ANTIMICROBIAL USE AND PATHOGENS SENSITIVITY AMONG SMALLHOLDER DAIRY HERDS AROUND NAKURU PERI-URBAN AREAS, KENYA

MARIAMA NJIE

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Livestock Production Systems of Egerton University

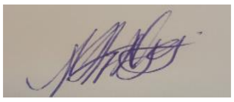
EGERTON UNIVERSITY

OCTOBER, 2024

DECLARATION AND RECOMMENTATION

Declaration

This research thesis is my original work and to the best of my knowledge has not been presented in this University or any other for the award of a degree.

Signature:……  Date: 14/10/2024

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Recommendation

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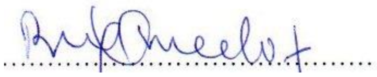
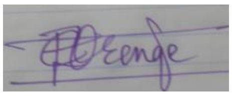
Signature:  …………………………………… Date:…14/10/2024

Prof. Bockline Omedo Bebe, PhD

Department of Animal Sciences

Faculty of Agriculture

Egerton University

Signature:  ………………… Date:..14/10/2024

Dr. Caleb Oburu Orenge, BVM, M.Sc., PhD

Department of Veterinary Anatomy and Physiology

Faculty of Veterinary Medicine and Surgery

Egerton University

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DEDICATION

I dedicate this work to those who have shaped my life and supported my journey. To my beloved parents, Alieu Badara Njie and Fatou Jobe Njie, whose love and guidance have been my foundation. To my dear siblings - Abdoulie Alieu Njie, Sophie Njie, and Jabou Njie - who have stood by me through thick and thin. To my precious children, Fatima Badjie and Muhammed Lamin Badjie, who inspire me to be better every day. To Professor Bockline Omedo Bebe, my academic father and mentor, whose wisdom has illuminated my path. And to the cherished memory of Lamin S. Jawla, a friend and brother whose presence I miss dearly. Your collective influence has been instrumental in shaping this work and the person I am today. Praise be to the Glory the Almighty Allah

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ABSTRACT

Inappropriate Antimicrobial Use (AMU) leads to antimicrobial residues and antimicrobial resistance (AMR) development. The risk can be high in peri-urban smallholder dairy herds, where with intensification, mastitis infections become prevalent, inducing frequent AMU, but sometimes inappropriately. This study (i) assessed farmers' Knowledge, Attitude and Practices (KAPs) about AMU and AMR, (ii) quantified use frequency and dosage of veterinary antimicrobial use, and (iii) tested for antimicrobial sensitivity levels of pathogenic bacteria species causing mastitis. A cross-sectional survey was conducted in four peri-urban wards of Nakuru city, Kenya where change from free-grazing to semi-zero-grazing and to zero-grazing dairy management represent increasing intensification. Data on KAPs was obtained from 124 farmers and a sample of 210 lactating cows positive for mastitis were examined for use frequency and dosage of antimicrobial drug, then milk samples bacteriologically cultured to test for antimicrobial sensitivity. The associations between KAPs and dairy intensification and antimicrobial sensitivity of different pathogenic bacteria species were tested using *Chi-square* test statistics. The drug use frequency and dosage were quantified using general linear models. Among the sample farmers, at least six in ten had intensified dairy management, marketed milk through informal outlets and were using antimicrobial drugs. Compliance with the withdrawal period, training on prudent antimicrobial use, positive attitudes and good practices in AMU increased ($p>0.05$) with increasing dairy intensification. Disease incidences decreased with intensification from free grazing (42.9) to semi grazing (29.4) and to zero grazing (25.8) percent. Farmers more frequently used antimicrobial drugs to treat mastitis (47.1) than ($p<0.05$) to treat East Coast fever (14.3), parasitic infections (11.8), or other diseases (26.8) percent. Only a few antimicrobials used were identified: gentamicin (13.9), tetracycline (11.5), penicillin G (4.9), and sulfamethoxazole (3.3) percent. The average use frequency of AMU was 2.2 times per year at a dose of 20mg/kg body weight and 8.405mg/cow/year. From the positive milk samples, *Staphylococcus aureus* (100), *Escherichia coli* (14.7), *Clostridum* (16.2), *Corynebacterium* (14.) and *Entetrococcus* (4.4) percent were isolated. *Staphylococcus aureus* and *Escherichia coli* showed high resistance to ampicillin (97.4), kanamycin (70.8), and penicillin G (91.7), and high susceptibility to ciprofloxacin (96.8), gentamicin (98.7), sulfamethoxazole (71.8), and streptomycin (79.5) percent. In conclusion, improving dairy management can lead to more responsible antibiotic use in livestock. The study findings indicate that milk from peri-urban cows may serve as a reservoir for multi-drug-resistant bacteria, calling for an urgent need for better stewardship and surveillance in smallholder dairy herds.

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LIST OF ABBREVIATIONS & ACRONYMS

AMD	Antimicrobial Drug
AMR	Antimicrobial Resistant
AMU	Antimicrobial Use
AST	Antimicrobial Sensitivity Test
DDD	Define Daily Dose
DFZ	Diseases-Free Zone
ECF	East Fever Coast
FAO	Food and Agriculture Organization of the United Nations
KAPs	Knowledge, Attitude and Practises
LMICs	Low Middle Income Countries
WOAH	World Organization for Animal Health
SDGs	Sustainable Development Goals
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Use of antimicrobial drugs in livestock production is on the increase with the increasing intensification of production systems, but it poses public health risks when use is inappropriate. In practice, antimicrobial drugs in livestock production can be used for disease treatment, or prophylactic to prevent disease infection or for promoting growth to improve health and attain production efficiency. However, the use of veterinary antimicrobial drugs for the treatment of sick animals must be informed by the diagnosis of a disease (Pozza *et al.,* 2020; Redding *et al.*, 2014). In dairy herds, use of antimicrobials for treatment is more frequent in cow udder for the treatment of mastitis and in calves to treat diarrhoea and respiratory diseases (Uyama *et al.,* 2022). Frequent and cumulative use of antimicrobials has been associated with increases in antimicrobial residues in milk, meat and products out of milk and meat. This becomes a public health concern because of associated side effects, which Bacanli and Başaran (2019) have enumerated. These include the transmission of antimicrobial-resistant pathogens to people, immunopathological effects, allergy, mutagenicity, nephropathy (from gentamicin), hepatotoxicity, reproductive problems, bone marrow toxicity (from chloramphenicol), and even carcinogenicity (sulphamethazine, oxytetracycline, furazolidone).

To avoid these side effects, prudent and responsible use of antimicrobials is advocated. When appropriately used, antimicrobial drugs enhance animal health optimize the production of wholesome food for humans and keep the environment healthy. However, misuse by farmers occurs in the form of underdosing, overdosing, indiscriminate use without disease diagnosis or failure to observe the recommended safe withdrawal period. The misuse subsequently leads to antimicrobial residues in milk (food) and faeces (soil and water environment). Consumption of milk with antimicrobial residues can transition into antimicrobial drug resistance in humans, of which *Salmonella* and *Escherichia coli* are the most common foodborne pathogens (Bantawa *et al.,* 2019; Menéndez *et al.,* 2010).

Development of antimicrobial drug resistance (AMR) can occur in both animals and humans when pathogens (bacteria, viruses, fungi, and parasites) no longer respond to medicines effectively, making it harder to treat infections. This subsequently increases the risk of disease spread, severe illness and death. This way, antimicrobial drug resistance impacts on good health and wellbeing 'Sustainable Development Goals' (SDG 3). The World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and

World Organisation for Animal Health (WOAH) in 2021 publication explained the AMR impacts beyond SDG 3. The impacts on SDG 2 (zero hunger) are through loss of livelihood assets when animals fail to respond to treatment. The AMR impacts on SDG 1 (no poverty) relate to high costs of treatment and chronic infections which is estimated to push additional 28.3 million people into extreme poverty by 2050. The impacts on SDG 8 (decent work and economic growth) relate to AMR increasing mortality and morbidity that comes with decline in labour supply and cause of a decrease in economic output that globally can amount to loss of USD 3.4 trillion (FAO, 2021).

With milk demand increasing in the lower middle-income countries including Kenya, antimicrobial drug use is projected to concomitantly increase by 67 percent between 2010 and 2030 (Cuong *et al.,* 2018). In Kenya, increased use of antimicrobial drugs can be expected in smallholder dairy herds where observed prevalence of mastitis pathogens can be as high as 93 percent (Ounah *et al.,* 2022) yet these herd also account for over 70 percent of the domestic marketed milk. Frequent treatment of mastitis can be associated with the observed high prevalence of antimicrobial residue reaching 31 percent (Bebe *et al.,* 2016) in marketed milk samples. Further, frequency of mastitis treatment can be associated with the observed antimicrobial resistance reaching over 90 percent for the commonly used antimicrobial drugs (Ounah *et al.*, 2022): gentamicin (94.2 percentage), ampicillin (81.7 percentage), penicillin (80.8 percentage), kanamycin (66.3 percentage) and sulfamethoxazole (60.6 percentage).

The antimicrobials can enter dairy herd through several pathways. The most frequent entry pathway is the treatment for cow mastitis and calf diarrhoea and respiratory diseases (Uyama *et al.,* 2022). The other pathways include failure to withhold milk for the appropriate withdrawal time, and possibly feeding feed contaminated with antibiotic residues. Generating data on these differential uses, residuals and pathogen sensitivity levels can inform prudent and responsible use to avoid antimicrobial residues accumulation and subsequent resistance development. In addition, this informs keeping the environment healthier whilst reducing the associated economic losses.

1.2. Statement of the Problem

Cases of inappropriate use of antimicrobial drugs is more likely to rise with increasing intensification of dairy management in the treatment of mastitis infections because prevalence is high. Resulting accumulated antimicrobial residues in milk and meat subsequently lead to development of antimicrobial resistance (AMR), which pose a public health concern because of a high probability of treatment failures in both animals and humans. Compared to the other dairy cows, those in peri-urban smallholder dairy herds could be at a higher risk of developing AMR from frequent treatment of mastitis infections, whose prevalence can exceed 80 percent on the peri-urban farms. Treating mastitis is a key entry point for AMR and it is likely to be exacerbated by easier access to a variety of antimicrobial drugs, just over the counters without prescription. This situation calls for avoidance measure practices at the farm level, but a major barrier to action is lack for evidence on farmers' Knowledge, Attitude and Practices regarding AMU; usage frequency and dosage of veterinary antimicrobial drugs; and antimicrobial sensitivity levels of pathogenic bacteria species. Without this evidence, it is a knowledge gap on how responsible and prudently farmers use antimicrobials for disease treatment, disease prevention or production performance enhancement. The AMR related studies report on a wide range of metrics but do not relate antimicrobials with the diseases commonly treated and the associated use frequency and dosage levels. The sensitivity of different pathogens is reported but such reports are scant on susceptibility along with specific resistance to the different antimicrobial drugs that can inform targeted prudent management measures. This study addresses these knowledge gaps to provide a more comprehensive understanding of antimicrobial use patterns, resistance levels, and the factors influencing judicious use in dairy production systems that are intensifying management.

1.3. General Objective

To contribute to good health and wellbeing (SDG 3) of both animals and humans by avoiding development of antimicrobial resistance through estimating the extent of usage of antimicrobial drugs and associated sensitivity levels of the common pathogens.

1.3.1 Specific objectives

i. To assess the Knowledge, Attitude and Practices (KAPs) of antimicrobial use in different smallholder dairy management systems around peri-urban areas of Nakuru city.
ii. To quantify the use frequency and dosage rate of antimicrobial drugs in smallholder dairy farms around peri-urban areas of Nakuru city.
iii. To determine antimicrobial sensitivity levels of common mastitis pathogenic bacteria in smallholder dairy farms around peri-urban areas of Nakuru city.

1.4 Hypotheses

i. Farmers 'knowledge, attitudes and practices are not significantly related to increasing dairy intensification around peri-urban areas of Nakuru city.
ii. The antimicrobial use frequency and dosage are not significantly different between treatment, prevention and production performance enhancement around peri-urban areas of Nakuru city.
iii. The sensitivity levels of common mastitis pathogenic bacteria to Tetracycline Sulfamethoxazole, Gentamycin and Penicillin G are not significantly different around peri-urban areas of Nakuru city.

1.5 Justification

Knowledge of why and how farmers use antimicrobial drugs in their dairy herds can inform options for prudent and responsible use of antimicrobial drugs to avoid development of antimicrobial residue, resistance and treatment failures associated with public health concerns, economic losses and environmental contamination. In comparison to human beings, there is paucity of information on antimicrobial use, residue, and resistance in dairy animals. Further, drug residue and resistance in dairy animals remains less documented in Nakuru peri-urban smallholder dairy farms. Evidence from this research gives a basis of advising farmers on interventions to minimise or properly use antimicrobial drugs without interfering with production and productivity of the animals, reduce economic loss, food with less antimicrobial residues and a healthier environment. This will support the long-term Sustainable development goals 2050 of (SDG 1) no poverty, (SDG 2) zero hunger, (SDG 3) good health and wellbeing and SDG 8 economic and also Kenya vision 2030 for Establishment of Disease-Free Zone (DFZ).

CHAPTER TWO
LITERATURE REVIEW

2.1 Antimicrobial Use in Livestock

The consumption and production of dairy products are rapidly increasing, especially in the developing world, due to population growth, an increase in income, urbanization, and the modernization of diets (Redding *et al.*, 2014). The world's population is predicted to grow to 9.7 billion people by the year 2050, necessitating urgent attention to issues of global food security and optimal health. Foods generated from dairy are a wholesome source of proteins, lipids, minerals, prebiotics, and probiotics, which can considerably improve both human health and food security (Garcia *et al.*, 2019). However, the use of antimicrobial medications in veterinary care revolutionized the way that animal diseases were treated after the Second World War (Prescott, 2018). Antimicrobial use has benefited the livestock sector by improving animal health, increasing production, and reducing foodborne pathogens. However, they also raise public health concerns. The concerns include the problem of antimicrobial residues in food products and in the environment, antimicrobial resistance leading to treatment failures in both animals and humans, and economic loss to farmers and the dairy industry (Redding *et al.*, 2014).

Global antimicrobial use (AMU) in animals represents 73 percent of all antimicrobials used worldwide, and it is projected to increase by 8 percent in 2030 (Mulchandani *et al.*, 2023). Global monitoring of antimicrobial use is essential, first to track progress in reducing the reliance of farming on antimicrobials. Second, to identify countries where antimicrobial-stewardship efforts should be targeted to curb antimicrobial resistance (Mulchandani *et al.*, 2023). Figure 1 presents global veterinary antimicrobial consumption. The defined daily doses (DDD) of antimicrobials on dairy farms range from 0.028 to 6.910. The most used antimicrobials, cephalosporin, penicillin G, and tetracycline, have a mean of 1.049, 0.667, and 0.275, respectively. (Werner *et al.*, 2023). It is possible to track AMU over time, establish benchmarks to encourage AMU reductions, and assess the associations between AMU and AMR by measuring AMU in animal production. The comparability of data across studies is, however, significantly hampered by the fact that AMU is evaluated using a wide range of metrics. Another challenge is the lack of resources and research capacities, a situation experienced more in many Low Middle-Income Countries (Cuong *et al.*, 2018).

Dairy product consumption has been proven to benefit the gut microbiome, cardiovascular health, and bone mass. For infants and young children, who require nutrient-and energy-rich diets for growth and cognitive development, milk is a crucial source of nourishment. Milk consumption has been linked to a decreased risk of stunting in numerous studies, and the World Health Organization (WHO) advises that 25–33percentage of dietary protein should come from milk (Garcia *et al.*, 2019).

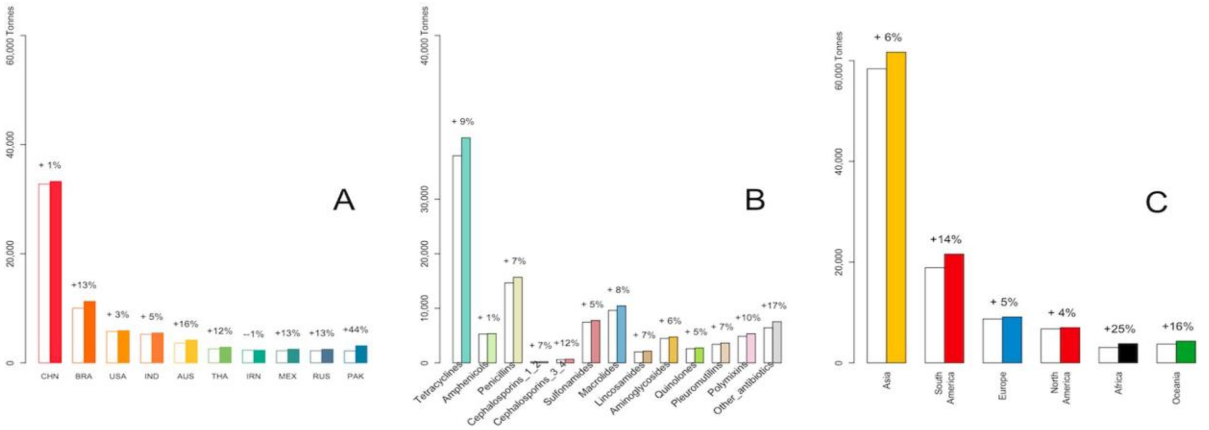

Figure 1: Veterinary antimicrobial consumption in 2020. Source: Mulchandani *et al.* (2023)

Veterinary antimicrobial consumption in 2020 (white bars) and their projected consumption for 2030 (coloured bars) by (A) country (top 10), (B) antimicrobial class, and (C) continent. CHN, China; BRA, Brazil; IND, India; USA, United States; AUS, Australia; IRN, Iran; THA, Thailand; PAK, Pakistan; JPN, Japan; MEX, Mexico However, dairy farms are the third most used antimicrobials in the livestock industry; Redding *et al.*(2014) has enumerated the antimicrobials frequently used to include oxytetracycline, penicillin+streptomycin, trimethoprim/sulfamethoxazole, cloxacillin, and antiparasitic drugs (fenbendazole, albendazole, levamisole, and triclabendazole). Omwenga *et al.* (2021) reported that tetracycline (95 percent) is the most common medication used for livestock in northern Kenya (Isolo and Marsabit), followed by sulphonamides (1.1percent), streptomycin (1.1percent), penicillin (1.1percent), tylosin (0.9 percent), and trimethoprim (0.9 percent). These research results reveal knowledge gap on the antimicrobials frequently used in peri-urban smallholder dairy farms.

