



Prevalence of antimicrobial resistance in *S. aureus* and *E. coli* from lactating dairy cows in Maasai Mara, Kenya

- A One Health perspective

Independent Project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme
Uppsala 2025



Prevalence of antimicrobial resistance in *S. aureus* and *E. coli* from lactating dairy cows in Maasai Mara, Kenya – A One Health perspective

Prevalens av antimikrobiell resistens i S. aureus och E. coli från lakterande mjölkkor i Maasai Mara, Kenya – Ett One Health perspektiv

Patricia Östling Vildhede

Supervisor:	Josef Dahlberg, Swedish University of Agricultural Sciences, Department of Clinical Sciences
Assistant supervisor:	Therese Hård, Borås Zoo, Sweden
Assistant supervisor:	Dinah Seligsohn, Swedish Veterinary Agency
Examiner:	Theodoros Ntallaris, Swedish University of Agricultural Sciences, Department of Clinical Sciences
Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent Project in Veterinary Medicine
Course code:	EX1003
Programme/education:	Veterinary Medicine Programme
Course coordinating dept:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2025
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	antimicrobial resistance, AMR, One Health, subclinical mastitis, <i>E. coli</i> , <i>S. aureus</i> , Maasai Mara, Kenya, milk, CMT, dairy cow

Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

Veterinary Medicine Programme

Abstract

Antimicrobial resistance (AMR) was found in an unexpectedly high prevalence in semi-domestic cats in Mararianta 2024. A suspected cause for this high prevalence of AMR in cats was milk from the Maasai dairy cows, because the milk is served to cats on a regular basis. The objective of this study was to investigate the prevalence of AMR against a number of antibiotic agents (Benzylpenicillin, Cefoxitin, Meropenem, Ciprofloxacin, Gentamicin and Tigecycline) using the disk diffusion test on *S. aureus* and *E. coli* isolated from milk. In this study a total of 124 cows were screened for subclinical mastitis (SCM) using the California Mastitis Test (CMT). In total 22 milk samples from udder quarters with SCM (CMT \geq 3) and 10 swab samples from cat bowls and milk containers were collected for cultivation and bacterial typing. In the tested population, 12.1% of the cows had SCM and 1.6% of the population had *S. aureus* induced SCM. The scarce number of cultivated bacteria made it impossible to draw a conclusion on the prevalence of AMR in *S. aureus* and *E. coli*. Although, the unexpectedly low prevalence of SCM is an indication that the milk from the Maasai dairy cows is not likely the cause of high AMR prevalence in the semi domestic cats in Mararianta. Further research to establish the cause of high AMR prevalence in the cats is needed.

Keywords: antimicrobial resistance, AMR, One Health, subclinical mastitis, *E. coli*, *S. aureus*, Maasai Mara, Kenya, milk, CMT, dairy cow

Table of contents

List of tables	9
List of figures	10
Abbreviations	11
1. Introduction	13
2. Literature Review	14
2.1 Antibiotics	14
2.1.1 WHO's list of essential medicines	15
2.1.2 Antibiotic substances used in this project	16
2.2 AMR	17
2.2.1 Mechanisms and transmission	17
2.2.2 Antimicrobial resistance in <i>Staphylococcus aureus</i> and Methicillin-Resistant <i>Staphylococcus Aureus</i> (MRSA)	17
2.2.3 Antimicrobial resistance in <i>Escherichia coli</i> and Extended spectrum beta- lactamases (ESBL)	18
2.2.4 Statistics and spreading of AMR globally and in Kenya	18
2.2.5 One Health and AMR	20
2.3 Milk in the local market	20
2.3.1 Clinical mastitis and subclinical mastitis	21
2.3.2 Bovine udder health in Kenya – risk factors	21
2.4 Maasai Mara	23
2.4.1 Pastoralist lifestyle and cattle in Maasai Mara	23
3. Material and Methods	26
3.1 Material collections	26
3.2 Bacterial growth	27
3.3 Typing of bacteria	28
3.4 Disk diffusion	28
3.5 Literature search and programs used	29
4. Results	30
4.1 Inhibition of bacterial growth	31
4.2 Prevalence of antibiotic resistance in milk from Mararanta	32
5. Discussion	33

5.1	Potential causes of SCM.....	33
5.2	One Health – The risk of AMR spreading	34
5.3	Limitations and bias	35
5.4	Source of error	35
6.	Conclusions.....	36
	References	37
	Popular science summary.....	44
	Acknowledgements.....	48

List of tables

Table 1: Categorization of antimicrobial behaviour. An explanation of the terms; spectrum of action, effect and relation between antimicrobial activity and drug concentration	14
Table 2. Summary of antibacterial categories.....	15
Table 3: CMT scoring among examined Maasai cows	30
Table 4: Bacterial isolates from milk samples.....	30
Table 5: Bacterial isolated from bulk tank containers and cat bowls	30
Table 6: Reference values for zone inhibition in the Disk Diffusion test as published by EUCAST	31
Table 7: Antimicrobial susceptibility testing, result of isolated strains of E. coli and S. aureus	31

List of figures

Figure 1: Maasai dairy cow with its calf in Mararianta. Picture by Patricia Östling Vildhede (2024).....	23
Figure 2: Mararianta, Kenya, is marked with red dot. Figure created with Mapchart.net by Emilia Schultz (2023).....	27

Abbreviations

AMR	Antimicrobial resistance
CMT	California Mastitis Test
<i>E. coli</i>	<i>Escherichia coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
ESBL	Extended spectrum beta-lactamase
FAO	Food and Agriculture Organization
KOH	Potassium hydroxide test
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MH	Mueller-Hilton agar
MIC	Minimum Inhibitory Concentration
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SCM	Subclinical mastitis
SLU	The Swedish University of Agricultural Sciences
SVA	Swedish Veterinary Agency
WHO	World Health Organization
WOAH	World Organisation for Animal Health

1. Introduction

Antimicrobial resistance is an ongoing problem and one of the top causes of human death worldwide (Murray *et al.* 2022). With increased prevalence of antimicrobial resistant bacteria, infectious diseases will become more difficult, if not impossible, to treat. This will result in increased expenses for healthcare and suffering for both humans and animals, affecting livelihood and food security. In Africa, there is limited availability of data regarding use of antibiotics for livestock. This is likely due to deficient coordination and/or cooperation between national authorities and livestock keepers together with poor regulation and surveillance systems. Although the available data gives grave concern with high levels of antimicrobial use and high antimicrobial resistance (AMR) prevalence, which is of great concern for human and animal welfare (Kimera *et al.* 2020; Mshana *et al.* 2021). A number of final year degree projects linked to the Swedish University of Agricultural Sciences (SLU) have been carried out in Maasai Mara since 2022, as a part of a bigger project, The Mara Cat Project (Karen Blixen Camp Trust 2023). A master thesis from 2024 found an unexpectedly high prevalence of antimicrobial resistant *Escherichia coli* in semi-domestic cats (Diamanti Barredal 2024). The cats live in close proximity to the local human population and are often fed milk from local cows (Byström 2023). Those results turned our interest towards the dairy cows and raised the question whether milk could be a source of AMR spreading to the cats. It is possible that a high prevalence of AMR in milk from dairy cows could spread further to the surrounding environment including wild animals and to the human population, thus making it of great importance for the One Health perspective.

2. Literature Review

2.1 Antibiotics

Antibiotics are substances of low molecular weight that in low concentrations kills or inhibits growth of microorganisms without causing substantial damage to the host (Giguère *et al.* 2013:3). If this substance is derived through another microorganism it is defined as an antibiotic, although these terms are in many cases used synonymously. Antimicrobial therapy is often an effective way to reduce severity of disease through decreased duration of infection as well as pain and further spread. Antimicrobial use is either therapeutical (treatment of illness), prophylactic (prevention of illness), metaphylactic (treatment of illness in both healthy and sick individuals to decrease infection spreading) or for growth promotion, although the latter is rapidly declining globally due to increased awareness of AMR development (Cockcroft 2015; WHO 2022). Antibiotics can be categorised through their spectrum of action, as bactericidal or bacteriostatic, and whether their effect is concentration dependent or time dependent (Mckellar *et al.* 2004; Giguère *et al.* 2013:5). Further explanation of these terms comes from “Antimicrobial Therapy in Veterinary Medicine” and are presented in table 1. There are numerous classes of antimicrobial substances, and a summary of their effect is presented in table 2.

Table 1: Categorization of antimicrobial behaviour. An explanation of the terms; spectrum of action, effect and relation between antimicrobial activity and drug concentration

Spectrum of action	Narrow-spectrum antibacterial agents are active against a smaller proportion of bacteria, for instant only gram-positive bacteria.	Broad-spectrum antibacterial agents often act against a larger spectrum of both gram-positive and gram-negative bacteria and could also act against intracellular bacteria.
Effect	Bactericidal: This means that the effect of the antimicrobial agent is to kill the microorganism. To determine if the antibiotic agent is able to kill the target microorganism, the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) is used. MBC determines the lowest acquired concentration of an antimicrobial agent to kill a target microorganism, and MIC determines the lowest required amount of an antimicrobial agent to prevent growth of a target microorganism. If the MBC is less than four times the MIC value, the antimicrobial is regarded as bactericidal.	Bacteriostatic: If the antimicrobial agent is regarded as bacteriostatic it means that the antimicrobial agents prevent the bacterial growth and proliferation.
Relation between antimicrobial activity and drug concentration	Time-dependent: If the antimicrobial agent is time dependent it means that the pathogen needs to be exposed to a concentration exceeding the MIC for a period of time for effect. Dosage is recommended to be just above MIC value with short duration between dosages to keep the concentration above MIC over time	Concentration dependent: This means that the microorganism will die when exposed to a high concentration of the substance rather than due to the time of exposure to the antimicrobial. Dosage is recommended as moderately higher than MIC and longer duration in between dosages.

