Goats and Sheep as Transmitters of Antibiotic-Resistant *Escherichia coli* and *Staphylococcus aureus* in Maasai Mara, Kenya

A One Health Perspective

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Independent Project • 30 credits
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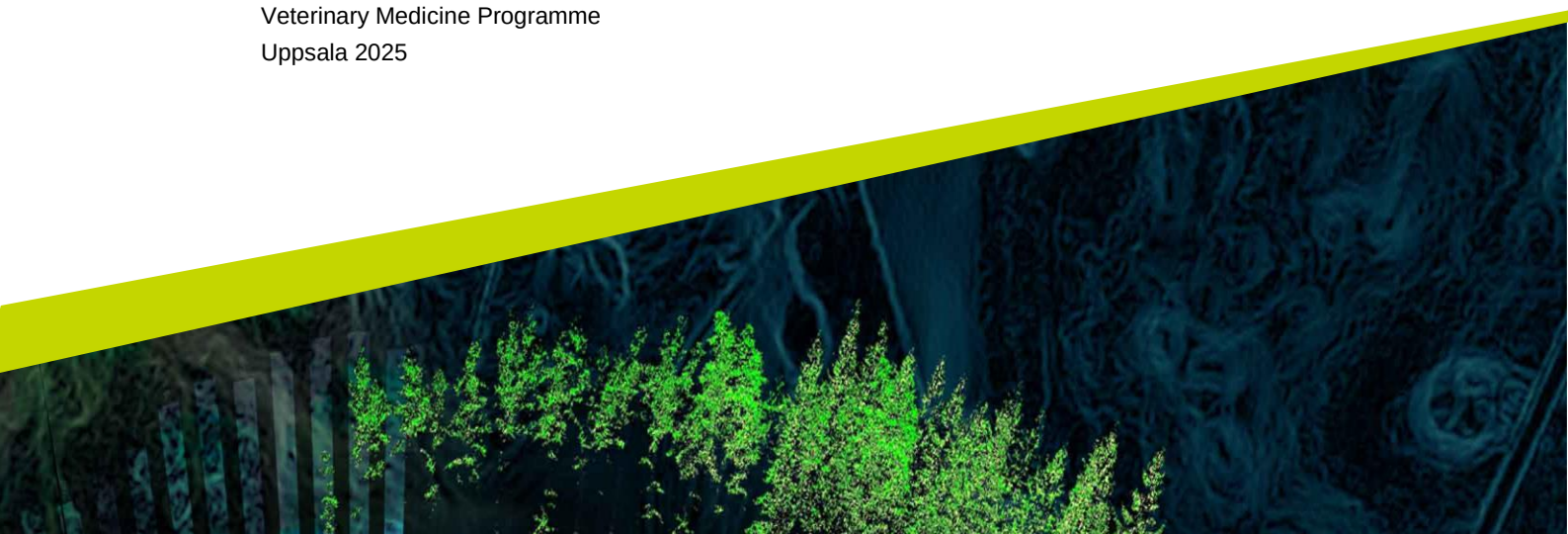
Uppsala 2025



Goats and Sheep as Transmitters of Antibiotic-Resistant *Escherichia coli* and *Staphylococcus aureus* in Maasai Mara, Kenya - A One Health Perspective

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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

Keywords:	antibiotic resistance, One Health, Maasai Mara, *Escherichia coli, Staphylococcus aureus*, subclinical mastitis, milk, sheep, goat

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Abstract

Antibiotic resistance is a pressing global health issue exacerbated by the global misuse of antibiotics across human, animal, and environmental interfaces. Antibiotic resistance is the phenomenon in which bacteria are no longer affected by the presence of antibacterial drugs. Resistant genes can occur naturally, as mutations in the bacterial genome or via acquired genes from other bacteria. Resistant bacteria can be transmitted between humans, animals and the environment via food, direct contact or waste. With increased antibiotic resistance, life-threatening diseases become more difficult to treat, leading to greater suffering and premature death.

In Kenya, the use of antibiotics is mostly unregulated and access to veterinary diagnostics is often limited. In a previous study, a high prevalence of antibiotic-resistant *E. coli* in domestic cats in Mararienta, Kenya, was discovered. Milk from livestock is a common food source for humans and cats in the region, making it a potential source of transmission of resistant bacteria.