2.2 Diseases Driving Antimicrobial Use

Diseases are the major causes of production losses (Bebe, 2003; Mudavadi *et al.*, 2001). The main diseases in dairy cattle in Kenya, in percentage frequency, are tick-borne diseases (43.1), pneumonia (22.0), anaplasmosis (13.0), mastitis (8.0), and foot and mouth disease (6.0), and eye conditions (4.0) (Odero-Waitituh, 2017). Infectious mastitis is treated with antimicrobial drugs in humans, companion animals, other livestock, and dairy cattle (Barlow 2011). Mastitis is one of the biggest threats to the health and wellbeing of livestock in intensive production, causing huge economic losses in the dairy industry (Gomes & Henriques 2016). The etiological agents of this disease are a wide range of Gram-positive and gram-negative bacteria, some of which are contagious (such as *Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma spp.*) and others of which are common (such as *Escherichia coli, Enterococcus spp., coagulase-negative Staphylococcus, and Streptococcus uberis*) (Kovačević *et al.,* 2023*).*

Unintentionally introducing milk from cows with subclinical mastitis into bulk milk exposes human health to risk and enters the food chain. *Staphylococcus aureus* is regularly found in milk and other dairy products (Gebremedhin *et al.*, 2022). However, *Streptococcus agalactiae* has been listed as one of the most prevalent causes of invasive infections (Hameed *et al.*, 2007). Enhancing biosecurity procedures, maintaining milking machines, maintaining teat cleanliness after milking, and increasing milking hygiene should all be part of control strategies to prevent mastitis Bacanli and Başaran (2019).

2.3 Knowledge, Attitude and Practices in Use of Antimicrobials in Livestock

Understanding key stakeholders' knowledge, attitudes, and practices (KAPs) about antimicrobial usage and resistance is one necessary step in the development of tailored response plans to address erroneous practices, a lack of awareness, and negative attitudes (Gemeda *et al*., 2020; Ozdikmenli 2023) reveal interesting results on KAPs among dairy farmers. The authors found that around 81.6 percent of livestock owners did have access to veterinary drugs through the government, veterinary officials, and private suppliers. The authors found that the drugs at hand were mostly stored under suboptimal conditions and exposed to changes in temperature, sunlight, and dust. Human-preparation antibiotics (tetracycline) were also being used for veterinary purposes by 18.5 percent. About 40.0 percent of veterinary antimicrobials were sold without a prescription, and knowledge of the dangers associated with AMR and AMU was mostly superficial. In another study, treatment failure occurred often, and there was a lack of differentiation between AMR and simply treatment failure (Kemp *et al.,* 2021). The use of antimicrobials for prophylactic purposes was common. For the most frequently used drugs, antibiotics were mainly used for treatment purposes, whereas anthelmintics were used for disease prevention and livestock fattening purposes. Respiratory diseases and digestive/internal parasitic infections were the main reasons for the therapeutic use of antimicrobials. In the mixed crop livestock system, the most frequently reported used drugs were anthelmintics (95 percent), antibiotics (24 percent), and acaricides (4.7 percent). Pastoralists mostly used antibiotics (86.7 percent, followed by anthelmintics 70.8 percent) (Gemeda *et al.,* 2020).

Mutua *et al.* (2023) reported that 78 percent of veterinary drugs are used for disease prevention, 41 percent for growth promotion, and 8 percent for treatment. However, the study was not in peri-urban smallholder dairy and did not relate the use to daily dose use and animal classes. Milk marketed in the formal and informal sectors in Kenya often does not meet the set microbial standards, posing a health hazard. Milk and dairy products are rich in nutrients, making them a good environment for the growth of both spoilage and pathogenic microorganisms. Nakuru has a Total Variable Count of 8.7 log10 cfu/ml with 4.449 log10 *Escherichia coli* and 5.092 log10 cfu/ml of *S. aureus* (Mogotu *et al.,* 2022). In Kenya dairy farms, there is low knowledge and negative attitudes regarding zoonoses and antibiotics residues as reflected by their disregard for milk regulations and standards (Nyokabi *et al.,* 2021)

2.4 Antimicrobial Residue in Animal Source of Food

After the Second World War, the use of antimicrobial medications in agriculture and veterinary care revolutionised the way that many animal diseases were treated (Prescott 2018). They are added to feed to help animals grow, are used to treat infections, and were important in the development of intensive methods of animal husbandry (dairy farms). There are significant risks that could be posed by antibiotic residues in foods like raw bulk milk, including allergic reactions, toxicity, carcinogenic effects, selection of resistant bacteria, disruption of human normal flora, provoking an immunological response, and inhibition of the starter culture. Significant financial losses can also result from antimicrobial contamination of milk for producers and manufacturers of milk and milk products (Kebede *et al*., 2014). Various side effects from these residues include the transmission of antibiotic-resistant bacteria to people, immunopathological effects, allergy, mutagenicity, nephropathy (from gentamicin), hepatotoxicity, reproductive problems, bone marrow toxicity (from chloramphenicol), and even carcinogenicity (sulphamethazine, oxytetracycline, and furazolidone). Bacanli and Başaran (2019). In contrast to the situation in Europe, the issue of antimicrobial residues in foods of animal origin has rarely been a substantial concern in developing countries. Even though the prevalence of veterinary drug residues in foods derived from animals is less than 1 percent in Europe, it can reach up to 94 percent in some African nations, with the two most frequent drugs, tetracycline and penicillin, at 89.9 percent and 97.3 percent, respectively.

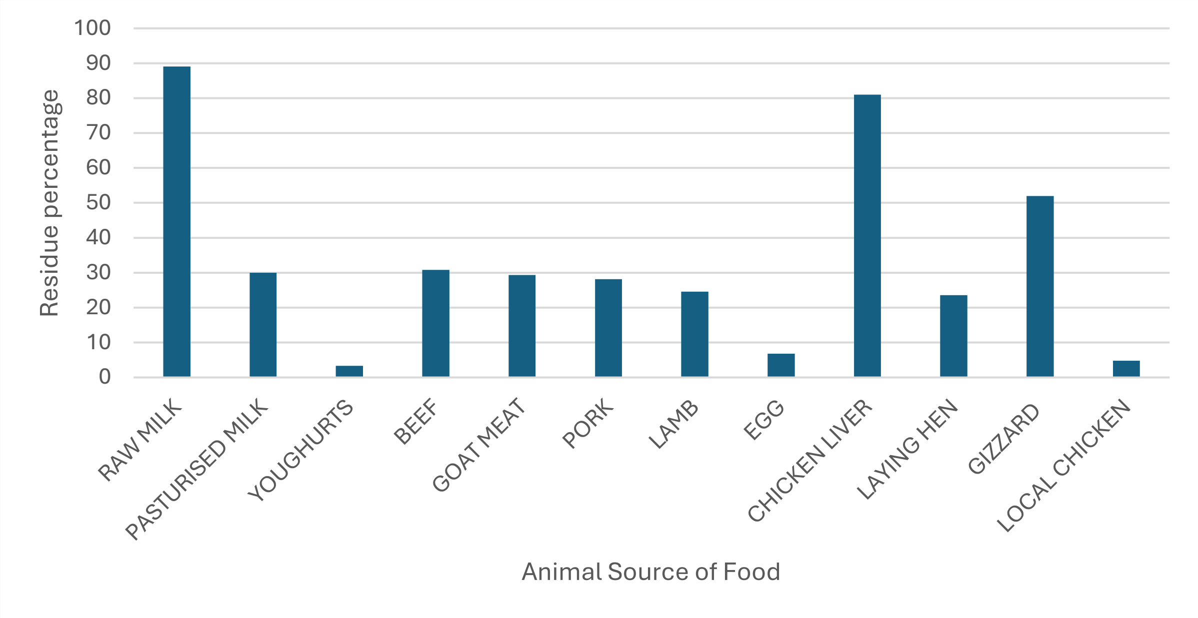

Figure 2: Antimicrobial residues in animal-source foods in Africa. Source: Sanders *et al.* (2014)

2.5 Antimicrobial Residue in the Environment

Pikkemaat and Yassin (2016) say that the main way that the use of antibiotics in veterinary medicine affects the environment is through drug residues in animal manure. Animals get rid of antibiotics in large amounts (17 percent to 90 percent for livestock), either in their original form or as active metabolites (epimers or isomers) of the parent species. While certain metabolites, like acetic conjugates of sulphonamides, can transform back into their parent compounds during manure storage, others, like some metabolites, are less powerful than their parent compounds (Massé *et al.,* 2014). Molecular markers have established that faces from livestock are the primary source of antibiotic residues in the environment, as humans routinely use considerably greater than recommended amounts. Many antibiotics and hormones given to animals are not absorbed or metabolized by the body and are instead discharged into the faces stream. As a result, wastewater systems play a crucial role in the cycle of antibiotic removal and distribution. Sewage sludge can be an important antibiotic reservoir since many antibiotics in wastewater are extracted and relocated to it throughout the sewage treatment process. Antibiotics may be released into the environment by sludge disposal practices such as agricultural application and landfilling, which may pose threats to animal and human health ecosystems (Robles *et al.,* 2022).

2.6 Antimicrobial resistance in livestock

Antimicrobial resistance is a global concern for both human and animal health (Menéndez *et al.,* 2010; Uyama *et al.,* 2022). Today, antimicrobial resistance has become more well-known in human medicine, and the understanding of the "One Health" approach among the stakeholders in the fight against antimicrobial resistance in livestock practices has begun to give the initiative a boost. The decrease in options to produce new antimicrobial drugs and the control of diseases has become a challenge, especially in Low- and Middle-Income Countries where infectious disease, poverty, and malnutrition are endemic (Omulo *et al.,* 2015). Due to the limited treatment options available, it is vital to manage food and animal producers in the antimicrobial resistance crisis, as they are one of the main stakeholders in the efforts to raise awareness of AMR in milk production and expand the use of appropriate antimicrobials. (Ozdikmenli 2023).

Increased AMR has a detrimental effect on the output of livestock, either by lowering farm productivity or by raising the expense of disease treatment. Growing amounts of evidence confirm the idea that AMU/AMR in animal production contributes to the overall burden of AMR in humans (Cuong *et al.,* 2018). An estimated 1.27 million deaths worldwide were attributed to antibiotic-resistant (AR) bacteria, and an additional 4.95 million deaths were linked to their presence. Unknown is the number of these that were connected to their presence. (Ozdikmenli 2023). Motivation for monitoring AMU/AMR in animal production has led to the implementation of several global, regional, and national efforts to encourage the responsible use of antibiotics and reduce the overuse of AMU in animal production. A supranational approach to tracking AMU in both people and animals across EU member states has been made possible by the European Union (EU). In comparison to the 3821 metric tonnes used for medical reasons, 8927 metric tonnes of antimicrobial active ingredients were used for animals across 28 EU member states, according to a 2014 surveillance report from the European Centre for Disease Control, the European Food Safety Agency, and the European Medicines Agency, 70 percent of all antimicrobials consumed in the USA in 2014 were sourced from the production of food animals (Cuong *et al.,* 2018).

The main driver behind the increasing rates of resistance is found to be the misuse of antimicrobials, both in human patients and livestock or released into the environment, which is no longer a medical issue but a global health threat that requires the collaboration of different shareholders to come together and fight AMR at the root cause (Roca *et al.,* 2015). Evidence has shown that human health can be at risk due to resistance originating from non-human use of antimicrobials such as zoonotic pathogens like *Salmonella spp*., *Campylobacter,* and *Enterococci* (Menéndez *et al.,* 2010), that is, zoonotic transmission of resistance bacteria to humans either through animal sources of food or physical contact with the animal (Uyama *et al.,* 2022).

2.7 Sensitivity levels of pathogens to antimicrobial drug

The most prevalent etiologic agents of mastitis, according to earlier investigations, were found to be 22 percent *Streptococcus*, 51.5 percent *Staphylococcus*, and 17.5 percent *Escherichia coli* with a high resistance level in Kenya, as represented in Table 1 (Ounah *et al.,* 2022). *Salmonella* was also found to be 4.2 percent (Kagira *et al.,* 2022). Antibiotic resistance patterns of *Staphylococcus aureus, Escherichia coli, Salmonella, Shigella, and Vibrio* isolated from different livestock species show that the prevalence of *Staphylococcus aureus, Escherichia coli, Salmonella, Shigella, and Vibrio* was 68 percent, 53 percent, 35 percent, 6 percent, and 6 percent, respectively. The resistance of *Salmonella* was most frequently observed to amoxicillin (100 percent), tetracycline (24 percent), chloramphenicol (11percent), and nalidixic acid (11percent). *S. aureus* was resistant to amoxicillin (100 percent), followed by tetracycline (63 percent), nalidixic acid (17 percent), and cefotaxime (13 percent), respectively. *Vibrio* isolates resisted amoxicillin (100 percent), tetracycline (40 percent), and chloramphenicol (20 percent). *Shigella* expressed the highest resistance to amoxicillin (100 percent), followed by chloramphenicol (80 percent), tetracycline (60 percent), and nalidixic acid (20 percent). There is dearth information about the sensitivity level of pathogens from raw milk in Nakuru County. However, a recent study shows that *Escherichia coli* isolated from red meat has a high prevalence resistance to co-trimoxazole, tetracycline, streptomycin, and ampicillin at 80 percent, 73 percent, 67 percent, and 67 percent, respectively (Ronald *et al.,* 2023).

Table 1: Resistance level of pathogens to antimicrobial drugs

Pathogens	Antimicrobial resistance level (%)				
	Ampicillin	Tetracycline	Penicillin	Sulfamethoxazole	Gentamycin
Staphylococcus	76.1	41.3	80.4	45.0	4.4
Streptococcus	80.0	55.0	90.0	80.0	1.0
Escherichia coli	100.0	90.9	63.6	81.0	9.1

Source: Ounah *et al.* (2022)

CHAPTER THREE

ANTIMICROBIALS USE BY SMALLHOLDER DAIRY FARMERS IN PERI-URBAN AREA OF NAKURU KENYA: KNOWLEDGE, ATTITUDES AND PRACTICES

Abstract

In dairy intensification, mastitis infections become prevalent and induce frequent Antimicrobial Use (AMU), sometimes inappropriately. This poses public health risks because of growing Antimicrobial Resistance (AMR), which calls for stewardship programs informed by Knowledge, Attitude and Practices (KAPs) about AMU and AMR to halt or reverse the worrying trend. Data was obtained in cross sectional survey conducted in four peri-urban wards around Nakuru city in Kenya. Randomly selected sample farmers (n=124) with free-grazing, semi-zero-grazing or zero-grazing dairy management, representing increasing dairy intensification levels provided data on KAPs. *Chi-square* test statistics was specified to establish associations between KAPs and dairy intensification levels. Among the sample farmers, six in ten (58.8 percent) had intensified dairy production, at least six in ten were marketing milk through informal outlets and were using antimicrobial drugs. Compliance with the withdrawal period was high and increased ($p<0.05$) with increasing intensification from free-grazing to zero-grazing. Within antimicrobial withdrawal period, at least seven in ten farmers did not sell milk, fewer than four in ten consumed their milk at home and fewer than three in ten fed the milk to calves. Though independent of dairy intensification level ($p>0.05$), using antimicrobials for mastitis treatment increased while sourcing of information on antimicrobial use from extension officers decreased with increasing dairy intensification. Farmers with some training on prudent antimicrobial use and with positive attitudes that milk from antimicrobial treated cows is unsafe, antimicrobial resistant pathogens and residues can be passed from milk to humans, mastitis can be treated without antimicrobial drugs, and that antimicrobial residues can end up accumulating in the soils increased ($p>0.05$) with increasing dairy intensification. These results show that intensification of dairy management motivates farmers to gain more knowledge, acquire positive attitudes and apply better practices towards responsible prudent use of antimicrobials in livestock. Therefore, strengthening stewardship with targeted training and sensitization can foster prudent and responsible antimicrobial use.

3.1 Introduction

Growing consumption demand for animal protein is driving intensification of livestock production systems in which antimicrobial use (AMU) is projected to increase by 67 percent between the year 2010 and 2030 (Gemeda *et al.,* 2020). In intensive dairy production, mastitis disease is prevalent and induces overdosing, underdosing or inappropriate use of veterinary antimicrobial drugs for treatment (Dankar *et al.,* 2023). Consequential to this is an occurrence of antimicrobial residue in food of animal origin and subsequent development of antimicrobial resistance (AMR), with impacts on food safety and public health (Sulis *et al.,* 2022). These present public health risks because of a high probability of future treatment failures in both animals and humans. This public health concern is increasing in countries where growth in consumption demand for animal protein is more rapid, yet consumption of antimicrobial veterinary drugs (AMD) is weakly regulated.

The growing public health concerns on AMU and AMR justify antimicrobial stewardship programs. Effective antimicrobial stewardship programs are evidence-informed with the Knowledge, Attitudes and Practices (KAPs) regarding AMU of the farmers. This is a critical step in developing antimicrobial stewardship programs (Hassan, 2022). The 2021–2025 action plan of the Food and Agriculture Organization (FAO) of the United Nations has proposed antimicrobial stewardship program actions. These include boosting stakeholder engagement and awareness, enhancing research and surveillance, encouraging good practices, and strengthening governance and sustainable resource allocation (FAO, 2021).

However, instance of inappropriate AMU frequently arises, which pose public health risks. This is a likely situation among the peri-urban smallholder dairy farmers in Kenya. They are intensifying their dairy management systems and so are more likely to engage in high AMU in treating mastitis infections, a prevalent intensification disease Geta and Kibret (2021). However, there is a dearth of information on KAPs regarding AMU and AMR among peri-urban smallholder dairy farmers, particularly in Kenya, a country with well-developed dairy industry in Africa (Hassan, 2020; Okello *et al.,* 2021).This knowledge gap is a barrier to evidence informed antimicrobial stewardship program actions that can reverse the worrying trends in antimicrobial resistance development (Hassan *et al.,* 2021).