Table 2: Summary of antibacterial categories*

Categories of antibacterial substances	A selection of agents	Effective against	Bactericidal or bacteriostatic effekt	Time- or concentration dependent effekt	Mechanism
Penicillins (beta-lactam)	Benzympenicillin , Ampicillin, Cloxacillin, Piperacillin	G+ and a selection of G- (anaerobic)	Bactericidal	Time dependent	Inhibits cell wall synthesis
Cephalosporins (beta-lactam) 1st to 4th generation	Cefoxitin , Cefaroline, Cefuroxime	G+ and moderat to good effect on G-	Bactericidal	Time dependent	Inhibits cell wall synthesis
Carbapenems (beta-lactam)	Doripenem , Meropenem	G+ and G-, aerobic and anaerobic, resistant to many betalactamases	Bactericidal	Time dependent	Inhibits cell wall synthesis
Monobactams (beta-lactam)	Aztreonam	G+ and G-, resistant to many betalactamases	Bactericidal	Time dependent	Inhibits cell wall synthesis
Fluoroquinolones	Ciprofloxacin , Levofloxacin, Ofloxacin	Aerobic G- and intracellular bacteria	Bactericidal	Concentration-dependent	Blocks DNA replication
Aminocyclcosides	Amikacin , Gentamicin	Aerobic G- and a selection of G+	Bactericidal	Concentration dependent	Interrupts normal protein synthesis
Glycopeptides and lipoglycopeptides	Teicoplanin, Vancomycin	G + (G+ cocci) as " last resort"	Bactericidal	Time- dependent	inhibit cell wall peptidoglycan synthesis
Makrolids, lincosamides and streptogramines	Azithromycin, Erythromycin, Clindamycin	G+, anaerobic and a selection of mycoplasma	Bacteriostatic/ bactericidal	Both time and concentration depending on substans	Inhibit protein synthesis
Tetracyclines	Doxycycline, Tetracycline, Tigecycline	G+, G-, anaerobic, aerobic, Intracellular bacteria and many protozoan parasites	Bacteriostatic	Time- dependent	Inhibit protein synthesis

*Note that these are the main categories of antibacterial substances and a selection of agents within each classification and therefore this table does not cover every antibacterial substance. "Effective against" is generalized and should not determine clinical use without additional antimicrobial susceptibility testing due to bacterial resistance development (Giguère et al. 2013; EUCAST 2024b). Substances used for this study is marked in red.

2.1.1 WHO's list of essential medicines

Antibiotics are classified under WHO into three groups; access group, watch group and reserve group antibiotics, as a tool to manage antibiotic utilization in an optimal manner (WHO 2023). The access group consists of antibiotics used daily in healthcare and a recommendation guide for these substances are provided by the WHO. The watch group consists of antibiotics that have the potential to select for AMR and is used for specific listed infectious syndromes. The reserve group is a last resort alternative for multidrug resistant pathogens and high priority pathogens, such as carbapenem resistant *Enterobacteriaceae*. The reserve group consists of quinolones, 3rd and higher generation cephalosporins, ketolides and macrolides, polymyxins and glycopeptides (WHO 2019).

2.1.2 Antibiotic substances used in this project

Benzylpenicillin, Cefoxitin and Meropenem

Benzylpenicillin, Cefoxitin and Meropenem are beta-lactam antibiotics. Beta-lactam antibiotics are bactericidal and time-dependent (Mckellar *et al.* 2004). They inhibit cell wall synthesis in the bacteria through inhibiting enzyme activity (penicillin binding proteins; transpeptidases, carboxypeptidases) and by that the final stage of peptidoglycan synthesis. Therefore, only cells with ongoing cell-wall synthesis will be affected (Blumberg & Strominger 1974). The effect of beta-lactam antibiotics is dependent on its ability to attach to the bacteria, penetrate the cell wall and resist beta-lactamase enzymes. Beta-lactamases are bacterial enzymes that break down penicillins and cephalosporins (Hall & Barlow 2004). Benzylpenicillin is a narrow-spectrum penicillin antibiotic with effect against gram-positive bacteria and moderate to good effect on gram-negative bacteria. Cefoxitin is a second generation cephalosporin and have a wide range of action against gram-positive and some gram-negative bacteria and it is resistant to most bacterial beta-lactamase enzymes. Meropenem is a carbapenem with a wide range of effects against gram-positive, gram-negative, anaerobic and aerobic bacteria and is resistant to most bacterial beta-lactamase enzymes (Bush & Bradford 2016).

Ciprofloxacin

Ciprofloxacin is a second-generation fluoroquinolone and is effective against aerobic, gram-negative and intracellular bacteria. The effect is bacteriostatic and bactericidal and concentration dependent (Roberts & Lipman 2006; Doble 2007). Fluoroquinolones is a synthetic antimicrobial agent that essentially blocks DNA replication through inhibition of DNA gyrase in the bacteria (Zhang *et al.* 2018).

Gentamicin

Gentamicin is an aminoglycoside. It has effect against aerobic, gram-negative bacteria and a selection of gram-positive bacteria, such as staphylococci. It has a bactericidal effect, and it is concentration dependent (Chaves & Tadi 2023). To assert effect, aminoglycosides must penetrate the bacteria, and they have a synergistic effect in the presence of beta-lactam antibiotics who interferes with the cell wall synthesis. Inside the cell, aminoglycosides bind to the 30S ribosomal subunit which causes a misreading of the genetic code which leads to interference in protein synthesis in the bacterium (Chen *et al.* 2014; Chaves & Tadi 2023).

Tigecycline

Tigecycline is a tetracycline which is a broad-spectrum antibiotic with effect against gram-negative, gram-positive, anaerobic, aerobic and intracellular bacteria and some protozoan parasites (Greer 2006). Its effect is bacteriostatic and time

dependent. Tetracyclines inhibit the protein synthesis in the bacterium through binding to the 30S ribosome which leads to an inhibition of tRNA to attach to the ribosome which ultimately disturbs the peptide synthesis and therefore bacterial growth. Tigecycline is especially interesting due to the wide use of tetracyclines among pastoralists in Kenya (Mburu *et al.* 2023). Tetracyclines are also one of the antibiotic agents that the WOH classifies as a Veterinary Critically Important Antimicrobial Agent and it is reported to be the most utilized agent for animal health globally (WOH 2022, 2024b).

2.2 AMR

2.2.1 Mechanisms and transmission

The mechanisms of antimicrobial resistance can be classified in four main categories (Cockcroft 2015):

1. Reduction of penetration of the antimicrobial agent into the bacterial cell.
2. Ejection by efflux pumps of the antimicrobial agent out from the bacterial cell.
3. Modification or degradation of the antimicrobial agent, before or after cell penetration, which inactivates it.
4. The target protein on the bacterial cell can be modified by another molecule which makes it inaccessible to the antimicrobial agent.

2.2.2 Antimicrobial resistance in *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

Staphylococcus aureus is a gram-positive, facultative anaerobic bacterium with zoonotic properties. It can cause infections in the skin, preexisting wounds and joints and cause abscesses and septicemia in a number of animals, including humans. It can also cause food poisoning in humans from contaminated food. It is often isolated from the milk from cattle with mastitis. (Algammal *et al.* 2020; VetBact 2023d)

The most common way for *Staphylococcus* spp. to become resistance against penicillin is through penicillinase formation. Penicillinase is an enzyme which breaks down penicillin and aminopenicillin (SVA 2024c). The combination of benzylpenicillin and cefoxitin is often used for screening, with disk diffusion tests, to determine if *Staphylococcus* spp. is sensitive to beta-lactam antibiotics or beta-lactamase producing and therefore resistant to benzylpenicillin and other narrow-spectrum penicillin (EUCAST 2024b). A majority of *S. aureus* strains are penicillinase producers, although if there is a susceptibility to cefoxitin they can

also be sensitive to beta-lactam beta-lactamase inhibitor combinations such as oxacillin. Unfortunately, penicillinase formation is not always discovered through a high MIC value or through a disk diffusion test and should therefore be evaluated through additional examination. Additional tests can be performed parallel to the AMR-testing, with for example a test with chromogenic cephalosporins (nitrocefin) (SVA 2024c). If the bacteria is resistant to ceftiofur or oxacillin according to the disk diffusion test, it is suspected to be methicillin-resistant and therefore resistant to other penicillins and cephalosporins as well (EUCAST 2024b). Methicillin-resistance can be confirmed using other molecular methods. Methicillin-resistance implies that beta-lactam antibiotics are ineffective due to a change in the cell wall structure in the bacteria which makes the antibiotic unable to attach to penicillin binding proteins within the bacteria.

2.2.3 Antimicrobial resistance in *Escherichia coli* and Extended spectrum beta-lactamases (ESBL)

Escherichia coli is a gram-negative, facultative anaerobic bacterium with zoonotic properties. Typically, *E. coli* is a non-pathogenic bacterium that is a part of the normal microbiome in the intestines. However, if the bacteria enter other parts of the body, it can cause infection and there are sub-species that can cause gastrointestinal infections. Uropathogenic *E. coli* can cause infections in the urinary tract in all susceptible animals. *E. coli* have sub-species such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) that infect the gastrointestinal system in humans but are part of the normal microbiome in cattle. In cattle verotoxinogenic *E. coli* (VTEC) can cause mastitis in cows and acute disease in calves resulting in septicemia and death. (VetBact 2023c)

Extended spectrum beta-lactamase (ESBL) are enzymes produced by bacteria to break down beta-lactams, subsequently making the bacteria resistant. The genes that code for ESBL can be transmitted to other bacteria which facilitates further spread. If a decreased susceptibility to cephalosporins with extended spectrum and/or carbapenems is exhibited in gram-negative intestinal bacteria such as *E. coli*, ESBL production should be suspected. (SVA 2024a; b)