This study investigated the prevalence of antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* in milk from goats and sheep within Mararienta, as well as in milk collection containers and feeding bowls for domestic cats. The method of choice for detecting antimicrobial resistance was disk diffusion, and the antibiotics used in this study were cefoxitin, tigecycline, gentamicin, ciprofloxacin, meropenem and benzylpenicillin. These antibiotics were chosen to be able to compare results to earlier studies done in the region and for their individual importance in human and veterinary therapy. A One Health perspective was employed, considering the close relations between humans, animals, and the environment in the region.

In total, 130 sheep and goats from 12 households were tested for subclinical mastitis using the California Mastitis Test (CMT), since only animals with subclinical mastitis were of interest. Milk samples (n=28) and swabs from milk containers (n=5) and feeding bowls (n=5) were analyzed for bacterial growth and antibiotic susceptibility. Despite occurrence of mixed bacterial flora in milk containers and feeding bowls, no antibiotic-resistant *E. coli* or *S. aureus* were isolated from milk samples or swabs. Only one *E. coli* strain was identified in a feeding bowl but was susceptible to all tested antibiotics.

The low prevalence of subclinical mastitis and minimal bacterial growth in milk samples suggest that milk is an unlikely source of antibiotic resistance transmitted to the domestic cats. However, it was noted that carcasses from livestock treated with antibiotics were often fed to cats and dogs. The unregulated use of antibiotics and lack of knowledge for the withdrawal period of antibiotics were therefore identified as potential risks for the spread of antibacterial resistance.

Keywords: antibiotic resistance, One Health, Maasai Mara, *Escherichia coli*, *Staphylococcus aureus*, subclinical mastitis, milk, sheep, goat

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Abbreviations

AMR	Antimicrobial Resistance
CMT	California Mastitis Test
ESBL	Extended Spectrum Beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ILCA	International Livestock Centre of Africa
KNBS	Kenya National Bureau of Statistics
KOH	Potassium Hydroxide
MIC	Minimum Inhibitory Concentration
MRL	Maximum Residue Limit
MRSA	Methicillin-resistant *Staphylococcus aureus*
OECD	Organization for Economic Cooperation and Development
PBP	Penicillin-binding Protein
PCR	Polymerase Chain Reaction
SLU	Swedish University of Agricultural Sciences
SVA	Swedish Veterinary Agency
WHO	World Health Organization

1. Introduction

Antibiotics are one of, if not the, most important group of drugs used in modern medicine today (WHO 2023a). Without the use of antibiotics, common infections that are treatable today and surgical procedures can become fatal. Unfortunately, globalization and misuse of antibiotics in humans, animals and agriculture has accelerated the development of antimicrobial and antibiotic resistance. In 2019, it is estimated that antimicrobial resistance (AMR) was directly responsible for 1.3 million deaths and contributed to 5 million deaths globally (Murray *et al.* 2022).

In Kenya, the problem of antibiotic resistance is exacerbated by inadequate healthcare systems, limited diagnostic capabilities, poor hygiene, lack of knowledge about correct use of antibiotics and their largely unregulated use (WHO 2022). Due to the lack of adequate surveillance and insufficient diagnostics, the prevalence of antibiotic resistance is mostly unknown in large areas of the country, including in the Maasai Mara.

A study from 2024 by Diamanti Barredal found that 22% of domestic cats in the Mararienta district in the north of the Maasai Mara conservatory were carriers of resistant *Escherichia coli* (Diamanti Barredal 2024). This prevalence is quite high, compared to a study by Albrechtova *et al.* (2012) in northern Kenya where only 4% of domestic cats were carriers. However, it is still unknown how the cats became carriers since the vast majority are not treated with antibiotics when sick (Diamanti Barredal 2024). In another study from 2023, conducted in the same village, they found that the cats were mostly fed with raw milk from livestock owned by the Maasai people (Schultz 2023). It is therefore of interest to investigate whether sheep and goats could be the primary carriers and transmitters of antibiotic-resistant bacteria. Since the livestock graze over large areas in the Maasai Mara, live in close contact with wildlife and provide milk not only to the cats but also their owners, a One Health and conservational approach is of interest in this study.