The goal of responsible antimicrobial stewardship is to prevent the emergence and spread of antimicrobial resistance, maintain the effectiveness of veterinary drugs, and promote a One-Health concept (Sweeney *et al.,* 2024). Antimicrobial stewardship program actions are being implemented in industrial livestock systems because data is available from effective monitoring of AMU and AMR trends in livestock production (Gemeda *et al.,* 2020). In order to track the susceptibility of the principal mastitis pathogens to antimicrobial medications used to treat the disease in North America, mastitis pathogen antimicrobial susceptibility surveillance program was established in 2002 and its implementation has continued (Sweeney *et al.,* 2024). This is yet to be achieved in peri-urban smallholder dairy systems in Kenya because data on KAPs regarding AMU and AMR remain scarce. This knowledge gap hinders good understanding of the association between dairy intensification and KAPs about AMU and AMR. Therefore, the objective of this study was to assess Knowledge, Attitudes and Practices of farmers that relate to antimicrobial use in different dairy production systems representing increasing intensification levels (free-, semi-zero and zero-grazing) among smallholder farmers in the peri-urban areas of Nakuru city in Kenya.

3.2 Materials and Method

3.2.1Study Area

The study was conducted in peri-urban areas of Nakuru city, specifically smallholder farms in Njoro, Lare, Lanet and Kabatini Wards. The area is located within Longitudes 35.41 º East or 35 º 24’ 36” East and 36.6 ºEast or 36 º36’ 0” East and Latitude 0.23 º North or 0 º 13’ 48” North and 1.16 º South or 1º 9`36” South (Figure1). In the four Wards selected for the study, dairy production is predominantly mixed crop-dairy farming, with strong historical linkage to White Settler farming heritage (Bebe *et al.,* 2003). Dairy production is a major productive economic activity, with developed supportive infrastructure. The supportive infrastructure includes facilities and institutions for dairy education, training and research and veterinary investigation laboratories, and feed and milk processing plants. These facilities and institutions are either public, farmer or private operation ownerships (County Government of Nakuru Integrated Development Plan, 2018).

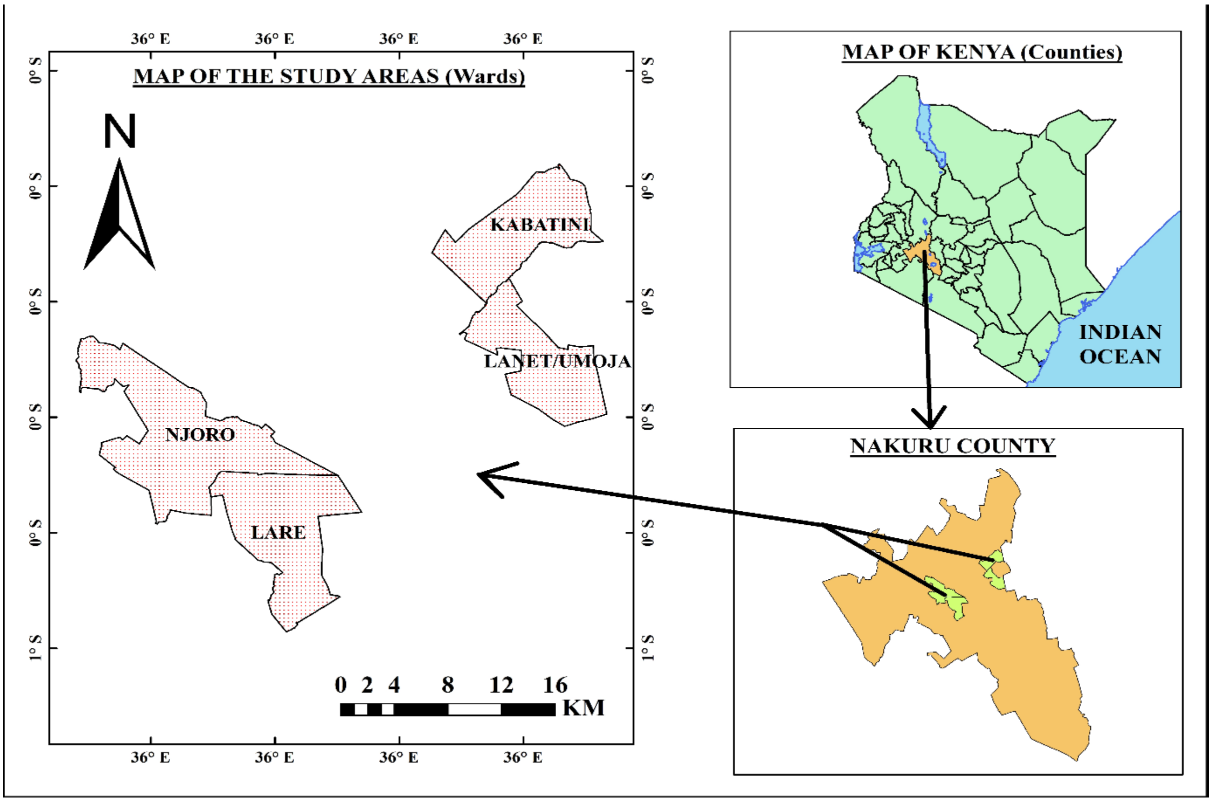

Figure 3: Map of the study area.

Source: Self QGIS

3.2.2 Data collection

A structured questionnaire was developed in Kobo tool kit with four sections (Appendix C). The first section captured demographical information, the second section captured farmers knowledge, the third section captured farmers attitudes, while the last section captured practices by farmers on antimicrobial use, antimicrobial drugs and antimicrobial resistance. The interest on farmers knowledge was to establish the purpose of using antimicrobials, whether antimicrobials are used for mastitis treatment and the recommended withdrawal periods are observed, and how farmers source information on antimicrobial residues and antimicrobial resistance. The interest on farmers' attitudes was assessed with nine questions for the degree of agreement or disagreement to reveal positive and negative attitudes that farmers have about antimicrobial use on animals and humans. The interest on practices that farmers deploy in using antimicrobials was to give insight into how farmers responsibly and prudently used the antimicrobials.

The questionnaire was pretested among 10 dairy farmers outside the four wards targeted for the study. Pre-testing was done to enhance the clarity and accuracy of the questions so that the intended information could be obtained. The adjusted questionnaire was administered to a random sample of 124 farmers who provided information on their KAPs regarding antimicrobial use, antimicrobial drugs and antimicrobial resistance.

3.2.3 Data analysis

Data in the Kobo tool kit was exported to Excel version 2016 for cleaning then processed for further descriptive and inferential statistics using SAS version 9.0 software. The descriptive and inferential statistics were generated by applying cross tabulation and *Chi-square* test statistics for association between KAPs and dairy intensification level. Rejection of the null hypothesis of independence between KAPs and dairy intensification levels was set to $p< 0.05$. In analysing the sampling distribution, Pearson's *Chi-square* was used when the expected frequencies in each cell were greater than five, otherwise Fisher's exact test probability was used when the expected frequencies were less than five (Field, 2009).

3.3 Results

3.3.1 Demographic characteristics of sample farmers

The demographic characteristics of the sample farmers (n=124) is presented in Table 2. The demographic statistics reveal that more than 70 percent of the sample farmers came from two neighbouring wards (Njoro and Lare). Among the farmers, male (56.5 percent; 70/124) dominated over female (43.5 percent; 54/124), and seven in ten (70.2 percent) had attained at least secondary level education. Observed frequencies show that six in ten (58.8 percent) of the farmers had intensified dairy production management by adopting semi-zero-grazing or zero-grazing dairy management. Though being in peri-urban area of the city is expected to present proximity advantage to formal milk market channels to these farmers, it was found that at least six in ten (63.7 percent) were marketing milk through informal market outlets.

Table 2: Demographic statistics of the sample farmers

Categories	Frequency	Percent
Wards		
Njoro	50	40.3
Lare	40	32.3
Lanet	17	13.7
Kabatini	17	13.7
Sex		
Male	70	56.5
Female	54	43.5
Education Level		
Adult Education	4	3.2
Primary	33	26.6
Secondary	59	47.6
Post-secondary	28	22.6
Production System		
Free Grazing	51	41.1
Semi Grazing	33	26.6
Zero Grazing	40	32.2
Milk market outlets		
Informal only	79	63.7
Both formal and informal	36	29.0
Formal only	9	7.3

3.3.2 Farmers' knowledge about antimicrobial use

The summary statistics of farmers' knowledge about antimicrobial use are presented in Table 3. Use of milk before the end of withdrawal period was associated with dairy intensification levels ($p<0.05$) as those selling milk decreased while those feeding milk to calves increased with increasing intensification levels. Before withdrawal period ended, farmers indicating that they were selling the milk decreased with increasing intensification from free-grazing (15.7 percent) to semi-zero-grazing (12.1 percent) and zero-grazing (0 percent) while those feeding milk to calves increased from free-grazing (9.8 percent) to semi-zero-grazing (21.2 percent) and to zero-grazing (25.0 percent). Overall, compliance with the recommended withdrawal period was high, with at least seven in ten farmers not selling such milk during the withdrawal period while less than four in ten consumed such milk at home.

Table 3: Association between farmers' antimicrobial use knowledge and dairy intensification levels

Question	Free grazing (n=51)	Semi grazing (n=33)	Zero grazing (n=40)	*Chi-square* test
Purpose of using antimicrobial (Percent)				*p=0.5110*[$]
Do not use	37.3	21.2	32.5	
Treatment	33.3	30.3	40.0	
Prevention	3.9	6.1	5.0	
Production	25.5	42.4	22.5	
Using antimicrobials for mastitis treatment (Percent)				*p*=0.1939
Do not use	43.1	39.4	25.0	
Use sometimes	37.3	27.3	35.0	
Use frequently	19.6	33.3	40.0	
Using milk from antimicrobial treated cows before end of withdrawal period (percent)				*p*= 0.0405[$]
Home consumption	3.9	0.0	2.5	
do not sale out	70.6	66.7	72.5	
Sell out	15.7	12.1	0.0	
Give to calves	9.8	21.2	25.0	
Sourcing information on antimicrobial residues in food and antimicrobial resistance (Percent)				*p*= 0.0655[$]
No	21.6	30.3	25.0	
Extension/ veterinary officers	68.6	66.7	47.5	
Fellow farmers, relatives	2.0	0.0	5	
Field days	0.0	3.0	7.5	
Media (radio, newspapers, TV)	7.8	0.0	15	

[$]*p* value from Fisher's exact test

Regardless of dairy intensification levels, up to four in ten (21.1 to 37.3 percent) farmers did not use antimicrobials. Though reasons for using antimicrobials were independent of ($p>0.05$) of dairy intensification levels, use of antimicrobials for treatment (33.3 to 40.0 percent) and for treating mastitis (19.6 to 40.0 percent) had a pattern of increasing with increasing intensification levels from free to zero-grazing. When frequently using antimicrobials, the reason was treatment (30 to 40 percent) or production (22 to 42 percent) and not prevention of disease (4 to 6 percent). Among the sample farmers, sourcing of information about antimicrobial use was independent ($p>0.05$) of their dairy intensification levels. However, those sourcing information from the extension and veterinary officers had a pattern of decreasing with increasing intensification levels from free- and semi-zero grazing (66.7 -68.6 percent) to zero-grazing (47.5 percent).

3.3.3 Farmers' attitudes towards antimicrobial use in dairy farming

The study identified the specific recommendations for antimicrobial use in animals and humans that farmers have positive and negative attitudes towards. Agreement with statement on a specific recommendation for antimicrobial use indicated positive attitude. The observed frequency statistics for which the association with dairy intensification levels was significant ($p<0.05$) are presented in Table 4.

Overall, farmers with positive attitude towards AMU recommendations increased with increasing dairy intensification levels. Farmers with the attitude that milk from antimicrobial treated cows is unsafe to human health increased from those practicing free-grazing (56.9 percent) through semi-zero-grazing (78.8 percent) to zero-grazing (82.5 percent). Also, farmers with the positive attitude that antimicrobial resistant pathogens and residue from milk can be passed to humans through the food chain increased from those practicing free-grazing (60.8 percent) through semi-zero-grazing (69.7 percent) to zero-grazing (70.0 percent). Further, it was found that more of farmers practicing zero-grazing (70.0 percent) than those practicing free-grazing (62.8 percent) had the attitude that mastitis can be treated without antimicrobial drugs. Similarly, more of farmers practicing zero-grazing (70.0 percent) than those practicing free-grazing (66.7 percent) had the attitude that antimicrobial residues can end up accumulating in the soils.

Table 4: Significant associations between farmers' attitudes towards antimicrobial use and dairy intensification levels

Production systems	Degree of agreement or disagreement (Percent)			*Chi-square* test
	Agree	Neutral	Disagree	
Mastitis can be treated without using antimicrobial drugs				*P*=0.0010
Free-grazing (n=51)	62.8	23.5	13.7	
Semi-grazing (n=33)	24.2	39.4	36.4	
Zero-grazing (n=40)	70.0	12.5	17.5	
Milk from antimicrobial treated cows is harmful to human health				P=0.0283$
Free-grazing (n=51)	56.9	35.3	7.8	
Semi-grazing (n=33)	78.8	21.2	0.0	
Zero-grazing (n=40)	82.5	17.5	0.0	
Antimicrobial residues can end up accumulating in the soils				P=0.0004
Free-grazing (n=51)	66.7	25.5	7.8	
Semi-grazing (n=33)	51.5	12.1	36.4	
Zero-grazing (n=40)	70.0	36.4	2.5	
Antimicrobial resistant pathogens and residue from milk can be passed to humans through the food chain				*P*=0.0089$
Free-grazing (n=51)	60.8	37.3	2.0	
Semi-grazing (n=33)	69.7	12.1	18.2	
Zero-grazing (n=40)	70.0	27.5	2.5	

$*p value from Fisher's exact test*

In Table 5 are the observed frequency statistics for farmer attitudes towards antimicrobial use recommendations which showed no association with dairy intensification levels (p>0.05). Farmer attitudes that were independent of their dairy intensification levels were whether any antimicrobial drug can be used to treat a lactating cow, withdrawal period should be observed to avoid antimicrobial drug residues in milk, relationship exists between antimicrobial use and antimicrobial resistance, and whether antimicrobial drug residues and drug resistance occurs when AMU is not prudent. Though independent of the dairy intensification levels, farmers with the positive attitude that sale and distribution of antimicrobial drugs be restricted to licensed persons had a pattern of increasing with increasing intensification levels. The proportion of farmers increased from free-grazing (58.8 percent) through semi-zero-grazing (63.6 percent) to zero-grazing (67.5 percent).

Table 5: Insignificant associations between farmers' attitudes towards antimicrobial use and dairy intensification levels

Production systems	Degree of agreement or disagreement (Percent)			*Chi-square* test
	Agree	Neutral	Disagree	
Any antimicrobial drug can be used to treat a lactating cow				*P=0.3076*[$]
Free-grazing (n=51)	70.6	25.5	3.9	
Semi-grazing (n=33)	54.6	30.3	15.2	
Zero-grazing (n=40)	72.5	20.0	7.5	
Withdrawal periods should be observed to avoid antimicrobial drug residues in milk				*P=0.8945*[$]
Free-grazing (n=51)	70.6	21.6	7.8	
Semi-grazing (n=33)	69.7	21.1	9.1	
Zero-grazing (n=40)	62.5	30.0	7.5	
Relationship exists between antimicrobial use and antimicrobial resistance				*P=0.0524*
Free-grazing (n=51)	66.7	27.5	5.9	
Semi-grazing (n=33)	39.4	45.6	15.2	
Zero-grazing (n=40)	67.5	30.0	2.5	
Sale and distribution of antimicrobial drugs be restricted to licensed persons				*P=0.6918*[$]
Free-grazing (n=51)	58.8	33.3	7.8	
Semi-grazing (n=33)	63.6	27.3	9.1	
Zero-grazing (n=40)	67.5	20.0	12.5	
Antimicrobial drug residues and drug resistance occurs when not prudently used				*P=0.7747*[$]
Free-grazing (n=51)	72.6	19.6	7.8	
Semi-grazing (n=33)	60.6	24.2	15.2	
Zero-grazing (n=40)	65.0	22.5	12.5	

[$]p value from Fisher's exact test

3.3.4 Farmer practices in administration and prescription of antimicrobial drugs

Table 6a and b presents the observed association between farmers' practices (in the administration and prescription of antimicrobial drugs) and dairy intensification levels. Regardless of their dairy intensification levels, at least seven in ten farmers had professional prescriptions by veterinarians or pharmacies, observed withdrawal period and had been trained

Table 6a: Association between farmers' practices (in the administration and prescription of antimicrobial drugs) and dairy intensification levels

Question	Free grazing (n=51)	Semi grazing (n=33)	Zero grazing (n=40)	*Chi-square* test
From where do you usually buy the antimicrobial drugs? (Percent)				*P= 0.1230*$^{\$}$
Extension/veterinary officer	80.4	81.8	85.0	
Pharmacy	5.9	18.1	12.5	
Fellow farmers	13.7	0.0	2.5	
Who often prescribes antimicrobial drugs for you? (Percent)				*P= 0.0124*$^{\$}$
Extension/veterinary officer	63.6	97.0	85.0	
Pharmacy	5.8	0.0	5.0	
Self	25.5	3.0	10.0	
Who administers antimicrobial drugs to your animals? (Percent)				*P = 0.0040*$^{\$}$
Extension/veterinary officer	66.7	97.0	67.5	
Fellow farmers	11.8	0.0	5.0	
Self	21.6	3.0	27.5	
How often do you call a veterinarian whenever an animal is sick? (Percent)				*P= 0.0524*$^{\$}$
Frequently	27.5	42.4	45.0	
Sometimes	51.0	48.5	52.5	
Do not	21.6	9.1	2.5	
What is the common disease condition(s) of lactating cows for which you administer antimicrobial drugs? (Percent)				*P= 0.4051*$^{\$}$
Mastitis	11.8	24.2	35.0	
Respiratory diseases	17.7	12.1	10.0	
Diarrhoea	27.5	21.2	20.0	
Udder injuries	7.8	12.1	10.0	
Others	35.3	30.3	25.0	

$p value from Fisher's exact test

Table 7b: Association between farmers' practices (in the administration and prescription of antimicrobial drugs) and dairy intensification levels

Question	Free grazing (n=51)	Semi grazing (n=33)	Zero grazing (n=40)	*Chi-square* test
Do you observe the withdrawal period after treating the animals with antimicrobials (Percent)				*P= 0.0316$*
Yes	86.3	78.8	97.5	
No	13.7	21.2	2.5	
Do you give subsequent doses after the administration of the first dose of the treatment. (Percent)				*P= 0.4861*
Yes	72.6	72.7	82.5	
No	27.5	27.3	17.5	
Do you stop giving treatment when an animal recovers? (Percent)				*P= 0.5867*
Yes	72.6	66.7	77.5	
No	27.5	33.3	22.5	
Have you had training on antimicrobial usage, AMR, and residue. (Percent)				*p= 0.0221*
Yes	68.6	60.6	87.5	
No	31.4	39.4	12.5	
Do you check for the expiry date before AMU (Percent)				*P= 0.0737*
Yes	82.4	66.7	87.5	
No	17.7	33.3	12.5	
Do you use human drugs on animals. (Percent)				*P= 0.5034*
Yes	66.7	54.6	65.0	
No	33.3	45.5	35.0	

$p value from Fisher's exact test

in antimicrobial use including residual effects and development of antimicrobial resistance. Farmers who self-prescribed and administered antimicrobial drugs declined ($p<0.05$) with increasing intensification of dairy management from free- to zero-grazing. Farmers who had most intensified their dairy management (zero-grazing) were the majority with some training on prudent antimicrobial use (87.5 percent) and in observing the withdrawal period (97.5 percent). There were several of farmers' practices in administration and prescription of antimicrobial drugs that were independent ($p>0.05$) of their dairy intensification levels. These included where farmers were buying the antimicrobial drugs, how often they called a veterinarian whenever an animal was sick, and common disease condition(s) of lactating cows for which they administered antimicrobial drugs. Other practices were administering a follow-up dose, stopping treatment when an animal recovers, checking for the expiry date before use, and using human drugs on animals. Though was independent of dairy intensification levels, the use of human drugs on animals was alarmingly prevalent (over 60.0 percent).