2.2.4 Statistics and spreading of AMR globally and in Kenya

Antimicrobial resistance is, according to WHO's criteria, one of the top 10 global health threats. Thus the organization have identified six specific pathogens (*E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) as priority pathogens because of their contribution to the burden of AMR in 2019 (WHO 2022). In 2019, an estimated 1.27 million human deaths were directly related to AMR and 4.95

million human deaths were associated to AMR globally, with the highest all-age death rate in western sub-Saharan Africa (Murray *et al.* 2022). Laxminarayan *et al.* (2016) point out that the low- and middle-income countries suffer the greatest loss due to AMR, although it is a problem in high income countries as well. They emphasize that the lack of access to antibiotics results in even more deaths than AMR. Providing access to appropriate antibiotics and restriction of inappropriate use of antibiotics remains a great challenge that requires alterations in finances and securing the ability to provide adequate healthcare (Laxminarayan *et al.* 2016). As described by the World Organisation for Animal Health (WOAH 2022), the global use of antimicrobial substances for food producing animals has declined over time (13% decrease from 2017-2019) with the exception of Africa (+45%) and America (+5%). It is not known whether the increase is due to an actual increase in use or increase in reporting the use, although it is worth noting that the use of antibiotics and prevalence of AMR is underreported in Kenya and other sub-Saharan countries in Africa (WOAH 2022; Sohaili *et al.* 2024). The greatest reduction is seen in the utilization of tetracyclines which still is the most utilized substance in animal health globally. Furthermore, tetracyclines is classified as a Veterinary Critically Important Antimicrobial Agent (VCIA) by the WOAH and is of great importance in the treatment of specific diseases with few alternative agents (WOAH 2024b). Data from a review article about antimicrobial use in food producing animals (Kimera *et al.* 2020) show that there is a high level of antibiotic use in animal production in Africa. The use of tetracycline, penicillin and aminoglycoside are especially high which most certainly makes a contribution to the continent's currently high AMR levels. In addition, there is a lack of documentation of antibiotic use in Kenya. A study by A. Shitandi and Å. Sternesjö found that, from a total of 500 herds, approximately 40% of dairy farmers had documented use of antimicrobial substances. They found that it was less likely to document drug use (<30 %) if the dairy farms were small-scale producers (<50 litres of milk per day) (Shitandi & Sternesjö 2004). Brown *et al.* (2020) states that in low-income countries, antibiotics can be used preventatively against livestock losses to disease. It is not uncommon for milk to be sold in informal food markets where there is little to no antibiotic regulation, enforcement of drug withdrawal periods or residue testing programs. The study investigated the presence of antibiotic residues and AMR bacteria in pasteurized and unpasteurized milk samples from shops, street vendors or vending machines in Nairobi, Kenya. Out of the 95 milk samples, 74 pasteurized and 21 unpasteurized, 7.4% tested positive for beta-lactam residues and 3.2% tested positive for tetracycline residues. Further, 67% of the unpasteurized milk samples contained *E. coli*, with a majority resistant to ampicillin and/or tetracycline (Brown *et al.* 2020). This is an example of how poor regulation and adherence control from authorities leads to the spread of AMR and exposure to antibiotic residues to consumers of milk from the local

markets in Kenya. Brown further emphasizes the difficulty to follow the existing recommended milk withdrawal period for ampicillin and oxytetracycline (two and four days) because of the economic loss for the financially vulnerable farmers.

2.2.5 One Health and AMR

One Health is a concept where the world is viewed as a unity between humans, animals and the environment. This means that we are dependent on each other and so is our health. Over 60% of pathogens causing disease in humans, derive from animals. Therefore, it is essential to work towards health not only in humans, but in the animal population as well (WOAH 2024a). WHO presents the importance of applying a One Health perspective regarding AMR development. The spreading of pathogens and AMR could occur between animals, human and the environment which makes our health interdependent (WHO 2017). The World Organization for Animal Health states that it is crucial to collaborate between sectors in charge of human, animal and environmental health to fight increasing AMR in the following statement:

“It is by reducing the overuse of antimicrobials in humans, animals, and plants that we will be able to achieve better global health.” (2024a).

2.3 Milk in the local market

Milk as a food product is of big importance to the Kenyan population. It is a source of nutrition and energy, especially for infants and children in low-income communities. On an average, people in Kenya consume milk every day to a majority of the weekdays. The milk is sold through both formal and informal channels, where informal channels constitutes a vast majority, channeling approximately 86% of the total milk volume (Mtimet & Karugia 2020). To distribute milk in Kenya, there is a requirement to have a permit obtained from Kenya Dairy Board, which is a corporation under the Ministry of Agriculture and Livestock Development. To receive this permit, the vendors and intermediaries are obliged to adhere to established standards regarding milk quality and safety (Kenya Dairy Board 2023). According to a study from 2021 (Blackmore *et al.* 2022), a majority of operating milk vendors have not obtained a permit. A common reason for this, according to the vendors and intermediaries, was that it is too difficult to live up to the standards and recommendations required for the permit. This leads to the distribution of a high quantity of milk that is lacking information about its quality and potential health risks for the consumers as well as potential bacterial content carrying AMR. The informal market consists mainly of raw, unprocessed milk which often is bought directly from the producer or from small shops. The raw milk is often preferred by the consumers due to its taste, lower price, availability

and higher content of butterfat. It is often presumed by consumers that processed milk have lower nutritional qualities (Mtimet & Karugia 2020). The safety measures taken by the consumers when buying and storing milk include testing the milk for bad smell and taste, using a clean container, a close contact to the producer building trust and boiling milk before intake. The general assumption by consumers is that milk is totally safe after boiling. Consumers who have a refrigerator use this as safety measure.

2.3.1 Clinical mastitis and subclinical mastitis

Mastitis is an inflammatory response in the udder most commonly derived from infection. Visibly abnormal milk is seen in clinical mastitis and with the inflammation there can also be changes in the udder (heat, swelling, redness, pain) and further systemic changes such as fever. Subclinical mastitis is an infection without visible signs of local or systemic inflammation. Subclinical mastitis causes losses in milk yield (Wieland 2024). There are two main groups of pathogens associated with mastitis: environmental and contagious pathogens. The major pathogens in the contagious group are *S. aureus* and *Streptococcus agalactiae* and the main pathogens in the environmental group are *E. coli* and *Streptococcus uberis*, although there are several other pathogens that could cause mastitis. The contagious pathogens commonly derive from the teat skin or the udder and are spread during the milking process and migrate into the udder through the teat canal. The environmental pathogens are mainly spread through poor milking hygiene, for example contact between the teat canal and with contaminated objects either during milking, by inserting teat canulae or by passive penetration after milking e.g. laying down on a dirty foundation. After entering the teat, the pathogens migrate into the udder through the teat canal. Coliform mastitis is commonly a sign of poor hygiene (Scott *et al.* 2011). The clinical manifestation of mastitis caused by *E. coli* is often of acute character and resolves relatively fast over a few days. Mastitis caused by *S. aureus* is commonly less severe although it can remain for a longer period of time and become chronic (Schukken *et al.* 2011).

2.3.2 Bovine udder health in Kenya – risk factors

Although there is limited data on udder health in Kenya, existing data indicates that mastitis is very common and especially subclinical mastitis. According to a review regarding bovine mastitis in Africa, subclinical mastitis has a prevalence of 60-80% in Kenya (Motaung *et al.* 2017). This is high in comparison to Sweden which had a prevalence of 23-24% from 2017-2023 (VÄXA 2023). *S. aureus* is the predominant pathogen occurring in milk samples on the African continent and it has the highest prevalence in Kenya compared to the rest of the continent. The economic consequences and milk losses due to mastitis in Kenya are not well

documented but are believed to be of great importance. Reasons for the high prevalence of *S. aureus* could be explained through many adding risk factors. These risk factors are mainly associated with hygiene and management. According to the Food and Agriculture Organization (FAO) several principals of management are recommended to reduce the risk of microbial contamination and the spread of disease between cows and to humans via consumption. To reduce these risk factors, FAO points out the importance of hygiene during the milking procedure and specifically regarding the animal's skin, the milking equipment, the milking personnel and the surrounding environment. If there are signs of illness in the animal, or in the milk, it should not be used for human consumption and the animal should be milked separately from the others or as the last one in line (FAO 2024). To prevent new infections with contagious bacterial mastitis, Moroni *et al.* (2018) recommend, in addition to the previously stated principals of management, the segregation of infected cows, dry cow therapy or culling of chronic carriers. They also emphasise the importance of udder hygiene and recommend washing and drying the teats with a separate towel for every animal and post milking dipping of the teats (Moroni *et al.* 2018). When evaluating risk factors for bovine sub-clinical and clinical mastitis in central Kenya, a study found that farms that did not milk the mastitic cows last were 2.3 times more likely to have a higher mastitis occurrence in the farm compared to farms that had a milking order as described above (Mbindyo *et al.* 2020). The most common pathogen found in their study was from the contagious group, *S. aureus*, which demonstrates the importance of measurements to counteract risk factors and maintain high-grade hygiene to prevent the spreading of mastitis. Other risk factors associated with mastitis prevalence in this study was a lack of a clean and dry udder towel for each cow and a previous history of mastitis.

Information about mastitis prevalence and hygiene management within the pastoralist system in the Maasai community is scarce, although a book by Garine and Ibrahim on the anthropological approaches on drinking ("Drinking: anthropological approaches"), describes the hygienic procedures by the Maasai women. They state that the women clean the gourds, which are used to contain milk, every night. This is done by washing it with water and stick-brushes and then drying it by shaking glowing embers from a sacred tree (*Olea Africana*) in the gourds and then pouring it out. In the morning, the women wash their hands with water and then walk out to the cow. They further disinfect the gourd through rinsing it with cow urine. The first draws of milk are thrown from the gourd into the sky (Garine & Ibrahim 2001). Information from this book is possibly outdated and it does not give information whether any measurement is taken to reduce the risk of pathogens spreading between the cows being milked e.g. washing of the udder and washing hands before moving on to the next cow.

2.4 Maasai Mara

Maasai Mara is a nature reserve located southwest in Narok County, Kenya. It is home to one of the world's highest densities of wildlife including the big five (lion, leopard, elephant, rhinoceros and buffalo), zebra, wildebeest, cheetah, giraffe and many more. The Mara Conservancy is a nonprofit organization operating in the Mara Triangle, which consists of about one-third of the total Maasai Mara National Reserve area. The Mara Conservancy has a partnership with the local Maasai community and works towards a sustainable environment and wildlife conservation. With a commission from the Narok County Government the Mara conservancy is to tackle all aspects of protected area management within the Mara Triangle which consists of development and maintenance of infrastructure, collection and distribution of revenue, security and management of tourism (Mara Conservancy 2024).

2.4.1 Pastoralist lifestyle and cattle in Maasai Mara

The Maasai population live in close proximity to their cattle and are dependent on their livestock for survival (Omondi *et al.* 2021). The Narok County population mainly consists of pastoralists. The main economic activities in the county consists of livestock management by the Maasai pastoralists, crop farming and tourism (Narok County Government 2024). There are three main categories of production systems in Kenya; small-scale dairy and meat, large-scale dairy and meat and small-scale dairy whereas the latter includes the Maasai pastoralists (Onono *et al.* 2013). The main cattle breed in Africa is Zebu (*Bos indicus*). Zebu cattle is well adapted to the harsh, hot climate and produce more than European breeds under these climate circumstances. Zebu cattle also endure insects and infectious diseases better than European breeds located in Africa (Hansen 2004).



Figure 1: Maasai dairy cow with its calf in Mararianta. Picture by Patricia Östling Vildhede (2024).