Escherichia coli and *Staphylococcus aureus* were used as indicator bacteria since they are two of the most prevalent opportunistic bacterial pathogens in both humans and animals and central components in the emergence of antibiotic resistance (WHO 2023a).

2. Literature Review

2.1 Antibacterial Drugs

Antibiotics are drugs used to treat bacterial infections (Patel *et al.* 2024). They are commonly classified into two main categories based on their *in vitro* effect on bacteria: bacteriostatic and bactericidal. Bacteriostatic agents prevent bacterial growth whereas bactericidal agents kill bacteria.

Antibacterial agents are also classified for their mechanism of action and/or chemical structure (Patel *et al.* 2024). Some of the most common groups are beta-lactams (e.g. penicillins, cephalosporins and carbapenems), aminoglycosides (e.g. gentamicin), tetracyclines (e.g. tigecycline) and fluoroquinolones (e.g. ciprofloxacin).

Additionally, antibiotics can be divided into groups according to their pharmacodynamic properties, having either concentration-dependent or time-dependent activity. Concentration-dependent activity means that the efficacy of bactericidal effect increases as the concentration of the drug increases. Time-dependent activity means that the efficacy of bactericidal activity is determined by the duration of time when the *in vivo* level of the antibiotic is higher than the minimum inhibitory concentration (MIC). That means the longer the *in vivo* concentration is higher than the MIC, the more effective the bactericidal activity is.

2.1.1 Antibiotic Substances

The antibiotics used in this study were tigecycline, gentamicin, ciprofloxacin, cefoxitin, meropenem and benzylpenicillin. Their group, activity, mechanism of action and common use in humans are shown in Table 1.

Table 1. Summary of the antibiotic substances used in this study.

Antibiotic	Group	Activity	Mechanism of action	Areas of use in humans
Tigecycline[1]	Tetracycline	Broad-spectrum, bacteriostatic, time-dependent	Inhibition of cell wall synthesis by binding the 30S ribosome	Last resort against multi-drug resistant *Enterobacterales*
Gentamicin[2]	Aminoglycoside	Gram-negative bacteria, bactericidal, concentration-dependent	Inhibition of cell wall synthesis by binding the 30S ribosome	Peritonitis, meningitis, septicaemia, urinary tract infections
Ciprofloxacin[3]	Fluoroquinolone	Broad-spectrum, bactericidal, concentration-dependent	Inhibition of DNA-replication	Pneumonia, skin infections, anthrax, salmonellosis, typhoid fever
Cefoxitin[4]	Beta-lactam; cephalosporin	Gram-negative bacteria, bactericidal, time-dependent	Inhibition of cell wall synthesis by blocking PBPs	Respiratory infections
Benzylpenicillin[5]	Beta-lactam; penicillin	Gram-positive bacteria, bactericidal, time-dependent	Inhibition of cell wall synthesis by blocking PBPs	Respiratory infections, syphilis
Meropenem[6]	Beta-lactam: carbapenem	Gram-negative bacteria, bactericidal, time-dependent	Inhibition of cell wall synthesis by blocking PBPs	Meningitis, pneumonia, peritonitis, urinary tract infections

PBP = Penicillin-binding Protein

[1] (Greer 2006; Korczak *et al.* 2024)
[2] (Chaves & Tadi 2024)
[3] (Patel et al. 2024; Thai *et al.* 2024)
[4] (Bui *et al.* 2024)
[5] (Gartlan *et al.* 2024)
[6] (Pandey & Cascella 2024)

2.2 Antibiotic Resistance

When antibacterial drugs no longer have an effect on bacteria, antibiotic resistance develops (WHO 2023a). The development of antibiotic resistance is a natural phenomenon used by bacteria to protect themselves against naturally occurring antibacterial substances such as penicillin. But with human activity, misuse and overuse of antibiotics, the spread and emergence of resistance has accelerated. The Organization for Economic Cooperation and Development (OECD) estimates that the resistance to last-resort antibacterial drugs will have doubled by the year 2035 compared to 2005 (OECD 2023).