3.4 Discussion

The distribution of farmers with free-, semi-zero and zero-grazing dairy management observed in this study support that intensification of dairy management is increasing in the peri-urban areas of Nakuru city. Though more than half of the sample farmers (58.8 percent) had intensified their dairy management, a larger majority marketed milk in the informal market outlets. Because follow up is difficult, participation in the informal milk market outlets present a weak link in implementing antimicrobial stewardship programs, boosting stakeholder engagement and awareness, enhancing research and surveillance, encouraging good practices, and strengthening governance and sustainable resource allocation (FAO, 2021).

Among the farmers in this study, seven in ten had attained at least secondary education. A higher level of education among dairy farmers and farm workers can play a significant role in promoting AMU stewardship practices. Better-educated individuals are more likely to understand the importance of prudent antimicrobial use, follow recommended guidelines, implement biosecurity measures, and adopt best management practices. Education can also enhance their ability to interpret diagnostic test results, maintain accurate treatment records, and make informed decisions about antimicrobial therapy. Consequently, increased education levels can encourage more responsible and sustainable AMU stewardship actions as dairy farming intensifies. This would support antimicrobial stewardships program as training of farmers can then enhance the practice of disease diagnosis to inform treatment. However, achieving impactful change necessitates a comprehensive approach (Portillo-Gonzalez *et al.*, 2024).

Regardless of the level of dairy intensification management, the study revealed that at least seven out of ten farmers were utilizing antimicrobial drugs, with up to eight out of ten employing these drugs specifically for the treatment of mastitis. This finding is not surprising, as mastitis is a highly prevalent infection in intensified smallholder dairy management (Abdi *et al.*, 2021). The observed therapeutic use of antimicrobials is consistent with the observations of many researchers. Farrell *et al.* (2021), Gemeda *et al.* (2020), Geta and Kibret (2021) and Hassan (2022) have all reported that antimicrobials are predominantly used for therapeutic purposes in livestock production systems. However, other researchers have reported contrasting observations, suggesting that the primary use of antimicrobials is for disease prevention rather than treatment. Kisoo *et al.* (2023), Mogotu *et al.* (2022), Nyokabi *et al.* (2021) and Omwenga *et al.* (2021), are among the researchers who have highlighted the preventive use of antimicrobials as a common practice in various livestock production management. The contrasting on purpose of use may be attributed to regional differences, variations in production systems. It is crucial to investigate the underlying factors contributing to the contrasting observations and tailor interventions accordingly to promote judicious antimicrobial use practices. The high prevalence of mastitis and the associated therapeutic use of antimicrobials observed in the present study underscore the need for effective disease management strategies and alternative approaches to minimize the reliance on antimicrobial treatments. Improved hygiene practices, vaccination programs, and the adoption of preventive measures could contribute to reducing the incidence of mastitis and, consequently, the need for antimicrobial therapy. Ongoing research, education, and collaboration among stakeholders, including farmers, veterinarians, and policymakers, are essential to address the challenges of antimicrobial resistance and promote sustainable livestock production practices.

The present study revealed a high level of compliance with the withdrawal period among farmers, which refers to the mandated time after administering antimicrobials to dairy animals before milk can be introduced into the food supply chain. Notably, this compliance increased significantly ($p<0.05$) as the level of dairy intensification increased. Specifically, during the antimicrobial withdrawal period, at least seven out of ten farmers refrained from selling the milk, fewer than four out of ten consumed the milk at home, and fewer than three out of ten fed the milk to calves. These findings align with the recommendations of Uyama *et* *al.*, (2022), who emphasized the importance of adhering to withdrawal periods to prevent antimicrobial residues from entering the food supply chain and safeguard public health. Their study highlighted the potential risks associated with the consumption of milk containing antimicrobial residues, including the development of antimicrobial resistance and adverse health effects. Similarly, Mogotu *et al.* (2022) reported a positive correlation between farmer knowledge of withdrawal periods and compliance with these guidelines in their study conducted in Cameroon. They stressed the need for continuous education and awareness campaigns to promote responsible antimicrobial stewardship practices among dairy farmers. The observed trend of increased compliance with withdrawal periods as dairy operations intensified could be attributed to factors such as improved access to veterinary services, better record-keeping, and enhanced awareness of food safety and public health concerns, as suggested by Kashongwe *et al.* (2020) in their study of antimicrobial use practices in intensive dairy farming systems.

However, it is of concern that a significant proportion of farmers still engaged in practices such as consuming or feeding milk to calves during the withdrawal period. These practices can contribute to the spread of antimicrobial resistance and pose potential health risks, as highlighted by Uyama *et al.* (2022) and Mogotu *et al.* (2022). Continued efforts are needed to address these practices through targeted education and extension programs, as recommended by organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO). These initiatives should emphasize the importance of strict adherence to withdrawal periods and promote alternative management strategies to minimize the need for antimicrobial treatment, ensuring food safety and public health throughout the dairy value chain (FAO 2021; WHO 2021).

Though was found to be independent of the level of dairy intensification ($p>0.05$), the use of antimicrobials for treating mastitis showed an increasing pattern, while the practice of sourcing information on antimicrobial use from extension and veterinary officers decreased as dairy operations became more intensive. The observed trend in the present study raises concerns about the potential for increased antimicrobial use and the risk of antimicrobial resistance as dairy operations intensify, especially if farmers rely less on professional advice and guidance from veterinary and extension services. It is crucial to address this issue by strengthening the collaboration between farmers, veterinarians, and extension services, in line with the recommendations of the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) in their guidelines for prudent and responsible use of antimicrobials in agriculture (OIE 2020; FAO 2021). Ongoing education and awareness campaigns, coupled with accessible and affordable veterinary services, can play a vital role in promoting sustainable antimicrobial use practices, even as dairy production systems get more and more intensive.

The present finding just observed contrasts with previous research that has emphasized the importance of veterinary guidance and extension services in promoting judicious antimicrobial use, particularly in intensive livestock production systems. For instance, Redding *et al.* (2014) highlighted the positive impact of veterinary-client relationships and education programs on reducing unnecessary antimicrobial use among dairy farmers in the United States. Similarly, a study by Higham *et al.* (2020) found that farmers who received training and support from extension services were more likely to adopt best practices for responsible antimicrobial stewardship, such as improved record-keeping and adherence to treatment protocols.

The present study found that farmers who received training on prudent antimicrobial use and held positive attitudes regarding the risks associated with antimicrobial overuse were more likely to adopt responsible practices as dairy production intensified. Specifically, those who believed that milk from antimicrobial-treated cows is unsafe for consumption, that antimicrobial-resistant pathogens and drug residues can transmit from milk to humans, that mastitis can be managed without antibiotics, and that antimicrobial residues accumulate in soil demonstrated a tendency to increased ($p>0.05$) toward judicious use as their dairy operations became more intensive. These findings align with previous research highlighting the importance of farmer education and awareness. Higham *et al.* (2020) emphasized that understanding antimicrobial resistance risks and residue entry into the food chain positively shapes attitudes toward antimicrobial stewardship. Similarly, Redding *et al.* (2014) found U.S. dairy farmers receiving prudent use training were more likely to implement best practices like selective dry cow therapy to reduce unnecessary antimicrobial administration. Moreover, Saini *et al.* (2012) observed farmers recognizing antimicrobial residue risks in milk and the environment were more receptive to alternatives such as improved hygiene and preventive measures to minimize treatment needs. These findings underscore the pivotal role of education in promoting responsible antimicrobial stewardship, especially as dairy production intensifies.

In the present study, it was observed that the majority of farmers obtained antimicrobial drugs through veterinarians, who not only prescribed but also administered the drugs themselves. This practice is considered beneficial and should be encouraged, promoted, and strengthened to mitigate concerns related to antimicrobial resistance (AMR) and contribute to the sustainable use of antimicrobials (Dankar *et al.,* 2022). This approach aligns with the Global Action Plan on AMR, which outlines strategies for combating the emergence and spread of AMR. The implementation of such strategies can check the threats posed by the emergence and spread of AMR to multiple Sustainable Development Goals, including those related to health, food security, environmental well-being, and socioeconomic goals (WHO 2019 and FAO, 2021).

However, our findings deviate from those reported by Gemeda *et al*. (2020) in their study conducted in Ethiopia. The researchers observed that antimicrobial drugs were primarily accessed from private suppliers in the context of their study. It is important to note that their study sample came from pastoral production system, which differs from the smallholder peri-urban dairy systems examined in the present research. The contrasting findings could be attributed to the differences in accessibility to veterinary services, which is often limited in pastoralist production settings. In the present study, a predominant reliance on veterinarians and extension worker for antimicrobial drug procurement was observed. This is expected since smallholder peri-urban dairy can more easily access veterinary services and guidance, unlike pastoral cattle systems where veterinary services would be less available. Under such pastoral conditions, public and NGO led veterinary service delivery would be expected to prevail and could influence the sources from which antimicrobial drugs are obtained.

The observed practice of farmers obtaining antimicrobial drugs through veterinarians, who also prescribed and administered the drugs, can be attributed to the education and guidance received from veterinary extension officers on prudent antimicrobial use and the relationship between antimicrobial use (AMU) and antimicrobial resistance (AMR). This educational intervention by veterinary officials likely played a crucial role in shaping the responsible antimicrobial procurement and administration practices among the farmers in this study. However, this finding stands in contrast to a study conducted in Ethiopia by Geta and Kibret (2021), where farmers reportedly administered antimicrobial drugs to sick animals on their own before seeking veterinary assistance. Furthermore, the same authors stated that farmers claimed they would continue to use antibiotics on animals even if they were aware of the potential negative impact on public health Geta and Kibret, (2021). This unfortunate practice can be attributed to inadequate delivery of veterinary services together with lack of education on prudent use of antimicrobials and the relationship between AMU and AMR by the veterinary officials from the private sector.

These results of this study show that farmers knowledgeable and with positive attitudes and good practices on AMU and AMR increased with increasing intensification of dairy management. The implication is that intensification of dairy management motivates farmers to gain more knowledge, have positive attitudes and good practices towards prudent use of antimicrobials in livestock. These needs strengthening with targeted training and sensitization to promote prudent and responsible antimicrobial use.

CHAPTER FOUR

ANTIMICROBIAL USE AND SENSITIVITY OF MASTITIS PATHOGENS AMONG SMALLHOLDER DAIRY COWS IN PERI-URBAN AREAS OF NAKURU CITY, KENYA

Abstract

Antimicrobial use (AMU) in lactating dairy cows for treatment or prevention of mastitis if not responsible and prudent can advance into antimicrobial resistance (AMR). This study is determining use frequency and dosage of antimicrobial drugs and antimicrobial sensitivity of common mastitis pathogens. The study was cross-sectional survey of smallholder dairy farms around peri-urban area of Nakuru city, Kenya. A sample of 210 lactating cows testing positive for mastitis were examined for antimicrobial drug use within past 12 months. Their milk samples were collected and processed for bacteriological culturing and Antimicrobial Susceptibility Testing (AST). The differences in means of drug use frequency and the Defined Daily Dose (DDD) were tested with general linear models. Prevalence of isolated mastitis pathogens and their sensitivity to different antimicrobial drugs were evaluated with *Chi-square* test statistics. Of the cows sampled, more than half (119/210; 56.7 percent) had been treated within past 12 months, mostly for mastitis (47.1) than ($p<0.05$) for East Coast Fever (14.3), parasitic infections (11.8) and other diseases (26.8) percent. The average drug use frequency was 2.2 per year with an average dose of 20mg/kg and 8.4 DDD mg/cow/year. The disease incidences decreased with increasing intensification from free-grazing (42.9) to semi-grazing (29.4) and to zero-grazing (25.8) percent. Though seven in ten (66.4 percent) farmers did not know the specific drugs used, some drugs were identified: gentamycin (13.4), tetracycline (11.8), penicillin G (5.0), and sulphamethoxazole (3.4) percent. The DDD varied with disease condition ($p<0.05$) and decreased with increasing intensification from free- grazing (10.9) to semi-grazing and zero-grazing (6.5 - 6.6) mg/cow/year. Of the pathogens isolated from milk samples (n=191), *Staphylococcus aureus* (100 percent) predominated over ($p<0.05$) Clostridium (16.2*), Escherichia coli* (14.7), *Corynebacterium* (14.0) and *Enterococcus species* (4.4) percent, while *Streptococcus agalactia* and *Salmonella typhi* were negative. Both *Staphylococcus aureus* and *Escherichia coli* exhibited a significant level ($p<0.05$) of resistance (97 to 100 percent) against Ampicillin and Penicillin G. In conclusion Milk from peri-urban dairy poses a risk for multidrug-resistant bacteria due to inappropriate antimicrobial use. Urgent antimicrobial stewardship and surveillance are needed in peri-urban smallholder dairy herds to prevent antimicrobial resistance.

4.1 Introduction

Use of antimicrobial drugs to treat bacterial diseases in livestock offers some benefits including reduced morbidity and mortality, improved production efficiency, and improved animal welfare (de Campos *et al.,* 2021). However, unregulated use leads to drug misuse and antimicrobial resistance. An estimated 11.25 million kilograms of antimicrobial drugs are used non-therapeutically, contributing to the problem of antimicrobial resistance development (Geta & Kibret, 2021). This can be associated with weak stewardship actions and low awareness among farmers, especially in the Low-Income Countries.

Antibiotic resistance has become a global health concern as it is attributed to the death of about 0.7 million people every year and is expected to rise to 10 million per year by 2050 (Shankar, 2016). Antibiotic resistance inhibits the effective treatment of infections by allowing microorganisms to evade killing. Antibiotic resistance has consequently been observed as one of the greatest threats to medicine (Bantawa *et al.*, 2019). Antimicrobials should be used prudently especially those classified as critically important antimicrobials by public health and animal health authorities, practicing physicians and veterinarians, and other interested stakeholders (One Health) involved in managing antimicrobial resistance antimicrobials (WHO 2019). Many of the commonly used antibiotics in dairy production are considered critically important by the World Health Organization (WHO 2018). Understanding how antimicrobials are used on farms and how that use may have evolved requires knowledge of antimicrobial use data. Additionally, these statistics are required to encourage the prudent use of antibiotics and to comprehend the connection between antibiotic use and antibiotic resistance (Redding *et al.*, 2019).

Antimicrobials may be used in livestock production for therapeutic purposes, prophylactic measures, or for growth promotion (Geta & Kibret, 2021). In dairy cattle, antimicrobials are administered for a variety of therapeutic purposes (e.g., mastitis, metritis, respiratory diseases, foodborne illnesses) and prophylactic purposes (e.g., dry cow therapy, medicated milk replacers for calves), as observed by Redding *et al.* (2019). The most prevalent use of antimicrobials in dairy cows is for the treatment and prevention of mastitis (Li *et al.*, 2023). However, there exists a significant disparity in the utilization of antibiotics across various production systems, for instance, between conventional and organic dairy herds. In conventional dairy production, larger herds appear to be associated with higher levels of antibiotic use, though this correlation is not observed in organic dairy production (Krogh *et al.*, 2020) and is unknown in peri-urban smallholder dairy herds.

The most important food-borne bacteria transmitted through consumption of animal source food include *Salmonella, Shigella, Staphylococcus aureus, Escherichia coli, Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica and Aeromonas hydrophila* (Bantawa *et al.*, 2019). These pathogens are also associated with mastitis infections (Mandefero and Yeshibelay 2018). However, research on their sensitivity to the different antimicrobials in use is scanty. An adequate understanding of the challenges surrounding AMR in the current context is impossible to achieve in the absence of documented empirical evidences relating veterinary antimicrobial medicines, systematic reporting of treatment failures, and AMR surveillance (Kemp *et al.*, 2021).