Cattle and milk have a major social and cultural value for the pastoralists of the Maasai community. The diet of the Maasai community consists almost exclusively from animal produce such as milk, meat and blood of the livestock, and the food has a great meaning in many rituals (Århem 1989; Garine & Ibrahim 2001). The women have full control over the distribution of milk to the household, milk that is obtained from the livestock. It is by tradition the women's responsibility to make sure the men and children receive milk according to their social values. Århem (1989) states that as tradition, men only drink cow's milk, preferably fresh, and if the supply is scarce the women reserve fresh milk to men. In this case, the women and children could drink milk from small ruminants and milk mixed with water and maize flour that is boiled into a thick gruel. The milking is done by women and by hand, often two times a day, in the morning before grazing and in the evening when the cattle return from pasture. The calf is often separated from the cow when grazing. During the time of milking, the women often let the calf suckle from one or two teats before milking and then collect the milk from the remaining teats. How much milk the calf receives depends on various factors including the age and physical health of the calf. According to a study by Lindell (2013), reasons for not milking the cows were if the cows were physically ill or if the women decided that the calf needed the milk. A majority of the participants in their study stated that the milk production depended on the availability of good grazing areas. The herdsmen often decided the grazing area in advance (Lindell 2013). The Maasai pastoralists can move over large areas to search for water and pasture for their cattle (Omondi *et al.* 2021). The availability of good grazing areas is more challenging during the drought period. Several interviewed pastoralists in Oloitoktok sub county Kajiado County, Kenya, witness that they were obligated to walk a greater distance to search for water and grazing areas during drought periods (Mburu *et al.* 2023). Greater walking distances also leads to loss of livestock as prey to wild animals and gives way for transmission of diseases between the cattle and wildlife. During the dry-season there is usually an increase in disease, especially Foot and Mouth disease (FMD), Contagious caprine pleuropneumonia (CBCP) and lumpy skin disease which is thought to be due to the greater number of animals meeting when searching for water and pasture (Mburu *et al.* 2023). Livestock diseases are a part of the pastoralists' life in Kenya and an increased mortality or reduction in live-stock productivity could be a great challenge for their economic situation and lead to poverty due to lack of resilience. The pastoralists use both traditional and conventional methods to treat illness although the typical treatment for various diseases in cattle are oxytetracycline, penicillin and various dewormers through trial and error. Professional advice for treatment is rarely sought out. When modern medicine is used without regulation and education it is likely that the administration, dosing and storage of

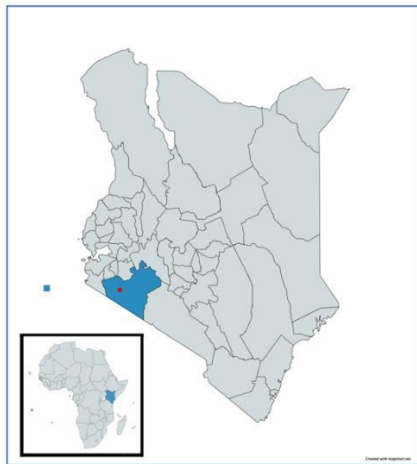
the used substance, duration of treatment, hygienic measures and withdrawal period are not being adequately fulfilled.

3. Material and Methods

3.1 Material collections

The sample collection was performed in the Mararianta district, Mara North Conservancy, in the national reserve Maasai Mara, Kenya (Figure 2). A number of samples were collected and divided as follows; milk samples from cows with subclinical mastitis, swab samples from milk containers and swab samples from cat bowls. The material was collected from Maasai family farms where both cats and cattle lived. The farms were selected through accessibility by foot from the field laboratory at Karen Blixen camp, and therefore 18 Maasai family farms within walking distance were visited. All samples were collected during milking in the morning. All cows that were being milked when present, were tested for subclinical mastitis (SCM). Milk from individual cows without clinical sign of mastitis were tested with California Mastitis Test (CMT) which is a cheap, accessible, and simple test to give an indication of the number of somatic cells a milk sample contains (Cockcroft 2015:142). An equal volume of milk, from all udder quarters separately, and CMT-liquid is added to four wells of a plastic paddle which then is gently swirled by hand. Increased viscosity is interpreted as an increase in somatic cell count (Cockcroft 2015). It is interpreted on a scale from 0-5 where a result of ≥ 3 indicates a cell count of $\geq 400\ 000$ cells/ml and the presence of SCM (SVA 2019). A total of 124 cows were CMT tested. Milk samples were collected in an aseptic manner for bacteriological examination from teats with SCM. This was done through washing hands and using new disposable gloves, thoroughly cleaning of the teat and especially the teat tip with surgical ethanol on cotton wool and discarding a minimum of two draws of foremilk before taking the sample (Cockcroft 2015). The milk samples were transported in a cooling bag back to the lab. Storing the milk sample in cold temperature, maximum 4 °C, when transporting is essential to prevent bacterial growth (Scott *et al.* 2011).

Antibiotic agents chosen to study in this project were cefoxitin, meropenem, ciprofloxacin, gentamicin and tigecycline for *E. coli* and benzylpenicillin, cefoxitin, ciprofloxacin, gentamicin and tigecycline for *S. aureus*. The antibiotics chosen for this project is based on EUCAST available cut-off values and on last year's project regarding AMR resistance found in cats in Maasai Mara (Diamanti Barredal 2024) in order to be able to compare the results between studies.



Coordinates (Karen Blixen Camp in
Mara North Conservancy):
1°11'14.2"S 35°03'26.6"E

Figure 2: Mararianta, Kenya, is marked with red dot. Figure created with Mapchart.net by Emilia Schultz (2023).

3.2 Bacterial growth

The milk samples were inoculated with a sterile 10 µl plastic loop onto a SELMA PLUS agar which is a selective cultivation plate for identification of growth of typical clinical mastitis pathogens. The SELMA PLUS agar plates were incubated for 18-24 hours in 37 °C aerobically. The bacterial growth on the SELMA PLUS agar plates were assessed according to an identification brochure from the Swedish Veterinary Agency (SVA 2020). SELMA PLUS agar consists of four different agar solutions on one plate: bovine blood agar with esculin, MacConkey agar, PGUA agar and Mannitol salt agar. On the SELMA PLUS plate, *E. coli* colonies grow on blood agar, MacConkey agar and PGUA agar. *E. coli* colonies are opaque and grey-white on blood agar. The colony size is approximately 2-3 mm, and some strains have a clear haemolysis zone. *E. coli* colonies are coloured red-violet on MacConkey agar and yellow-green on PGUA agar (SVA 2020; VetBact 2023a). On the SELMA PLUS plate, *S. aureus* colonies grow on blood agar and Mannitol salt agar. *S. aureus* colonies are opaque and white or yellow on blood agar. The colony size is 2-3 mm and double haemolysis is often seen, consisting of a clear haemolysis zone near the colony and a diffuse haemolysis zone beyond the first. *S. aureus* colonies are coloured lemon yellow on Mannitol salt agar (SVA 2020; VetBact 2023b). Suspected colonies of *E. coli* and *S. aureus* cultures were re-cultured upon blood agar plates and incubated for 18-24 hours in 37 °C for further identification.

3.3 Typing of bacteria

The suspected *E. coli* colonies were confirmed with a positive potassium hydroxide test (KOH) and a positive spot indole test (VetBact 2017b; c). A KOH test is used to identify gram-negative bacteria. Potassium hydroxide is mixed with one to two colonies of bacteria from the blood agar plate and stirred with a sterile inoculating plastic loop against a microscope slide. Potassium hydroxide dissolves the cell wall of gram-negative bacteria, releasing cell contents including DNA. The solution will then become viscous and stick to the plastic loop which represents a positive test result. The spot indole test is used to identify bacteria which expresses the enzyme tryptophanase, such as *E. coli*. Tryptophanase hydrolyses tryptophan to indole inter alia. A few drops of indole reagent (p-Dimethylamino-cinnamaldehyde) are placed on filter paper. Bacteria from one colony from the blood agar plate is then rubbed on to the filter paper on the reagent with a sterile plastic loop. If indole is present the reagent produces a blue color within ten seconds which indicates a positive test result.

The suspected *S. aureus* colonies were confirmed with a positive latex slide agglutination test and a positive catalase test (VetBact 2017a). The catalase test is used to identify bacteria that produces the enzyme catalase, such as *S. aureus*. Bacteria with the catalase enzyme produce oxygen in the presence of hydrogen peroxide. One drop of 3% H₂O₂ is put on a microscope slide and bacteria from one colony on the blood agar plate is rubbed in the solution with a sterile inoculating plastic loop. If the test is positive, gas formation will be seen in form of bubbles. The latex slide agglutination test is used to differentiate *S. aureus* from other *Staphylococci* by detecting clumping factor, capsular polysaccharides and Protein A. The test was performed by using DrySpot Staphytest Plus, which is a “reaction card” with rabbit IgG and porcine fibrinogen coated on blue latex particles. One drop of saline solution is put on the reaction card but not onto the blue latex particles. Five colonies are dissolved in the saline solution with a sterile plastic loop until the suspension is smooth. The suspension is then rubbed on the test latex. The test is positive if agglutination is seen in the blue suspension within 20 seconds (OXID 2012).

3.4 Disk diffusion

Disk diffusion is a quality method to test antimicrobial susceptibility on a great number of bacterial pathogens and it covers a large range of antimicrobial agents that can be tested. It requires no special equipment which makes it suitable for this project as it can be performed in a field laboratory. The disk diffusion technique used in this project was the EUCAST method, which is a standardized method

(EUCAST 2024a). A few single colonies of *S. aureus* and *E.coli*, verified with the techniques described above, were separately mixed with a sterile inoculating plastic loop in sterile saline solution until the density of 0.5 McFarland (0.4 -0.6 McFarland is acceptable) was achieved, measured with a photometric device. The inoculum was streaked with a sterile cotton swab upon Mueller-Hilton agar (MH) in six columns and then spread evenly over the whole surface of the agar plate in six directions. The inoculum suspension was used within 60 minutes. Antimicrobial discs were applied onto the inoculated agar within 15 minutes, with help from a template to ensure the same distance between the discs. The recommended incubation conditions for *Staphylococcus* spp. and *Enterobacterales* are 35 ± 1 °C in air for 18 ± 2 h. The zone of inhibition was assessed using a ruler and measured to the nearest millimeter from the edges where bacterial growth were not present. The reading was done directly from above with a light source from above and with the plate against a dark background. If the measurement is below the reference value by EUCAST, it should be interpreted as resistant (EUCAST 2024b). Interpretation of benzylpenicillin inhibition is an exception and should be examined from the front of the plate while held up to a transmitted light. If the zone is measured to ≥ 26 mm and if the edges of the inhibition zone is sharp, it should be interpreted as resistant (EUCAST 2024a).

