more susceptible to mastitis (Kibebew, 2017).

2.6.4 Poor Hygiene

Poor hygiene which includes inadequate cleanliness in the milking environment also contributes to mastitis risk. This includes dirty stalls, soiled bedding, and improper udder preparation before milking. Proper cleaning and sanitation protocols are essential to minimize bacterial exposure (Weigel, 2018).Inadequate milking procedures like incomplete udder stimulation, inadequate teat disinfection and over milking which can also destroy teat tissues and increase susceptibility to mastitis. Maintaining proper milking practices, including appropriate milking machines and trained personnel, is crucial (Bhosale *et al*., 2014).

2.6.5 Physical Factors

Physical factors such as trauma to the udder caused by injuries from kicks, bites or excessive pressure during milking, can create opening for bacterial entry and increase mastitis risk (Haghkhah *et al*., 2011, Khasapane *et al*., 2024). Finally, environmental stressors such as overcrowding, transportation, abrupt changes in diet or extreme weather conditions can compromise the cow's immune function and increase mastitis risk (Hogan and Smith, 2012).

2.7 Management of Mastitis

Currently, the most common treatment of mastitis is the use of antibiotics, particularly β-lactams (Gomes *et al*., 2016).The ideal antibiotic for systemic treatment of mastitis would be weakly basic, poorly bound to plasma proteins, and lipid soluble. It would retain activity in inflammatory secretions and have antimicrobial activity against mastitis pathogens. Systemically administered sulfonamides, penicillins, aminoglycosides and cephalosporins do not readily penetrate the mammary gland. Macrolides (erythromycin, tilmicosin), trimethoprim, tetracyclines, and fluoroquinolones distribute well to the mammary gland (Erskine *et al.*, 1993). Some of the drugs used in treating dairy mastitis, their classes and mode of action are provided in Table 2.1.

Table 2.1: Examples of Drugs Used in Treatment of Mastitis, Their Classes and Mode of Action

Examples of Drugs	Class	Mode of action
Cloxacillin, dicloxacillin, oxacillin and flucloxacillin	Penicillin	Binds beta-lactam ring to DD-transpeptidase inhibiting its cross-linking activity and preventing new cell wall formation
Ceftiofur, cefquinome, cefotaxime and cefuroxime	Cephalosporins	Inhibits bacterial cell synthesis
Ciprofloxacin	Fluoroquinolones	Interrupts DNA reunion step by binding DNA-gyrase or topoisomerase II and topoisomerase IV.
Clavulanic acid	Beta-lactamase inhibitors	Inhibits/binds to beta-lactamase enzymes
Trimethoprim and oxytetracycline	Tetracyclines	Inhibits bacterial growth by interfering with folate synthesis Prevents protein production
Erythromycin and azithromycin	Macrolides	Binds the 50S ribosomal subunit preventing protein synthesis
Sulphanilamide and sulphadimidine	Sulphonamides	Blocks the biosynthesis of folate coenzyme in bacteria thus stopping growth and division
Gentamycin, kanamycin and neomycin	Aminoglycosides	Inhibits protein synthesis Disrupts the integrity of bacterial cell membrane

Source: (Prescott, 2013; Dowling *et al*., 2017)

2.8 Control Strategies for Mastitis

Over the past 40 years, antibiotics ranging from narrow to broad spectrum have been used in the treatment of mastitis (Barkema *et al*., 2006). However, due to the emerging antibiotic resistance believed to be as a result of their overuse and misuse, the effectiveness of antibiotic therapy has been compromised. Therefore, the control of bovine mastitis has become one of the most challenging problems on dairy farms today (Tiwari *et al*., 2013). Selective antibiotic therapy has been found to cause a reduction of manifestation of clinical mastitis (Petersson *et al*., 2010). Intramammary infections have traditionally been treated using systemic or intramammary antibiotic therapy (Barlow, 2011).

Animals with chronic mastitis are unlikely to recover and should be culled to prevent transmission of bacteria to other animals which are the future of the herd. On its own, culling isn't a way of controlling contagious mastitis. Dairy farmers are often reluctant to cull the affected animals due to the devastating financial impact on backyard farmers due to losses in milk production as is often the case in developing countries (Petersson *et al*., 2010). During the dry period, the cow is at greatest risk of acquiring new intramammary infections with both gram positive and gram negative bacteria. Dry cow therapy with antibiotics has been suggested as one of the options to control intramammary infections and prevent development of mastitis (Ziv *et al*., 1981).

2.8.1 Husbandry Measures to Control Mastitis

Clean and dry housing provide clean, dry and well-ventilated housing for dairy goats. Regularly cleaning and disinfecting pens, stalls and bedding minimizes bacterial contamination and reduces the risk of mastitis transmission (Iraguha, 2023).It is important that good milking practices be implemented to minimize the risk of mastitis (Mahlangu *et al*., 2018). This would include thorough udder cleaning and sanitization before milking, using clean and well-maintained milking equipment, and ensuring gentle and efficient milking techniques to avoid teat damage. Another measure to combat mastitis is to have regular udder health monitoring of dairy goats by visually inspecting the udder and checking for signs of inflammation, swelling or abnormalities. Regular udder health screening, such as California Mastitis Testing, should be conducted to detect subclinical mastitis early and appropriate interventions initiated (Jesse *et al*., 2023).

A hygienic milking environment should be maintained by keeping milking areas clean and free from contamination. This ensures that there are no pathogens in the milking area that would otherwise cause mastitis. In addition, overcrowding should be avoided during milking sessions to minimize stress on goats and reduce risk of mastitis transmission between animals. The dairy goats should be provided with a balanced diet comprising of clean water and high-quality forage, supplement concentrates, vitamins and minerals to support udder health and immune function (Shahudini *et al.*, 2018). Good pasture management should be practiced to minimize exposure to environmental pathogens and reduce risk of mastitis. It is also important to rotate pastures regularly, maintain adequate grazing areas and avoid grazing goats on wet or muddy terrain, especially during periods of high rainfall (Shahudini *et al.*, 2018).

Minimizing stress factors that weaken the immune system and increase the susceptibility of goats to mastitis should be taken into consideration. This includes providing a low-stress environment with adequate space, comfortable housing and appropriate social interactions to promote overall health and well-being. Biosafety measures needs to be instituted in order to prevent the introduction and mastitis-causing pathogens spread within the goat herd. This includes quarantine procedures for new animals, limiting contact with outside animals, and controlling visitors' access to the farm to minimize the risks of disease transmission (Delabbio, 2006).

There should be prompt treatment of clinical mastitis with appropriate antibiotics under the guidance of a veterinarian. Treatment protocols should be followed carefully and complete recovery should be ascertained in order to curb the infection spread within the herd and minimize the development of antibiotic resistance (Ruegg, 2018). Establishment of a comprehensive herd health management plan that includes routine vaccinations, deworming and regular health screening to detect and address potential health issues before they escalate into larger problems including mastitis (Shahudini *et al.*, 2018).

2.8.2 Vaccines

There are experimental approaches undertaken for development of vaccines against bovine mastitis caused by major pathogens (Tiwari *et al.*, 2013). The vaccines are being formulated with the hope of reducing the incidence mastitis on-farm or backyard farming, and promising prototype vaccine candidates of the mastitis-associated pathogens. There are several efforts made to develop a vaccine against mastitis but only few have claimed satisfactory outcomes (Leitner *et al.*, 2000, El-Din *et al.*, 2006, Xu *et al.*, 2011). It is clear that a single vaccine will not prevent mastitis caused by the plethora of pathogens and their different mechanisms of pathogenesis (Tiwari *et al.*, 2013). Some of the mastitis vaccines developed include; vaccines against *S. aureus,* Coliforms,*Streptococcus uberis*, *Streptococcus agalactiae* and *Streptococcus dysagalactiae* (Tiwari *et al*., 2013).

2.8.3 Breeding of Mastitis-Resistant Animals

Breeding of production animals that are resistant to infectious diseases like mastitis should also be enhanced (Adam *et al*., 1998). Researchers use traditional breeding methods and genetic techniques (Hu *et al*., 2020) to identify animals within a population that show resistance to diseases by studying the immune response, genetic markers and other relevant traits. Once the resistant traits are identified, selective breeding programs are established to propagate those trains within the population. The specific genes associated with disease resistance are identified which allows for more precise selection of breeding stock based on their genetic makeup. This can speed up the breeding process by allowing breeders to identify desirable traits without waiting for phenotypic expression. By crossing animals from different genetic backgrounds, breeders can introduce novel combinations of genes that confer resistance. There are several countries that have made major strides in breeding disease-resistant cattle. An example is Australia and New Zealand that have sheep breeds that are resistant to nematode infections (Stear *et al*., 2012). In United States, there is an ongoing program to breed cattle with improved resistance to bovine mastitis, bovine respiratory disease (BRD) and other common ailments (Rothrock., *et al*. 2020).Selective breeding for immune response which focuses on selecting animals with strong immune response is done. Continuous monitoring and evaluation of breeding stock and their offspring are essential to assess the effectiveness of breeding programs. This allows breeding breeders to make adjustments as needed to further enhance disease resistance.

2.8.4 Bacteriophage Therapy

Bacteriophage therapy employs pathogen specific bacteriophages in the treatment of a bacterial infection. Recent interest in phage therapy in veterinary medicine was sparked by some early success in the treatment of *E. coli* infections in animal models including a chicken model for respiratory diseases (Huff *et al*., 2002), a mouse model for meningitis (Smith *et al*., 1982) and a calf model for diarrhoea (Smith *et al*., 1983).

However, the few studies that have been carried out using bacteriophages to treat mastitis caused by *S. aureus* infection have yielded variable results. While intramammary infusion of bacteriophage into *S. aureus* infected quarters of lactating dairy cattle did not show significant protection (Gill *et al*., 2006), Kwiatek *et al* (2012) isolated and characterized a bacteriophage from the milk of cows suffering from mastitis with broad-spectrum activity against MRSA. It is therefore suggested that additional research is required to explore the therapeutic potential of bacteriophages to treat clinical and subclinical mastitis associated bacterial infections (Tiwari *et al*., 2013).

2.9 Antimicrobial Resistance

Antimicrobial resistance (AMR) is a big challenge in the health sector and deaths associated to it could rise to 10 million by 2050 (WHO, 2021). Antibiotics are indispensable in treatment of bacterial diseases in both humans and animals but the prevailing remedies that are used to combat infections have been rendered inefficient due to rising AMR cases. According to Habboush and Guzman, (2018), antibiotic resistance arises when bacteria evolve and develop multiple different mechanisms to escape the effectiveness of antibiotics. The use of antibiotics in food animals is a foremost cause of evolving AMR in humans (Manyi-Loh *et al*., 2018). In livestock farming, animals expel antibiotic-resistant bacteria (ARB) in their gastrointestinal track; consequently, Antibiotic – resistant genes (ARGs) are spread into the environment including soil and water bodies (Manyi-Loh *et al*., 2018). The contaminated water and water bodies harbor resistant pathogens and resistant genes that may enter the food chain (Bürgmann *et al*., 2018), thus transferring ARB to humans via direct interface with animals, exposure to animal waste and consumption of contaminated foods of animal origin and fresh produce (Khan *et al*., 2020, Founou *et al*., 2021). Transfer of AMR from animals to humans and the environment is not only limited to foodborne pathogens, but also to commensal bacteria. Antimicrobial resistant *Enterococcus* spp., *Salmonella* spp., *Pantoea* spp., *Yersinia enterocoliticas*, *Enterobacter cloacae*, *Serratia marcascens*, *Enterobacter aerogenes* and *Serratia odorifera* and other commensal bacteria have been isolated from manure and soil in dairy farms (Mukuna *et al*., 2023). Some of these bacteria isolated have been found to be resistant to some antibiotics (Najeeb *et al.*, 2013). *Enterobacteriaceae* was found to be 100% resistant to novobiocin, erythromycin and vancomycin. Resistance to tetracycline ranged between 75% and 100%. *Enterobacteriaceae* isolates also displayed low resistance (≤ 25%) to cefpodoxime and nalidixic acid (Mukuna *et al.*, 2023). Some of the isolates from dairy farms have been found to have multidrug resistance. According to a study done by Mukuna *et al* (2023), *E. coli*, *Salmonella* spp., *Enterococcus* spp and *Listeria monocytogenes* were found to have multidrug resistance.

Antimicrobial resistance arises when microorganisms such as bacteria, viruses, fungi and parasites evolve in ways that render the medications used to treat them ineffective (Baquero *et al*., 2021). There are several interconnected factors that contribute to the development and spread of antimicrobial resistance.

2.9.1 Food Production and Animal Husbandry

The development and spread of antimicrobial resistance (AMR) have been linked to certain practices in animal agriculture and food production systems (Allcock et al., 2017). Among these are the incorporation of antimicrobials into animal feeds, not only to promote growth but also as a preventive measure against infections (Landers et al., 2012). According to Basulira et al. (2019), residues of antimicrobials—particularly beta-lactam antibiotics—were found in higher concentrations in meat from adult cattle compared to that from younger animals. This is indicative of the inappropriate use of these drugs, which significantly contributes to the development of AMR in both commensal and pathogenic bacterial populations in ruminants (Pehrsson et al., 2013). Martinez (2009) further emphasized that approximately 75–90% of administered antimicrobials are excreted unmetabolized, leading to the accumulation of drug residues in the environment surrounding livestock operations. These resistant bacteria can be transmitted to humans either through the consumption of contaminated animal products, such as meat and milk, or via environmental exposure through direct or indirect contact with animals or their waste (Hassell et al., 2019) as shown in Fig 2.2

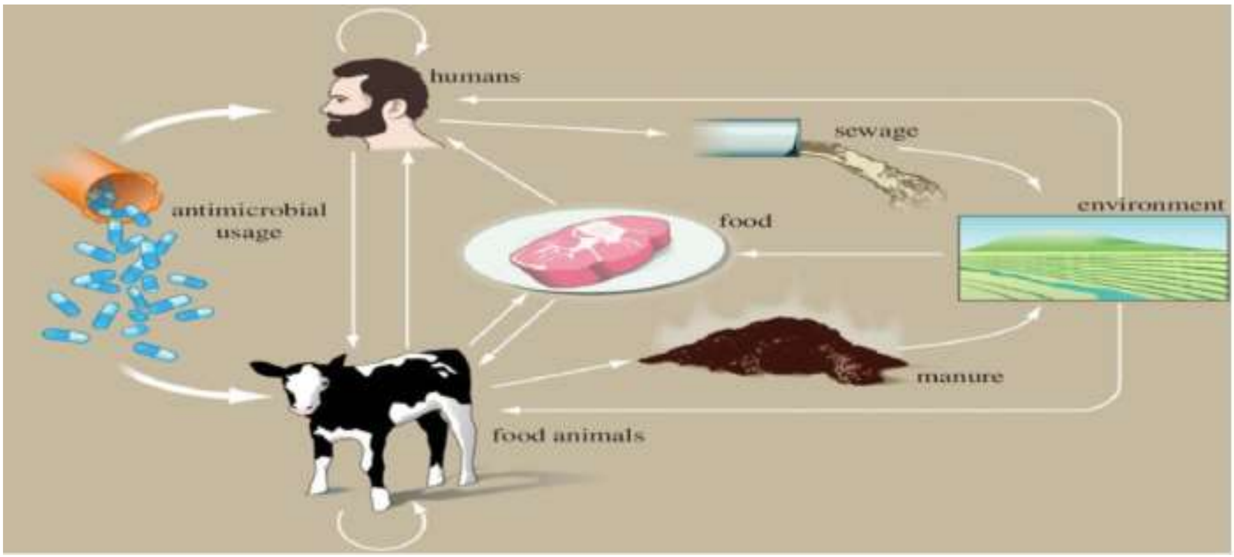

Figure 2.2: Routes of Transmission of Drug-Resistant between Different Farm Animals, Environment and Humans

Source: (Hassel *et al*., 2019).