Though Kenya possesses of a relatively well-developed dairy industry in the Sub-Saharan Africa (Kashongwe *et al.*, 2017), research relating mastitis infections with the prevalence of AMR and AMU is scanty. Surveillance of AMR that is carried out has not been in a systematic manner. This has created a dearth of information, even though other research has revealed a high frequency of AMR in humans and livestock in other Low Middle Income Countries (LMICs) Pearson and Chandler (2019). There are reports of food-borne pathogens being significant cause of illness and death Lee and Yoon (2021). In dairy cows, mastitis pathogens can result in significant financial losses due to treatment costs, veterinary care, microbiological testing, extra management investments, culling and replacing diseased animals (Kashongwe *et al.*, 2017).This study targeted lactating cows testing positive for mastitis in peri-urban smallholder dairy farms for two objectives. One, to quantify the use frequency and dosage of antimicrobial drugs. Two, to determine antimicrobial sensitivity levels of *Staphylococcus aureus, Streptococcus agalactiae, Salmonella typhi and Escherichia. coli* to tetracycline Sulfamethoxazole, gentamycin and penicillin.

4.2 Materials and Methods

4.2.1 Study Area

The study was conducted in smallholder dairy farms in Nakuru peri-urban area. Four wards were targeted for the study. Informed by the recent census statistics (KNBS 2020), the four wards have total dairy cattle population of 7,302, but unevenly distributed; Njoro 4,524 (62 percent); Lare 1,405 (19 percent); Kabatini 720 (10 percent) and Lanet 652 (9 percent).

The detailed description of the study area is presented in chapter three section 3.3.1. Therefore, only a brief description is presented in this chapter from findings in chapter three. In this peri-urban area, more than half of the dairy farms have intensified production management, either adopting semi-zero-grazing or zero-grazing management. Milk is predominantly marketed in the informal market outlets. Use of antimicrobials for treatment and prevention of mastitis show a pattern of increasing with increasing intensification levels from free to zero-grazing. Farmers indicate that they practice high compliance with the antimicrobial withdrawal period. During the antibiotic withdrawal period, at least seven in ten farmers indicate that they do not sell milk, fewer than four in ten consume such milk at home and fewer than three in ten feed such milk to calves.

4.2.2 Sampling process

A sample of lactating cows testing positive for mastitis was targeted. The sample size was determined using the formula of Thrufield (2005):

$$n = \frac{1.96^2 * P(1 - P)}{d^2}$$

Where n= required sample size, P = mastitis prevalence, set to 83.6 percent (Mogotu *et al.*, 2022), and d = desired absolute precision, set to 0.05. The computation returned that the minimum needed sample is 211 lactating cows.

4.2.3 Data collection

A cross-sectional survey of smallholder dairy farms was carried out. The sample farms were randomly selected from a list obtained from the local livestock and veterinary extension offices. On every farm visited, random sampling of lactating cows was not feasible because the herds have a mode of one cow kept (Kagira *et al.*, 2022). So, all lactating cows present were first subjected to mastitis positive test, and those testing positive further subjected to historical examination for drug usage, use frequency, dosage, disease condition in the last 12 months.

Milk samples were collected hygienically from lactating cows testing positive for mastitis. Hygiene process involved cleaning hands and udder with 70 percent ethanol and a wiping paper towel when obtaining milk sample into sample bottles before transferring to a cool box containing ice packs (Byaruhanga *et al.*, 2022). The samples were transported to the laboratory and stored at -20 degree census before it was analysed.

Media Preparation

Various growth media for culturing bacterial samples were prepared followed with careful measuring out precise amounts of dehydrated powder for Blood Agar, MacConkey Agar, Mannitol Salt Agar, Nutrient Agar, Eosin Methylene Blue Agar, and Muller Hinton Agar into separate flasks. To each flask, a 1000mL of distilled water was added and swirled gently to dissolve the powders. The media solutions were then covered and autoclaved at 121°C for 15 minutes. The media were allowed to cool down to 50°C then carefully pouring the steaming agar into separate sterile Petri dishes, allowing it to solidify into a nutrient-rich gel. However, for the Xylose Lysine Deoxycholate Agar, a different process was used. This media was heated on a hot plate, stirred frequently until it reached a boiling point, then transferred to a water bath maintained at temperature of 50°C while being dispensed into Petri dishes. The agar plates prepared were then ready for use in isolating and identifying various bacterial species based on their growth patterns and reactions to the selective media.

Enumeration of Escherichia coli

Milk samples were evenly spread on MacConkey agar plates using sterile cotton swabs. These specialized plates were designed to allow *Escherichia. coli* colonies to grow in distinctive colours based on their ability to ferment lactose. The inoculated plates were then incubated at 37°C for 24 hours to encourage bacterial growth. Following incubation, the plates were examined for pink colonies which could indicate the presence of the *Escherichia coli*. Any suspect pink colonies were carefully transferred to tubes containing a nutrient-rich peptone water broth and incubated again to increase the bacterial *(Escherichia coli*) population. After this enrichment step, a few drops of Kovac's indole reagent were added to each tube. A bright red ring forming at the top of the broth confirmed the production of indole by the bacteria, providing evidence that the pink colonies were indeed *Escherichia coli*. For those samples testing positive for *Escherichia coli*, a portion was streaked onto another differential agar: Eosin Methylene Blue (EMB). Colonies of Escherichia coli develop a striking green metallic sheen when growing on this medium, allowing confirmation of their identity. Finally, pure isolated colonies were transferred to a general nutrient agar to provide a pipeline of cultures for subsequent antimicrobial susceptibility testing.

Enumeration of Staphylococcus aureus and Streptococcus agalactia

Even spreading the milk samples onto blood agar plates using sterile cotton swabs was done. The inoculated plates were then incubated at 37°C for 24 hours to allow the growth of bacterial colonies (*staphylococcus aureus and streptococcus agalactia)* to grow. After incubation, plates were carefully observed, looking for distinctive colony morphologies that could indicate the presence of *Staphylococcus aureus and Streptococcus agalactia*. Grayish-white colonies without a pinpoint appearance were considered potential *Staphylococcus aureus*, while grayish-white pinpoint colonies could represent *Streptococcus agalactiae*. Any suspected *Staphylococcus. aureus or Streptococcus. agalactiae* colonies were further sub-cultured onto Mannitol Salt Agar (MSA), a selective medium. On MSA, *Staphylococcus. aureus* colonies appeared yellow, while *Streptococcus. agalactiae* formed pink colonies, providing further confirmation of their identities. Before proceeding, biochemical tests were performed on the suspected colonies to validate their identities. This involved Gram staining to determine if the bacteria were Gram-positive cocci, as well as a catalase test to detect the enzyme catalase produced by *Staphylococci aureus*. Once confirmed, pure isolated colonies of the *Staphylococcus. aureus and Streptococcus. agalactiae* strains were transferred and streaked onto nutrient agar plates. These provided refreshed cultures for subsequent antimicrobial susceptibility testing against various antibiotics.

Enumeration of Salmonella typhi

Milk sample was spread evenly across the surface of pre-poured XLD (xylose lysine deoxycholate) agar plates using sterile cotton swabs to investigate for the presence of pathogenic bacterium *Salmonella typhi* in the milk samples. The XLD is a selective and differential medium specifically designed to allow *Salmonella species* to produce distinctive colony morphologies. After ensuring an even distribution of the milk samples on the agar surfaces, the inoculated plates were placed into an incubator set to 37°C, for 24 hours to allow any *Salmonella* present sufficient time to multiply and form visible colonies. Following incubation, plates were meticulously examined to identify colonies exhibiting the characteristic appearance of *Salmonella typhi* on XLD agar - red colonies with black centres. This distinctive bull's eye morphology results from the ability of *Salmonella typhi* to ferment xylose and decarboxylate lysine. Any suspects fitting this description were marked and presumptively identified as potential Salmonella typhi isolates to be confirmed through subsequent biochemical testing procedures. Isolates confirmed as *Salmonella typhi* would undergo further characterization and antimicrobial susceptibility profiling.

Antimicrobial Sensitivity Test

To assess the bacteria's susceptibility to different antimicrobial agents, Kirby-Bauer disk diffusion test was conducted. First, isolated colonies growing on a nutrient agar plate were selected and used to prepare a bacterial suspension in 0.85 percent saline solution. The turbidity of this suspension was adjusted to match the 0.5 McFarland standard, to ensure appropriate concentration of bacterial cells is attained. Next, was dipping a sterile cotton swab into the standardized bacterial suspension and using it to evenly inoculate the entire surface of a Mueller-Hinton agar plate. Sterile forceps were used to carefully place antibiotic impregnated paper disks at spatially separated points on the inoculated agar surface, gently pressing down on each disk to ensure complete contact with the agar. With the disks in place, the plate was incubated at 37°C for 24 hours to allow the antibiotics to diffuse outwards, inhibiting bacterial growth in their vicinity. After incubation, the plate was retrieved and examined for zones of inhibited growth surrounding each antibiotic disk. Using a ruler, precise measurements of the diameter of these clear zones was taken, which correlates with how susceptible, intermediate or resistant the bacterial isolate is to that antimicrobial agent based on standardized interpretive criteria (CISI).

4.2.4 Data and statistical analysis

The data capture was recorded in Kobo tool kit then exported to Excel version 2016, subsequently cleaned and subjected to statistical analyses using SAS version 9.0. The data captured included dairy management, antimicrobial use frequency and dosage, and diseases treated within past 12 months period, and disease conditions. Descriptive statistics generated included the frequencies and percentages and means of use frequency and dosage and diseases treated within past 12 months period.

Drug dosage was computed for each cow using different metrics. The dosage concentration for each drug class (Gentamycin, Penicillin, Sulphamathoxazole and Tetracycline) was multiplied by 10 to obtain the drug concentration (mg/ml) (Mills *et al.*, 2018). For the unknown drug, the average concentration (percentage) of the drugs was used:

$$Drug\ concemtration\ \left(\frac{mg}{ml}\right) = concentration\ (percentage)\ *\ 10$$

The total amount of active ingredient (mg/cow/year) was calculated as the number of units (ml) multiplied by the drug concentration (mg/mL) × dosage units per treatment (mL) × frequency of treatment:

$$Total\ Active\ ingredient\ \left(\frac{\frac{mg}{cow}}{year}\right) = drugconc.\left(\frac{mg}{ml}\right) *\ dosage\ units\ per\ treatment(ml) *\ frequency\ of\ treatment$$

The Define Daily Dosage (DDD mg/cow/year) was calculated as Total active ingredients (mg/cow/year) divided by the liveweight of the animal.

$$DDD\left(\frac{\frac{mg}{cow}}{year}\right) = \frac{total\ active\ ingreadient\left(\frac{\frac{mg}{cow}}{year}\right)}{liveweight\ of\ the\ animal}$$

Chi-Square or Fisher's exact tests was performed to determine if there were significant distribution differences in antimicrobial resistance patterns between different pathogens and the antimicrobial drugs.

A general linear model was fitted to examine the relationship of the use frequency and the Defined Daily Dose (DDD) with the antimicrobial drugs (AMD), dairy intensification management and disease conditions.

For the AST test, all data were entered into Microsoft Excel Spread Sheet before further statistical analyses. Descriptive statistics were generated to show the distribution of mastitis pathogens isolated from milk samples and *Chi-square* test independence performed for the antimicrobial drugs by the sensitivity level of the pathogens at $p < 0.05$.

4.3. Results

4.3.1. Disease cases treated among lactating cows.

Figure 4 presents disease distribution that farmers indicated treating in lactating cows. Of the cows sampled, more than half (119/210; 56.7 percent) had been treated within the past 12 months, most frequently for mastitis (47.1 percent) relative to ($p<0.05$) East Coast Fever (14.3 percent), or parasitic infections (11.8 percent) or any other disease.

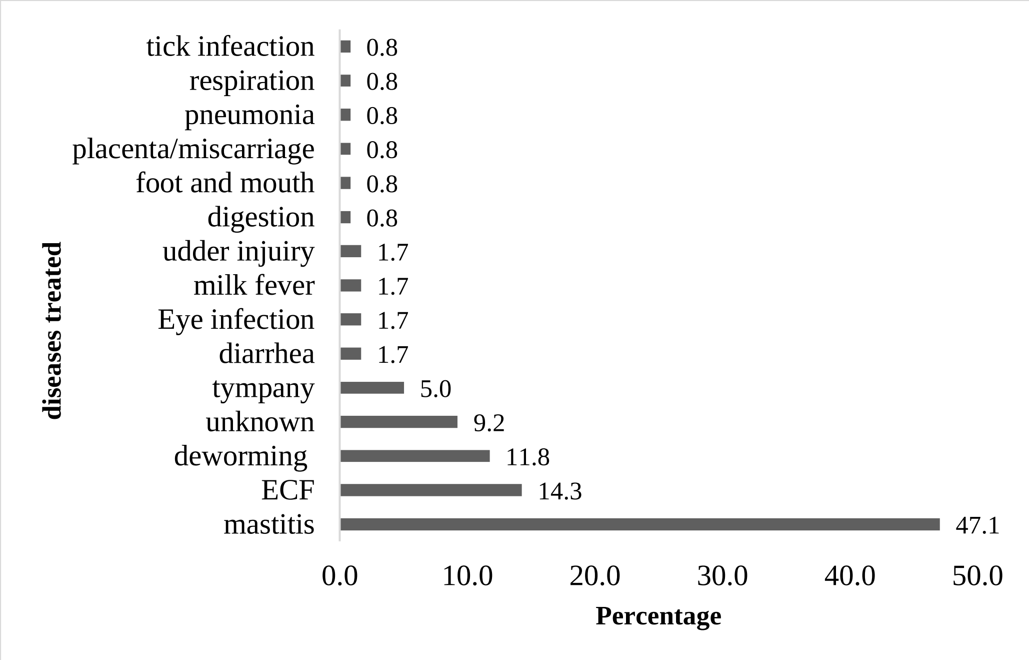

Figure 4: Distribution of diseases that farmers indicated treating in lactating cows.

The distribution of diseases by dairy intensification presented in Table 7 show that in general, disease incidences decreased with increasing dairy intensification from free grazing (42.9 percent) to semi grazing (29.4 percent) and further to zero grazing (27.7 percent). However, while incidences of mastitis were higher at high intensification (zero-grazing; 57.6 percent) than at low intensification (free-grazing; 47.1 percent), the incidences of ECF and parasitic infections were lower at high intensification (zero-grazing; 3.0 to 12.1 percent) than at low intensification (free-grazing; 15.7 to 17.7 percent). It is noticeable that the antimicrobial drug treatment was most frequent at low intensification level (free grazing; 42.9 percent).

Table 8: Distribution of diseases by dairy intensification levels

Diseases	Free grazing	Semi grazing	Zero grazing	*Chi-square* test
Parasitic Infection	17.7	17.1	3.0	*P*=0.4484$
East Coast Fever	15.7	17.1	12.1	
Mastitis	47.1	42.9	57.6	
Other	19.6	22.9	27.3	
All diseases	42.9	29.4	27.7	

$p value from Fisher's exact test

For the cows treated (Figure 5), the antimicrobial drugs used were mostly unknown (66.4 percent) to the farmers, but they could identify Gentamycin (13.4 percent), tetracycline

(11.8 percent), Penicillin G (5.0 percent), and sulphamethoxazole (3.4 percent). For treatment of mastitis, more than a third (37.5 percent) of the antimicrobial drugs used were unknown to farmers while they knew of Gentamycin (26.8 percent), tetracycline (19.6 percent), Penicillin G (8.9 percent), and Sulphamethoxazole (7.1).

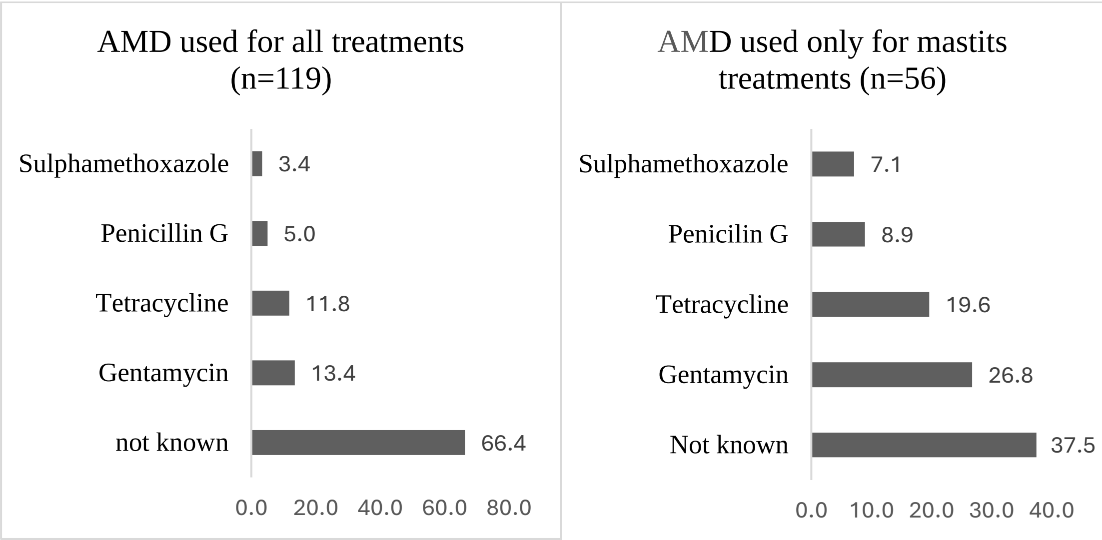

Figure 5: Distribution of antimicrobial drugs that farmers used for all treatments and treating mastitis in lactating cows.