3.5 Literature search and programs used

Relevant literature was searched for in google scholar, pubmed and primo using the search words: Mastiti*, SCM, Subclinical mastitis, Dairy, Cow, Cattle, Zebu, Milk, Udder health, CMT, Kenya, Africa, Maasai, Massai Mara, Masai, Pastoralist, Grazing, AMR, Antimicrobial resistance, Resistance mechanism, Hygiene, Antibiotic, Data, Guidelines, Prevalence, Management, *S. aureus*, *E.coli*, and One Health in different combinations. Data was compiled in Microsoft Excel and KoboToolbox and the paper was written in Microsoft Word.

4. Results

A total of 124 cows from 18 different farms were CMT tested. 109 cows had a CMT score of 0-2 on all udder quarters. 15 cows had one or more udder quarters with a CMT score of 3 or above. From the 15 cows, 22 milk samples were collected, three of them from quarters with CMT score 4 and 19 from quarters with a CMT score of 3 (table 3). Additionally, ten swab samples were collected from bulk tank containers and cat bowls from five different farms. Bacterial culturing was performed on 32 samples in total.

In the tested population, 12.1% of the cows and 4,4% of the tested quarters, had subclinical mastitis (table 3). *S. aureus* was found to be the predominant bacteria for subclinical mastitis in 1.6% of the population (table 4).

Table 3: CMT scoring among examined Maasai cows

CMT score among 124 tested	Number of cows	Prevalence rate
0-2	109	0.879
3	15	0.121
4	3	0.024
5<	0	0

Table 4: Bacterial isolates from milk samples

Bacterial isolates	Number of isolates	Prevalence rate amongst CMT tested cows
<i>Escherichia coli</i>	0	0
<i>Staphylococcus aureus</i>	2	0.016
Mixed flora/ miscellaneous agents	15	0.12
No growth	5	0.04

Table 5: Bacterial isolated from bulk tank containers and cat bowls

Origin	Bacterial isolates	Number of isolates
Cat bowl	<i>Escherichia coli</i>	1
	<i>Staphylococcus aureus</i>	0
	Mixed flora/ miscellaneous agents	3
	No growth	1
Bulk tank container	<i>Escherichia coli</i>	0
	<i>Staphylococcus aureus</i>	0
	Mixed flora/ miscellaneous agents	5
	No growth	0

4.1 Inhibition of bacterial growth

Three collected samples had growth of either *E. coli* or *S. aureus* and were analysed for antimicrobial resistance. The *E. coli* was isolated from a swab sample collected from a cat bowl and the *S. aureus* isolates came from cows suffering from subclinical mastitis. The reference values for the selected antimicrobials for respective bacteria is presented in table 6. The measured zone of inhibited growth for each tested bacterial isolate is presented in table 7. The *E. coli* isolate, and one *S. aureus* isolate were susceptible to all tested antimicrobial agents. One *S. aureus* was susceptible to all tested agents except Benzylpenicillin which indicates that it is penicillinase producing.

Table 6: Reference values for zone inhibition in the Disk Diffusion test as published by EUCAST

Reference values for <i>Enterobacteriales</i> , including <i>Escherichia coli</i> , for chosen antimicrobial substances as published by EUCAST (EUCAST 2024b). A zone of inhibition below the reference value confirms resistance to this type of antibiotic.	Reference values for <i>Staphylococcus</i> spp., including <i>Staphylococcus aureus</i> , for chosen antimicrobial substances as published by EUCAST (EUCAST 2024b). A zone of inhibition below the reference value confirms resistance to this type of antibiotic.
<ul style="list-style-type: none"> • Cefoxitin, FOX: 19 mm. • Ciprofloxacin, CIP: 22-25 mm. • Gentamicin, CN: 17 mm. • Meropenem, MEM: 16-22 mm • Tigecycline, TGC: 18 mm. 	<ul style="list-style-type: none"> • Benzylpenicillin, P: 26 mm • Cefoxitin, FOX: 22 mm • Ciprofloxacin, CIP: 17-50 mm. • Gentamicin, CN: 18 mm. • Tigecycline, TGC: 19 mm.

Table 7: Antimicrobial susceptibility testing, result of isolated strains of *E. coli* and *S. aureus*

Bacterial isolates	Tested antimicrobial agents						
	S/I/R	P	FOX	CIP	CN	MEM	TGC
<i>E. coli</i> (n = 1)	S	-	22	27mm	19mm	29mm	19mm
<i>S. aureus</i> (n = 2)							
<i>S. aureus</i> 1	S	29mm	32mm	30mm	23mm	-	20mm
<i>S. aureus</i> 2	I	12mm	30mm	27mm	23mm	-	21mm

P = benzylpenicillin

FOX = cefoxitin

CIP = ciprofloxacin

CN = gentamicin

MEM = meropenem

TGC = tigecycline

4.2 Prevalence of antibiotic resistance in milk from Mararianta

The number of bacterial isolates were not sufficient to make a statement on prevalence of AMR in *S. aureus* and *E. coli* in milk from cows in Mararianta. Two isolates from milk samples were identified as *S. aureus* where one was resistant to benzylpenicillin and susceptible to all other tested antibiotics and the other isolate was susceptible to all tested antibiotics. *E. coli* was not found in the milk samples collected directly from dairy cows. One isolate from one cat bowl was identified as *E. coli* and this isolate was susceptible to all tested antibiotics. Due to the scarce number of isolates, the AMR testing cannot be extrapolated to *S. aureus* or *E. coli* induced SCM in Mararianta.

5. Discussion

The purpose of this study was to determine the potential risk of transmission of AMR bacteria from milk to the Maasai cats along with the Maasai human community and the nearby environment. This project has been among the first to examine prevalence of SCM and the potential spread of AMR bacteria through milk by the Maasai cows in Kenya. Among 124 CMT tested cows, 12.1% of the cows had SCM. This is a prodigiously low prevalence in comparison to the findings by Motaung *et al.* (2017) where 60-80 % of cows in Kenya had SCM. The predominant pathogen in their study was *S. aureus*. This project focused on AMR in isolated *S. aureus* and *E. coli*. 9,1 % of the analyzed milk samples had growth of *S. aureus* in this study, miscellaneous bacterial growth or growth of a mixed flora were much more common. These results indicate an overall better udder health status on the Maasai cows in comparison to cows held under other production systems in Kenya. Although, the scarce number of isolates found in this study makes it hard to draw conclusions about AMR prevalence in *S. aureus* or *E. coli* found in milk or milk bulk tanks/cat bowls. This could indicate that the high AMR levels found in the Maasai cats derive from other sources than milk due to the lack of bacterial content. The source of high AMR presence in cats is yet to be investigated.

5.1 Potential causes of SCM

The prevalence of SCM found in this study was surprisingly low. The development of SCM is influenced by many different risk factors including type of production system, quantity of production, exposure to environmental pathogens, hoof health, energy balance, previous illness and genetics (Arnott *et al.* 2017; Cheng & Han 2020). The Maasai cattle herds that were investigated in this study were free roaming during the day and kept within a fence during the night. This way of keeping the cattle is most likely one part of the unexpectedly low prevalence of mastitis found. Dairy cows kept within a grazing production system have a lower frequency of mastitis than those kept within a confinement system (Washburn *et al.* 2002; Arnott *et al.* 2017). More studies on a larger scale are desired to retrieve information about prevalence on SCM, bacterial contents and AMR in the Maasai cows. Likewise, it would be desirable to further investigate if and why the

prevalence of SCM is lower among Maasai pastoralist cows than cows held under other production systems in Kenya and learn from the protective management implemented by the Maasai.

5.2 One Health – The risk of AMR spreading

The risk of AMR spreading from the milk from Maasai cows is, based on this study, not a probable cause for the high prevalence of AMR among cats in Mararianta. However, the studied population is a small portion of the total number of Maasai cows and restricted to a small area in comparison to the entire Maasai owned land and it cannot be excluded that prevalence of AMR bacteria in milk is higher in other parts of Mararianata. Based on the findings in this study and observations during sample collection a potential source of AMR bacteria previously found in cats was identified. During sample collection a sick lamb, with poor prognosis, was being treated with oxytetracycline by a local veterinarian. The animal was given one chance and if the treatment would prove un-successful, the meat would be given to the dogs and cats of the household. When asked if this was a common solution with severely sick animals, the answer was that it happens often. This is a possible contribution to how cats become carriers of AMR bacteria. It is not known to which extent this occurs although it could be a basis for further research to examine to which extent withdrawal periods is being implemented in the area, both for human and animal consumption. The exposure to antibiotic residues is high when milk and meat is consumed within the withdrawal period (Anika *et al.* 2019; Vougat Ngom *et al.* 2020). Antibiotic use is the main driver of the development of antibiotic resistance. With increased use of antibiotics the exposure to antibiotic residues increases as well. Antibiotic residues are easily spread as it is secreted through faeces and urine into the environment, contaminating the soil, water, plants and wildlife as a few examples. When consuming meat and milk with a high content of antibiotic residues or being exposed to antibiotic residues from the environment, the microbiome within the gastrointestinal system could be altered and a development of genes coding for AMR is a possible outcome. This may cause failure of antibiotic treatment if an infection develops (Polianciuc *et al.* 2020). This emphasizes the importance of avoiding misuse of antibiotic agents in order to impede the rapid AMR progression (Barbosa & Levy 2000). In the Maasai community antibiotics are used without regulation and prior to seeking professional advice (Mangesho *et al.* 2021; Mburu *et al.* 2023). This lack of regulation represents a probable cause for selection of resistance in the area which is of great concern regarding the One Health perspective. In the work towards a sustainable use of antimicrobial substances it is important to consider the effort that is put in by every community, sector and country.

5.3 Limitations and bias

The largest limitation in this study was the unexpectedly low prevalence of cows suffering from subclinical mastitis and by that the low prevalence of *S. aureus* and *E. coli* in milk. The project was limited geographically to the area adjacent to Karen Blixen Camp due to lack of transportation availability. If a greater distance could be traveled, it would have improved the research's applicability to a larger area. The time limit for the sample collection and culturation of bacteria was 16 days, which made the total number of samples lower than desirable. The milk samples were collected in an as aseptic manner that was allowed according to the conditions. It could affect the result of bacterial growth with eventual contamination of the samples.

5.4 Source of error

No quality control bacterial strains were brought to the field laboratory due to regulations when transporting biological materials across country borders. There was therefore no quality control testing when doing the disk diffusion test or Staphylect test.