2.9.2 Antibiotic Overuse and Misuse

The excessive and improper use of antibiotics remains a major contributor to the development of antimicrobial resistance (AMR). In the management of mastitis, repeated or unregulated antibiotic application can foster the emergence of resistant bacterial strains within the goat's udder as well as in the wider farm environment (Mbidyo et al., 2021). Subclinical mastitis, which often lacks visible symptoms, is common in goat herds. As a result, farmers may resort to preventive antibiotic use, even in the absence of clinical disease. This approach can favor the selection of resistant bacterial populations within the udder and may further propagate resistance on the farm (Ajose et al., 2022). Additionally, improper treatment protocols—such as incomplete courses or incorrect dosages can allow partially resistant bacteria to survive, increasing the risk of persistent infections and future treatment failure (Kovačević, 2022). Resistant mastitis-causing bacteria may be excreted into milk, contaminating dairy products and potentially posing a risk to consumers. Moreover, infected animals can act as reservoirs, transmitting resistant pathogens to healthy herd members and thereby facilitating the spread of resistance within the farm ecosystem (Widianingrum, 2022). The slowdown in the discovery and development of new antibiotics over recent decades driven by scientific, regulatory, and economic constraints has further limited treatment options. This situation increases reliance on existing antibiotics and heightens the risk of resistance development (Tang et al., 2023). Additionally, antibiotics and resistant microbes can be introduced into the environment via sewage, runoff from agricultural lands, and waste from pharmaceutical production facilities.

Antibiotics overuse and misuse has been one of the primary drivers of AMR. In mastitis management the frequent and sometimes indiscriminate use of antibiotics may result into bacteria that are resistant to antibiotics within the goat's udder and the broader farm environment (Mbidyo *et al*., 2021). The non-existence of clinical symptoms can also be prevalent in goat herds due to subclinical mastitis. Farmers may use antibiotics prophylactically or as a preventative measure to address subclinical mastitis. This practice may result into selection of bacteria that are resistant to antibiotics within the udder and potentially contribute to the spread of resistance (Ajose *et al*., 2022). In addition, inadequate treatment of mastitis cases, such as incomplete or improper administration of antibiotics, can promote the survival of bacteria with resistance to the antibiotic used. It may increase the persistence of antibiotic-resistant bacterial strains within the udder and increase the likelihood of treatment failure in subsequent mastitis cases (Kovačević, 2022). Bacteria that are resistant to antibiotics and connected with mastitis can be shed into the milk, contaminating milk products and potentially posing a risk to human health. Additionally, infected goats can transmit resistant bacteria to other animals within the herd, contributing to the dissemination of resistance to antibiotics in the farm surroundings (Widianingrum, 2022).The development of new antibiotics has significantly slowed down in recent decades due to scientific, regulatory and economic challenges. Without effective new antibiotics, veterinary officers have fewer options to treat infections, leading to increased reliance on existing drugs and emergence of resistance (Tang *et al*., 2023). Antibiotics and resistant bacteria can also be found in the surroundings through sewage, agricultural runoff and pharmaceutical manufacturing waste.

2.9.3 Environmental Exposure

Exposure of the environment to antibiotics can facilitate the emergence and persistence of antibiotic-resistant bacteria in soil, water bodies, and even among wildlife populations (Larsson, 2014).

2.10 Mechanisms of AMR

Antibacterial agents exert their effects by disrupting key bacterial processes, including inhibition of cell wall synthesis, alteration of cell membrane integrity and function, blockage of protein synthesis, or interference with the replication and transcription of genetic material such as DNA and RNA (Munita and Arias, 2016).

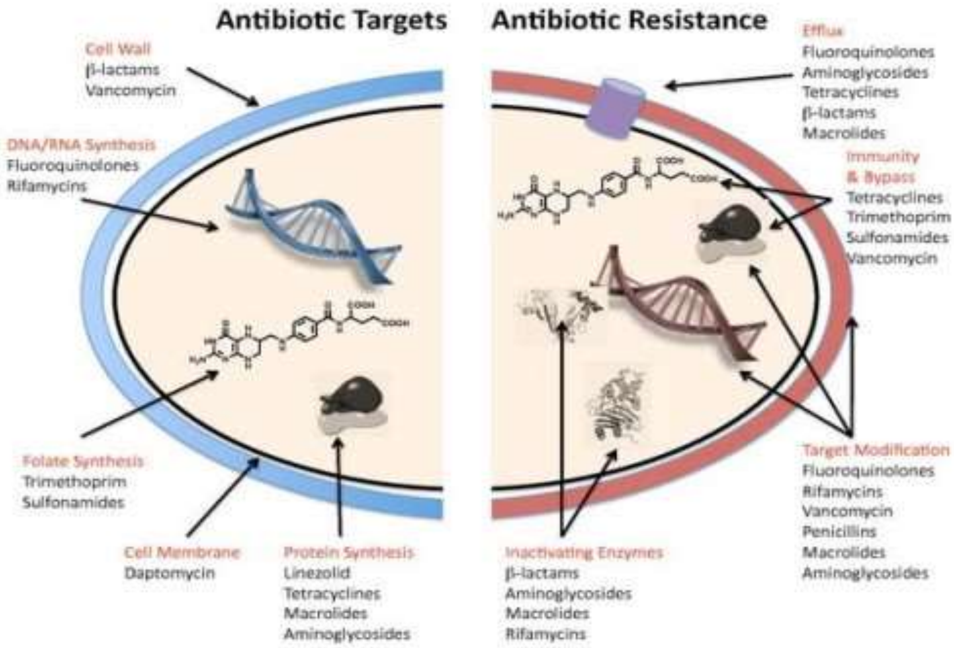

Figure 2.3: Antibiotic Targets and Mechanism of Resistance

Source: (Munita and Arias, 2016).

Bacterial resistance to antimicrobials can generally be grouped into four main mechanisms. One involves limiting the antibiotic's access to its target site, as seen in *Pseudomonas aeruginosa* and *Klebsiella* species resistant to β-lactam antibiotics, as well as in vancomycin-intermediate resistant *Staphylococcus aureus*, which produces a thickened cell wall that sequesters the antibiotic before it reaches its target (Munita and Arias, 2016). A second mechanism is the activation of efflux pumps, which actively expel antibiotics such as streptogramins, tetracyclines, macrolides, and lincosamides from the bacterial cell, reducing intracellular drug concentrations. The third strategy involves enzymatic inactivation or modification of the antibiotic, such as the hydrolysis of the β-lactam ring in penicillins and cephalosporins by bacterial β-lactamases (King et al., 2016). The fourth mechanism entails structural changes to the antibiotic's target within the bacterial cell. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) expresses altered penicillin-binding proteins, while vancomycin-resistant *Enterococcus* species exhibit genetic changes affecting the synthesis of cell wall components, membrane integrity, autolysis, and teichoic acids (Blair et al., 2015). In addition, bacteria can acquire resistance through genetic mutations or by horizontal gene transfer, facilitating the spread of resistance traits among susceptible species (Wintersdorff et al., 2016).

2.11 AMR Genes in Bacteria

Antimicrobial resistance (AMR) in mastitis-causing bacteria is primarily driven by specific genes that confer resistance to various antimicrobial agents (Boireau *et al*., 2018). These genes are acquired as transfers of genes horizontally, mutations and mobile genetic presence through genetic elements such as integrons, transposons and plasmids. The genes responsible for AMR in mastitis - causing bacteria are dependent on the species and strain (Xue *et al*., 2011). Some examples of genes associated with AMR in bacteria commonly implicated in mastitis include;

2.11.1 *Staphylococcus aureus*

mecA gene confers resistance to beta-lactam antibiotics including methicillin and other penicillins, by encoding penicillin-binding protein 2a (PBP2a) with reduced affinity for beta-lactams (Pu *et al*., 2014). *blaZ* gene encodes beta-lactamase enzyme, which hydrolyzes beta-lactam antibiotics such as penicillins and cephalosporins, rendering them ineffective (Yang *et al*., 2015). *Erm* gene family confers resistance to macrolide antibiotics including erythromycin, by encoding rRNA methyltransfererases that modify the ribosomal target site (Spiliopoulou *et al*., 2004). *Tet* gene family confers resistance to tetracycline antibiotics by encoding efflux pumps or ribosomal protection proteins that prevent tetracycline binding to the ribosome (Zhu *et al*., 2021).

2.11.2 *Streptococcus agalactiae*

Streptococcus agalactiae poses as a contagious pathogen that is persistent in the mammary glands and healthy cow's transmission is through unhygienic methods of milking, (Gao *et al*., 2012). Resistance determinants that are identified in *S.agalactiae* include *erm* genes that confer resistance to macrolide antibiotics, *tet* gene family confer resistance to tetracycline antibiotics and aphA-3, aad-6 which are resistant to aminoglycosides (Gao *et al*., 2012).

2.12.3 *Streptococcus uberis*

Resistance determinants identified in *Streptococcus uberis* include *Inu* gene which encodes a nucleotidyltransferase that modifies lincosamide antibiotics, such as lincomycin and clindamycin thereby reducing their efficacy (Vezina *et al*., 2021).

2.11.4 *Escherichia coli*

blaTEM, *blaCTX-M*, *blaSHV* genes encode extended-spectrum beta-lactamases (ESBLs) enzymes that are capable of hydrolyzing extended-spectrum cephalosporins and other beta-lactam antibiotics (El-Mohandes *et al*., 2022). *Gnr* gene family confers resistance to quinolone antibiotics including fluoroquinolones by protecting DNA gyrase and topoisomerase IV from drug inhibition (Solano-Gálvez *et al*., 2020). Aac(6')- *Ib-cr* gene confers resistance to fluoroquinolones by encoding a variant of the aminoglycoside acetyltransferase that modifies fluoroquinolones, reducing their activity. *Sul* gene family confers resistance to sulfonamide antibiotics by encoding dihydropteroate synthase with reduced affinity for sulfonamides. *tet* gene family confers resistance to tetracycline antibiotics (Rana *et al*., 2022).

2.11.5 *Klebsiella pneumoniae*

blaKPC, *blaNDM*, *blaOXA*-48 genes encode carbapenemases which are enzymes capable of hydrolyzing carbapenem antibiotics that lead to carbapenem resistance.

blaSHV and *blaCTX-M* genes encode extended-spectrum beta-lactamases (ESBL), conferring resistance to extended cephalosporins and other beta-lactam antibiotics (Koovapra *et al*., 2016).

2.12 Detection for Antimicrobial Resistance (AMR) Genes in Mastitis-Causing Bacteria

Detection for AMR genes in mastitis-causing bacteria involves molecular techniques aimed at detecting specific genetic elements associated with resistance to antimicrobial agents. A general outline of how to assay for AMR genes in mastitis involves the extraction of the DNA (genomic) from the bacterial isolates using a suitable an extraction DNA kit or protocol. The DNA extraction method used should be compatible with downstream molecular assays. Polymerase chain reaction (PCR) should be performed in order to amplify the target AMR gene of interest (Mahuku, 2004). Primers should be designed which should be specific to the target genes or gene families based on available sequence information (Borah, 2011). PCR conditions should be optimized for efficient and amplified specifically. In order to verify amplification, agarose gel electrophoresis analysis needs to be done on PCR products and determine the size of the PCR products (Ruiz-Villalba *et al*., 2017). The gel should be stained using DNA-binding dye (e,g. ethidium bromide or SYBR safe) and bands visualized under ultraviolet (UV) light. The specificity of PCR products should be validated by sequencing or other appropriate methods to be congruent with the genes of amplified AMR. This step is important as it ensures that the detected genes are indeed the target resistance genes and not non-specific amplification products. If quantification information is required, quantitative PCR (qPCR) or digital PCR (dPCR) can be used to quantify the abundance of AMR genes in the sample. Standard curves using known concentrations of target gene templates could be generated for absolute quantification. Data analysis where the PCR results are analyzed including gel images, sequencing data and quantification data to establish existence or non-existence of AMR genes and their relative abundance in the sample. The results are then compared with the known reference sequences and databases to identify the detected genes and assess their clinical significance, (Abboud *et al*., 2021).

2.13 Methods of Detecting Antibiotic Resistance

Bacterial culture-based methods as well molecular methods are used in detection of antibiotic resistance. Antimicrobial susceptibility testing method is one of the majorly used *in vitro* procedures used to detect antimicrobial resistance in individual bacterial isolates. These methods can be used for monitoring the emergence and spread of resistant microorganisms in the population. Guidelines and recommendations for the cultured based methods which specify antimicrobial testing methods and interpretative criteria for veterinary pathogens are: the Clinical and Laboratory Standards Institute (CSLI) in the United States of America (USA), World Organization of Animal Health (OIE) in European Union (EU) and Calibrated Dichotomous Sensitivity – Antimicrobial Sensitivity Test (CDS-AST) in Australia. Agar disk-diffusion and the broth micro dilution methods are the two most commonly used methods in veterinary laboratories (Yalew, 2020).

2.13.1 Disk Diffusion Method

Disk diffusion is also known as Kirby-Bauer antibiotic testing method (Osman *et al*., 2014). Disk diffusion test is performed by applying a bacterial inoculum of approximately $1–2×10^8$ CFU/ml to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone shows the susceptibility of the isolate and the diffusion rate of the drug through the agar medium. The drug diffuses radially through the agar, the concentration of the drug decreasing logarithmically as the distance from the disk increases and results in a circular zone of growth inhibition around the disk, the diameter of which is inversely proportional to the MIC (Minimum Inhibitory Concentration). The zone diameters are interpreted on the basis of guidelines published by Clinical & Laboratory Standards Institute (CLSI), and the organisms are reported as susceptible, intermediate, or resistant (Humphries *et al*., 2021). Disk diffusion can only be used to test rapidly growing organisms.

2.13.2 Broth Dilution Method

Broth dilution is the most commonly used method to determine the minimal concentration of antimicrobial agents that kill (bactericidal activity, minimum bactericidal concentration (MBC) or inhibit the growth (bacteriostatic activity, minimum inhibitory concentration (MIC) of microorganisms. Dilution methods are performed when quantitative methods are required for microorganisms with a variable growth rate. The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. The MIC is thus the minimum concentration of the antibiotic that will inhibit this isolate. The broth dilution technique of antibiotic susceptibility testing is also known as the minimal inhibitory concentration (MIC) technique (Yalew, 2020).