4.3.2 Antimicrobial use frequency by dairy intensification

The average frequency of antimicrobial use (AMU) was further examined across levels increasing dairy intensification management. The results are summarised in Table 8, showing that the overall average AMU frequency was 2.2 instances per year, but was higher at low intensification (2.4 instances per year in free-grazing) than at high intensification (2.04 to 2.2 times per year). At low intensification management, disease exposure is higher relative to high intensification where disease control and monitoring is better, potentially leading to a reduced necessity for antimicrobial use to treat infections,

Table 9: Average frequency of AMU by dairy intensification

Intensification levels	Average frequency of AMU
Free-grazing	2.40
Semi-zero-grazing	2.04
Zero-grazing	2.20
Overall	2.20

4.3.2 The Define Daily Dosage (DDD) by disease, drug and dairy intensification.

The mean Define Daily Dosage (DDD mg/cow/year) by diseases, antimicrobial drugs used, and dairy intensification levels estimates from a general linear model explained 26.7 percent of the variation (Table 9). The DDD estimates was highest for mastitis and varied by

Table 10: The mean Define Daily Dosage (DDD mg/cow/year) by diseases, drugs and dairy intensification levels

Variables	Category	Mean	Standard error
	Overall mean	8.4[a]	0.6021
Diseases	Mastitis	16.6[a]	0.097
	ECF	8.2[a]	0.184
	Parasitic Infection	7.9[a]	0.196
	Others	3.4[a]	0.165
Drugs	Tetracycline	13.2[a]	0.180
	Sulphamethoxazole	13.1[a]	0.330
	Gentamycin	5.5[a]	0.180
	Penicillin	4.9[a]	0.259
	Unknown	5.9[a]	0.070
Intensification	Free-grazing	10.9[a]	0.124
	Semi-zero-grazing	6.5[a]	0.149
	Zero-grazing	6.6[a]	0.158

R^2 =0.267; p=0.001; Means with different letter superscripts differ at p<0.05

disease condition (p<0.05) but by the antimicrobial drug used nor dairy intensification level (p>0.05). Though not significantly variable by dairy intensification, the DDD was higher at low intensification (free-grazing; 10.9 DDD mg/cow/year) than at high dairy intensification (semi-zero-grazing and zero-grazing; 6.6 DDD mg/cow/year). Also, though not significantly variable by the drugs used, the DDD was higher for Tetracycline and Sulphamethoxazole (13.2 DDD mg/cow/year) than for Gentamycin and penicillin G (4.9 -5.5 DDD mg/cow/year). For all antimicrobial treatments, the average frequency of therapy was 1.1 per year with average dose of 20.6 mg/kg and 8.4 DDD mg/cow/year.

4.3.3. Antimicrobial Sensitivity Test

Of all the milk samples (n=191) collected from lactating cows, at least seven in ten (71.2 percent) tested positive for mastitis pathogens. The relative distribution (Figure 6) of the isolated pathogens from the milk samples reveal that *Staphylococcus aureus* was present in all the samples (100.0 percent), while the next most frequent pathogens were less than 20 percent (16.2 percent *Clostridium,* 14.7 percent *Escherichia coli* and 14.0 percent *Corynebacterium, Enterococcus* were fewer (4.4 percent) while *Salmonella* and *Streptococcus agalactia* were absent in the milk samples examined.

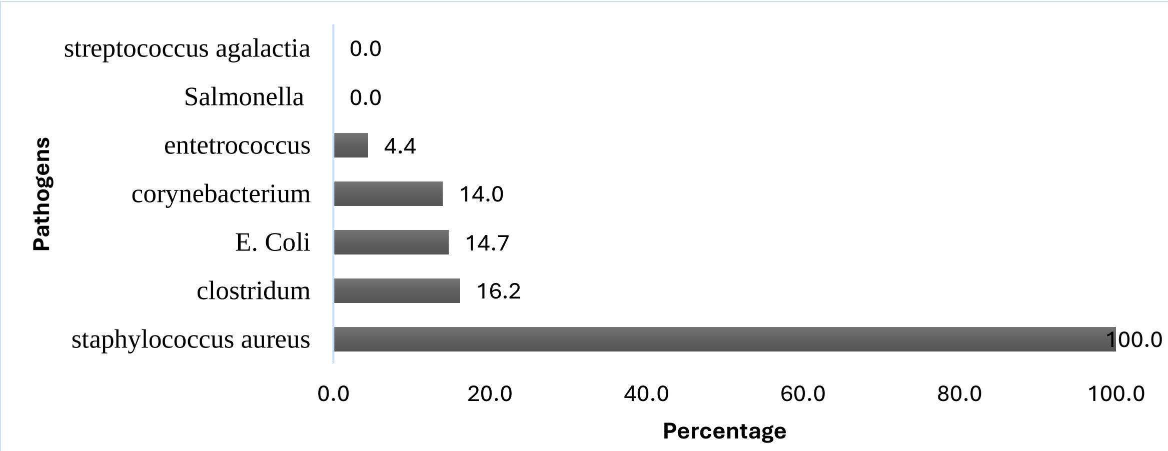

Figure 6: Distribution of mastitis pathogens in the milk samples

Antimicrobial Sensitivity Test was performed on only two pathogens associated with mastitis infections: *Staphylococcus aureus* and *Escherichia. coli*. The results are summarised in Table 10 from a total of 156 isolated pathogen with 136 *Staphylococcus aureus* and 20 *Escherichia coli* and their levels of sensitivity. The sensitivity of *Staphylococcus aureus* and *Escherichia. coli* were tested against various antibiotics, Ampicillin 30ug, Ciprofloxacin 5ug, Gentamycin 10ug, Kanamycin 30ug, Sulphamethoxazole 25ug, Streptomycin 10ug, Tetracycline 30ug and Penicillin G 10ug. While *Staphylococcus aureus and Escherichia. coli* showed no differential sensitivity (p>0.05) to Ampicillin, Penicillin G and tetracycline, both showed an alarming high resistance to Ampicillin and Penicillin G (p>0.05). *Staphylococcus aureus* exhibited 97.1 percent resistance to Ampicillin and 90.4 percent resistance to Penicillin G, while *Escherichia coli* showed 100 percent resistance to Ampicillin and 97.1 percent resistance to Penicillin G. Both *Staphylococcus. aureus* and *Escherichia coli* demonstrated

Table 11: Antimicrobial sensitivity test results of *Staphylococcus aureus* and *Escherichia coli* isolated from milk samples Testing positive for mastitis

Antimicrobials	Sensitivity level	Pathogens (%)		Total (%) (n=156)	*Chi-square* test
		Staphylococcus aureus (n=136)	*Escherichia coli* (n=20)		
Ampicillin	Susceptible	2.9	0.0	2.6	*P=1.000*
	Intermediate	0.0	0.0	0.0	
	Resistance	97.1	100.0	97.4	
Ciprofloxacin	Susceptible	98.5	85.0	96.8	*P=0.001*
	Intermediate	0.7	15.0	2.6	
	Resistance	0.7	0.0	0.6	
Gentamycin	Susceptible	100.0	90.0	98.7	*P=0.016*[$]
	Intermediate	0.0	10.0	1.3	
	Resistance	0.0	0.0	0.0	
Kanamycin	Susceptible	8.1	28.0	11.2	*P=0.001*
	Intermediate	8.8	68.0	18.0	
	Resistance	83.1	4.0	70.8	
Sulphamethoxazole	Susceptible	75.7	45.0	71.8	*P=0.009*
	Intermediate	5.1	5.0	5.1	
	Resistance	19.1	50.0	23.1	
Streptomycin	Susceptible	81.6	65.0	79.5	*P=0.044*
	Intermediate	7.4	25.0	9.6	
	Resistance	11.0	10.0	10.9	
Tetracycline	Susceptible	67.6	65.0	67.3	*P=0.258*
	Intermediate	8.8	20.0	10.3	
	Resistance	23.5	15.0	22.4	
Penicillin G	Susceptible	9.6	0.0	8.3	*P=0.221*[$]
	Intermediate	0.0	0.0	0.0	
	Resistance	90.4	100.0	91.7	

[$]p value from Fisher's exact test

similar susceptibility rates (65-67 percent) to tetracycline ($p>0.05$), with resistance rates of 23.5 percent and 15 percent, respectively. The antimicrobial drugs Ciprofloxacin, Gentamicin, Kanamycin, Sulfamethoxazole, and Streptomycin have been found to have a significant impact on the sensitivity levels of the pathogens. Very low resistance level was observed for *Staphylococcus aureus* (0.6 percent) and for *Escherichia coli* (0.0 percent) against Ciprofloxacin and Gentamycin. However, *Staphylococcus* showed a higher resistance level against Kanamycin (83.1 percent), Sulphamethoxazole (19.1 percent) and to streptomycin (11.0 percent) compared to *Escherichia coli* against Kanamycin (4.0 percent), Sulphamethoxazole (50.0 percent) and streptomycin (10.0 percent).

4.4. Discussion

The objective of this study was to generate estimates of the usage of veterinary antimicrobial drugs and associated antimicrobial sensitivity levels of common mastitis pathogens. The results generated are of relevance to informing avoidance of the development of antimicrobial resistance in livestock production and further in humans and in the environment. Antimicrobials are utilized in animals worldwide for both prevention and therapy. Whereas the use of antibiotics for growth promotion is prohibited in the majority of high-income countries (Mulchandani *et al.,* 2023), many farmers in low- and middle-income countries (LMICs) continue to use antibiotics indiscriminately for the same reasons.

The present study revealed a strikingly high prevalence of bovine mastitis among the sample dairy herds surveyed. The prevalence of mastitis was significantly higher ($p<0.05$) than that of other important bovine diseases like East Coast Fever (14.3%) and parasitic infections (11.8%). East Coast Fever, a tick-borne protozoal disease, and parasitic infestations have been documented as prevalent constraints on economic loss to cattle production (Rashid *et al.,* 2019). However, the current study highlights mastitis as dairy farmers' most pressing health concern. In a Canadian study, Uyama *et al.* (2022) reported that mastitis and diarrhoea are the most common diseases prompting antimicrobial use (AMU) in livestock production. This finding underscores the significant impact of Mastitis and highlights the importance of addressing them through appropriate antimicrobial usage strategies, as it is the main source of antimicrobial use in dairy production.

Mastitis, an inflammatory condition of the mammary gland, was diagnosed in nearly half (47.1%) of the cows examined. This finding corroborates previous reports from other regions of Europe and Asia which identified mastitis as a major endemic disease affecting dairy cattle production and productivity (Bag *et al.*, 2022; Verteramo *et al.*, 2019). This study confirms that the high use of antimicrobials is linked to the incidence of diseases, like mastitis, East Coast Fever, and parasitic infection being the most reported, and the increased use of antimicrobials was observed in free grazing with the use frequency of 2.4 per year. The findings align with a study conducted in Kenya by Kisoo *et al.* (2023), which observed a higher incidence of diseases and increased antimicrobial utilization at low intensification in pasture based grazing systems. This challenges the prevailing notion that the intensification of dairy production systems has positively influenced the excessive use of antimicrobials in dairy production, attributable to the increased demand for animal protein in LMIC (Clay *et al.*, 2020). It may be contended that in this study, farmers resorted to antimicrobial drugs only for treatment, as opposed to prevention and production efficiency, in contrast to the findings of (Kisoo *et al.*, 2023; Mogotu *et al.*, 2022; Nyokabi *et al.*, 2021; Omwenga *et al.*, 2021),

In this study, it was found that farmers in Nakuru peri-urban area only use antimicrobial drugs on lactating cow for therapeutic purpose only. In contrast to the findings of the present study, earlier research in Kenya indicated that farmers primarily use antimicrobials for disease prevention and control (Kisoo *et al.*, 2023; Mutua *et al.*, 2023). Though farmers were using antimicrobials, a larger proportion (66.4 percent) were not knowledgeable of the specific antimicrobial drugs they were administering, indicating that decisions on which antimicrobial to use was made by others for them and their record-keeping practice is inadequate for stewardship actions. Among the antimicrobials that farmers could identify, the most used were gentamicin (13.9 percent), tetracycline (11.5 percent), penicillin G (4.9 percent), and sulfamethoxazole (3.3 percent). This finding corroborates with previous studies by Redding *et al.* (2019) and Geta and Kibret (2021), who reported widespread use of these antimicrobials in dairy production.

The findings of the present study revealed that disease incidences decreased with increasing intensification, but cases of mastitis infections were most frequent at high intensification (zero-grazing). An explanation for this observation is that at high intensification, mastitis cases were more frequent but the cases of other diseases like ECF and parasitic infections were lower. This also explains the observation that the DDD was lowest at high intensification and highest for mastitis disease compared to other diseases because antimicrobial usage decreased with increasing intensification. The drug use at higher intensification therefore was for mastitis treatment but higher DDD at low intensification was associated with treatment of the other diseases, mainly ECF and parasitic infections. Although no significant differences were observed with increasing intensification levels, the highest dosage of 10.90063 DDD mg/cow/year was recorded at low intensification (free-grazing).

The DDD significantly varied by disease condition but was independent to the type of antimicrobial drug and dairy intensification levels. Notably, mastitis exhibited the highest DDD compared to other disease conditions, suggesting a higher antimicrobial usage for treating this prevalent disease in dairy cattle is the main driver of antimicrobial use. This observation supports those of Jong *et al.* (2023) who observed that mastitis treatment and dry cow therapy accounted for increased antimicrobial use. These findings are consistent with the existing literature, which emphasizes the importance of prudent antimicrobial use, especially for critically important antimicrobials, to combat the growing threat of antimicrobial resistance (Kemp *et al.,* 2021; WHO *et al.,* 2019).

The average dosage in the dairy sample dairy cows was 8.41 DDD mg/cow/year, with tetracycline and sulfamethoxazole exceeding the average DDD (13.2 DDD mg/cow/year) compared to gentamycin and penicillin (4.9 -5.5 DDD mg/cow/year). The DDD estimates in smallholder herds are much higher than the estimates recently published in the USA where dairy management is more intensive and in Australia where dairy management is pasture-based. In more intensive dairy herds of the USA, the mean DDD of the commonly used drugs is less than 6 mg/cow/year. For instance, cephalosporin (5.9 mg/cow/year), penicillin (5.2 mg/cow/year), tetracyclines (0.4 mg/cow/year, lincosamides (0.2 mg/cow/year), and sulfonamides (0.1 mg/cow/year) from reports of (Portillo-Gonzalez *et al.*, 2024). In Australian pasture-based dairy herds, the mean DDD is even much lower, for cephalosporins (1.049 mg/cow/year), penicillin (0.667 mg/cow/year) and tetracycline (0.275 mg/cow/year) in a recent publication (Werner *et al.,* 2023).

While AMU practices vary significantly across regions and production systems, due to differences in disease prevalence, management practices, and regulatory frameworks, higher levels observed in smallholder dairy herds necessitates a call for strengthened stewardship. Continuous monitoring and targeted interventions are necessary to promote responsible antimicrobial stewardship and ensure the sustainable production of safe and high-quality animal-derived products. The judicious use of antimicrobials in livestock production is crucial to preserve their effectiveness and mitigate the emergence and spread of antimicrobial-resistant pathogens. Furthermore, the results indicate that smallholder dairy farmers in the Nakuru peri-urban region have an average antimicrobial usage of 20 mg/kg, which is substantially lower than the global average of 45 mg/kg and the reported estimate of 66 mg/kg in Pakistan (Umair *et al.*, 2020).

The research identified several bacterial pathogens associated with mastitis, that included *Staphylococcus aureus* (found in all instances), *Clostridium spp*. (16.2 percent), *Escherichia coli* (14.7 percent), *Corynebacterium species*. (14.0 percent), and *Enterococcus spp*. (4.4 percent). There was a high occurrence (71.2 percent) of mastitis-causing pathogens observed in the present study. This diverse range of pathogens provides valuable insights into the complexities of mastitis and underscores the need for comprehensive strategies to address this issue. It is worth noting that several studies have consistently highlighted the high prevalence of mastitis in dairy cattle, especially within smallholder farming systems (Bantawa *et al.*,2019; Kashongwe *et al.*,2017). Additionally, Mogotu *et al.* (2022) emphasized the need for improved hygiene management practices among farmers, as they reported a mastitis prevalence of 83.6 percent. Moreover, various studies have documented high mastitis prevalence in Kenya (Kagira *et al.*, 2022; Omwenga *et al.*,2021; Ounah *et al.*, 2022), Uganda (Byaruhanga *et al.*,2022; Miyama *et al.*,2020), and Ethiopia (Amin *et al.*, 2017; Gebremedhin *et al.*,2022). Conversely, there are studies in Ethiopia that have reported lower mastitis prevalence, highlighting the need for further investigation into the factors contributing to these variations (Balemi *et al.*,2021; Gemechu *et al.*, 2018; Hailay *et al.*,2023; Tesfaye and Gizaw 2021).

In various studies conducted in Kenya, different areas showed varying prevalences of mastitis pathogens. In the Kabete area of Kiambu county Ounah *et al.* (2024) found moderate prevalence of *Staphylococcus aureus* (31.7 percent), *Escherichia coli* (17.2 percent), *Streptococcus species* (10.3 percent), *Klebsiella species* (9.7 percent), and *Pseudomonas aeruginosa* (7.6 percent). Meanwhile, in Juja sub-county, Kagira *et al.* (2022) reported different prevalences, including *Staphylococcus spp*. (41.7 percent), *Klebsiella spp.* (24.5 percent), *Pseudomonas spp*. (22.1 percent), *Escherichia coli* (6.8 percent), *Shigella spp.* (1.8 percent), and *Salmonella spp.* (3.1 percent). Ounah *et al.* (2022) also found varying prevalences in Moiben and Kapseret, including *Staphylococcus epidermidis* (9.3 percent), *Escherichia coli* (5.1 percent), *Citrobacter freundii* (2.8 percent), and *Streptococcus species* and *Micrococcus species* at 2.3 percent. These findings highlight the need to consider environmental, management, and hygiene factors when addressing mastitis pathogens in different regions.

The study has confirmed that both *staphylococcus aureus* and *Escherichia coli* exhibit multidrug resistance to several antimicrobial drugs including Ampicillin, Penicillin G, and Tetracycline, at a significant level of $p>0.05$ and to Kanamycin, sulfamethoxazole, and streptomycin at a significant level of $p<0.05$. Conversely, they have shown high susceptibility to Gentamycin and Ciprofloxacin with a $p< 0.05$, also statistically significant. This underscores the pressing need for enhanced antimicrobial stewardship and surveillance programs in smallholder dairy farming systems. It emphasizes the critical importance of judicious antimicrobial use, implementation of good farming practices, and regular monitoring of antimicrobial resistance patterns to address this escalating concern.