6. Conclusions

The result of this study indicates that the Maasai cows within the studied area have a lower prevalence of SCM than cows in conventional farms in Kenya. Although 33% of the examined bacterial isolates were resistant to one or more antibiotics, the number of tested isolates were too few (n=3) to make conclusions about the importance of milk as a source of AMR bacteria. Based on the low prevalence of SCM and low prevalence of *S. aureus* and *E. coli* bacteria in milk it is not likely that resistant bacteria from milk is a major source of AMR in the region.

References

- Algammal, A.M., Hetta, H.F., Elkelish, A., Alkhalifah, D.H.H., Hozzein, W.N., Batiha, G.E.-S., El Nahhas, N. & Mabrok, M.A. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and Drug Resistance*, 13, 3255–3265. <https://doi.org/10.2147/IDR.S272733>
- Anika, T.T., Al Noman, Z., Ferdous, Most.R.A., Khan, S.H., Mukta, M.A., Islam, Md.S., Hossain, Md.T. & Rafiq, K. (2019). Time dependent screening of antibiotic residues in milk of antibiotics treated cows. *Journal of Advanced Veterinary and Animal Research*, 6 (4), 516–520. <https://doi.org/10.5455/javar.2019.f376>
- Århem, K. (1989). Maasai food symbolism: The cultural connotations of milk, meat, and blood in the pastoral Maasai diet. *Anthropos*, 84 (1/3), 1–23
- Arnott, G., Ferris, C.P. & O’Connell, N.E. (2017). Review: welfare of dairy cows in continuously housed and pasture-based production systems. *Animal*, 11 (2), 261–273. <https://doi.org/10.1017/S1751731116001336>
- Barbosa, T.M. & Levy, S.B. (2000). The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates*, 3 (5), 303–311. <https://doi.org/10.1054/drup.2000.0167>
- Blackmore, E., Guarin, A., Vorley, W., Alonso, S. & Grace, D. (2022). Kenya’s informal milk markets and the regulation–reality gap. *Development Policy Review*, 40 (3), e12581. <https://doi.org/10.1111/dpr.12581>
- Blumberg, P.M. & Strominger, J.L. (1974). Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. *Bacteriological Review*, 38 (3), 291–335. <https://doi.org/10.1128/br.38.3.291-335.1974>
- Brown, K., Mugoh, M., Call, D.R. & Omulo, S. (2020). Antibiotic residues and antibiotic-resistant bacteria detected in milk marketed for human consumption in Kibera, Nairobi. *PLoS One*, 15 (5), e0233413. <https://doi.org/10.1371/journal.pone.0233413>
- Bush, K. & Bradford, P.A. (2016). β -lactams and β -lactamase inhibitors: An overview. *Cold Spring Harbor Perspectives in Medicine*, 6 (8), a025247. <https://doi.org/10.1101/cshperspect.a025247>
- Byström, R. (2023). *The seropositivity of Toxoplasma gondii in free-roaming domesticated cats in Masai Mara Kenya*. Swedish University of Agricultural Sciences. Veterinary Medicine Programme. <http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-18620>

- Chaves, B.J. & Tadi, P. (2023). Gentamicin. In: *StatPearls*. StatPearls Publishing.
<http://www.ncbi.nlm.nih.gov/books/NBK557550/> [2025-01-16]
- Chen, C., Chen, Y., Wu, P. & Chen, B. (2014). Update on new medicinal applications of gentamicin: Evidence-based review. *Journal of the Formosan Medical Association*, 113 (2), 72–82. <https://doi.org/10.1016/j.jfma.2013.10.002>
- Cheng, W.N. & Han, S.G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments — A review. *Asian-Australasian Journal of Animal Sciences*, 33 (11), 1699–1713. <https://doi.org/10.5713/ajas.20.0156>
- Cockcroft, P. (2015). *Bovine Medicine*. 3. ed. John Wiley & Sons, Incorporated.
<http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1998778>
 [2024-08-14]
- Diamanti Barredal, A. (2024). *Prevalence of antibacterial resistance in domestic cats in Masai Mara, Kenya - A One Health perspective*. Swedish University of Agricultural Sciences. Veterinary Medicine Programme.
<http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-19850>
- Doble, A. (2007). Quinolones. In: Enna, S.J. & Bylund, D.B. (eds) *xPharm: The Comprehensive Pharmacology Reference*. Elsevier. 1–3.
<https://doi.org/10.1016/B978-008055232-3.61012-8>
- EUCAST (2024a). *Antimicrobial susceptibility testing EUCAST disk diffusion method*. European Committee on Antimicrobial Susceptibility Testing. www.eucast.org
 [2024-08-14]
- EUCAST (2024b). *Breakpoint tables for interpretation of MICs and zone diameters*. European Committee on Antimicrobial Susceptibility Testing.
https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf
- FAO (2024). *Code of Hygienic Practice for Milk and Milk Products*. Food and Agriculture Organization.
https://www.fao.org/fileadmin/user_upload/livestockgov/documents/CXP_057e.pdf
- Garine, I. de & Ibrahim, N. (2001). *Drinking: Anthropological Approaches*. Berghahn Books.
https://books.google.se/books?hl=sv&lr=&id=AHIBpn_fCTMC&oi=fnd&pg=PA87&dq=milk+ritual+maasai&ots=ce3cpWAjIZ&sig=ape4LelqoTJ_CY_B3qTS7UHde0w&redir_esc=y#v=onepage&q=milk%20ritual%20maasai&f=false
- Giguère, S., Prescott, J.F. & Dowling, P. (2013). *Antimicrobial Therapy in Veterinary Medicine*. 5. ed John Wiley & Sons, Incorporated.
<http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1324465>
 [2024-08-14]
- Greer, N.D. (2006). Tigecycline (Tygacil): the first in the glycylicycline class of antibiotics. *Proceedings (Baylor University. Medical Center)*, 19 (2), 155–161
- Hall, B.G. & Barlow, M. (2004). Evolution of the serine β -lactamases: past, present and future. *Drug Resistance Updates*, 7 (2), 111–123.
<https://doi.org/10.1016/j.drug.2004.02.003>

- Hansen, P.J. (2004). Physiological and cellular adaptations of zebu cattle to thermal stress. *Animal Reproduction Science*, 82–83, 349–360.
<https://doi.org/10.1016/j.anireprosci.2004.04.011>
- Karen Blixen Camp Trust (2023). *The Mara Cat Project*.
<https://karenblixencamptrust.org/program/mara-cat-project/> [2024-08-16]
- Kenya Dairy Board (2023). *Dairy Regulatory Services*.
<https://www.kdb.go.ke/index.php/dairy-regulatory-services/> [2024-09-03]
- Kimera, Z.I., Mshana, S.E., Rweyemamu, M.M., Mboera, L.E.G. & Matee, M.I.N. (2020). Antimicrobial use and resistance in food-producing animals and the environment: an African perspective. *Antimicrobial Resistance and Infection Control*, 9, 37. <https://doi.org/10.1186/s13756-020-0697-x>
- Laxminarayan, R., Sridhar, D., Blaser, M., Wang, M. & Woolhouse, M. (2016). Achieving global targets for antimicrobial resistance. *Science*, 353 (6302), 874–875. <https://doi.org/10.1126/science.aaf9286>
- Lindell, J. (2013). *Maasai herding and milking strategies – A case study of goals and decision-making within the household*. (452). Swedish University of Agricultural Sciences. Agricultural Science Programme, Animal Science.
<http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-2131>
- Mangesho, P.E., Caudell, M.A., Mwakapeje, E.R., Ole-Neselle, M., Kabali, E., Obonyo, M., Dorado-Garcia, A., Valcarce, A., Kimani, T., Price, C., Eckford, S. & Fasina, F.O. (2021). “We are doctors”: Drivers of animal health practices among Maasai pastoralists and implications for antimicrobial use and antimicrobial resistance. *Preventive Veterinary Medicine*, 188, 105266.
<https://doi.org/10.1016/j.prevetmed.2021.105266>
- Mara Conservancy (2024). *Mara Conservancy - Protecting the Mara Triangle*.
<https://www.maratriangle.org> [2024-08-19]
- Mbindyo, C.M., Gitao, G.C. & Mulei, C.M. (2020). Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajiado Counties, Kenya. *Veterinary Medicine International*, 2020, 8831172. <https://doi.org/10.1155/2020/8831172>
- Mburu, C.M., Bukachi, S., Majiwa, H., Ongore, D., Baylis, M., Mochabo, K., Fevre, E. & Howland, O. (2023). Prioritization of livestock diseases by pastoralists in Oloitoktok Sub County, Kajiado County, Kenya. *PLoS One*, 18 (7), e0287456.
<https://doi.org/10.1371/journal.pone.0287456>
- Mckellar, Q.A., Sanchez Bruni, S.F. & Jones, D.G. (2004). Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics*, 27 (6), 503–514. <https://doi.org/10.1111/j.1365-2885.2004.00603.x>
- Moroni, P., Nydam, D.V., Ospina, P.A., Scillieri-Smith, J.C., Virkler, P.D., Watters, R.D., Welcome, F.L., Zurakowski, M.J., Ducharme, N.G. & Yeager, A.E. (2018). 8 - Diseases of the Teats and Udder. In: Peek, S.F. & Divers, T.J. (eds) *Rebhun's Diseases of Dairy Cattle*. 3. ed. Elsevier. 389–465.
<https://doi.org/10.1016/B978-0-323-39055-2.00008-5>