2.14 Strategies of Controlling AMR in Animals

Controlling antimicrobial resistance (AMR) in animals is a critical component of ensuring both animal welfare and public health. Several strategies have been proposed and implemented globally to mitigate the emergence and spread of resistant pathogens in livestock production systems. One of the fundamental approaches to AMR control is the judicious use of antimicrobials in animal agriculture. This includes ensuring that antimicrobials are administered only when necessary and under the supervision of a qualified veterinarian. Preference should be given to narrow-spectrum antibiotics where applicable, as they are less likely to disrupt the natural microbiota (Kasimanickam et al., 2021). For example, the United States Food and Drug Administration (FDA) has emphasized the prudent use of medically important antibiotics in food-producing animals to minimize resistance development.

Biosecurity measures are another critical strategy in reducing antimicrobial reliance. Effective biosecurity protocols, such as regulating the movement of animals, personnel, and equipment, help prevent the spread of infectious agents on farms (Koutsoumanis et al., 2022). Maintaining hygienic housing conditions, regular cleaning routines, and implementing proper waste management practices can significantly reduce pathogen loads in the environment, thereby lowering infection risks. Preventive health measures such as vaccination programs also contribute to the reduction of antibiotic use. By minimizing disease incidence, the need for therapeutic interventions is reduced.

In addition, comprehensive animal health management programs, which include routine health monitoring and prompt isolation of sick animals, have been shown to improve herd health outcomes and reduce the burden of infectious diseases. Improved animal nutrition and husbandry practices are essential in promoting immune competence and disease resilience.

Adequate nutrition enhances the overall health status of animals, making them less vulnerable to infections that might otherwise necessitate antibiotic treatment (Mudenda et al., 2023). Monitoring and surveillance systems are necessary for tracking antimicrobial usage and resistance patterns in livestock. Regular susceptibility testing provides data for informed decision-making in veterinary practice. Surveillance also facilitates early detection and response to emerging resistance threats, supporting the implementation of targeted control measures (Kasimanickam et al., 2021). Awareness creation and education of key stakeholders, including farmers and veterinary professionals, play a pivotal role in promoting responsible antimicrobial use. Knowledge on the implications of AMR and the importance of antimicrobial stewardship is essential in changing attitudes and practices. Farmers should be encouraged to adopt alternative strategies such as improved hygiene and biosecurity instead of routine prophylactic use of antibiotics (Mudenda et al., 2023).

Investment in research and innovation is critical for the development of new antimicrobial compounds and alternative interventions. There is a growing need for agents that are selectively active against pathogens, environmentally safe, minimally disruptive to gut flora, and effective as growth promoters (Sharma et al., 2018). Additionally, the development of rapid diagnostic tools can enable targeted therapy and reduce empirical antibiotic use. The advancement of vaccines and other non-antimicrobial interventions also offers promising alternatives for disease prevention (Kasimanickam et al., 2021).

Finally, the success of AMR control depends on strong regulatory frameworks and international cooperation. Enforcing regulations on antimicrobial usage in animals, preventing illegal drug use, and promoting compliance with stewardship guidelines are essential (Kasimanickam et al., 2021). Cross-border collaboration among countries and international organizations enhances the coordination of surveillance, policy harmonization, and resource mobilization in combating AMR on a global scale.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Mukurwe-ini Sub County in Nyeri County (fig 3.1) which is a high agricultural potential sub-county with a population of 89,137 people as per the 2019 national census (Kenya National Bureau of Statistics, 2019). The study was conducted in Rugi, Central, West and Gikondi wards. Due to land fragmentation most farmers are small scale farmers and practice mixed farming. Dairy goat farmers are organized into Farmers groups. The livestock population of Mukurwe-ini Sub-County is 168,685 (Kenya National Bureau of Statistics, 2019), comprising of exotic cattle, indigenous cattle, sheep, goats, donkeys, pigs and chicken. The goat population was 10,379 as per the 2019 national census (Kenya National Bureau of Statistics, 2019).

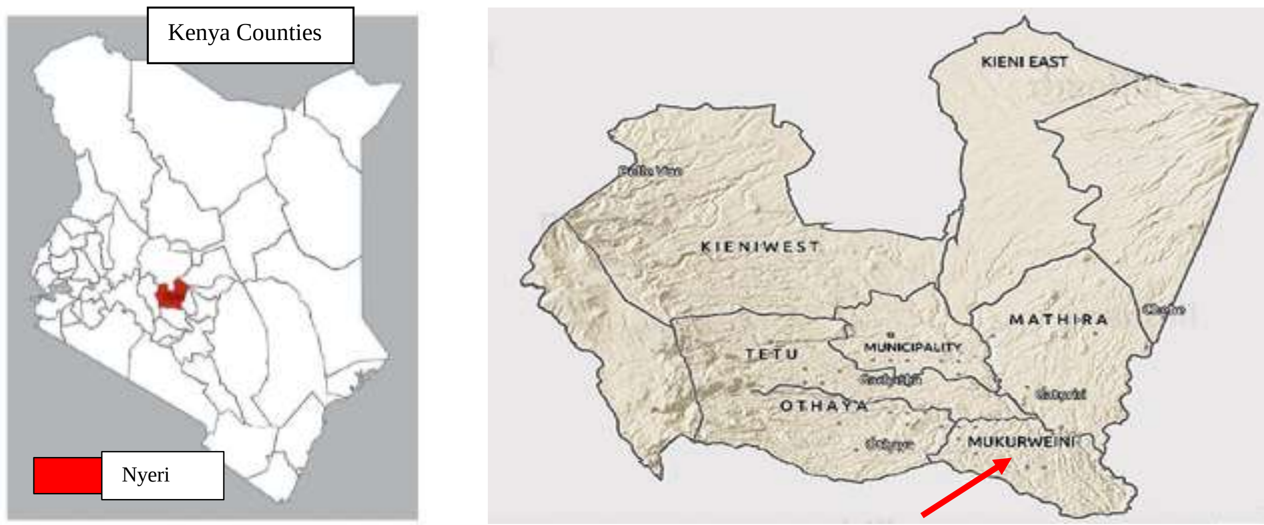

Figure 3.1: Map of Kenya Showing the Location of Nyeri County and Study Sub-County

Mukurwe-ini Sub-County has an area of 171.1 km^2 with a human population of 89,137 people and a density of 498 people per square kilometer. The coordinates for Mukurwe-ini Sub -County are

0.5609 ^{o}S, 37.0488 ^{o}E. The average temperature of Mukurwe-ini is a high of 24 ^{o}C and a low of 13 ^{o}C. The average annual rainfall in Mukurwe-ini is 1206 mm and occurs in two seasons, which are short rains and long rains.

3.2 Study Design

A cross-sectional study was carried out between August 2021 to April 2022.

3.3 Study Population

The study population was conducted on lactating dairy goats whose owners had given consent, and 40 goats were sampled from each of the four wards earlier mentioned.

3.4 Sample Size Calculation

The sample size was calculated using the formula by Dohoo *et al*., (2003): n= $(Z^2\alpha pq)/L^2$, where n is the required sample size, Zα is the value of Z that provides 95% confidence intervals (1.96), p is a priori estimate of the prevalence at the time of the study. With an expected prevalence of 28.6% based on a previous study (Ndegwa *et al*., 2000) in a similar sub-county, a sample size of 314 was obtained. The minimum sample size of 160 was calculated using the adjusted formula for small animal populations (less than 10,000) (Thrusfield, 2018).

In the current study, 189 animals were sampled from a total of 56 farms, each owning 1-10 dairy goats. The sample size of 189 was adopted in order to cater for any losses or damage during handling, transportation or storage.

3.5 Sampling Technique

Since there was no formal list of dairy goat farmers in Mukurwe-ini Sub County available in the study area, the snowball technique was used to identify farmers with lactating dairy goats. The initial farmers were identified by the local extension officers and then these farmers helped in identifying the others until the sample size was achieved. A maximum of 5 goats were sampled on one farm.

3.6 Questionnaire Survey

During the farm visits, data was collected using a semi structured questionnaire (Appendix 1) administered on each farm through personal interview by the investigator. The information obtained from the respondents included farm bio-data and farm management practises such as whether the does were housed, floor type, use of beddings, shed cleaning frequency, washing of udder before milking, drying of udder during milking and use of separate drying towel for each goat during milking. The doe factors which were obtained included breed, history of mastitis, production and supplements given to the does.

3.7 Milk Sampling and California Mastitis Test (CMT)

With help of a veterinarian, a detailed visual inspection and systemic palpation of the udder and teats of the lactating doe was done and the milk observed for clinical signs of mastitis which includes swollen udder, flakes in the milk, discoloured milk. Milk samples were collected aseptically from each teat of 189 lactating dairy goats as described by Sánchez *et al*., (2004). For milk collection, the first three streams of milk were discarded and 3ml of milk were directly put into a California Mastitis Test paddle (ImmuCell RP, USA) and an equal amount of commercial CMT reagent added (ImmuCell RP, USA). The California Mastitis Test was observed within 20 seconds and the results read on a score of 0-3. A score of 0 was considered negative, 1 as trace while a score of 2 and 3 were considered as positive for mastitis. Ten milliliters of milk were then collected into labeled sterile universal bottles for further laboratory analysis. The samples were placed in cool boxes with ice packs and transported to the JKUAT Microbiology Laboratory, within 24 hours.

3.8 Culture and Identification of Bacteria

The milk samples were enriched with alkaline peptone water at 37^{o}C overnight. A hundred microliters of the enriched milk samples were inoculated onto both sheep blood agar (Himedia Laboratories, India) and McConkey agar (Oxoid, UK), respectively. The plates were incubated at 37^{o}C for 24-48 hours. Plates which had mixed growth were sub-cultured to obtain pure colonies. Further characterization was done for the beta hemolytic bacteria obtained from sheep blood agar by catalase test (Reiner, 2013) to differentiate *Streptococcus* from *Staphylococcus* spp.

Bacteria identification was confirmed using MALDI-ToF technique. Briefly, forty (40) mg/ml alpha-cyano-4-hydroxycinnamic acid (matrix) (Sigma-Aldrich, St. Louis, USA) was prepared in LC-MS grade solvents; acetonitrile, ethanol and water in the ratio of 3:3:3 in 3% trifluoroacetic acid. The 25% formic acid overlay method was used for spotting. Using a sterile microcentifuge tip, 0.5µl bacterial colonies was transferred onto the target plate and each spot overlaid with 0.5µl of 25% formic acid (Sigma-Aldrich, St. Louis, USA). This was followed by application of 0.5µl of α-cyano-4-hydroxycinnamic acid matrix onto each spot and thoroughly mixing before MALDI-MS measurements in Shimadzu Axima Confidence (Shimadzu, Kyoto, Japan). A spectrum was acquired for each spot and species identification of each spectrum was compared to the ribosomal marker based database, SARAMIS.

3.9 Antibiotic Susceptibility Testing

Antibiotic susceptibility test was performed using the disc diffusion method (CSLI, 2019). One hundred isolates comprising of *E.coli*, *Enterobacter* spp., *E. vulneris*, *Pseudomonas* spp., *Proteus vulgaris*, *K. oxytoca*, *S. aureus* and CoNS were randomly selected, suspended in growth media, and standardized through a turbidity test (0.5 McFarland's standard). The hundred isolates were considered as possible causes of mastitis based on literature (Mahlangu *et al*., 2018, Cobirka *et al*., 2020). A hundred µl of the standardized suspension of the test organism was then inoculated on Mueller Hinton Agar (Himedia, Ltd) plates. Eight antibiotics (Himedia, Ltd) commonly used for treatment of mastitis in Kenya were selected for antibiotic susceptibility testing in the study: Cefuroxime (30µg), Cefotaxime (30µg), Amoxicillin and Clavulanic acid (10µg), Oxacillin (10µg), Azithromycin (15µg, Meropenem (10µg), Ciprofloxacin (10µg) and Nitrofurantoin (300µg). The discs were placed on the media surface and plates incubated at 37^{o}C for 24 hours. The respective zones of inhibition were measured and results interpreted according to the CLSI, (2019). Results were recorded as resistant, intermediate or susceptible to specific antibiotics.

3.10 Biochemical Identification of Bacteria

Forty (40) mg/ml alpha-cyano-4-hydroxycinnamic acid (matrix) (Sigma-Aldrich, St. Louis, USA) was prepared in LC-MS grade solvents; acetonitrile, ethanol and water in a ratio of 3:3:3 in 3% trifluoroacetic acid. The 25% formic acid overlay method was used for spotting. Briefly, using a sterile microcentifuge tip, 0.5 µl bacterial colonies was transferred onto the target plate and overlaid each spot with 0.5 µl of 25% formic acid (SigmaAldrich, St. Louis, USA). This was followed by applying 0.5 µl of α- CHAC matrix onto each spot and thorough mixing and left to air dry completely before MALDI-MS measurements in Shimadzu Axima Confidence (Shimadzu, Kyoto, Japan). Spectra was acquired for each spot and species identification of each spectrum was compared to the ribosomal marker based database, SARAMIS. Methicillin resistant *Staphylococcus aureus* was used as a positive control.

3.11 Molecular Detection of Antibiotic Resistant Genes

Thirty five isolates of *E. coli* (18), *S. aureus* (12), *Enterobacter* spp (3) and *K. oxytoca* (2) with multiple antibiotic resistance index were selected for molecular detections of antibiotic resistant genes. Plasmid DNA was extracted from 35 isolates using plasmid DNA extraction kit (Bioline) following the manufacturer's instructions. Amplification was done using specific primers for detection of genes conferring resistance to Oxacillin (*mecA*), and Amoxicillin and Clavulanic acid (bla_{TEM}) (Mehrotra *et al*., 2000, Monstein *et al*., 2007). The primer sequences and the expected fragment size of each gene are listed in Table 3.1. The PCR amplicons were quantified using a Nanodrop spectrophotometer (Jenway, Genova Nano, London, UK) followed by the confirmation of a successful recovery by resolving 10 µl of product on 1.5% (w/v) agarose gel and visualizing under UV trans-illuminator (UVITEC Cambridge, Japan) against a 100bp molecular size ladder.