The research conducted in Kenya indicates that mastitis pathogens have varying resistance level against multiple antimicrobial drugs, with Ampicillin reaching 81.7 percent, Tetracycline 51.9 percent, Cotrimoxazole 52.9 percent, Streptomycin 58.7 percent, Sulfamethoxazole 60.6 percent, and Penicillin 80.8 percent. It's worth noting that Gentamicin reached a high cumulative susceptibility of 94.2 percent Furthermore, Michira *et al.* (2023) reported resistance of *Staphylococcus aureus* against Ceftazidime at 75 percent, Amoxicillin 50 percent, Streptomycin 46.9 percent, Oxacillin 34.4 percent, and Cefoxitin 12.5 percent.

Following a study in Ethiopia, Hailay *et al.* (2023) found that *Staphylococcus aureus* exhibited a 100 percent susceptibility to amoxicillin, kanamycin, and penicillin, while 95.5% of *Escherichia coli* were susceptible to kanamycin, oxytetracycline, and streptomycin. Meanwhile, in Australia, Werner *et al.* (2023) reported that *Escherichia coli* exhibited resistance to tetracyclines, sulphonamides, and penicillin. The indiscriminate and imprudent use of antimicrobial agents has been associated with high levels of antibiotic resistance. This underscores the critical need to assess the in vitro susceptibility of commonly used antimicrobial drugs before applying them in animals to prevent the emergence of antibiotic resistance and to provide effective antimicrobial therapy (Ounah *et al.*, 2022). The high levels of resistance observed in this study emphasize the necessity for enhanced antimicrobial stewardship and surveillance programs in smallholder dairy farming systems. Thoughtful use of antimicrobials, implementation of good farming practices, and regular monitoring of antimicrobial resistance patterns are essential to address this growing threat (Kemp *et al.*,2021; WHO 2019). In summary, the study highlights the intricate relationship between antimicrobial use, disease incidence, and production systems, while also shedding light on the knowledge gap and potential lack of record-keeping practices among farmers regarding the specific antimicrobials used in livestock management.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Rationale of the study

This study was motivated by a growing need to avoid the development of antimicrobial residue, resistance and treatment failures in livestock production associated with public health concerns, economic losses and environmental contamination. Empirical knowledge of why and how farmers use antimicrobial drugs in their dairy herds can inform options for effecting prudent and responsible use of antimicrobial drugs. The target study population of dairy cows in peri-urban herds are at high risk of inducing the development of antimicrobial residue, resistance and treatment failures due to frequent treatment of prevalent mastitis infections. The study hypotheses that guided the study were:

i. whether the KAPs vary with dairy intensification levels
ii. whether antimicrobial use frequency and dosage are different when farmers use antimicrobials for treatment, prevention or production performance enhancement, and
iii. whether there are differences in sensitivity levels of common mastitis pathogens to tetracycline, sulfamethoxazole, gentamycin and penicillin G antimicrobial drugs.

5.2 Methodology

5.2.1 Sampling procedures

To answer the three research questions outlined in section 5.1, different samples were obtained. The samples were farmers for data on the KAPs, mastitis positive cows for data on antimicrobial use frequency and dosage, and milk samples for data on sensitivity levels of common mastitis pathogens to antimicrobial drugs. The tested antimicrobial drugs were tetracycline, sulfamethoxazole, gentamycin and penicillin. Farmers were obtained randomly while all cows in a farm testing positive for mastitis were sampled because herd sizes are small, often a median of one cow. This approach in sampling enabled access to adequate number and right samples needed for the specific research questions.

5.2.2 Data collection and measurement

The data needed to answer the research questions specific to KAPs, and the antimicrobial use frequency and dosage were collected in a cross-sectional survey while the data needed to answer research questions on sensitivity levels of common mastitis pathogens to antimicrobial drugs was collected in laboratory procedures. In cross sectional survey, a sample of 124 farmers were interviewed using a structured questionnaire. From these 124 farmers, a total of 210 lactating cows were obtained on which data was collected on drug usage and milk samples collected. Of the 210 cows, 191 tested positives for mastitis. Milk samples from these mastitis positive lactating cows was processed for bacteriological culturing to identify mastitis pathogens and determine their resistance levels through Antimicrobial Sensitivity Tests.

Data was captured and stored in Kobo tool kit for later cleaning in Excel 2016 before subjecting to descriptive and inferential statistics using different statistical software packages: SPSS version 26, SAS 9.0 and R version 4.3.2. This systematic approach enabled the research to gain a deeper understanding of antimicrobial use and resistance in smallholder dairy farming, thereby contributing to the discussion on this important topic.

5.2.3 Statistical Procedure

Statistical tests were set at significance level of $p < 0.05$ for rejection of the null hypothesis. The data on KAPs and on sensitivity levels of common mastitis pathogens to antimicrobial drugs were counts or categorical in nature. The statistical approach adopted was first to cross tabulate and fit a *Chi-square* test statistic for test of independence. For these, analysis of the sampling distribution involved using Pearson’s *Chi-square* for expected frequencies greater than five and Fisher’s exact test for frequencies less than five.

The frequency and dosage data were treated as scale data and so were fitted to a General Linear Model to estimate mean differences between the disease conditions, antimicrobial drugs, and dairy intensification levels. The DDD was quantified by multiplying dosage concentration by 10 (100mg/ml) for specific drug classes (Mills *et al.,* 2018).

5.3 Implication of the findings

The evidence presented in Chapter Three Section 3.3.2 (Table 3) and Section 3.3.4 (Table 6) reveals that the purpose of antimicrobial use (do not use, treatment, prevention, growth promotion) and mastitis treatment was independent of the level of dairy intensification ($p>0.05$). However, in chapter four section 4.3.1, it was found that common diseases were significantly linked to intensification. Mastitis was most observed at high intensification (in zero-grazing systems), while diarrhoea, respiratory issues, and other diseases were more prevalent at low intensification (in free-grazing systems). In Chapter Four section 4.3.1 results show that of the lactating animals sampled, more than half (56.7 percent) had been treated with antimicrobial drugs for therapeutic purposes only. Table 7 results demonstrate a decrease in disease incidence with intensification. Although high intensification (zero-grazing) had the highest incidence of mastitis, low intensification (free grazing) was associated with a higher overall incidence of all diseases, which consequently led to the highest utilization and DDD of antimicrobial drugs. It is recommended that farmers should receive more training on good management practises regardless of dairy intensification to minimise disease incidences as it is the gateway for antimicrobial use in dairy cow production in the Nakuru peri-urban area.

The study revealed that knowledge, attitudes, and practices significantly differ by dairy intensification levels. A general pattern was that the more the farmers intensified their dairy management from free-grazing to zero-grazing the better was their knowledge, attitudes, and practices about AMU, AMR and AMD. Though most farmers were knowledgeable about antimicrobial use, residues and AMR, there was evidence of imprudent and irresponsible use of antimicrobials, which pose a risk of residue accumulation in food and subsequent development of resistance pathogens, regardless of dairy intensification level. There were concerning gaps in knowledge, such as the use of milk from treated cows before the withdrawal period and the use of human drugs on animals. This calls for an urgent need for a multi-faceted approach involving education, regulatory measures, promotion of best practices, socio-economic considerations, and collaborative efforts to ensure responsible AMU in the dairy value chain and to mitigate the risks of AMD misuse that result in AMR development.

The attitudes of farmers towards antimicrobial use varied with the level of dairy intensification. Farmers practising zero-grazing (higher intensification) generally expressed more positive attitudes towards antimicrobial use and its associated risks. This is exemplified by their acknowledging that mastitis can be treated without antimicrobials, recognizing the harmful effects of antimicrobial residues on human health, and accepting the potential for AMR pathogens to be transmitted through the food chain. While compliance with observing withdrawal periods and following up with treatments was generally high, alarming practices were noted. These include self-prescription of antimicrobials, administration of drugs by non-professionals, and the purposive use of human drugs on livestock, which raise concerns about the prudent use of antimicrobials. In chapter three section 3.3.4, Table 6, a significant proportion of farmers concurred that the sale and distribution of antimicrobial drugs should be limited to licensed individuals. Over 80 percent of the farmers procure antimicrobial drugs from veterinary and extension workers under the assumption that they are licensed, despite this not aligning with the regulations stipulated in the Kenya Veterinary Act. These practices have the potential to continue the inappropriate use of antimicrobials, which subsequently contribute to the development of antimicrobial resistance (AMR).

The findings suggest that knowledge gaps, coupled with negative attitudes and inappropriate practices, potentially can lead to imprudent antimicrobial use and development of AMR. Therefore, targeted training and sensitization of farmers and animal health professionals on prudent antimicrobial use, antimicrobial residue accumulation, and the associated risks of AMR in both animal and human health are crucial. Effective training, sensitisation, with effective antimicrobial stewardship programs is needed that is aligned with SDG 3, which emphasizes the importance of combating antimicrobial resistance. Promoting judicious antimicrobial use, minimizing the emergence and spread of AMR, and safeguarding public health contribute to the overall goal of ensuring healthy lives and well-being for all. Such programs should focus on enhancing knowledge through education and awareness campaigns, fostering positive attitudes towards responsible antimicrobial use, and promoting best practices in antimicrobial administration and record-keeping.

In Chapter four section 4.3 Table 7, the results demonstrate a decrease in disease incidence with increasing dairy intensification. Mastitis was the most frequent disease at high dairy intensification (zero-grazing) and was associated with the highest DDD. In contrast, at low dairy intensification (free-grazing), disease incidences were highest, which can explain the highest use of antimicrobial drugs. By implementing a comprehensive approach that addresses targeted AMU, biosecurity, antimicrobial stewardship, surveillance, education, and research, the livestock industry can mitigate the risks of antimicrobial residues, resistance development, and treatment failures to ensure a sustainable and responsible approach to livestock production.

The primarily use of antimicrobials in dairy farms in peri-urban areas of Nakuru was for therapeutic purpose only (chapter four), with mastitis the most common disease condition for which antimicrobial treatment was made. The average frequency of antimicrobial use was 2.2 times per year, with an average dosage of 8.4 DDD mg/cow/year which is higher than what is observed in more intensive dairy of the USA at 5.9 DDD and pasture-based dairy of Australia at 1.049 DDD. There is a potential issue of over-usage or higher-than-recommended dosages of antimicrobials in these dairy farms, which could contribute to the development of antimicrobial resistance (AMR). This highlights the importance of responsible and judicious use of antimicrobials, especially in settings where there may be a higher risk of overuse or misuse. Mastitis had the highest DDD (mg/cow/year) compared to other conditions, suggesting higher antimicrobial usage for treating this disease. This finding is consistent with the literature, which highlights the importance of prudent antimicrobial use, especially for critically important antimicrobials, to combat antimicrobial resistance (AMR). A field veterinary practitioner consulted by the author explained that under dosage is common in the field, mostly related to treatment of other diseases except for mastitis which overdose occurs with the belief that the animal will recover faster. This could explain why the dosage for mastitis was higher (16.6 DDD mg/cow/year). It is important to note that a significant proportion (66.4percentage) of the antimicrobial drugs used were unknown to the farmers, indicating a lack of knowledge or record-keeping practices. This lack of information could potentially lead to inappropriate dosing or misuse of antimicrobials, contributing to the development of AMR. Therefore, options to reverse of contain this trend and practice are urgent.

The highest frequency use and dosage of antimicrobials was observed at low dairy intensification (free-grazing). The higher AMD usage may be linked to the management of concurrent conditions such as ECF and parasitic infections. Possible causative factors include the pasture grazing behaviour of animals at low intensification, potentially facilitating the acquisition of parasites from the common pasture fields. Conversely, high intensification (zero-grazing) demonstrates the highest prevalence of mastitis cases, attributable to diseases of intensification. By adopting a comprehensive approach that encompasses targeted antimicrobial usage, biosecurity measures, antimicrobial stewardship, surveillance, education, and research, the livestock industry can effectively mitigate the potential hazards associated with antimicrobial residues, resistance development, and treatment failures. This strategy promotes sustainable and responsible practices in livestock production.

This study as is many others also found that common mastitis pathogens are *Staphylococcus aureus, Escherichia coli, Clostridium species., Corynebacterium species*, and *Enterococcus species, Streptococcus species, Klebsiella species, Pseudomonas aeruginosa*. Multiple studies conducted in Kenya revealed varying prevalences of mastitis pathogens in different regions but the most common in all are *Staphylococcus aureus, Escherichia coli Streptococcus species and Klebsiella species.* These findings highlight the need to consider environmental, management, and hygiene factors when addressing mastitis pathogens in dairy management.

Antimicrobial susceptibility testing revealed that the common pathogens exhibited significant resistance to commonly used antimicrobials, such as ampicillin, penicillin G, kanamycin, and tetracycline showing a multi-drug resistance of pathogens. However, the pathogens showed susceptibility to gentamicin and ciprofloxacin. Antimicrobial resistance (AMR) poses a significant threat to livestock production, which is essential for achieving food security and ending hunger (SDG 2). The study found that antimicrobials were primarily used for therapeutic purposes in dairy farms, with mastitis being the most common condition requiring treatment. However, the high frequency of antimicrobial use, inappropriate dosing practices, and the presence of resistant pathogens could compromise the effectiveness of antimicrobial treatments in livestock, potentially leads to reduced productivity and economic losses for smallholder dairy farmers. The study highlights the potential public health risks associated with AMR in livestock production. Resistant pathogens can be transmitted to humans through the food chain or direct contact with animals, posing a significant threat to human health. The development of AMR could render antimicrobial treatments ineffective, making it challenging to combat infectious diseases and ensure good health and well-being for all (SDG 3). AMR in livestock production can adversely impact the economy of smallholder dairy farmers, impacting their ability to achieve decent work and economic growth (SDG 8). Reduced productivity due to ineffective antimicrobial treatments could lead to economic losses, threatening the livelihoods of smallholder farmers and their families. Improved antimicrobial stewardship programs in smallholder dairy farming systems to promote prudent and judicious use of antimicrobials, particularly those considered critically important for human health. Such programs can help mitigate the development of AMR, contributing to the achievement of SDGs 2, 3, and 8 by ensuring food security, maintaining public health, and supporting the economic well-being of smallholder dairy farmers. The varied resistance profiles observed across different antimicrobials reinforce the critical need for ongoing surveillance, judicious antibiotic use, and the development of new therapeutic approaches to combat the evolving challenge of multi-drug resistant pathogens in dairy herds.

Additionally, the establishment of monitoring and surveillance programs to track antimicrobial usage patterns and the emergence of AMR is crucial. These programs can provide valuable data to inform evidence-based policies and interventions aimed at reducing inappropriate antimicrobial use and mitigating the risk of AMR development, ultimately contributing to sustainable achievement of the mentioned SDGs

5.4 Conclusions

i. Farmers' knowledge is not significantly associated with intensification except for Milk sales observe withdrawal period, Attitudes and Practices are significantly associated with intensification except for Attitude, any antimicrobial drug can be used to treat a lactating cow, and Practices, do you use human drugs on animals regarding antimicrobial use.

ii. The Antimicrobial Use frequency and dosage significantly differ between treatment, prevention and production performance enhancement.

iii. The sensitivity levels of *Staphylococcus aureus and Escherichia coli* are not significantly different to Ampicillin, Penicillin G and Tetracycline but are significantly different among Ciprofloxacin, Gentamycin, Kanamycin, Streptomycin, Sulfamethoxazole, are different.

5.5 Recommendations

i. A. Farmers to promote good farming practices, including improved hygiene and biosecurity measures, to reduce the overall need for antimicrobial treatment and minimize the risk of pathogen transmission and good record-keeping

B. Livestock government institutions and stakeholders to improve farmers' and animal health personnel knowledge and awareness about antimicrobial use, AMR, and the potential consequences through educational programs and training initiatives for both human and animal health

ii. A. Livestock institutes and Public Health Authorities implementing antimicrobial stewardship programs that promote judicious use of antimicrobials, particularly those considered critically important for human health in the dairy farmer.

B. Livestock government Institutions and stakeholders to establish monitoring and surveillance systems to track antimicrobial usage patterns, monitor the emergence of AMR, and provide data-driven guidance for antimicrobial stewardship efforts.

C. Stakeholders' collaborative and multidisciplinary approach involving farmers, veterinarians, public health authorities, and policymakers to safeguard the efficacy of antimicrobials and protect animal human and environmental health (One Health).

iii. A. Strengthening regulations and policies to ensure prudent antimicrobial use, including restrictions on over-the-counter sales and self-medication practices in both animals and humans.

B. Develop and implement a national antimicrobial stewardship program of monitoring and surveillance system that promotes judicious use of antimicrobials in both animal and human sectors.

C. Further research on antimicrobial residue on both dairy products and the environment, bovine mastitis pathogens at the animal-human interface and resistance strains at the molecular level

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APPENDICES

Appendix A

ETHICAL CLEARANCE

EGERTON **UNIVERSITY**

TEL: (051) 2217808
FAX: 051-2217942

P. O. BOX 536
EGERTON

EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DIR/009
Approval No. EUISERC/APP/303/2024 ***19th February 2024***

Mariama Njie
P.O. Box 536-20115
Egerton.
Telephone: +254- 0718232973.
E-mail: M21411012n@gmail.com.

Dear Mariama,
RE: ETHICAL APPROVAL: ASSESSMENT OF ANTIMICROBIAL USE AND ASSOCIATED RESIDUAL AND RESISTANCE LEVEL IN PERI-URBAN SMALLHOLDER DAIRY HERD ANIMALS NAKURU. KENYA

This is to inform you that the ***Egerton University Institutional Scientific and Ethics Review Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUISERC/APP/303/2024.*** The approval period is ***19th February 2024 – 20th February, 2025***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. You are required to adhere to the Institutional Experimental Animals Use and Care policy.
iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Egerton University Institutional Scientific and Ethics Review Committee.***
iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours of notification
v. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours.