The different mechanisms of resistance bacteria exhibit can be classified as intrinsic, acquired and adaptive (Garneau-Tsodikova & Labby 2016). Intrinsic resistance is when bacteria express naturally occurring genes which make them able to withstand antibacterial exposure, e.g. gram-negative bacteria that are intrinsically resistant to penicillins. Acquired resistance is when bacteria gain certain genes and characteristics over time that enable their survival. Acquired resistance is often caused by incorporating exogenous genes into the bacterial genome, or mutations to already existing genes (van Hoek *et al.* 2011). Exogenous genes are often contained in plasmids, which are mobile DNA segments able to easily move from one bacterium to another. Adaptive resistance is a type of acquired resistance and is a result of environmental stimuli which causes temporary changes in genes and protein expression, e.g. bacteria in biofilm (Garneau-Tsodikova & Labby 2016).

2.2.1 Mechanisms of Resistance

Low Cell Wall Permeability

One common intrinsic mechanism is low cell wall permeability (Darby *et al.* 2023). Many antibacterial drugs need to penetrate the cell wall to be able to exert their antibacterial activity. If the cell wall is impermeable to the antibiotic, the bacteria can withstand exposure. This is especially important in gram-negative bacteria since they naturally express a double membrane which is impermeable to many antibiotics. Gram-positive bacteria on the other hand, can acquire this mechanism of resistance, for instance by changing the fatty acid composition in the cell wall, which makes it harder for some antibiotics to penetrate (Mishra *et al.* 2012). Furthermore, alterations to porin structures have also been identified as an important mechanism of resistance, especially in multidrug-resistant *E. coli* where this is common (Lou *et al.* 2011). Porins are protein-channels in the cell wall, which are important for uptake of nutrients and are expressed by all bacteria (Darby *et al.* 2023).

Efflux Pumps

Another mechanism of resistance is the expression of efflux pumps, which leads to active transport of antibiotics out of the cytoplasm (Darby *et al.* 2023). Efflux pumps are energy-dependent transmembrane proteins that transport different toxic materials out of the cell, including antibiotics. All bacteria express efflux pumps designed to pump out specific or non-specific substances. Overexpression, especially of non-specific pumps, is a common mechanism in multi-drug resistance. Just like reduced cell wall permeability, efflux pumps can be an intrinsic or acquired mechanism.

Alteration of Target Site

Alteration of the target site is an additional mechanism of resistance used by many different bacteria (Darby *et al.* 2023). To exert their antibacterial activity, many antibiotics bind to a primary target site, which often inhibits an important cellular function, leading to cellular death or stunted bacterial growth. Alterations of the target site can be achieved by random mutation and natural selection, which leads to, for example, changes in the conformation of the site or added protective components. This results in lower binding affinity for the antibiotic while still allowing cellular functions. These types of mechanisms can often be seen in bacterial strains resistant to beta-lactams and macrolides.

Modification of Antibacterial Drugs

Bacteria can also inactivate or modify the antibacterial drug itself, often via enzymes (Darby *et al.* 2023). This mechanism is widely spread and plays a major role in transmitting antibiotic resistance among bacteria. Some of the most-known enzymes are beta-lactamases, which initiate hydrolysis of beta-lactams, causing inactivation and therefore resistance to the drug (Naas *et al.* 2017). Some beta-lactamases have high specificity, e.g. penicillinases, whereas others have low specificity and can provide resistance to almost all beta-lactams, e.g. carbapenemases and extended spectrum beta-lactamases (ESBL).

Drug-modifying enzymes, on the other hand, do not cause hydrolysis of the antibiotic but rather inhibit their action by transferring a chemical group or modifying their conformation (Darby *et al.* 2023). Aminoglycosides and macrolides are often targets for drug-modifying enzymes. Drug-modifying and hydrolysing enzymes can be found in both gram-positive and gram-negative bacteria.

Target Bypass

Target bypass is another mechanism of resistance used by some bacteria to withstand exposure to antibiotics (Darby *et al.* 2023). By making the original target of the antibiotic redundant, via producing a new cellular pathway and therefore retaining its cellular function, the bacteria can continue to thrive in the

presence of antibacterial drugs. One of the best-known examples of this mechanism is methicillin-resistant *Staphylococcus aureus* (MRSA). These bacteria produce a separate homologous enzyme to the original target site for the antibiotic, but with lower binding affinity, and can therefore continue their cellular functions and survive.