Table 3.1: Primer Sequences Used in the Study

Target Gene	Primer Sequence	Expected amplicon size	Source
bla_{TEM}	F_TCGCCGCATACACTATTCTCAGAATGA R_ACGCTCACCGGCTCCAGATTTAT	445bp	Monstein *et al.*, (2007)
MecA	F_TGG TAT GTG GAA GTT AGA TTG G R_GGA TCT GTA CTG GGT TAA TCA G	166bp	Mehrotra *et al.*, (2000)

Source: (Monstein *et al*., 2007, Mehrotra *et al*., 2000)

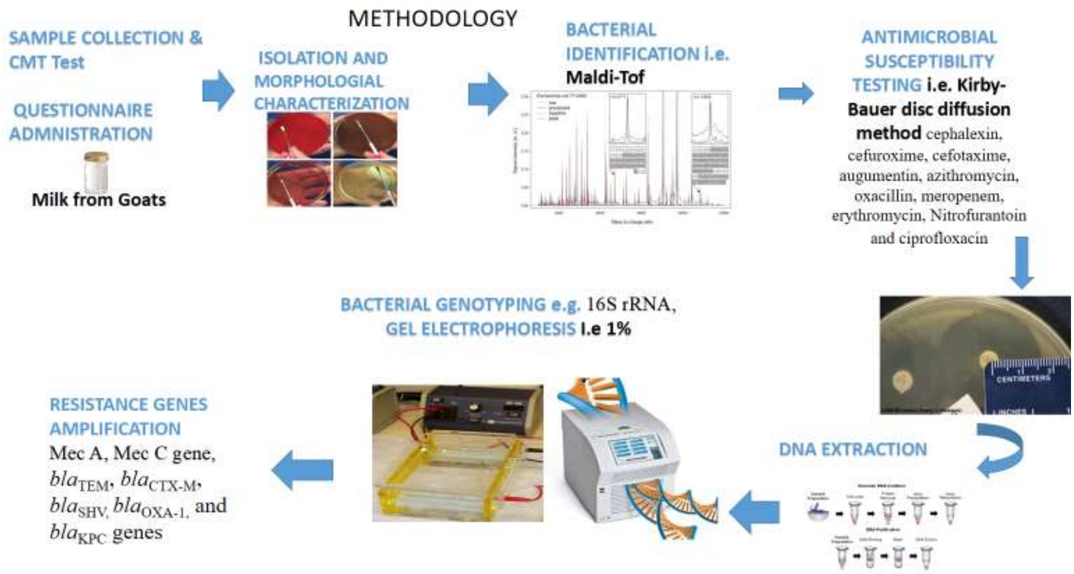

Figure 3.2: Flow Chart Showing Activities Undertaken in the Study

3.12 Data Analysis

Data entry and management was done using Microsoft Excel 2010 (Microsoft, USA), while data analysis was done using SPSS v26 (Microsoft, USA). Descriptive statistical data was presented in tables. Chi square was used to evaluate the association between occurrence of risk factors and prevalence of mastitis ($p<0.05$). A doe was assumed to be infected with mastitis if it had clinical or sub-clinical mastitis based on CMT results. Logistic regression was used to test individual risk factors and their strength of association in mastitis infection. The odds ratio was used to determine the strengths of association identified in logistic regression.

3.13 Ethical Consideration

For this study, ethical approval was obtained from Institute of Primate Research (IPR) (Appendix 2). Milk samples were obtained with the help of the farmers who volunteered to participate in the study under the supervision of veterinary officers from the county veterinary office. The protocols used for sampling, bacterial isolation, and antibiotic susceptibility testing were performed in agreement with the Animal Diseases act, Section 20, Act of 1984 (Act No. 35 of 1984).

CHAPTER FOUR

RESULTS

4.1 Prevalence of Clinical and Sub-Clinical Mastitis

The overall prevalence of mastitis was 85.7% (162/189), with most (160/189, 84.7%) of them having subclinical disease and (2/189, 1.06%) having clinical mastitis. Clinical mastitis was characterized by presence of swollen and inflamed udder, presence of flakes in the milk and discoloured milk.

4.2 Relationship between Prevalence of Mastitis and Origin of Goat, Breed, Milking Practices

The results of the CMT were used to evaluate the relationship between mastitis prevalence and risk factors. The highest prevalence of mastitis was in lactating does originating from Central Ward. However, there was no significant difference (P=0.520) between prevalence of mastitis in does originating from the different wards. In terms of breeds, the highest prevalence of mastitis was found in crosses (36.4%), followed by German Alpine (30.9%) and the least affected were the local breeds (1.9%). Nonetheless, there was no significant (p=0.28870) difference in the prevalence of mastitis in the different breeds (Table 3). The highest prevalence of mastitis was in lactating does whose houses were cleaned weekly (p= 0.001) compared to those cleaned daily. The highest prevalence of mastitis was found in lactating does where farmers did not wash their hands before milking (p=0.01) compared to those who cleaned their hands. The lowest prevalence of mastitis was found in milk from lactating does whose udder were dried with individual towel (p=0.04) compared to those from farms where towels were shared between different goats (Table 4.1).

Table 4.1: Relationship between Origin of Goat, Breed and Milking Practices and Prevalence of Mastitis in Dairy Goat in Mukurwe-ini Sub-County as Identified by CMT (N=189)

Factor	Number Positive	Prevalence of Mastitis %
Ward of Origin		**(p=0.52)**
Rugi	41	21.69
Central	42	22.22
West	40	21.16
Gikondi	39	20.63
Breed of Goat		**(p=0.2887)**
Toggernburg	28/30	14.81
Crosses	59/65	31.22
German alpine	50/54	26.46
Saanen	7/10	3.70
Kenyan alpine	15/23	7.94
Local	3/7	1.59
Pen Cleaning schedule		**(p=0.001)**
Weekly	71	37.57
Fortnightly	60	31.75
Daily	12	6.35
Irregular	19	10.05
Hand washing		**(p=0.01)**
Yes	142	75.13
No	20	10.58
Separate drying towel for each goat		**(p=0.04)**
Yes	30	15.87
No	132	69.84
Floor type		(p=0.35)
Wooden	123	65.08
Earthen	39	20.63
Use of beddings		
Yes	0	0
No	162	85.71
Washing of udder before milking		**(p=0.42)**
Yes	153	80.95
No	9	4.76
Housing		
Yes	162	85.71
No	0	0

4.3 Spectrum/Profile of Bacteria in the Sampled Milk

All the 189 milk samples were cultured. A total of 162 (85.7%) milk samples positive for clinical and sub-clinical mastitis yielded bacteria in culture while the 27 samples that were negative for mastitis did not yield any bacteria. The most prevalent species isolated included *S. aureus* (21.98%), Coagulase – negative Staphylococcus (20.33%), *E. coli* (18.13%), *Pseudomonas* spp. (14.29%) and *Enterobacter* spp. (10.44%). Other identified species of bacteria included *K. Oxytoca*, *Proteus vulgaris*, *E. vulneris*, *Raoutella ornithinolytica* and *Serratia marcescens* and their prevalence are as summarized in Table 4.2.

Table 4.2: Bacteria Isolated from Dairy Goats (n=189) in Mukurwe-ini Sub-County, Kenya

Bacteria species	Number of isolates	Percentage
Staphylococcus aureus	40	22.0
Coagulase-negative *Staphylococcus*	37	20.3
Escherichia coli	33	18.1
Pseudomonas spp	26	14.3
Enterobacter spp	19	10.3
Klebsiella oxytoca	11	6.0
Proteus vulgaris	3	1.7
Raoutella ornithinolytica	3	1.7
Escherichia vulneris	3	1.7
Pantoea agglomerans	2	1.1
Serratia marcescens	2	1.1
Sterotrophom maltophilia	2	1.1
Cedeceus spp.	1	0.6
Total	182	100

4.4 Antimicrobial Susceptibility Pattern

Antibiotic sensitivity was under taken for the following bacteria; *E. coli*, *Enterobacter* spp., *K. oxytoca*, *E. vulneris*, *Pseudomonas*, *P. vulgaris*, *S. aureus* and Coagulase-negative Staphylococcus (CoNS). A plate with Muller Hinton medium with inoculum was used as a control. The sensitivity of these bacteria was compared with CLSI, 2025 and classified as either susceptible, intermediate or resistant. The tested bacteria were highly resistant to Oxacillin (93.9%-100%). *Staphylococcus aureus*, which was the most prevalent species, was highly susceptible to Ciprofloxacin (100%), Nitrofurantoin (87.5%) and Azithromycin (82.5%) and highly resistant (97.5%) to Oxacillin. Coagulase – negative Staphylococcus was 100% susceptible to Ciprofloxacin, 82.2% susceptible to Azithromycin, 68.5% susceptible to Nitrofurantoin and 100% resistant to Oxacillin.

All (100%) *K. oxytoca* isolates were categorized as having intermediate form of resistance. Susceptibility to Amoxicillin/Clavulanic acid was highest in *E. vulneris*

(100%), *Pseudomonas* spp. (100%) and *E. coli* (87.9%) and lowest in *K. oxytoca* (72.7%) and *Enterobacter* spp. (57.9%). Strains susceptible to Meropenem included *Proteus vulgaris* (100%), *E. vulneris* (100%), *K. oxytoca* (90.9%) and *E. coli* (87.9%). *Pseudomonas* spp isolates were resistant to Azithromycin (100%), Oxacillin (100%) and Ciprofloxacin (100%) and 100% susceptible to Amoxicillin/Clavulanic acid. A summary of the antibiotic susceptibility profiles is given in Table 4.3 and 4.4.

Table 4.3: Antibiotic Susceptibility Profiles of *E. coli*, *Enterobacter* spp., *K. oxytoca*, *E. vulneris*, *Pseudomonas* spp. and *Proteus vulgaris* (percentages) Isolated from Lactating Does in Mukurwe-ini Sub-County (n=95) (CLSI, 2025)

Antibiotics	***E. coli* (n=33)**			***Enterobacter* spp. (n=19)**			***K. oxytoca* (n=11)**			***E. vulneris* (n=3)**			***Pseudomonas* spp. (n=26)**			***Proteus vulgaris* (n=3)**		
	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
AMC	0	87.9	12.1	21.1	57.8	21.1	9.1	72.7	18.2	0	100	0	0	100	0	0	66.7	33.3
CXM	12.1	15.2	72.7	26.3	15.8	57.9	18.2	18.2	63.6	66.7	33.3	0	50	50	0	33.3	0	66.7
CTX	27.3	57.6	15.1	26.3	47.4	26.3	18.2	72.7	9.1	66.7	33.3	0	50	50	0	33.3	66.7	0
Mpm	6.1	87.8	6.1	5.3	15.8	78.9	0	90.9	9.1	0	100	0	0	50	50	0	100	0
FT	3.0	51.5	45.5	26.3	42.1	31.6	18.2	63.6	18.2	66.7	33.3	0	50	50	0	66.7	0	33.3
AZM	30.3	69.7	0	10.5	31.6	57.9	36.4	63.6	0	33.3	66.7	0	100	0	0	66.7	0	33.3
OX	93.9	6.1	0	100	0	0	0	0	100	100	0	0	100	0	0	100	0	0
CIP	12.1	69.7	18.2	15.8	15.8	68.4	0	90.9	9.1	33.3	66.7	0	100	0	0	0	100	0

Key: S. susceptible: I. intermediate: R. resistant: AMC. Amoxicillin + Clavulanic acid: CXM. Cefuroxime: CTX. Cefotaxime: Mpm. Meropenem: FT. Nitrofurantoin: AZM. Azithromycin: OX. Oxacillin: CIP. Ciprofloxacin.

Table 4.4: Antibiotic Susceptibility Profiles of *Staphylococcus aureus* and Coagulase Negative Staphylococcus (CoNS) (in Percentages) Isolated from lactating Does in Mukurwe-ini Sub-County (n=77) (CLSI, 2025)

Antibiotics	S. aureus n= 40			CoNS n=37		
	R	S	I	R	S	I
Nitrofurantoin	12.5	87.5	0	32.4	67.6	0
Azithromycin	17.5	82.5	0	18.9	81.1	0
Oxacillin	97.5	2.5	0	100	0	0
Ciprofloxacin	0	100	0	0	100	0

Key: S- susceptible, I- intermediate, R- resistant, CoNS- Coagulase negative Staphylococcus

Table 4.5: Multidrug resistance among bacteria isolated from milk of dairy goats with mastitis in Mukurwe-ini Sub County, Kenya.

Bacterial isolate	Number of resistant drugs		
	1	2	3
E.coli (n=33)	20	8	5
K. oxytoca (n=11)	6	4	1
E. vulneris (n=3)	1	2	0
Pseudomonas spp (n=26)	13	4	9
Proteus vulgaris (n=3)	2	1	0
S. aureus (n=40)	29	4	7
CoNs (n=37)	21	10	6

4.5 Occurrence of Antimicrobial Resistance Genes in Mastitis Pathogens Isolated from Dairy Goats in Mukurwe-ini Sub County

blaTEM antibiotic resistance genes were detected in the plasmid DNA of *E. coli*, *Enterobacter* spp. and *K. oxytoca* (Fig 4.1). *mecA* and *blaTEM* antibiotic resistance genes were detected in *S. aureus* isolates. The bands on the agarose gel gave the expected sizes of 445bp and 225bp, respectively. A total of 18 *E. coli* isolates (78.26 %) showed the presence of *blaTEM* while no (0%) isolates had *mecA* gene. MecA and *blaTEM* genes were detected in 10 (83%) and 3 (25%) *S. aureus* isolates, respectively as shown in figure 4.1.