- Motaung, T.E., Petrovski, K.R., Petzer, I.-M., Thekiso, O. & Tsilo, T.J. (2017). Importance of bovine mastitis in Africa. *Animal Health Research Reviews*, 18 (1), 58–69. <https://doi.org/10.1017/S1466252317000032>
- Mshana, S.E., Sindato, C., Matee, M.I. & Mboera, L.E.G. (2021). Antimicrobial use and resistance in agriculture and food production systems in Africa: A systematic review. *Antibiotics*, 10 (8), 976. <https://doi.org/10.3390/antibiotics10080976>
- Mtimet, N. & Karugia, J. (2020). Consumer perception of milk safety in Kenya. *Nairobi, Kenya: ILRI*. <https://cgspace.cgiar.org/server/api/core/bitstreams/89b9de08-bf24-454a-9dc2-e41b06b109f8/content>
- Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S.C., Browne, A.J., Chipeta, M.G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B.H.K., Kumaran, E.A.P., McManigal, B., Achalapong, S., Agarwal, R., Akech, S., Albertson, S., Amuasi, J., Andrews, J., Aravkin, A., Ashley, E., Babin, F.-X., Bailey, F., Baker, S., Basnyat, B., Bekker, A., Bender, R., Berkley, J.A., Bethou, A., Bielicki, J., Boonkasidecha, S., Bukosia, J., Carvalho, C., Castañeda-Orjuela, C., Chansamouth, V., Chaurasia, S., Chiurchiù, S., Chowdhury, F., Donatien, R.C., Cook, A.J., Cooper, B., Cressey, T.R., Criollo-Mora, E., Cunningham, M., Darboe, S., Day, N.P.J., Luca, M.D., Dokova, K., Dramowski, A., Dunachie, S.J., Bich, T.D., Eckmanns, T., Eibach, D., Emami, A., Feasey, N., Fisher-Pearson, N., Forrest, K., Garcia, C., Garrett, D., Gastmeier, P., Giref, A.Z., Greer, R.C., Gupta, V., Haller, S., Haselbeck, A., Hay, S.I., Holm, M., Hopkins, S., Hsia, Y., Iregebu, K.C., Jacobs, J., Jarovsky, D., Javanmardi, F., Jenney, A.W.J., Khorana, M., Khusuwan, S., Kisson, N., Kobeissi, E., Kostyanov, T., Krapp, F., Krumkamp, R., Kumar, A., Kyu, H.H., Lim, C., Lim, K., Limmathurotsakul, D., Loftus, M.J., Lunn, M., Ma, J., Manoharan, A., Marks, F., May, J., Mayxay, M., Mturi, N., Munera-Huertas, T., Musicha, P., Musila, L.A., Mussi-Pinhata, M.M., Naidu, R.N., Nakamura, T., Nanavati, R., Nangia, S., Newton, P., Ngoun, C., Novotney, A., Nwakanma, D., Obiero, C.W., Ochoa, T.J., Olivas-Martinez, A., Olliaro, P., Ooko, E., Ortiz-Brizuela, E., Ounchanum, P., Pak, G.D., Paredes, J.L., Peleg, A.Y., Perrone, C., Phe, T., Phommasone, K., Plakkal, N., Ponce-de-Leon, A., Raad, M., Ramdin, T., Rattanavong, S., Riddell, A., Roberts, T., Robotham, J.V., Roca, A., Rosenthal, V.D., Rudd, K.E., Russell, N., Sader, H.S., Saengchan, W., Schnall, J., Scott, J.A.G., Seekaew, S., Sharland, M., Shivamallappa, M., Sifuentes-Osornio, J., Simpson, A.J., Steenkeste, N., Stewardson, A.J., Stoeva, T., Tasak, N., Thaiprakong, A., Thwaites, G., Tigoi, C., Turner, C., Turner, P., Doorn, H.R. van, Velaphi, S., Vongpradith, A., Vongsouvath, M., Vu, H., Walsh, T., Walson, J.L., Waner, S., Wangrangsimakul, T., Wannapinij, P., Wozniak, T., Sharma, T.E.M.W.Y., Yu, K.C., Zheng, P., Sartorius, B., Lopez, A.D., Stergachis, A., Moore, C., Dolecek, C. & Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399 (10325), 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)

- Narok County Government (2024). *About Narok County*. <https://narok.go.ke/about-narok/> [2024-08-16]
- Omondi, G.P., Obanda, V., VanderWaal, K., Deen, J. & Travis, D.A. (2021). Animal movement in a pastoralist population in the Maasai Mara Ecosystem in Kenya and implications for pathogen spread and control. *Preventive Veterinary Medicine*, 188, 105259. <https://doi.org/10.1016/j.prevetmed.2021.105259>
- Onono, J.O., Wieland, B. & Rushton, J. (2013). Productivity in different cattle production systems in Kenya. *Tropical Animal Health and Production*, 45 (2), 423–430. <https://doi.org/10.1007/s11250-012-0233-y>
- OXID (2012). *DrySpot Staphylect Plus*. https://assets.fishersci.com/TFS-Assets/MBD/Instructions/X5241D.pdf?_ga=2.14100471.1740118901.1727707038-815164632.1726842045
- Polianciuc, S.I., Gurzău, A.E., Kiss, B., Ştefan, M.G. & Loghin, F. (2020). Antibiotics in the environment: causes and consequences. *Medicine and Pharmacy Reports*, 93 (3), 231–240. <https://doi.org/10.15386/mpr-1742>
- Roberts, J.A. & Lipman, J. (2006). Antibacterial dosing in intensive care. *Clinical Pharmacokinetics*, 45 (8), 755–773. <https://doi.org/10.2165/00003088-200645080-00001>
- Schukken, Y.H., Günther, J., Fitzpatrick, J., Fontaine, M.C., Goetze, L., Holst, O., Leigh, J., Petzl, W., Schuberth, H.-J., Sipka, A., Smith, D.G.E., Quesnell, R., Watts, J., Yancey, R., Zerbe, H., Gurjar, A., Zadoks, R.N. & Seyfert, H.-M. (2011). Host-response patterns of intramammary infections in dairy cows. *Veterinary Immunology and Immunopathology*, 144 (3), 270–289. <https://doi.org/10.1016/j.vetimm.2011.08.022>
- Scott, P., D. Penny, C. & Macrae, A. (2011). *Cattle Medicine*. 1. ed. Taylor & Francis Group. <http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1407686> [2024-08-14]
- Shitandi, A. & Sternesjö, Å. (2004). Prevalence of multidrug resistant *Staphylococcus aureus* in milk from large- and small-scale producers in Kenya. *Journal of Dairy Science*, 87 (12), 4145–4149. [https://doi.org/10.3168/jds.S0022-0302\(04\)73557-2](https://doi.org/10.3168/jds.S0022-0302(04)73557-2)
- Sohaili, A., Asin, J. & Thomas, P.P.M. (2024). The fragmented picture of antimicrobial resistance in Kenya: A situational analysis of antimicrobial consumption and the imperative for antimicrobial stewardship. *Antibiotics*, 13 (3), 197. <https://doi.org/10.3390/antibiotics13030197>
- SVA (2019). *California Mastitis Test (CMT)*. Statens veterinärmedicinska anstalt. <http://www.juverportalen.se/media/1136/cmt-20190412.pdf>
- SVA (2020). *SELMA och SELMA PLUS*. Statens veterinärmedicinska anstalt. https://www.sva.se/media/en1cowjt/selmabroschyr_171019.pdf
- SVA (2024a). *ESBL*. Statens veterinärmedicinska anstalt. <https://3b06bbe9-73a1-4198-b7fd-a3d5e6f3220c.azurewebsites.net/amnesomraden/antibiotika/anmalningspliktig-resistens/esbl> [2024-08-21]

- SVA (2024b). *Resistensbestämning – ESBL*. Statens veterinärmedicinska anstalt. <https://www.sva.se/amnesomraden/antibiotika/laboratoriediagnostik/resistensbestamning-esbl/> [2024-08-21]
- SVA (2024c). *Resistensbestämning – stafylokocker*. Statens veterinärmedicinska anstalt. <https://www.sva.se/amnesomraden/antibiotika/laboratoriediagnostik/resistensbestamning-stafylokocker/> [2024-08-14]
- VetBact (2017a). *Catalase test*. <https://www.vetbact.org/index.php?displayextinfo=30&vbsearchstring=katalas> [2024-09-30]
- VetBact (2017b). *Indole test*. <https://www.vetbact.org/index.php?displayextinfo=35&vbsearchstring=Indole> [2024-09-30]
- VetBact (2017c). *Potassium hydroxide test*. <https://www.vetbact.org/index.php?LANG=en&displayextinfo=117&vbsearchstring=KOH> [2024-09-30]
- VetBact (2023a). *Escherichia coli*. <https://www.vetbact.org/index.php?artid=68&vbsearchstring=E%20coli> [2024-11-07]
- VetBact (2023b). *Staphylococcus aureus subsp. aureus*. <https://www.vetbact.org/index.php?artid=20&vbsearchstring=s.%20aureus> [2024-11-07]
- VetBact (2023c). *Veterinärmedicinsk bakteriologi; Escherichia coli*. <https://www.vetbact.org/?artid=68> [2024-08-14]
- VetBact (2023d). *Veterinärmedicinsk bakteriologi; Staphylococcus aureus subsp. aureus*. <https://www.vetbact.org/?artid=20#> [2024-08-14]
- VÄXA (2023). *Cattle health statistics 2022–2023*. VÄXA. <file:///Users/danielmac/Downloads/Djurh%C3%A4lsostatistik%202022-2023.pdf>
- Vougat Ngom, R.R.B., Foyet, H.S., Garabed, R. & Zoli, A.P. (2020). Human health risks related to penicillin G and oxytetracycline residues intake through beef consumption and consumer knowledge about drug residues in Maroua, Far North of Cameroon. *Frontiers in Veterinary Science*, 7. <https://doi.org/10.3389/fvets.2020.00478>
- Washburn, S.P., White, S.L., Green, J.T. & Benson, G.A. (2002). Reproduction, mastitis, and body condition of seasonally calved Holstein and Jersey cows in confinement or pasture systems. *Journal of Dairy Science*, 85 (1), 105–111. [https://doi.org/10.3168/jds.S0022-0302\(02\)74058-7](https://doi.org/10.3168/jds.S0022-0302(02)74058-7)
- WHO (2017). *Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis*. World Health Organization. <file:///Users/danielmac/Downloads/WHO-EMP-IAU-2017.12-eng.pdf>
- WHO (2019). *Critically important antimicrobials for human medicine*. 6th rev. World Health Organization. <https://iris.who.int/handle/10665/312266> [2024-08-20]

- WHO (2022). *Global antimicrobial resistance and use surveillance system (GLASS) report*. World Health Organization, Licence: CC BY-NC-SA 3.0 IGO. <https://www.who.int/publications/i/item/9789240062702> [2024-08-13]
- WHO (2023). *World Health Organization Model List of Essential Medicines - The selection and use of essential medicines 2023: Web Annex A.*, World Health Organization. <https://iris.who.int/bitstream/handle/10665/371090/WHO-MHP-HPS-EML-2023.02-eng.pdf?sequence=1>
- Wieland, M. (2024). Mastitis in Cattle - Reproductive System. *MSD Veterinary Manual*. <https://www.msdrveterinary.com/reproductive-system/mastitis-in-large-animals/mastitis-in-cattle> [2024-11-07]
- WOAH (2022). *Annual report on antimicrobial agents intended for use in animals*. (7). <https://www.woah.org/app/uploads/2023/05/a-seventh-annual-report-amu-final.pdf> [2024-08-13]
- WOAH (2024a). *Antimicrobial resistance*. World Organisation for Animal Health. <https://www.woah.org/en/what-we-do/global-initiatives/antimicrobial-resistance/> [2024-08-23]
- WOAH (2024b). *List of Antimicrobial Agents of Veterinary Importance*. World Organisation for Animal Health. <https://www.woah.org/app/uploads/2021/06/amended-91gs-tech-03-amr-working-group-report-en.pdf>
- Zhang, G.-F., Liu, X., Zhang, S., Pan, B. & Liu, M.-L. (2018). Ciprofloxacin derivatives and their antibacterial activities. *European Journal of Medicinal Chemistry*, 146, 599–612. <https://doi.org/10.1016/j.ejmech.2018.01.078>