2.2.2 Resistance to Specific Antibiotics

Resistance to cefoxitin is often caused by changes in the target protein, i.e. penicillin-binding proteins (PBPs) (Bui *et al.* 2024). As mentioned previously, this mechanism is a major cause of resistance in methicillin-resistant *Staphylococcus aureus* (MRSA), and cefoxitin is therefore often used as a surrogate marker for its detection (Fernandes *et al.* 2005). Another common mechanism for resistance to cefoxitin is the expression of modifying enzymes, i.e. beta-lactamases such as ESBL (Bui *et al.* 2024).

Resistance to benzylpenicillin is most often caused by modification of the antibiotic by beta-lactamases, the most common group being penicillinases (Gartlan *et al.* 2024). Another common mechanism is target-site alterations.

Resistance to meropenem is, as for most other beta-lactams, most often caused by beta-lactamases produced by the bacteria (Pandey & Cascella 2024). Other common methods of resistance are decreased penetration of the drug, mutation of the target site, or efflux of the drug out of the bacterial cell via increased expression of efflux pumps in the cell wall membrane.

Resistance to ciprofloxacin can be seen in many bacterial strains, such as *E. coli* (Thai *et al.* 2024). Resistance is often caused by mutations to the DNA-gyrase, expression of efflux pumps or other plasmid-mediated mechanisms.

The most common mechanism of resistance to gentamicin is inactivation of the antibiotic by aminoglycoside-modifying enzymes (AMEs) produced by the bacteria (Garneau-Tsodikova & Labby 2016). Other mechanisms seen are expression of efflux pumps, mutations to the target site, and intrinsic or acquired low cell wall permeability.

Tigecycline is often considered as being one of the last resort antibacterial drugs against multi-drug resistant bacteria (Korczak *et al.* 2024). Unfortunately, resistant strains belonging to the *Enterobacteriaceae*-family have emerged during recent years with efflux pumps being one of the most common mechanisms (Korczak *et al.* 2024).

2.3 Indicator Bacteria for Antibiotic Resistance

Bacteria used in this study were *Escherichia coli* and *Staphylococcus aureus*. They are two of the most prevalent opportunistic bacterial pathogens in both

humans and animals, and are central components in the emergence of antibiotic resistance (WHO 2023a)

2.3.1 *Escherichia coli* and ESBL

Escherichia coli is a gram-negative bacteria belonging to the family *Enterobacteriaceae* (VetBact 2023a). It is a short motile rod and facultatively anaerobic. Colonies on blood agar are 2-3mm in diameter, sticky, with an opaque, greyish colour, have a characteristic odour and sometimes give a narrow zone of clear haemolysis on blood agar.

Most strains of *E. coli* are non-pathogenic and naturally occurring intrinsic bacteria (VetBact 2023a). Some strains are, however, pathogenic and can cause mild to severe systemic disease in humans and animals. Despite this, antibiotics are usually not recommended as treatment and should only be used in life- threatening cases since the development of antibiotic resistance happens quickly.

Extended-spectrum beta-lactamases (ESBL) are a group of enzymes produced by some bacteria and used as a resistance mechanism against beta-lactams (Folkhälsomyndigheten 2016). They are most often found in strains of *Klebsiella pneumoniae* and *E. coli*. Similar to other beta-lactamases, ESBL use hydrolyzation as a mechanism to break down beta-lactams, causing inactivation of the drugs (Naas *et al.* 2017). Most ESBL-producing strains are, therefore, resistant to these beta-lactams but can be treated with carbapenems. An alarming development in recent years is the emergence of ESBL-CARBA which are ESBLs that can also inactivate carbapenems (Nordmann *et al.* 2011). A positive correlation was seen between high usage of antibiotics, especially broad-spectrum cephalosporins, and the development and spread of resistance caused by ESBL-producing bacteria (Folkhälsomyndigheten 2016).