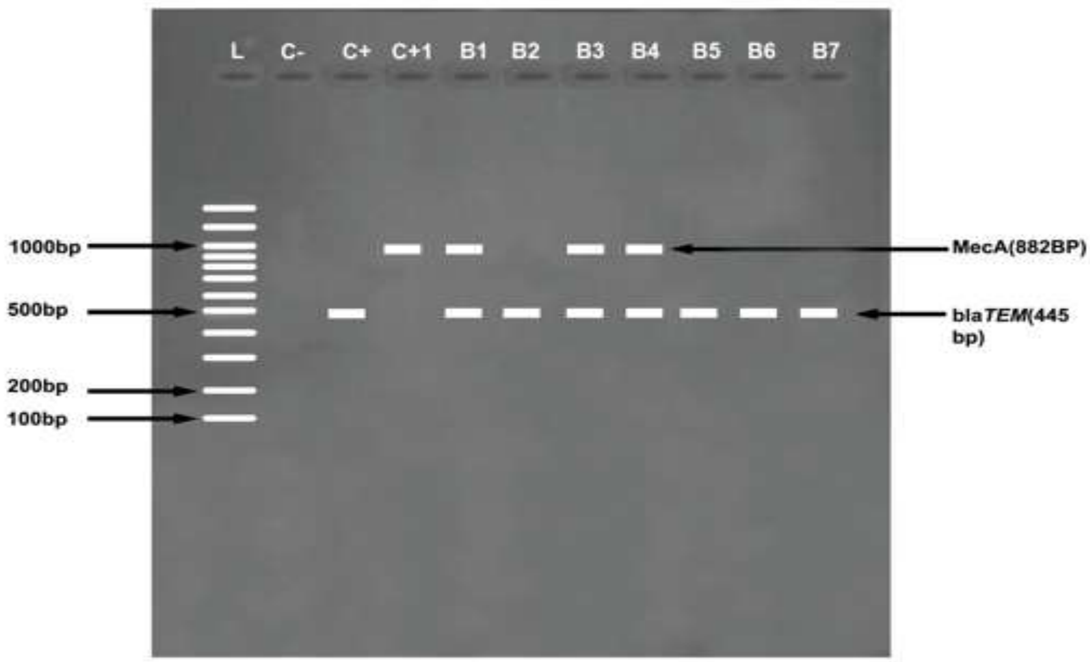

Figure 4.1: Agarose Gel Electrophoresis for Resistant Genes

Key: C- is negative control, C+ is positive control (blaTEM), C+1 is positive control (mecA), B1, B3 & B4 is *S. aureus*, B2 is *E. coli*, B5 & B6 is *Enterobacter* spp. B7 is *Klebsiella oxytoca* and L is 100bp Bioline hyper ladder

CHAPTER FIVE

DISCUSSION

The current study sought to characterize mastitis-causing pathogens and their antibiogram in dairy goats reared by small-scale farmers from Mukurwe-ini Sub-County in Nyeri County, Kenya. The study found the prevalence of sub-clinical mastitis to be higher than clinical mastitis, which is expected because sub-clinical mastitis cases are not detected by physical examination of milk or udder (Radostits *et al*., 2007). The current study agrees with a study in Pakistan (Mirza *et al*., 2019), which reported a prevalence of mastitis to be 75-85% over a period of three years. The findings are also close to the findings of Jabbar *et al*., (2020), which reported the overall prevalence of mastitis in Pakistan to be 61.8%. Compared to other Kenyan studies, the prevalence of mastitis in the current study area was higher than that reported both by Ndegwa *et al*, (2000) (9.8%) and Mahlangu *et al*., (2018) (50.9%) in dairy goats from Thika East sub-County, Kenya. The rise in the prevalence of mastitis could be attributed to concomitant changes in the environment, climate, land use, human behavior and demographics. The emergence of more virulent bacteria strains, poor nutrition or other concurrent diseases which could reduce immunity further contributing to the rise in prevalence of mastitis. This study revealed that farms that did not use an udder drying towel for each cow had significantly higher prevalence of mastitis than farms that used a drying towel for each cow. This agrees with finding by Mbidyo *et al*., (2020) from Kenya and Mekonnen *et al*., (2017) from Ethiopia who reported that use of the same drying towel for the herd was responsible for the spread of mastitis-causing pathogens. This can explain the high prevalence of *Staphylococcus* spp. reported in this study. Since these organisms are part of the normal flora residing in the udder and the teats, they can spread through the use of same drying towel during milking. In this study there was significantly higher prevalence of mastitis in Does whose houses were cleaned weekly compared with does whose houses were cleaned more frequently. These results are in agreement with those by others (Mahlangu *et al*., 2018, Mbidyo , 2014). The study showed hygiene plays a major role in occurrence of subclinical mastitis. Farms where the hands were not washed before milking showed significantly higher prevalence of mastitis compared with farms where farmers washed hands before milking. (Gianneechini *et al*., 2002). In contrast to other studies (Mahlangu *et al*., 2018, Mbidyo *et al*., 2014), the current one found that cross-breeds had higher prevalence of mastitis compared to others breeds of goats. The causes of variation in the breed differences are not clear and should be studied further.

The current study found coagulase negative Staphylococci [CoNS], *S. aureus, E. coli* and *Enterobacter* spp. to be the predominant bacteria causing mastitis in goats. The findings are consistent with those of Mahlangu *et al*., (2018) who found CoNS (20.7%) and *S. aureus* (10.7%) as the commonest causative agents of mastitis in dairy goats kept by farmers in Thika Sub-County, Kenya. Our findings are also consistent with the findings of Gelbi *et al*., (2018) which found CoNS (20.7%), *S. aureus* (10.7%) among the Gram-positive bacteria, *Enterobacter* spp. (6.5%) and *E. coli* (5.9%) among the Gram-negative bacteria as major causative agents of mastitis in Indonesia. Altaf *et al*., (2019) and Jabbar *et al*., (2020) also found *Staphylococcus* spp. to be the predominant bacteria causing subclinical mastitis in goats in Pakistan. Pisanu and colleagues stated that *Staphylococcus* spp. are the most prevalent intramammary pathogens in dairy goats causing subclinical, clinical, acute and gangrenous mastitis (Pisanu *et al*., 2020). Further, in recent years it is important to note that CoNS have emerged as a significant threat to food safety since they have been shown to harbor numerous enterotoxins and antimicrobial resistance genes (Gizaw *et al*., 2020).

Coliforms, including *Klebsiella* spp*., E. coli* and *Enterobacter* spp. were also observed in dairy goats in the current study. These bacteria are normally associated with environmental contamination of the udder and establishment of intramammary infection. The presence of coliforms may be linked with poor hygiene practices in dairy goat farms (Okoko *et al*., 2020). In our study, the sharing of towels to dry the udders, failure to wash hands before milking and infrequent cleaning of goats' houses were found to be risk factors for the development of mastitis in the lactating goats. This finding agrees with the study done by Mbidyo *et al* (2020) in Embu and Kajiado Counties in Kenya which showed that the use of same drying towel for the herd was responsible for spreading mastitis pathogens.

In the current study, the isolated *E. coli* showed high antibiotic resistance to oxacillin but high susceptibility to Amoxicillin/Clavulanic acid, Meropenem, Azithromycin and Ciprofloxacin. Multidrug resistance has been observed in the gram- negative bovine mastitis pathogens (Saini *et al*., 2012). In the current study, multidrug resistance was found in *E.coli* isolates (resistant to Oxacillin, Cefotaxime, Azithromycin, Cefuroxime, Meropenem, Nitrofurantoin and Ciprofloxacin), *Enterobacter* species (resistant to Cefuroxime, Cefotaxime, Amoxicillin/Clavulanic acid, Meropenem, Nitrofurantoin, Azithromycin, Ciprofloxacin and Oxacillin) and *Klebsiella oxytoca* isolates (resistant to Cefuroxime, Cefotaxime, Amoxicilli/Clavulanic acid, Nitrofurantoin and Azithromycin). Coliform contaminations rank high among the most types of contamination in the dairy industry. Microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter* spp., *Klebsiella* spp. and *Proteus mirabilis* can multiply in the normal summer temperatures and hence unpasteurized milk has every chance of containing *E. coli* (Dhanashekar *et al*, 2012). Amoxicillin/Clavulanic acid was also found to be effective against *E. vulneris*, *Pseudomonas* spp. and *Proteus vulgaris*. This can be attributed to limited use of Amoxicillin/Clavulanic acid in the treatment of sub-clinical mastitis in the current study area.

Most bacteria isolates from the current study area were resistant to oxacillin. Most *S. aureus* isolates were resistant to oxacillin. *Staphylococcus aureus* is an important pathogen because of a combination of toxin-mediated virulence , invasiveness and antibiotic resistance (Guimarães *et al*., 2017). Moreover, the bacterium is a significant cause of human nosocomial infections (Guimarães *et al*., 2017). On the contrary, *S. aureus* showed high antibiotic susceptibility to Ciprofloxacin, Nitrofurantoin and Azithromycin. The findings on susceptibility agrees with a study by Upadhyay and others that reported high susceptibility of *S. aureus* to Azithromycin (100%) and Ciprofloxacin (76.67%) (Upadhyay *et al*., 2009).

The current study focused on detection of antimicrobial resistance genes in dominant species causing mastitis. *Escherichia coli* has the ability to produce ß-lactamase enzyme and modified penicillin- binding proteins that hydrolyze or inhibit the binding of ß-lactam antibiotics (Okoko *et al*., 2020). In *S. aureus,* the current study reported the presence of *mecA* gene while in *E. coli* bla$_{TEM}$ antibiotic resistance gene was identified. Similar findings in *E. coli* and *S. aureus* were reported by other authors (Fawzy *et al*., 2022, Gelbı *et al*., 2018). These resistant genes are common and have been shown to spread across the ecosystem (man- animal- environment) through horizontal gene transfer (Todorović *et al*., 2018). *Enterobacter* spp. are common human gut micro flora but have also been isolated from milk (Hogan & Smith, 2012). In the study, *Enterobacter* spp. was found in goat milk, suggesting the possible transmission of the bacteria to goat udders through water, soil or fecal contamination. *Enterobacter* spp. and *E.coli* are highly resistant to Oxacillin. The study agrees with a study done in India which showed *E.coli* and other mastitis bacteria to be highly resistant to Oxacillin (Fahim *et al*., 2019).

Some bacteria that were found in the milk samples such *S. maltophilia* are environmentally acquired opportunistic pathogens which are not known to cause mastitis. Other studies have found this bacteria to carry resistance genes to most classes of antibiotics including beta-lactamases, aminoglycoside inactivating enzymes and efflux pumps (Gil-Gil *et al*., 2020). Its presence in milk, therefore, is a major public health concern as it is known to cause a number of infections mainly at hospitals (among immunocompromised hosts) as well as in cystic fibrosis patients (Gil-Gil *et al*., 2020) and can potentially contribute to transfer of AMR genes from *S. maltophilia* to humans through milk consumption. In recent years, *K. oxytoca* has been reported as a significant opportunistic pathogen causing nosocomial infections in neonates as well as adults (Neog *et al*., 2021). *Klebsiella* species have been found to harbor many AMR genes including *blaTEM*, *bla OXA* and *blaCTM-M-15* (Muraya *et al*., 2022). The most prevalent ESBL-producing Enterobacterales in Kenya are *CTX-M-15* and *TEM* which is associated with use of third generation Cephalosporins in a previous study (Muraya *et al*., 2022). In our study, *K. oxytoca* was found to be resistant to Amoxicillin/Clavulanic acid, Cefuroxime and Cefotaxime which agrees with a study done by Neog *et al* (2021) in India.

CHAPTER SIX

CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusions

The study concludes the following:

1. There was high (84.7%) prevalence of sub-clinical mastitis and a low (1.06%) prevalence of clinical mastitis in dairy goats in the study area. Poor hygiene where farmers did not wash their hands before milking and also where udder/teat drying towels were shared between does was found to be a major predisposing factor to goats getting mastitis.
2. The main pathogenic bacteria isolated from dairy goats included; *S. aureus*, Coagulase – negative Staphylococcus, *E. coli*, *Pseudomonas* spp. and *Enterobacter* spp. (10.44%).
3. Majority of the bacteria causing mastitis were resistant to the common antibiotics used to treat mastitis. The resistant bacteria included *S. aureus*, *E. coli*, *Enterobacter* spp., *K. oxytoca*, E. vulneris, *Pseudomonas* spp., *Proteus vulgaris* and CoNS. These bacteria were majorly resistant to Oxacillin, Cefuroxime, Cefotaxime and Azithromycin. Some bacteria were found to have MDR and these included *S. aureus*, CoNS and *E.coli*. Some were found to have high efficacy and thus could be recommended for treatment of mastitis in the study area, and these include Ciprofloxacin, Meropenem, Nitrofurantoin and Amoxillin and Clavulic acid.
4. Bla_{TEM} antibiotic resistance genes were detected in the plasmid DNA of *E. coli, Enterobacter* spp. and *K. oxytoca*. MecA and Bla_{TEM} antibiotic resistance genes were detected in *S. aureus* isolates.

6.2 Recommendations

1. Farmers in the study area should be trained on proper hygiene practices while handling goats to mitigate mastitis. There is also need to train farmers on use of CMT in order to have early detection and treatment of mastitis.
2. Further studies should be undertaken to determine the causes of the high burden of AMR and whether there could be cross-transmission of these bacteria to humans in the study area. Further molecular study should be conducted on the zoonotic potential of these isolates.
3. Some drugs were found to have high efficacy and thus could be recommended for treatment of mastitis in the study area and include; Ciprofloxacin, Meropenem, Nitrofurantoin and Azithromycin.
4. Further screening of AMR genes other than blaTEM, blaOXA and MecA should be done in the study area. Moreover, more drugs should be tested in order to create awareness on effective drugs for mastitis treatment. Genome sequencing to determine the clonal relationship of the circulating bacterial strains in the study population.

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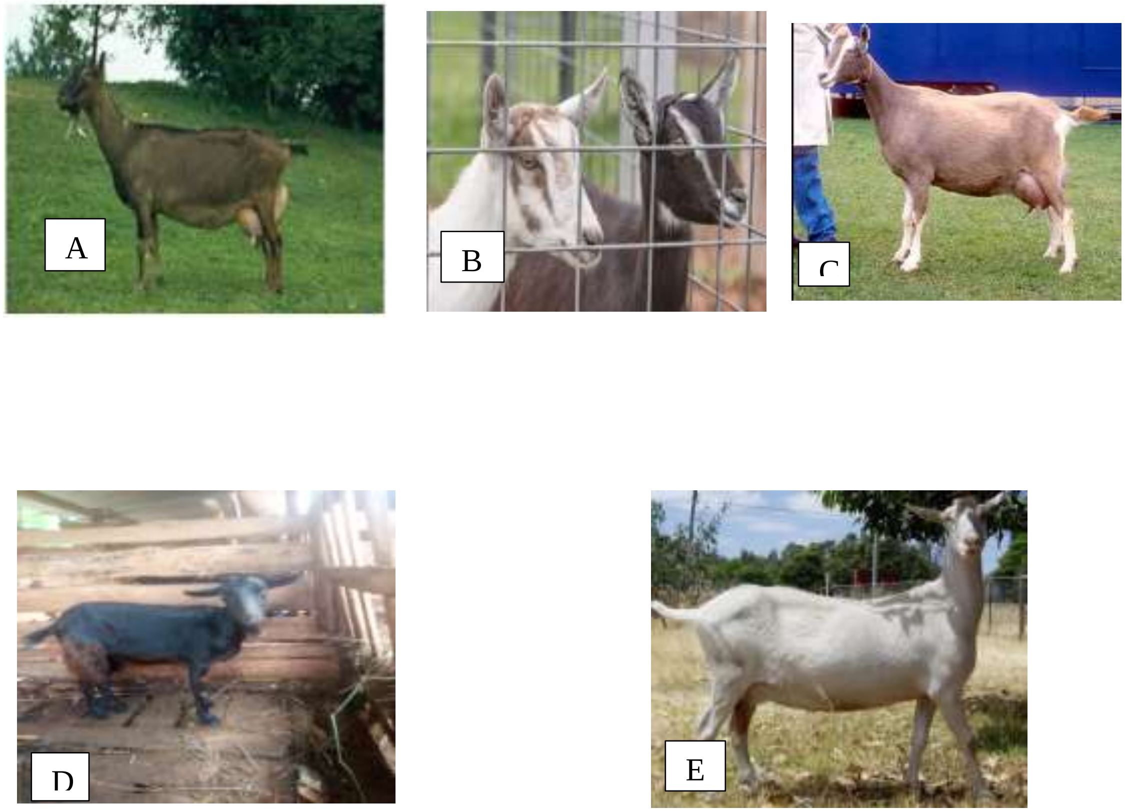
APPENDICES

Appendix I: Goat Breeds Sampled for the Study



Key: A-Alpine, B-Cross-breed, C-Toggernburg, D-Local breed, E-Saanen

Appendix II: Questionnaire

Introduction; Thank you for taking your time to meet us today. We are from Jomo Kenyatta University of Agriculture and Technology (JKUAT) and are interested in improving the management of mastitis by understanding the risk factors associated with mastitis and antibiotic resistance.