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vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
viii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Institutional Scientific and Ethics Review Committee.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

EGERTON UNIVERSITY — Date. 19/02/2024 — RESEARCH ETHICS

Prof. Raphael M. Ngure
CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE
RMN/BK/

Appendix B

NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **306914**

Date of Issue: **09/March/2024**

RESEARCH LICENSE

This is to Certify that Ms.. Mariama Njie of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: ASSESSING ANTIMICROBIAL USE, RESIDUES AND RESISTANCE LEVELS ASSOCIATED WITH MASTITIS IN PERI URBAN SMALLHOLDER DAIRY COWS, NAKURU COUNTY, KENYA for the period ending : 09/March/2025.

License No: **NACOSTI/P/24/33414**

306914

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix C

DATA COLLECTION QUESTIONNAIRE

Consent seeking.

This questionnaire is meant to assess existing knowledge and practices, and antimicrobial usage in different classes of dairy animals in Nakuru peri urban area. The data collected is purely for academic purposes towards MSc study programme requirements. Participation is voluntary. I personally commit to strictly observe confidential treatment to all information offered.

I, ……………………………………………………, the rightful owner of dairy cattle, hereby do voluntarily give consent to participate in the study as explained to me.

Signature:……………………………………………… Date:……………….

Name of interviewee:………………………………… Date:………………..

A: Farmer identity

<table>
<tr><td>Ward</td><td>Gender of herd owner</td><td>Education level</td><td>Total cattle</td><td>Total milk (litres/day)</td><td>Milk outlet</td><td>Production systems</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">Wards:
1=Lare |
2=Njoro |
3=Lanet
4=Kabatini</td><td rowspan="2">1=Male
2= Female</td><td rowspan="2">1= Adult education
2= primary
3=secondary
4=post-secondary
5=uneducated</td><td>Total cows (≥2 parity)</td><td>Total heifers (1st parity)</td><td rowspan="2">1=Informal only
2=Informal and formal
3= Formal only</td><td rowspan="2">1=free grazing entirely
2=semi zero grazing
3= zero grazing entirely</td></tr>
<tr><td></td><td></td></tr>
</table>

B: Knowledge about Antimicrobial usage in dairy animals

	Question	Response	
1	For what purpose do you use antimicrobial drugs in your animals?		1=No antimicrobial treatment 2=treating mastitis (udder infections) 3=preventing mastitis (udder infections) 4=improving performance like growth and milk yield
2	How do you treat udder diseases (mastitis)?		1=frequently with antimicrobial drugs 2=only sometimes with antimicrobial drugs 3=totally No use of antimicrobial drugs 4= Use ethnoveterinary /herbs
3	What do you do with milk from cows that have been treated with antimicrobial drug before withdrawal period?		1=sale out 2= do not sale out 3=give to calves 4=consume at home 5=give gift to others
4	Have you heard of antimicrobial resistance and antimicrobial residue before?		1=Yes, from extension/veterinary officers 2=Yes, from the media (radio, newspaper, TV) 3=Yes, from field days 4= Yes, from fellow farmers, relatives 5=No

C: Attitudes regarding Antimicrobial usage in dairy animals

To what extent do you agree with the following statements?		Response	
5	Udder diseases (mastitis) can be treated without using antimicrobial drug		1=strongly agreed 2=agreed 3=neutral 4=disagreed
6	Any antimicrobial drug can be used to treat a lactating cow		
7	Cow's milk used during antimicrobial treatment is harmful to human health		
8	Treating cows and calves with antimicrobial can end up accumulating in the soils		

9	Antimicrobial resistance and residue in lactating cows can be passed to humans through food chain		5=strongly disagreed
10	Drug withdrawal periods should be adhered to as per the prescription to avoid drug residues in milk		
11	There is relationship between antibiotic use in animals and development of resistance		
12	Sale and distribution of AMD shall only be done by persons permitted to do so by law		
13	AMD residues and drug resistance will occur when AM are not used prudently		

D: Practices regarding Antimicrobial usage in dairy animals

	Practice	Response	
1	Where do you usually buy the antimicrobials from?		1= A veterinarian /animal health professional outlet 2=Pharmacy 3=Fellow farmers 4= Drug hawkers 5=Self
2	Who often prescribes antimicrobials drugs for you?		
3	Who administer antimicrobials used on the animals in your herd?		
4	How often do you call a veterinarian /animal health professional whenever an animal is sick?		1=frequently 2=only sometimes 3=totally No
5	What common disease condition(s) of lactating cows do you use antimicrobials for in your herd?		1=Mastitis 2=Respiratory diseases 3=Diarrhoea 4= Udder injuries 5= Other diseases
6	Do you observe withdrawal period after treating the animals with antimicrobials?		1=Yes 2=No
7	Do you give the subsequent doses of antimicrobial after the administration of the first dose of the treatment?		

8	Do you stop giving treatment when the recovers?		
9	Have you attended any trainings or conferences to update your knowledge on antimicrobial usage and antimicrobial resistance and residue?		
10	Do check the expiry date of the antibiotics before use		
11	Do you use drugs purposely for humans on animals?		

E: Administration of antimicrobials to different classes of animals in the farm

Animal ID #	1	2	3	4	5	6	7	8	9	10
Name of the animal /Identity used										
1=lactating cow 2= lactating heifer										
Animal Live weight (kg)										
Months in the herd in the last 12 months										
Has the animal been treated with antimicrobial drug in the last 12 months? (1=yes; 2=no)										
What was the reason for using the drug?										
Type of drug used										
Dosage (g) of the drug given										
Number of times the drug has been administered in last 12 months										
If different drug, use next rows										

	Type of drug 1=Tetracycline	**Reasons for using antimicrobial drug administered in last 12 months.**

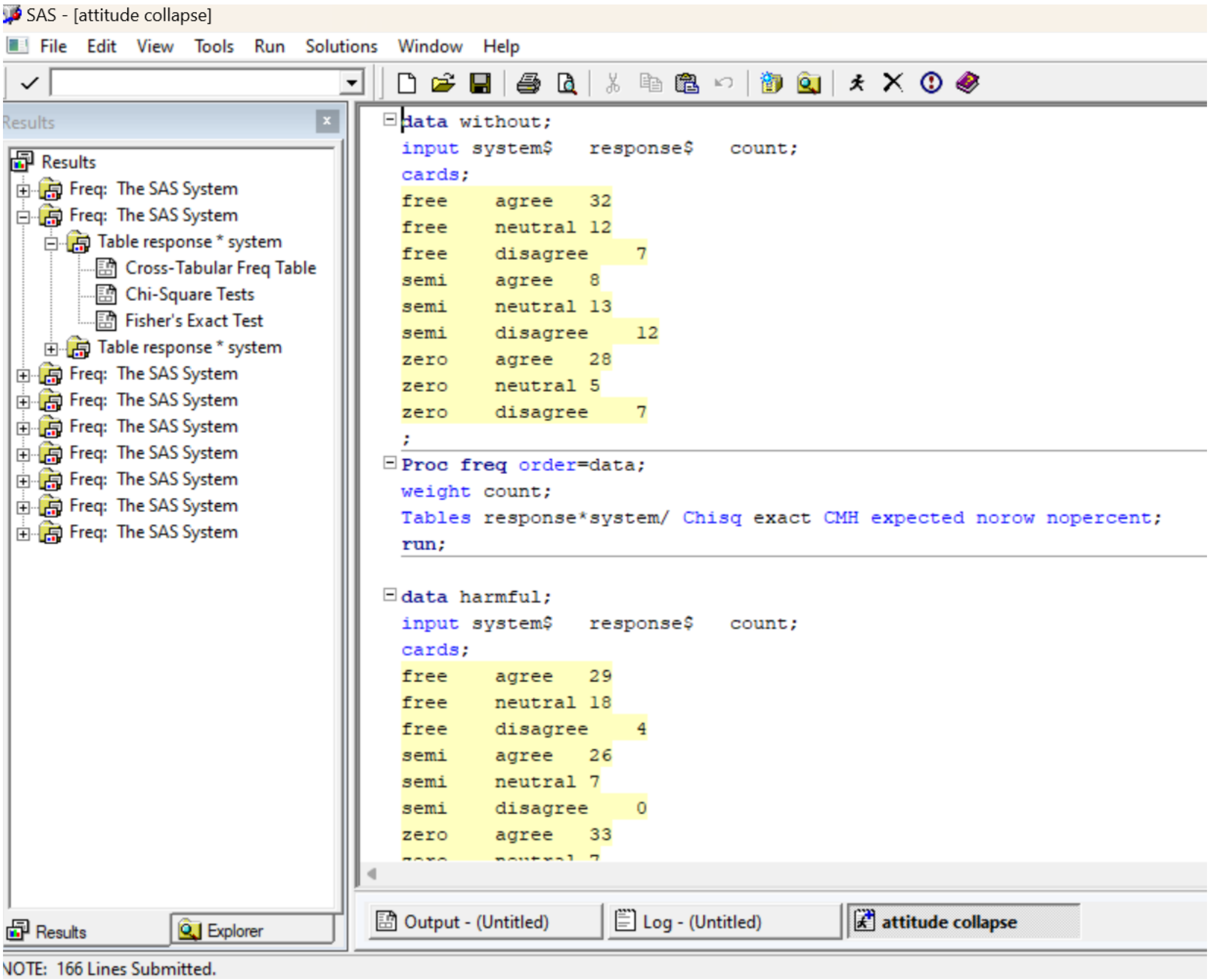
	2= sulphonamides 4=Gentamycin 5=Penicillin G 6= Others (specify) 7=Not known	1=Treating mastitis (udder infections) 2= Preventing mastitis (udder infections) 3=Improving production efficiency

F: Sensitivity level of the pathogen to antimicrobial drug

Animal ID Milk sample. *Per ID in section D*	**Pathogen detected.** 1=Staphylococcus aureus 2= Streptococcus agalactiae 3= Salmonella typhi 4=Escherichia coli 5=None	**Sensitivity level of the pathogen to antimicrobial drug** 1=resistance, 2=susceptible, 3=intermediate			
		Tetracycline	Sulphonamides	Gentamycin	Penicillin G

Appendix D

STATISTICAL ANALYSIS



The SAS System 07:54 Monday, October 4, 2024 1

The FREQ Procedure

Table of response by system

response / system Frequency Expected Col Pct	free	semi	zero	Total
agree	32 27.968 62.75	8 18.097 24.24	28 21.935 70.00	68
neutral	12 12.339 23.53	13 7.9839 39.39	5 9.6774 12.50	30
disagree	7 10.694 13.73	12 6.9194 36.36	7 8.3871 17.50	26
Total	51	33	40	124

Statistics for Table of response by system

Statistic	DF	Value	Prob
Chi-Square	4	18.5486	0.0010
Likelihood Ratio Chi-Square	4	19.3835	0.0007
Mantel-Haenszel Chi-Square	1	0.0013	0.9714
Phi Coefficient		0.3868	
Contingency Coefficient		0.3607	
Cramer's V		0.2735	

Fisher's Exact Test

Class Level Information

Class	Levels	Values
condition	4	Dewormin ECF Mastitis Others
drug	5	Gentamyc Penicill Sulphame Tetracyc notknown
system	3	free semi zero

Mariama Njie

Number of observations 119

The SAS System 07:37 Monday, March 15, 2024 7

The GLM Procedure

Dependent Variable: dddmg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	14.44729443	1.60525494	4.43	<.0001
Error	109	39.52719039	0.36263477		
Corrected Total	118	53.97448482			

R-Square	Coeff Var	Root MSE	dddmg Mean
0.267669	65.13079	0.602192	0.924588

Source	DF	Type I SS	Mean Square	F Value	Pr > F
condition	3	10.96597953	3.65532651	10.08	<.0001
drug	4	2.16015340	0.54003835	1.49	0.2104
system	2	1.32116150	0.66058075	1.82	0.1667

Appendix: E

CONFERENCE

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Knowledge, attitude and practices of antimicrobials in dairy herds in Nakuru Peri-Urban Area, Kenya

[1]Njie, M., [1]Bebe O. B. and [2]Orenge O. C.

[1]Department of Animal Science, Egerton University

[2]Veterinary Medicine Department, Egerton University

Corresponding Author: m21411012n@gmail.com

ABSTRACT

High prevalence of mastitis infections induces frequent use of antimicrobials Antimicrobial Use (AMU) and this poses public health risks and potential economic losses. This justifies developing stewardship programs for Antimicrobial Resistance (AMR) that is informed by Knowledge Attitude and Practices (KAPs) about the AMU. This study explored the KAPs about AMU in different production systems of smallholder dairy farmers in four peri-urban wards of Nakuru city in Kenya. In a cross-sectional survey with a questionnaire, a random of 124 farmers provided data on KAPs. The data was subjected to chi-square statistics to establish associations between the KAPs and AMU. Results revealed that seven in ten (68.5%) farmers used Antimicrobial Drug (AMD) and four in ten (34.7%) used the drugs for treatment. Eight in ten (75%) farmers were knowledgeable of antimicrobial residues and development of AMR, regardless of the production system ($p>0.05$) they practiced. An exception was the use of milk from treated cows before the withdrawal period ($p<0.05$). Six in ten (54.8%) farmers agreed that mastitis can be treated without use of AMD while seven in ten (62.9%) held that lactating cows can be treated with any type of AMD. Those who disagreed that human health risks can be attributed to consuming milk from antimicrobial treated cows before the withdrawal period were fewer (three in ten; 28.2%). Majority of the farmers used AMD that were prescribed (81.5%), purchased over the counters without prescription (82.3%) or self-administered (75.0%) on the advice of extension and veterinary officers. Compliance was high with the recommended withdrawal period (87.9%) and follow-up treatments (75.8%), but purposive use of human drugs on livestock was alarmingly high (62.9%). These results suggest further need for more targeted training and sensitization of farmers on the prudent AMU, antimicrobial residue accumulation and subsequent risk of AMR in both animal and human health.

Keywords: Antimicrobial resistance, antimicrobial use, Kenya, knowledge attitude and practices, mastitis,

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CERTIFICATE

OF ATTENDANCE AND PARTICIPATION

This is to certify that

MARIAMA NJIE

Attended and Presented a Paper Titled "**Knowledge, Attitude and Practices of Antimicrobials in Dairy Herds in Nakuru Periurban Area, Kenya**" at the **15^{th} Biennial International Conference** held at **ARC Hotel, Njoro Campus**, from 19^{th} - 21^{st} March, 2024.

Prof. Nancy W. Mungai
Ag. Director, Research and Extension

Dr. Mariam K. Charimbu
Chair,
Conference Committee

Transforming Lives Through Quality Education

The 4th International Nutrition and Dietetics Scientific Conference

ABSTRACT: Antimicrobial use and sensitivity of mastitis pathogens among smallholder dairy cows in peri-urban areas of Nakuru City, Kenya


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CERTIFICATE
— OF PARTICIPATION —

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Mariama Njie
(PRESENTER)

"ANTIMICROBIAL USE AND SENSITIVITY OF MASTITIS PATHOGENS AMONG SMALLHOLDER DAIRY COWS IN PERI-URBAN AREAS OF NAKURU CITY, KENYA"

who presented at the
4th International Nutrition and Dietetics Scientific Conference
held from 12th to 15th August, 2024

CONFERENCE CHAIR
Prof. Gordon Nguka

CONFERENCE DIRECTOR
Dr. David Omondi Okeyo

Appendix F

PUBLICATION

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Research Article

Antimicrobials Use by Smallholder Dairy Farmers in Peri-Urban Area of Nakuru Kenya: Knowledge, Attitudes and Practices

Mariama Njie[1, 3, *], Bockline Omedo Bebe[1], Caleb Oburu Orenge[2]

[1]Department of Animal Science, Faculty of Agriculture, Egerton University, Njoro, Kenya
[2]Veterinary Medicine Department, Egerton University, Njoro, Kenya
[3]Department of Livestock Services, Ministry of Agriculture, Abuko, The Gambia

Abstract

In dairy intensification, mastitis infections become prevalent and induce frequent Antimicrobial Use (AMU), sometimes inappropriately. This poses public health risks because of growing Antimicrobial Resistance (AMR), which calls for stewardship programs informed by Knowledge, Attitude and Practices (KAPs) about AMU and AMR to halt or reverse the worrying trend. Data was obtained in cross sectional survey conducted in four peri-urban wards around Nakuru city in Kenya. Randomly selected sample farmers (n=124) with free-grazing, semi-zero-grazing or zero-grazing dairy management, representing increasing dairy intensification levels provided data on the KAPs. Chi-square test statistics was fitted to establish associations between KAPs and dairy intensification levels. Among the sample farmers, six in ten (58.8 percent) had intensified dairy production, at least six in ten were marketing milk through informal outlets and were using antimicrobial drugs. Compliance with the withdrawal period was high and increased ($p<0.05$) with increasing intensification from free-grazing to zero-grazing. Within antibiotic withdrawal period, at least seven in ten farmers did not sell milk, fewer than four in ten consumed their milk at home and fewer than three in ten fed the milk to calves. Though independent of dairy intensification level ($p>0.05$), using antimicrobials for mastitis treatment increased while sourcing information on antimicrobial use from extension and veterinary officers decreased, with increasing intensification level. Farmers with some training on prudent antimicrobial use and with positive attitudes that milk from antimicrobial treated cows is unsafe, antimicrobial resistant pathogens and residues can be passed from milk to humans, mastitis can be treated without antimicrobial drugs, and antimicrobial residues can end up accumulating in the soils increased ($p>0.05$) with increasing dairy intensification levels. These results show that regarding AMU and AMR, farmers become more knowledgeable, with positive attitudes and good practices as they intensify their dairy management. The implication is that intensification of dairy management motivates farmers to gain more knowledge, acquire positive attitudes and apply good practices towards responsible prudent use of antimicrobials in livestock. Therefore, strengthening stewardship with targeted training and sensitization can foster prudent and responsible antimicrobial use.

Keywords

Antimicrobial Resistance, Antimicrobial Use, Kenya, Knowledge Attitude and Practices, Mastitis

*Corresponding author: m21411012n@gmail.com (Mariama Njie)

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