2.3.2 *Staphylococcus aureus* and MRSA

Staphylococcus aureus is a gram-positive non-motile facultatively anaerobic coccus (VetBact 2023b). Colonies are 2-3mm in diameter, opaque, white or yellow in colour and often give a double haemolysis on blood agar. *Staphylococcus aureus* is a commensal found on the skin of most mammals and can cause skin- and wound infections, abscesses, septicaemia, food poisoning and mastitis.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* that have developed resistance to almost all beta-lactams (Peacock & Paterson 2015). They utilize a resistance mechanism known as “target bypass”. These bacteria are not affected by the presence of most beta-lactams, since they produce a similar protein to the native penicillin-binding protein (PBP). The non-native protein, known as PBP2a, has a lower affinity to beta-lactams and can, therefore, withstand exposure to the antibiotic. The gene encoding for the necessary enzyme,

known as the *mecA* gene, is located on a mobile genetic element, which enables it to be easily transferred to other strains, making it possible to spread rapidly. Methicillin-resistant *Staphylococcus aureus* can only be treated with a few antibiotics and since resistance is becoming more prevalent, gaining information about the existence of MRSA is therefore important.

Another common mechanism of resistance in *S. aureus* is the synthesis of beta-lactamases, most commonly penicillinases (Mlynarczyk-Bonikowska *et al.* 2022).

2.4 Maasai Mara

The Maasai Mara is a national reserve located in the south-west of Kenya (Global Alliance of National Parks n.d., Figure 1). It is a continuation of the neighbouring Serengeti National Park in Tanzania and covers an area of approximately 1500 square kilometres. The Maasai Mara provides a home to an abundance of wildlife, including over 400 species of birds, and the "Big five", i.e. lions, leopards, elephants, buffalo and rhinoceros. Each year the Maasai Mara welcomes over 1.5 million wildebeests as they migrate north from the south of Tanzania to the south of Kenya. This world renowned natural spectacle is recognized as one of the "Seven Wonders of the Natural World" (National Geographic Society n.d.).

The Maasai Mara is also home to the Maasai people, which give the name to the national reserve (Cartwright 2019). The Maasai people are an indigenous east African tribe known for their animal husbandry and success in warfare. They are pastoralists who mostly herd cattle, but sheep and goats are also common. It is estimated that there are 1-2 million Maasai people in Kenya according to the 2019 census, but the numbers are believed to be higher (KNBS 2019).

Mararienta is a village in the northwest of the Mara North Conservatory located in the Narok County (Figure 1), which also contains the Maasai Mara national reserve. The population size is unknown and consists mostly of Maasai people. The population of small ruminants is also unknown. Wild animals are free to roam and thus create an interface between wild animals, domestic animals and the people.

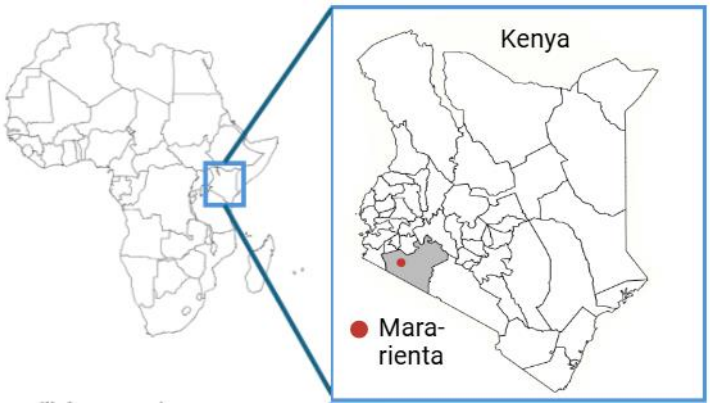

Figure 1. Map of Kenya. Grey area shows Narok county, where the Maasai Mara is located. The red dot indicates the location of Mararienta and Karen Blixen Camp. Maps are generated with Mapchart.net and Biorender.com.

2.4.1 Sheep and Goats in Maasai Mara

Sheep and goats are of great importance to the Maasai people. Livestock, i.e. cattle and small ruminants, are used as a source for food, as currency, to maintain social ties and as insurance in case of crisis. Milk is a major food for the local population and because goats produce milk almost all year round, they are an important and valuable food source. Goats and sheep are also important financially, their milk and/or wool can be sold to provide an income for the families. Livestock is also used directly as currency and often given away as gifts to create and maintain relationships and social status (Bekure & ILCA 1991).

The most common breeds of sheep owned by the Maasai are the Red Maasai and the Black-headed Somali, or crosses between them, and the most common breed of goat is the Small East African goat (Bekure & ILCA 1991). More than 2.6 million sheep and 1 million goats are kept by the Maasai people in the entire Maasai Mara (KNBS 2019).