We appreciate your cooperation.

Location: ……………………………….. Date: ……………………….

PART I: BACKGROUND INFORMATION (Please tick one)

NAME: ________________________FARM NUMBER : _______________

COUNTY: ____________________ VILLAGE: _______________________

MOBILE: ______________________ GENDER: [ ] Male [ ] Female

1. What is your age?
   - [ ] Under 18 years
   - [ ] 18 -30 years
   - [ ] 31 - 40 years
   - [ ] 41 -50 years
   - [ ] Over 50 years
2. What is your highest level of education?
   - [ ] Post University
   - [ ] University degree
   - [ ] College diploma
   - [ ] Certificate
   - [ ] Primary school
   - [ ] Others, Specify:

   ____

____

____

3. What is your employment status?

   [ ] Self-employed [ ] Employee

4. Which economic activity do you partake?

   [ ] Cash crop farming

   [ ] Dairy cattle farming

   [ ] Dairy goat farming

   [ ] Horticulture

   [ ] Selling goat meat

   [ ] Other, Specify: ____

5. How long have you been farming dairy goats?

   [ ] Less than 5 years

   [ ] 6 to 10 years

   [ ] 11 to 15 years

   [ ] 16 to 20 years

   [ ] More than 20 years

6. What is the size of your farm under dairy goat farming?

   [ ] Less than 1 acre

   [ ] 1 to 3 acres

   [ ] 4 to 6 acres

   [ ] 7 to 9 acres

   [ ] More than 9 acres

7. How many dairy goats do you own?

[ ] Less than 10 goats

[ ] 11 to 20 goats

[ ] 21 to 30 goats

[ ] 31 to 40 goats

[ ] More than 40 goats

8. Which type of goat breed do you own?

[ ] German alpine

[ ] Toggenberg

[ ] Saneen

[ ] Not sure

[ ] Other, Specify:

____

____

____

9. Do you keep records?

[ ] Yes [ ] No

If yes specify:

[ ] Production

[ ] Breeding

[ ] Others (specify):

____

____

____

10. Indicate the production costs and inputs of dairy goat farming in the table below.

S. No	Input type	Quantity used

11. Where do you source your inputs from?

[ ] Local source [ ] International source

12. What is the source of water for your dairy goats?

____

____

____

____

____

13. Any given to the dairy goats' supplements?

[ ] Yes [ ] No

14. Are the dairy goats housed with a roof?

[ ] Yes [ ] Yes

15. If housed, what is the nature of the floor where the dairy goats lie down?

[ ] Concrete [ ] Earthen

16. Do the animal houses have beddings?

[ ] Yes [ ] No

17. How often is the floor cleaned?

[ ] Daily

[ ] Weekly

[ ] Monthly

[ ] Others (specify):

____

18. What do you use to clean the floor of the goat pens?

19. Which milking practices do you use?

[ ] Milking using Hand [ ] Milking using machines

20. If you milk by hand, do you wash hands before milking the goats?

[ ] Yes [ ] No

If yes, what do you use to wash your hands?

21. If you milk using machines, are the machines washed?

[ ] Yes [ ] No

If yes, how often are they washed?

[ ] Immediately after use

[ ] Daily

[ ] Weekly

[ ] Monthly

[ ] Others (specify):

____

____

22. What do you use to clean the machines?

____

____

____

23. Do you process any of the dairy goat milk?

[ ] Yes [ ] No

If yes, what do you process dairy goat milk into?

____

____

Do you wash the udder before milking?

[ ] Yes [ ] No

24. What do you use to wash the udder?

____

____

____

____

____

25. Is the udder dried before milking with a clean cloth/towel/paper?

[ ] Yes [ ] No

26. Do you use a different clean drying cloth/towel/paper used for each dairy goat?

[ ] Yes [ ] No

27. Have you ever received training on dairy goat rearing?

[ ] Yes [ ] No

If yes, from which institution?

____

____

____

PART II: MASTITIS – SIGNS AND TREATMENT

28. Have you observed any of the following signs or symptoms on the dairy goat for the past year?

    [ ] Swollen udder

    [ ] Change of milk color to either yellow or red

    [ ] Loss of appetite

    [ ] Other, Specify: ____

29. How often have you been observing these symptoms?

    [ ] Monthly

    [ ] Quarterly

    [ ] Annually

    [ ] Biannually

    [ ] Any other____

30. Have you heard about mastitis before?

    [ ] Yes [ ] No

31. How do you treat/manage mastitis in your dairy goats?

    [ ] Using Conventional drugs (Antibiotics)

    [ ] Using herbal plants

    [ ] Other, Specify: ____

    If the answer to question 32 is Conventional drugs, which drugs are administered and how?

    ____

____

____

If the answer to question 32 is Herbal Plants, which plants do you use and in what form?

____

____

____

____

____

32. Who treats your dairy goats?

[ ] Vet Surgeon

[ ] Animal Health Assistant

[ ] Self

[ ] Others (specify):

____

____

____

33. Do they perform any tests to confirm mastitis?

[ ] CMT

[ ] Alcohol test

[ ] Culture

[ ] Other (specify):

____

____

____

34. Have you observed recurrence of symptoms after treatment of mastitis?

[ ] Yes [ ] No

If the answer to question 35 is yes, for how long?

____

____

____

35. Do you cull chronically infected dairy goats?

[ ] Yes [ ] No

If yes, are they quarantined/isolated/put in separate goat pens?

[ ] Yes [ ] No

36. Did any of your family members have any effects/symptoms from consuming milk from the infected dairy goat?

[ ] Yes [ ] No

If yes, what was the effect?

____

____

____

____

____

If yes, how were the effects/symptoms treated?

____

____

____

____

____

37. How much did it cost in treating the effects/symptoms experienced by the family members?

Appendix III: Ethical Consideration Proposal Approval Form

INSTITUTE OF PRIMATE RESEARCH

P.O. Box 24481 - 00502 Karen, Nairobi. Tel: (+254-20) 2606235 Fax: (+254-20) 2606231
URL: www.primateresearch.org | Email: directoripr@primateresearch.org

NATIONAL MUSEUMS OF KENYA

INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE (ISERC)

FINAL PROPOSAL APPROVAL FORM

Our ref: ISERC/08/2021

Dear Dr. Caroline W. Ngugi

It is my pleasure to inform you that your proposal entitled **"PREVALENCE, RISK FACTORS AND ANTIMICROBIAL RESISTANCE PROFILES OF MASTITIS-CAUSING BACTERIA IN DAIRY GOATS IN SELECTED SUB-COUNTIES IN NYERI AND KIAMBU COUNTY, KENYA"** has been reviewed by the Institutional Scientific Ethics Review Committee (ISERC) following a meeting held on 19th August 2021. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes.

The committee is guided by the Institutional guidelines as well as international regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed..LOhole For....Chairman IRC: Dr. Lucy Ochola

Signed ..[signature].......... Secretary IRC: DR Faith Onditi

Date INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED..5th Oct. 2021......

IPR is ISO 9001: 2008 Certified, a WHO Collaborating Centre, an AMDI African Centre of Excellence in Preclinical Research, an Associate Partner of the EUPRIM-Net and has Statutory Registration with the NIH-Office of Laboratory Animal Welfare.

Appendix IV: Publication

Check for updates

DOI: 10.1002/vms3.1420

ORIGINAL ARTICLE

RUMINANTS

WILEY

Prevalence and antimicrobial resistance profiles of mastitis causing bacteria isolated from dairy goats in Mukurweini Sub-County, Nyeri County, Kenya

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Funding information
Pan African University; Japan International Cooperation Agency

Abstract

Background: Ruminant mastitis continues to be a cause of economic losses in the dairy industry and remains a major public health hazard globally.

Objectives: This cross-sectional study was carried out in Mukurweini Sub-County of Nyeri County, Kenya, to investigate the prevalence of bacteria causing mastitis, risk factors associated with goat mastitis and the antibiotic resistance profiles of bacteria isolated from the goat milk.

Methods: Farm level data on risk factors for mastitis was obtained from 56 farmers using a semi structured questionnaire. A total of 189 goat milk samples were collected. The goat's udder was observed for signs of clinical mastitis and the California Mastitis Test (CMT) used to test the milk for sub-clinical mastitis. All samples were then cultured for morphological identification of bacteria and strain typing by Matrix Assisted Laser Desorption/Ionization (MALDI)-Time of Flight (ToF) technique. Antimicrobial susceptibility of the isolated *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (CoNS), *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas* spp., *Enterobacter* spp., *Proteus vulgaris* and *Escherichia vulneris* to eight commonly used antibiotics was done by the disc diffusion method and validated by determining the presence of antibiotic resistance genes (*mecA* and *blaTEM*) using polymerase chain reaction method.

Results: The prevalence of clinical mastitis was 1.1% (2/189) while that of sub-clinical mastitis was 84.7% (160/189). Higher ($p < 0.05$) prevalence of mastitis was observed in goats whose houses were cleaned fortnightly and in cases where farmers used same towel to dry different does' udders during the milking process. Thirteen different bacterial species were isolated from the milk samples and identified by MALDI-ToF, and these included *S. aureus* (22.0%), CoNS (20.3%), *E. coli* (18.1%), *Pseudomonas* spp. (14.3%), *Enterobacter* spp. (10.4%), *K. oxytoca* (6.0%), *E. vulneris* (1.7%), *P. vulgaris* (1.7%), *Raoutella ornithinolytica* (1.7%), *Stenotrophomonas maltophilia* (1.1%), *Pantoea agglomerans* (1.1%), *Serratia marcescens* (1.1%) and *Cedeceas* spp. (0.6%). One hundred pathogenic bacterial isolates were randomly selected and tested for antibiotic

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sensitivity to eight antibiotics out of which *S. aureus* were 97.5% resistant to Oxacillin and 100% sensitive to Ciprofloxacin. The CoNSs were 100% resistant to Oxacillin and 100% sensitive to Ciprofloxacin. *E. coli* were 93.9% resistant to Oxacillin, 69.7% sensitive to Ciprofloxacin and 87.9% sensitive to both Amoxicillin/Clavulanic acid and Meropenem. The antimicrobial resistant genes detected in *S. aureus* and *E. coli* were *mecA* [66.7%, 0%], and bla_{TEM} [20% and 78.3%], respectively.

Conclusion: In conclusion, the study showed that most of the does were affected by subclinical mastitis with the main causative bacteria being *Staphylococci* spp. and coliforms. Farmers need to be trained on improved control of mastitis by adoption of good milking practices and use of CMT kit for early detection of mastitis. Occurrence of multidrug resistance by key mastitis causing pathogens was shown to be prevalent and therefore there is need for development of intervention strategies.

KEYWORDS
antimicrobial resistance, coagulase-negative staphylococcus, mastitis, Mukurweini, risk factors

1 | INTRODUCTION

In Kenya, the dairy goat population is about 200,000, of which 80% are reared by small-scale farmers in the Mount Kenya region Counties, including Meru, Embu, Nyeri, Kirinyaga and Murang'a (Mbindyo et al., 2018). Mukurweini Sub-County in Nyeri County has a higher population of dairy goats owing to the combined effort between government and Non-Governmental Organizations to improve food and nutritional security in the region through dairy farming (Mburu et al., 2014). The social-economic studies done by Kinyanjui et al. (2008) showed that 57% of the dairy goats' milk produced was consumed in households, whereas the surplus was sold to specific individuals mainly suffering from ailments, such as diabetes, AIDS and those sensitive to cow milk (Mburu et al., 2014). The same study further noted that in Kenya, there is a high potential of dairy goat farming where each goat can yield up to 2.96 litres of milk daily. However, goat farming is affected by a number of challenges, the main one being infectious diseases such as mastitis (Kagucia et al., 2020; Mahlangu et al., 2018).

Mastitis is the most common disease that affects dairy goats (Gazzola et al., 2021), leading to heavy economic losses associated with poor milk quality, reduced milk yield, high cost of treatment, discarded milk while the animal is on treatment, early culling and sometimes death of the animal (Zigo et al., 2022). Globally, losses due to dairy goat sub-clinical mastitis have not been well document, but figures range from 65 to 80 Euros (Sánchez et al., 1997; Oosterhuis, 2010, https://aboutsmallruminants.com/analytics-costs-mastitis-sheep-goats/). The costs are mainly due to production, reproduction losses and treatment costs.

The disease results in the inflammation of the mammary glands as a result of infection by pathogenic agents such as bacteria, fungi or viruses and spreads mainly through unhygienic conditions in dairy farms (Zigo et al., 2022). Contagious mastitis is caused by bacteria residing on the skin of the teat and inside the udder such as *Staphylococcus aureus* and *Streptococcus agalactiae* and is transmissible from one goat to another. Environmental mastitis is caused by environmental pathogens such as *Escherichia coli*, *Streptococcus uberis* and *Klebsiella* spp., usually found on bedding, feed, manure and soil. Mastitis is, therefore, the outcome of interplay between three major factors: infectious agents, host resistance and environmental factors (Verma et al., 2018). Early detection and effective treatment of mastitis are of great importance to curb losses incurred by farmers while providing nutritional security.

Mastitis is treated by antibiotics, mainly penicillins (Younan, 2002). Although coliform mastitis may sometimes be treated using cephalosporins and fluoroquinolones (Aqib et al., 2019), their use should strictly be based on bacteriological diagnosis (Suojala et al., 2013). Early treatment with effective antibiotics significantly limits the severity of mastitis, economic loss and development of antimicrobial resistance (AMR) (Verma et al., 2018). The overuse and misuse of veterinary antibiotics by farmers has been associated with infection resurgence due to development of AMR in mastitis-causing pathogens (Aqib et al., 2019). Cloxacillin, for example, an analog of Methicillin/oxacillin, which is used in dry cow therapy, has been implicated in development of methicillin-resistant *S. aureus* (MRSA) isolates (Saini et al., 2012). The latter has been isolated from raw milk samples and is a leading cause of dairy mastitis. In Kenya, antibiotics are used routinely to treat mastitis in goats (Mahlangu et al., 2018). Worldwide, there has been reduced efficacy of drugs used to treat infections caused by MRSA due to its increased resistance to glycopeptides (Tarai et al., 2013). Further, extended-spectrum beta-lactamases (ESBL) antibiotic-resistant *E. coli* and *Klebsiella* species have been isolated from dairy milk (Badri et al., 2017).

Livestock bacteria can be reservoirs of antibiotic resistance genes such as those associated with ESBL in *Enterobacteriaceae*, which could be transferred to human beings through multiple routes (Ljungquist et al., 2016). In addition to expanded-spectrum cephalosporins (ceftriaxone, ceftiofur, cefotaxime and ceftazidime). ESBL producers often carry determinants that confer resistance to fluoroquinolones, aminoglycosides and trimethoprim – sulfamethoxazole combination (Saini et al., 2012).