Popular science summary

One Health is a concept where the world is viewed as a unity between humans, animals and the environment. This means that we are dependent on each other and so is our health. The purpose of this study was to investigate if antimicrobial resistant bacteria occur in milk from the cows of the Maasai community in Kenya, using a One Health perspective approach. The study was done through the Mara Cat Project based on a previous master thesis from 2024, where an unexpectedly high prevalence of antimicrobial resistant *Escherichia coli* in semi-domestic cats was found. The Maasai cats live close to the local human population and are often fed milk from cows. Could milk be a source of antimicrobial resistance spreading to the cats? If this is the case, it is possible that a high prevalence of antimicrobial resistance in milk from dairy cows could spread further to the surrounding environment including wild animals and also to the human population. This is one of the first studies investigating antimicrobial resistant bacteria in milk from the cows in the Maasai pastoralist production system. The Maasai cows are held very traditionally, being hand milked twice a day and walk great distances for grazing every day.

Healthy udders are very important for a cow to be able to produce milk. One of the most usual diseases linked to the cows' udder is mastitis. Mastitis is an inflammatory response in the udder most commonly derived from infection. Visibly abnormal milk is seen in clinical mastitis and with the inflammation there can also be changes in the udder (heat, swelling, redness, pain) and further systemic changes such as fever. Subclinical mastitis is an infection without visible signs of local or systemic inflammation although it causes losses in milk yield. The infection often derives from bacteria and one of the most common bacteria found in subclinical mastitis in Kenya is *Staphylococcus aureus* (*S. aureus*). One of the most common bacteria found in clinical mastitis is *Escherichia coli* (*E. coli*). There are several other bacteria that cause mastitis, but these are the ones that this study is focused on. *S. aureus* is contagious and can spread between cows if there is poor hygiene when milking. *E. coli* is often spread when the cows' surrounding environment is of poor hygiene. In conclusion, hygiene management is very important to prevent mastitis, both clinical and subclinical.

Information about udder health and hygiene management within the pastoralist system in the Maasai community is hard to come by. There is some information about udder health to be found in Kenya, although this is from cows held under other production systems or other species of animals such as camels or goats. In Kenya there is limited data on how common mastitis is, although there have been studies that show pessimistic results, where subclinical mastitis has a prevalence of 60-80%. This is very high in comparison to for example Sweden where the prevalence is approximately 23-24%. *S. aureus* is the predominant pathogen occurring in milk samples on the African continent and it has the highest prevalence in Kenya compared to the rest of the continent. There is limited documentation on how much this impacts the economy and how much milk is being lost because of mastitis, although it is believed to be of great importance.

Antibiotics is in some cases used to treat mastitis in cows. Antibiotics is an antimicrobial substance, meaning it is a substance that kills or inhibit the growth of microorganisms (bacteria). Treatment with antimicrobial substances is often an effective way to reduce severity, length and further spread of a disease. There are many different types of antimicrobial substances with different effects on different bacteria. When treating a bacterial infection, it is important to use the “right” antibiotics, meaning one that will work on that specific bacteria for that specific infection.

The antimicrobial substances used in this study were benzylpenicillin, ceftiofur, meropenem, ciprofloxacin, gentamicin and tigecycline. Benzylpenicillin, ceftiofur and meropenem are one of the more commonly used antibiotic groups for treating common infections. They inhibit cell wall synthesis in the bacteria. ciprofloxacin is a synthetic antimicrobial agent that essentially blocks DNA replication in the bacteria. Gentamicin works through interference in protein synthesis in the bacteria. Tigecycline is a tetracycline which is a broad-spectrum antibiotic and works through interference in protein synthesis in the bacterium. Tetracyclines are the most used antimicrobial agent for treating animals globally and it is also classified as a Veterinary Critically Important Antimicrobial Agent meaning it is of great importance in the treatment of specific diseases with few alternative options.

Antimicrobial resistance is one of the biggest health threats in the world and is one of the top causes for human death worldwide every year. In 2019 there were approximately 1.27 million human deaths directly caused by antimicrobial resistance globally, and as high as 4.95 million human deaths were associated to antimicrobial resistance. The highest all-age death rate was in western sub-Saharan Africa. Antibiotic use is the main driver of the development of antibiotic resistance which makes it important to only use antibiotics when needed and for the

right indication, meaning a bacterial infection that is susceptible to the antibiotic of choice. Antimicrobial resistance works through four main ways; the bacteria inhibits the antibiotics from penetrating it, the bacteria pushes the antibiotic out from the bacteria, the bacteria inactivates/destroys the antibiotic before or after it entering the bacteria or the bacteria changes its surface making it impossible for the antibiotic to attach to and affect the bacteria. Bacteria can sometimes spread this knowledge to each other through for example genes coding for antimicrobial resistance.

Maasai Mara is a nature reserve located in southwestern Kenya. It is home to one of the world's highest densities of wildlife including the big five (lion, leopard, elephant, rhinoceros and buffalo) and many more. The Maasai population, often called Maasai pastoralists, live among the wildlife with their livestock. Their cattle mostly consist of the Zebu breed (*Bos indicus*) which is a popular breed in Africa because of its endurance to the hot climate, infectious diseases and insects. Cattle and milk have a major social and cultural value for the pastoralists of the Maasai community. The Maasai diet consists almost exclusively of milk, meat and blood of the livestock. The milking is done by women and by hand, often two times a day, in the morning before grazing and in the evening when the cattle return from pasture. The calves get to suckle during the milking.

The Maasai pastoralists can move over large areas to search for grass and water for their livestock. This is a crucial way of infectious diseases spreading between wildlife and the livestock because of a greater number of animals meeting. Livestock diseases are a part of the pastoralists' life in Kenya and the loss of animals or the loss of production could be a great challenge for their economic situation and lead to poverty. The pastoralists use both traditional and conventional methods to treat sick animals, although the typical treatment for various diseases are oxytetracycline (a broad spectrum antibiotic), penicillin and various dewormers through trial and error. They rarely seek guidance by veterinarians before treatment.

The result of this study shows that the cows had an unexpectedly low prevalence of subclinical mastitis. Among 124 tested cows, 12.1% of the cows had SCM. This is a prodigiously low prevalence in comparison both to Swedish data (23-24%) and other data from Kenya (60-80%). The number of bacterial isolates within this study were not sufficient to make a statement on prevalence of antimicrobial resistance in *S. aureus* and *E. coli* in milk from cows in Mararianta. Two isolates from milk samples were identified as *S. aureus* where one was resistant to benzylpenicillin and susceptible to all other tested antibiotics and the other isolate was susceptible to all tested antibiotics. One *E. coli* was found in a cat bowl from which also is too low a number to make a conclusion. Ultimately,

the scarce number of bacteria found and the low prevalence of subclinical mastitis tells us that the risk of antimicrobial resistance spreading from the milk from Maasai cows, is not a probable cause for the high prevalence of antimicrobial resistance among cats in Mararianta. However, the studied population is a small portion of the total number of Maasai cows in a small area in comparison to the entire Maasai owned land. It cannot be excluded that prevalence of antimicrobial resistant bacteria in milk is higher in other parts of Mararianta. A different source to high antimicrobial prevalence in the cats was identified during sample collection. A sick lamb, about to die, was being treated with a broad-spectrum antibiotic called oxytetracycline by a local veterinarian. The animal was given one last chance and if it were to die, the meat would be given to the dogs and cats of the household. It is not known how often this happens although it could be a basis for further research to examine to which extent withdrawal periods is being implemented in the area. A withdrawal period is the amount of time were antibiotic residues is high within the animal. Within the withdrawal period, antibiotic residues are high in milk, meat, faces and urine. When consuming meat and milk with a high content of antibiotic residues or being exposed to antibiotic residues from the environment, the microbiome within the gastrointestinal system could be altered and a development of genes coding for antimicrobial resistance could occur. This may cause failure of antibiotic treatment if an infection develops. The lack of regulation regarding antibiotics represents a probable cause for selection of resistance in the area which is of great concern regarding the One Health perspective. In the work towards a sustainable use of antimicrobial substances it is important to consider the effort that is put in by every community, sector and country. Further research is needed to establish the prevalence of both mastitis and antimicrobial resistance on a wider scale in the Maasai community. It is important for the community to be able to make informed decisions regarding their livestock and veterinary treatment, both for their own sake and for the One Health perspective at large.

Acknowledgements

First of all, I would like to thank my supervisor Josef Dahlberg, and assistant supervisor Dinah Seligsohn and Therese Hård for their invaluable guidance and support through the preparations, fieldwork, compilation and presentation of my thesis project.

I am deeply thankful toward Karen Blixen Camp for the privilege to stay in their camp and the possibility to conduct our research in their facilities. I would like to thank VH-fakultetens stiftelse and Kungliga skogs- och lantbruksakademin for their financial support that made it possible to perform the fieldwork in Kenya.

I would like to thank the team involved in making the cat project possible. Therese Hård who is the founder of the cat project and guided us during the castrations and spaying of the Maasai cats. Christophe Bujon and Esther Macharia for their guidance during surgery. I want to thank everyone who was present at the castrations and vaccinations of the cats in this year's cat project.

I am most grateful for the Maasai community for trusting us to do research on their cattle and trusting us with their cats. I am grateful to David Noosaron and Jaqueline for all the help during our sample collection. It would not have been possible without their navigation and interpretation. Thank you to my fellow master students from SLU; Annika Seeliger, Linnea Lindström and Azadeh Gholami for amazing company and all your help with the sample collection.

Finally, I would like to thank everyone else who had part in making this possible. Marina Falk for her help with the laboratory training. SVA, Next2vet and Thermo Fisher Scientific for material support.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. You will find a link to SLU's publishing agreement here:

- <https://libanswers.slu.se/en/faq/228318>

YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.