The management of livestock is family-based, with older children and men caring for the cattle, whereas women and young children care for the sheep and goats. Most often, children herd the sheep and goats during the day, or care for the young. Women often care for young stock and do the milking. During the night, the livestock are kept in *bomas*, i.e. a cluster of houses and pens belonging to one family (Bekure & ILCA 1991).

Sheep and goats are often herded over large areas in the Maasai Mara (Bekure & ILCA 1991). Grazing patterns are determined by the location of the village and by the climate, how much it has rained and the access to water and pasture. The average herd size for sheep and goats is 400 for households considered rich and 70-100 for households considered poor (Tiampati 2015).

2.5 Antibiotic Resistance in Kenya

Antibiotic usage for livestock in Kenya is mostly unregulated (Rware *et al.* 2024). Antibiotics are often bought without a prescription and used for both therapeutic and non-therapeutic purposes. Even though Kenya has made notable progress towards the fight against antibiotic resistance, the emergence and spread of resistant bacteria is still a major problem.

Socio-economic and environmental factors, such as a warmer climate, increased urbanization and poor administrative governance, contribute to greater use of antibiotics, thus driving the development of resistance (Sohaili *et al.* 2024). Another factor is the increased demand for animal protein which has led to routine use of antibiotics in livestock production. Furthermore, the Kenya Veterinary Association estimates that 78% of veterinary medicine outlets are illegal with little or no regulations on sales and proper use of antibiotics (Kenya Veterinary Association 2016).

Even though Kenya has developed several national surveillance plans to gain knowledge about the spread of antibiotic resistance in the country, there is still limited knowledge and limited access to microbiological services (WHO 2022). Especially in rural areas, where most Maasai people live, access to veterinary care, adequate microbiological resources and obtaining cultures for testing for resistance in a timely manner is often difficult.

2.5.1 Sheep and Goats as Transmitters of Antibiotic Resistance

In a review article by Wee *et al.* (2020), livestock were identified as potential reservoirs and transmitters of antibiotic resistance between other animals and humans. Humans are at great risk of acquiring resistant bacteria through close contact with livestock and/or ecological overlaps, meaning direct or indirect contact. Thus, handling animals, their manure or their milk is a potential risk. Indirect contact through contaminated soil, sewage, surface water or wildlife is also of great importance.

Milk has been identified as a source for transmitting antibiotic-resistant bacteria. A study by Ngaywa *et al.* (2019) investigated the prevalence of antibiotic-resistant *E. coli* in raw milk in northern Kenya. They found that 95% of the isolated *E. coli* were resistant to at least one antibiotic, the majority being beta-lactams and tetracyclines, and that 14% were multidrug resistant. Another study by Omwenga *et al.* (2021) found that 88% of *S. aureus* isolates, also from milk samples of livestock in northern Kenya, were resistant to at least one antibiotic and 22% of them were classified as MRSA. Kabui *et al.* (2024) showed in a recent study that the most prevalent bacteria for subclinical mastitis in dairy goats in Nyeri County, north of Maasai Mara, were *S. aureus*. The prevalence of

subclinical mastitis was 84.7% and of *S. aureus* was 22%, of which 97.5% were resistant to penicillins. These figures also indicate a high prevalence of MRSA. In the same study, *E. coli* was also found to be common in goats with subclinical mastitis, with a prevalence of 18.1%. They found that 93.9% of the isolated *E. coli* strains were resistant to beta-lactams, also indicating a high prevalence of ESBL. All studies conclude that the consumption of raw milk contaminated with resistant *E. coli* and MRSA, respectively, could pose a serious public health risk.

Mastitis is a condition in which one or more mammary glands are inflamed (Wieland 2024). It is most often caused by bacteria and occasionally by mycotic infections. Mastitis can range from a subclinical to a life-threatening condition, and often results in a decrease in milk production. Subclinical mastitis is indicated by an increased number of cells in the milk. Signs of clinical mastitis can be discoloured milk, clumping or pain in the udder.

Besides milk, the close contact of sheep and goats to the environment and wildlife also poses a possible risk for transmitting resistance. As mentioned previously, the goats and sheep are allowed to graze over large areas and in close proximity to wildlife (Bekure & ILCA 1991). Their faeces are also spread over this considerable area. As a result, sheep and goats are possible important cornerstones in transmitting antibiotic resistance, not only to humans but also to wildlife and the environment.