AMR is a public health threat and can potentially cause mortalities approximated to 1 million per annum by 2050 if new effective antimicrobials are not developed (O'Neill, 2016). The mortality rates of infections associated with multidrug-resistance microorganisms have consistently increased over the last two decades across different populations (Ali et al., 2021). Over time the bacteria that cause mastitis have developed resistance to the antibiotics administered. Knowledge on prevalence of mastitis in dairy goats, microbial diversity, risk factors associated with mastitis development and AMR patterns would greatly improve prevention and guide treatment of the disease.

From the above, it is clear there is an urgent need for extensive research on the status of mastitis and mastitis-causing pathogens in Kenya so as to improve the existing control measures and to guide treatment. The current study investigated the prevalence and aetiology of clinical and subclinical mastitis, AMR profiles and associated risk factors in dairy goats kept by small-scale farmers in Mukurweini Sub-County, Nyeri County, Kenya.

2 | MATERIALS AND METHODS

2.1 | Description of the study area

This study was conducted in Mukurweini Sub-County, Nyeri County, Kenya. The study was conducted in Rugi, Central, West and Gikondi wards.

2.2 | Study design and sample size calculation

A cross-sectional study was carried out between August 2021 and April 2022. The sample size was calculated using the formula by Dohoo et al. (2003): $n = (Z^2\alpha pq)/L^2$, where n is the required sample size, $Z\alpha$ is the value of Z that provides 95% confidence intervals (1.96), p is a priori estimate of the prevalence at the time of the study. With an expected prevalence of 28.6% based on a previous study (Ndegwa et al., 2000), a sample size of 314 was obtained. The minimum sample size of 160 was calculated using the adjusted formula for small animal populations (Thrusfield et al., 2018). In the current study, 189 animals were sampled from a total of 56 farms, each owning 1–10 dairy goats. As there was no formal list of dairy goat farmers available in the study area, the snowball technique was used to identify farmers with lactating dairy goats. The initial farmers were identified by the local extension officers.

2.3 | Questionnaire survey

During the farm visits, data was collected using a semi structured questionnaire administered on each farm through personal interview by the investigators. The information obtained from the respondents included farm bio-data and farm management practises such as if the does are housed, floor type, use of beddings, cleaning frequency, washing of udder before milking, drying of udder during milking and use of separate drying towel for each goat during milking. The doe factors which were obtained included breed and history of mastitis.

2.4 | Milk sample sampling and California Mastitis Test (CMT)

Milk samples were collected aseptically from each teat of 189 lactating dairy goats as described by Deka et al. (2020). With the help of a veterinarian, a detailed visual inspection and systemic palpation of the udder and teats of the lactating doe was done and the milk observed for clinical signs of mastitis. For milk collection, the first three streams of milk were discarded and 3 mL of milk were directly put into a CMT paddle and an equal amount of commercial CMT reagent added (Immu-Cell RP). The CMT was observed within 20 s and the results read on a score of 0–3. A score of 0 was considered negative, 1 was considered trace while a score of 2 and 3 was considered positive (Contreras et al., 1996). Ten millilitres of CMT-positive milk were then collected into labelled sterile universal bottles for further laboratory analysis. The samples were placed in cool boxes with ice packs and transported to the microbiology laboratory within 24 h.

2.5 | Culture and identification of bacteria

The milk samples were enriched with alkaline peptone water at 37°C overnight. A hundred microliters of the enriched milk samples were inoculated onto both sheep blood agar (Himedia Laboratories) and McConkey agar (Oxoid), respectively. The plates were incubated at 37°C for 24–48 h. Plates which had mixed growth were sub-cultured to obtain pure colonies. Further characterization was done for the beta haemolytic bacteria obtained from sheep blood agar by catalase test (Reiner, 2010) to differentiate *Streptococcus* from *Staphylococcus* spp.

Strain typing was done using Matrix Assisted Laser Desorption/Ionization (MALDI)-Time of Flight (ToF) technique. Briefly, forty (40) mg/mL alpha-cyano-4-hydroxycinnamic acid (matrix) (Sigma-Aldrich) was prepared in LC–MS grade solvents; acetonitrile, ethanol and water in the ratio of 3:3:3 in 3% trifluoroacetic acid. The 25% formic acid overlay method was used for spotting. Using a sterile microcentifuge tip, 0.5 µL bacterial colonies was transferred onto the target plate, and each spot was overlaid with 0.5 µL of 25% formic acid (Sigma-Aldrich). This was followed by application of 0.5 µL of α-cyano-4-hydroxycinnamic acid matrix onto each spot and thoroughly mixing before MALDI-MS measurements in Shimadzu Axima Confidence (Shimadzu). A spectrum was acquired for each spot and species identification of each spectrum was compared to the ribosomal marker based database, SARAMIS.

TABLE 1 Primer sequences.

Target gene	Primer sequence	Expected amplicon size (bp)	Source
bla_{TEM}	F_TCGCCGCATACACTATTCTCAGAATGA R_ACGCTCACCGGCTCCAGATTTAT	445	Monstein et al. (2010)
MecA	F_TGG TAT GTG GAA GTTAGATTGG R_GGATCTGTACTG GGT TAA TCAG	166	Mehrotra et al. (2000)

2.6 | Antibiotic susceptibility testing

Antibiotic susceptibility test was performed using the disc diffusion method (Pum, 2019). One hundred isolates were randomly selected, suspended in growth media, and standardized through a turbidity test (0.5 McFarland's standard). The hundred isolates were considered possible causes of mastitis based on literature. A hundred microlitres of the standardized suspension of the test organism was then inoculated on Mueller Hinton Agar (Himedia, Ltd) plates. Eight antibiotics (Himedia, Ltd) commonly used for treatment of mastitis in Kenya were selected for antibiotic susceptibility testing in the study: Cefuroxime (30 μg), Cefotaxime (30 μg), Amoxicillin and Clavulanic acid (10 μg), Oxacillin (10 μg), Azithromycin (15 μg), Meropenem (10 μg), Ciprofloxacin (10 μg) and Nitrofurantoin (300 μg). The discs were placed on the media surface and plates incubated at 37°C for 24 h. The respective zones of inhibition were measured and results interpreted according to the CLSI (2019) table. Results were recorded as resistant, intermediate or susceptible to specific antibiotics.

2.7 | Molecular detection of antibiotic resistant genes

Biochemical test was done to identify the bacteria and identity confirmed through MALDI-ToF technique. Methicillin resistant *S. aureus* was used as a positive control. A total of 35 isolates with multiple antibiotic resistance indexes were selected for molecular detection of antibiotic-resistant genes. Plasmid DNA was extracted from 35 isolates using plasmid DNA extraction kit (Bioline) following the manufacturer's instructions. Amplification was done using specific primers for detection of genes conferring resistance to Oxacillin (*mecA*), Amoxicillin and Clavulanic acid (*bla_{TEM}*) (Mehrotra et al., 2000; Monstein et al., 2010). The primer sequences and the expected fragment size of each gene are listed in Table 1. The polymerase chain reaction amplicons were quantified using a Nanodrop spectrophotometer (Jenway, Genova Nano) followed by the confirmation of a successful recovery by resolving 10 μL of product on 1.5% (w/v) agarose gel and visualizing under UV trans-illuminator (UVITEC Cambridge) against a 100 bp molecular size ladder.

2.8 | Data analysis

Data entry and management were done using Microsoft Excel 2010 (Microsoft), whereas data analysis was done using SPSS v26 (Microsoft). Descriptive statistical data was presented in tables. Chi square was used to evaluate the association between occurrence of risk factors and prevalence of mastitis ($p < 0.05$). A doe was assumed to be infected with mastitis if it had clinical or sub-clinical mastitis based on CMT results. Logistic regression was used to test individual risk factors and their strength of association in mastitis infection. The odds ratio was used to determine the strengths of association identified in logistic regression.

3 | RESULTS

3.1 | Characteristics of farms and goats sampled

A total of 56 farms were sampled from the study area. The average size of the farms was half an acre with the sizes ranging from quarter acre to three acres majority of the goat houses were raised with timber while some were earthen (35%). Some (54%) farmers practiced zero grazing, whereas others practiced open grazing (20%) and tethering (26%). The goat breeds kept by the farmers were crosses (34.4%), German Alpine (28.6%), Toggenburg (15.9%), Kenya Alpine (12.2%), Saanen (5.3%) and local breeds (3.7%). The average number of goats kept by farmers was 3 with the number of goats ranging from 1 to 10. Most (84%) of the farmers were not aware of occurrence of mastitis in their dairy goats.

3.2 | Prevalence of clinical and sub-clinical mastitis

The overall prevalence of mastitis was 85.7% (162/189) with most (160/189, 84.7%) of them having subclinical disease and a few having clinical mastitis (2/189, 1.1%). Clinical mastitis was characterized by presence of swollen and inflamed udder, presence of flakes in the milk and discoloured milk.

3.3 | Relationship between prevalence of mastitis and origin of goat, breed milking practices (Table 2)

The results of the CMT were used to evaluate the relationship between mastitis prevalence and risk factors. The highest prevalence of mastitis was in lactating does originating from Central Ward. However, there was no significant difference ($p = 0.520$) between prevalences of mastitis in does originating from the different wards. In terms of breeds, the highest prevalence of mastitis was found in crosses (36.4%), followed

TABLE 2 Effect of origin of goat, breed and milking practices on prevalence of mastitis in dairy goat in Mukurweini Sub-County as identified by California Mastitis Test (CMT) ($n = 189$).

Factor	Number positive	Prevalence of mastitis %
Ward of origin		($p = 0.52$)
Rugi	41	21.69
Central	42	22.22
West	40	21.16
Gikondi	39	20.63
Breed of goat		($p = 0.2887$)
Toggenburg	28/30	14.81
Crosses	59/65	31.22
German Alpine	50/54	26.46
Saanen	7/10	3.70
Kenyan Alpine	15/23	7.94
Local	3/7	1.59
Pen cleaning schedule		($p = 0.001$)
Weekly	71	37.57
Fortnightly	60	31.75
Daily	12	6.35
Irregular	19	10.05
Hand washing		($p = 0.01$)
Yes	142	75.13
No	20	10.58
Separate drying towel for each goat		($p = 0.04$)
Yes	30	15.87
No	132	69.84
Floor type		($p = 0.35$)
Wooden	123	65.08
Earthen	39	20.63
Use of beddings		
Yes	0	0
No	162	85.71
Washing of udder before milking		($p = 0.42$)
Yes	153	80.95
No	9	4.76
Housing		
Yes	162	85.71
No	0	0

by German Alpine (30.9%) and the least affected were the local breeds (1.9%). Nonetheless, there was no significant ($p = 0.28870$) difference in the prevalence of mastitis in the different breeds (Table 3). The highest prevalence of mastitis was in lactating does whose houses were cleaned weekly ($p = 0.001$) compared to those cleaned daily. The highest prevalence of mastitis was found in lactating does where farmers did not wash their hands before milking ($p = 0.01$) compared to those who cleaned their hands. The lowest prevalence of mastitis was found in milk from lactating does whose udder were dried with individual towel ($p = 0.04$) compared to those from farms where towels were shared between different goats (Table 2).

TABLE 3 Bacteria isolated from dairy goats ($n = 182$) in Mukurweini Sub-County, Kenya.

Bacteria species	Number of isolates	Percentage
Staphylococcus aureus	40	22.0
Coagulase-negative *Staphylococcus*	37	20.3
Escherichia coli	33	18.1
Pseudomonas spp.	26	14.3
Enterobacter spp.	19	10.4
Klebsiella oxytoca	11	6.0
Proteus vulgaris	3	1.7
Raoutella ornithinolytica	3	1.7
Escherichia vulneris	3	1.7
Pantoea agglomerans	2	1.1
Serratia marcescens	2	1.1
Stenotrophomonas maltophilia	2	1.1
Cedecea spp.	1	0.6
Total	182	100

3.4 | Prevalence of bacteria in the sampled milk

All the 189 samples were cultured. A total of 162 samples positive for clinical and sub-clinical mastitis yielded bacteria in culture, whereas the 27 samples that were negative for mastitis did not yield any bacteria. The most prevalent species isolated included *S. aureus* (22.0%), coagulase-negative *Staphylococcus* (CoNS) (20.3%), *E. coli* (18.1%), *Pseudomonas* spp. (14.3%) and *Enterobacter* spp. (10.4%). Other identified species of bacteria and their prevalences are as summarized in Table 3.

3.5 | Antimicrobial susceptibility pattern

Antibiotic sensitivity was under taken for the following bacteria; *E. coli*, *Enterobacter* spp., *Klebsiella oxytoca*, *Escherichia vulneris*, *Pseudomonas*, *Proteus vulgaris*, *S. aureus* and CoNS. The tested bacteria were highly resistant to Oxacillin (93.9%–100%). *S. aureus*, which was the most prevalent species, was highly susceptible to Ciprofloxacin (100%), Nitrofurantoin (87.5%) and Azithromycin (82.5%) and highly resistant (97.5%) to Oxacillin. CoNS was 100% susceptible to Ciprofloxacin, 82.2% susceptible to Azithromycin, 68.5% susceptible to Nitrofurantoin and 100% resistant to Oxacillin.

All (100%) *K. oxytoca* isolates were categorized as having intermediate form of resistance. Susceptibility to Amoxicillin/Clavulanic acid

TABLE 4 Antibiotic susceptibility profiles of *Escherichia coli*, *Enterobacter* spp., *Klebsiella oxytoca*, *Escherichia vulneris*, *Pseudomonas* spp. and *Proteus vulgaris* (percentages) isolated from lactating does in Mukurweini Sub-County (*n* = 95).

	E. coli (*n* = 33)			*Enterobacter* spp. (*n* = 19)			*K. oxytoca* (*n* = 11)			*E. vulneris* (*n* = 3)			*Pseudomonas* spp. (*n* = 26)			*Proteus vulgaris* (*n* = 3)		
Antibiotics	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*
AMC	0	87.9	12.1	21.1	57.9	21.1	9.1	72.7	18.2	0	100	0	0	100	0	0	66.7	33.3
CXM	12.1	15.2	72.7	26.3	15.8	57.9	18.2	18.2	63.6	66.7	33.3	0	50	50	0	33.3	0	66.7
CTX	27.3	57.6	15.2	26.3	47.4	26.3	18.2	72.7	9.1	66.7	33.3	0	50	50	0	33.3	66.7	0
Mpm	6.1	87.9	6.1	5.3	15.8	78.9	0	90.9	9.1	0	100	0	0	50	50	0	100	0
FT	3.0	51.5	45.5	26.3	42.1	31.6	18.2	63.6	18.2	66.7	33.3	0	50	50	0	66.7	0	33.3
AZM	30.3	69.7	0	10.5	31.6	57.9	36.4	63.6	0	33.3	66.7	0	100	0	0	66.7	0	33.3
OX	93.9	6.1	0	100	0	0	0	0	100	100	0	0	100	0	0	100	0	0
CIP	12.1	69.7	18.2	15.8	15.8	68.4	0	90.9	9.1	33.3	66.7	0	100	0	0	0	100	0

Abbreviations: AMC, Amoxicillin + Clavulanic acid; AZM, Azithromycin; CIP, Ciprofloxacin; CTX, Cefotaxime; CXM, Cefuroxime; FT, Nitrofurantoin; *I*, intermediate; Mpm, Meropenem; OX, Oxacillin; *R*, resistant; *S*, susceptible.

TABLE 5 Antibiotic susceptibility profiles of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) (in percentages) isolated from lactating does in Mukurweini Sub-County (*n* = 77).

	S. aureus (*n* = 40)			CoNS (*n* = 37)		
Antibiotics	*R*	*S*	*I*	*R*	*S*	*I*
Nitrofurantoin	12.5	87.5	0	32.4	67.6	0
Azithromycin	17.5	82.5	0	18.9	81.1	0
Oxacillin	97.5	2.5	0	100	0	0
Ciprofloxacin	0	100	0	0	100	0

Abbreviations: CoNS, coagulase-negative *Staphylococcus*; I, intermediate; S, susceptible; R, resistant.

was highest in *E. vulneris* (100%), *Pseudomonas* spp. (100%) and *E. coli* (87.9%) and lowest in *K. oxytoca* (72.7%) and *Enterobacter* spp. (57.9%). Strains susceptible to Meropenem included *P. vulgaris* (100%), *E. vulneris* (100%), *K. oxytoca* (90.9%) and *E. coli* (87.9%). *Pseudomonas* spp. isolates were resistant to Azithromycin (100%), Oxacillin (100%) and Ciprofloxacin (100%) and 100% susceptible to Amoxicillin/Clavulanic acid. A summary of the antibiotic susceptibility profiles is given in Tables 4 and 5.

3.6 | Detection of antimicrobial resistance genes

Bla_{TEM} antibiotic resistance genes were detected in the plasmid DNA of *E. coli*, *Enterobacter* spp. and *K. oxytoca* (Figure 1). MecA and Bla_{TEM} antibiotic resistance genes were detected in *S. aureus* isolates. The bands on the agarose gel gave the expected sizes of 445 and 225 bp, respectively. A total of 18 *E. coli* isolates (78.3%) showed the presence of bla_{TEM}, whereas no (0%) isolates had *MecA* gene. *MecA* and bla_{TEM} genes were detected in 10 and 3 *S. aureus* isolates, respectively, as shown in Figure 2.

4 | DISCUSSION

The current study sought to characterize mastitis-causing pathogens and their antibiogram in dairy goats reared by small-scale farmers from Mukurweini Sub-County in Nyeri County, Kenya. Our study found the prevalence of sub-clinical to be higher than clinical mastitis, which is expected because sub-clinical mastitis cases are not detected by physical examination of milk or udder. The current study agrees with a study in Pakistan (Mirza et al., 2017), which reported a prevalence of mastitis to be 75%–85% over a period of 3 years. The findings are also close to the findings of Jabbar et al. (2020), which reported the overall prevalence of mastitis in Pakistan to be 61.8%. Compared to other Kenyan studies, the prevalence of mastitis in the current study area was higher than that reported by both Ndegwa et al. (2000) (9.8%) and Mahlangu et al. (2018) (50.9%) in dairy goats from Thika East Sub-County, Kenya. This study revealed that farms that did not use an udder drying towel for each cow had significantly higher prevalence of mastitis than farms that used a drying towel for each cow. This agrees

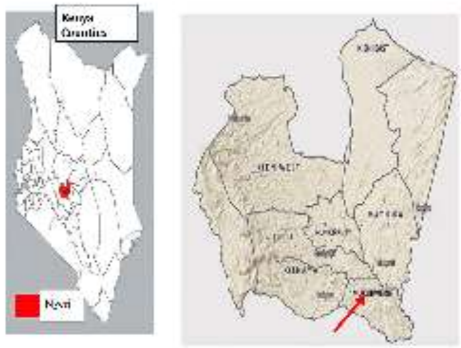

FIGURE 1 Map of Kenya showing the location of Nyeri County and study Sub-County. Mukurweini Sub-County has an area of 171.1 km^2 with a population of 89,137 people and a density of 498 people per square kilometre. The coordinates for Mukurweini Sub-County are 0.5609° S, 37.0488° E. The average temperature of Mukurweini is a high of 24°C and a low of 13°C. The average annual rainfall in Mukurweini is 1206 mm and occurs in two seasons. The livestock population of Mukurweini Sub-County is 168,685 (Kenya National Bureau of Statistics, 2019), comprising of exotic cattle, indigenous cattle, sheep, goats, donkeys, pigs and chicken. The goat population was 10,379 as per the 2019 national census (Kenya National Bureau of Statistics, 2019).

with finding by Mbindyo et al. (2020) from Mekonnen et al. (2017) from Ethiopia who reported that use of the same drying towel for the herd was responsible for the spread of mastitis-causing pathogens. This can explain the high prevalence of *Staphylococcus* spp. reported in this study. As these organisms are part of the normal flora residing in the udder and the teats, they can spread through the use of same drying towel during milking. In this study, there was significantly higher prevalence of mastitis in does whose houses were cleaned weekly compared with does whose houses were cleaned more frequently. These results are in agreement with those by others (Mahlangu et al., 2018; Mbindyo et al., 2014). The study showed hygiene plays a major role in occurrence of subclinical mastitis. Farms where the hands were not washed before milking showed significantly higher prevalence of mastitis compared with farms where farmers washed hands before milking (Gianneechini et al., 2002). In contrast to other studies (Mahlangu et al., 2018; Mbindyo et al., 2014), the current one found that cross-breeds had higher prevalence of mastitis compared to others breeds of goats. The causes of variation in the breed differences are not clear and should be studied further.

The current study found CoNS, *S. aureus*, *E. coli* and *Enterobacter* spp. to be the predominant bacteria causing mastitis in goats. Our findings are consistent with those of Mahlangu et al. (2018) who found CoNS (20.7%) and *S. aureus* (10.7%) as the commonest causative agents of mastitis in dairy goats kept by farmers in Thika Sub-County, Kenya. Our findings are also consistent with the findings of Saidani et al. (2018) which found CoNS (20.7%), *S. aureus* (10.7%) among the Gram-positive bacteria, *Enterobacter* spp. (6.5%) and *E. coli* (5.9%) among the Gram-negative bacteria as major causative agents of mastitis in Indonesia. Altaf et al. (2019) and Jabbar et al. (2020) also found *Staphylococcus* spp. to be the predominant bacteria causing subclinical mastitis in goats in Pakistan. Pisanu et al. (2020) stated that *Staphylococcus* spp. are the most prevalent intramammary pathogens in dairy goats causing subclinical, clinical, acute and gangrenous mastitis. Further, in recent years, it is important to note that CoNS have emerged as a significant threat to food safety since they have been shown to harbour numerous enterotoxins and AMR genes (Gizaw et al., 2020).

Coliforms, including *Klebsiella* spp., *E. coli* and *Enterobacter* spp. were also observed in dairy goats in the current study. These bacteria are normally associated with environmental contamination of the udder and establishment of intramammary infection. The presence of coliforms may be linked with poor hygiene practices in dairy goat farms (Okoko et al., 2020). In our study, the sharing of towels to dry the udders, failure to wash hands before milking and infrequent cleaning of goats' houses were found to be risk factors for the development of mastitis in the lactating goats. This agrees with the study done by Mbindyo et al. (2020) in Embu and Kajiado Counties in Kenya, which showed that the use of same drying towel for the herd was responsible for spreading mastitis pathogens.

In the current study, the isolated *E. coli* showed high antibiotic resistance to oxacillin but high susceptibility to Amoxicillin/Clavulanic acid, Meropenem, Azithromycin and Ciprofloxacin. Multidrug resistance has been observed in the Gram-negative bovine mastitis pathogens (Saini et al., 2012). In the current study, multidrug resistance was found in *E. coli* isolates (resistant to Oxacillin, Cefotaxime, Azithromycin, Cefuroxime, Meropenem, Nitrofurantoin and Ciprofloxacin), *Enterobacter* species (resistant to Cefuroxime, Cefotaxime, Amoxicillin/Clavulanic acid, Meropenem, Nitrofurantoin, Azithromycin, Ciprofloxacin and Oxacillin) and *K. oxytoca* isolates (resistant to Cefuroxime, Cefotaxime, Amoxicilli/Clavulanic acid, Nitrofurantoin and Azithromycin). Coliform contaminations rank high among the most types of contamination in the dairy industry. Microorganisms, such as *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter* spp., *Klebsiella* spp. and *Proteus mirabilis*, can multiply in the normal summer temperatures, and hence unpasteurized milk has every chance of containing *E. coli* (Dhanashekar et al., 2012). Amoxicillin/Clavulanic acid was also found to be effective against *Enterobacter* spp., *E. vulneris*, *Pseudomonas* spp. and *P. vulgaris*. This can be attributed to limited use of Amoxicillin/Clavulanic acid in the treatment of sub-clinical mastitis in the current study area.

Most bacteria isolates from the current study area were resistant to Oxacillin. Most *S. aureus* isolates were resistant to Oxacillin. *S. aureus* is an important pathogen because of a combination of toxin-mediated virulence, invasiveness and antibiotic resistance (Guimarães et al., 2017). Moreover, the bacterium is a significant cause of human nosocomial infections (Guimarães et al., 2017). On the contrary, *S. aureus* showed high antibiotic susceptibility to Ciprofloxacin, Nitrofurantoin and Azithromycin. The findings on susceptibility agrees with a study by Upadhyay and others that reported high susceptibility of *S. aureus* to Azithromycin (100%) and Ciprofloxacin (76.67%) (Upadhyay & Kumar Kataria, 2009).

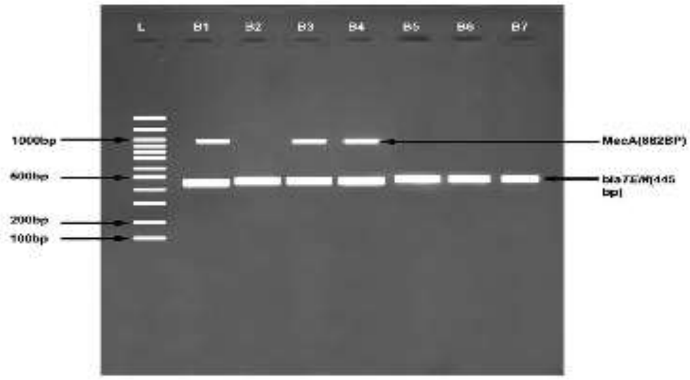

FIGURE 2 Gel image showing MecA and blaTEM: (B1 and B2) *Escherichia coli*, (B3 and B4) *Staphylococcus aureus*, (B5 and B6) *Enterobacter*, (B7) *Klebsiella oxytoca* and (L) hyperladder 100 bp Bioline.

The current study focused on detection of AMR genes in dominant species causing mastitis. *E. coli* has the ability to produce ß-lactamase enzyme and modified penicillin-binding proteins that hydrolyse or inhibit the binding of ß-lactam antibiotics (Okoko et al., 2020). In *S. aureus*, the current study reported the presence of *mecA* gene while in *E. coli* bla_{TEM} antibiotic resistance gene was identified. Similar findings in *E. coli* and *S. aureus* were reported by other authors (Eltaweil et al., 2022; Milk & Gelbu, 2018). These resistant genes are common and have been shown to spread across the ecosystem (man-animal-environment) through horizontal gene transfer (Todorović et al., 2018). *Enterobacter* spp. are common human gut micro flora but have also been isolated from milk (Hogan & Smith, 2012). In our study, *Enterobacter* spp. was found in goat milk, suggesting the possible transmission of the bacteria to goat udders through water, soil or faecal contamination. *Enterobacter* spp. and *E. coli* are highly resistant to Oxacillin. Our study agrees with a study done in India which showed *E. coli* and other mastitis bacteria to be highly resistant to Oxacillin (Fahim et al., 2019).

Some bacteria that were found in the milk samples such *Stenotrophomonas maltophilia* are environmentally acquired opportunistic pathogens which are not known to cause mastitis. Other studies have found this bacteria to carry resistance genes to most classes of antibiotics, including beta-lactamases, aminoglycoside inactivating enzymes and efflux pumps (Gil-Gil et al., 2020). Its presence in milk, therefore, is a major public health concern as it is known to cause a number of infections mainly at hospitals (among immunocompromised hosts) as well as in cystic fibrosis patients (Gil-Gil et al., 2020) and can potentially contribute to transfer of AMR genes from *S. maltophilia* to humans through milk consumption. In recent years, *K. oxytoca* has been reported as a significant opportunistic pathogen causing nosocomial infections in neonates as well as adults (Neog et al., 2021). *Klebsiella* species have been found to harbour many AMR genes, including *blaTEM*, *bla OXA* and *blaCTM-M-15* (Muraya et al., 2022). The most prevalent ESBL-producing Enterobacterales in Kenya are *CTX-M-15* and TEM which is associated with use of third generation Cephalosporins in a previous study (Muraya et al., 2022). In our study, *K. oxytoca* was found to be resistant to Amoxicillin/Clavulanic acid, Cefuroxime and Cefotaxime which agrees with a study done by Neog et al. (2021) in India.

5 | CONCLUSIONS

The current study found a high prevalence of sub-clinical mastitis in dairy goats in the study region. The major predisposing factors to goats contracting mastitis were poor hygiene factors including the use of one towel to dry several udders during milking and not cleaning the goat pens frequently. To mitigate emergence and spread of mastitis, farmers should be trained on proper hygiene practices including daily cleaning of goat pens and using one towel per doe to clean the udders during milking. There is also need to train farmers on use of CMT in order to have early detection of mastitis. Majority of the bacteria causing mastitis were resistant to the common antibiotics used to treat mastitis, particularly to Oxacillin. Other drugs were found to have high efficacy and thus could be recommended for treatment of mastitis in the study area, and these include Ciprofloxacin, Meropenem, Nitrofurantoin and Azithromycin. Further studies should be undertaken to determine the causes of the high burden of AMR and whether there could be cross-transmission of these bacteria to humans.

AUTHOR CONTRIBUTIONS

Designing of the study, data collection and analysis, writing the original draft, review and editing of the manuscript: Sarah Kabui. *Funding acquisition and study supervision, analysis of molecular study data and critical revision of the manuscript for important intellectual content:* Josephine Kimani. *Funding acquisition and study supervision, analysis of the microbiology data and critical revision of the manuscript for important intellectual* content: Caroline Ngugi. *Funding acquisition and study supervision, designing the study, analysis of the mastitis and antibiotic sensitivity data and critical revision of the manuscript for important intellectual content*: John Kagira.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

DATA AVAILABILITY STATEMENT

The data collected and analysed during this study are available from the corresponding author on reasonable request.

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PEER REVIEW

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ETHICS STATEMENT

Ethical approval was obtained from the Ethics Committee of Institute of Primate Research, Kenya. Milk samples were obtained with the help of the farmers who volunteered to participate in the study under the supervision of veterinary officers from the county veterinary office. The protocols used for sampling, bacterial isolation and antibiotic susceptibility testing were performed in agreement with the Animal Diseases act, Section 20, Act of 1984 (Act No. 35 of 1984).

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