2.5.2 Previous Study on Antibiotic Resistance in Domestic Cats

Diamanti Barredal (2024) studied the prevalence of antibiotic-resistant faecal *E. coli* in domestic cats in Mararienta. They found that 22% of the cats were carriers of at least one type of resistant strain of *E. coli*, compared to a study by Albrechtova *et al.* (2012) who found that only 4% of house cats in northern Kenya are carriers of resistant bacteria. Antibiotics tested in the study by Diamanti Barredal (2024) were cefotaxime, tigecycline, meropenem, gentamicin and ciprofloxacin. The examined cats were almost exclusively fed unpasteurized milk from livestock, including from goats and sheep (Schultz 2023). Few of the cats were treated with antibiotics.

2.6 One Health

One Health is a unifying multisectoral approach to achieve optimal health of humans, animals and the environment (WHO 2023b). By using already existing links between the different disciplines and collaborations across sectors, sustainnable global health can be achieved. The One Health approach is important in many sectors, especially for antimicrobial and antibacterial resistance. A One

Health approach is needed to combat these threats because of the growing human population, globalization and emerging health challenges.

Antibiotics are not only used for humans, but also for animals and in agriculture (WHO 2023a). The development of resistance is accelerated by the misuse of antibiotics in humans and animals, e.g. as treatment for viral infections, or the unnecessary use of broad-spectrum antibiotics. In animal production, antibiotics are often used as growth stimulants with sub-therapeutic doses and long-exposure periods, which create ideal conditions for bacteria to develop resistance (Robinson *et al.* 2016). The environment has a key role in spreading antibiotic resistance, through natural development of resistance or as a transmitter of resistance by influx of resistant bacteria from livestock or human waste.

The environmental impact is likely more prominent in developing countries, such as Kenya. Antibiotic resistance does not discriminate between rich and poor countries, different human populations, animals or flora (WHO 2023a). Global cooperation is, therefore, necessary to stop the spread and further development of antibiotic resistance.

2.7 Objectives

Since milk from sheep and goats is an important food source for humans and domestic cats, it is of great importance to gain knowledge about the status of resistant bacteria in milk from small ruminants. The aim of this present study is therefore to gain knowledge about the potential role these animals play by studying the prevalence of antibiotic-resistant *E. coli* and *S. aureus* in milk and the "milk chain", i.e. the handling of the milk between milking and feeding of the cats, in Mararienta, Kenya. Samples collected from milk, milk-collecting jars and milk-feeding bowls will hopefully provide answers to the following questions:

- What is the prevalence of antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* in milk from goats and sheep in Mararienta, Kenya?
- What is the prevalence of antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* in the milk-collecting jars and milk-feeding bowls used for the domestic cats in Mararienta, Kenya?
- Are the cats exposed to the resistant bacteria directly via the milk, through contamination after milking or from elsewhere?

Since the livestock graze over large areas, live in close contact with the wildlife and provide food not only to the cats but also humans, this study is of interest from both a One Health and a conservational perspective.

3. Material and Methods

3.1 Material Selection

Samples were obtained from both goats and sheep in the northwest of Mararienta, a village in the northwest of the Mara North Conservatory. No discrimination was made between the two species since they are commonly kept together and are therefore identified as one epidemiological unit. Only households that owned domestic cats and used sheep and/or goats' milk regularly as food were selected. Only sheep and/or goats that were lactating were tested with the Californian Mastitis Test (CMT). Only animals with a score of 3 or higher in one or both udders were selected and the milk samples collected. Before collection, the purpose of the study was explained, and oral consent was obtained by the help of an interpreter. No additional ethical approval was required.

Samples were collected by manual milk sampling from animals with a CMT score of 3 or higher without signs of clinical mastitis (i.e. discoloured milk, clumps or inflammation in the udder) since that milk is not consumed. The CMT is an indirect and subjective measurement of the number of somatic cells in milk. A higher score means more inflammatory cells, which is indicative of inflammation and consequently a higher chance of the presence of an intramammary infection and bacteria. The samples were scored subjectively according to the Scandinavian scoring system with scores 1-5, where score 5 is the highest with the highest amount of cells (SVA 2019).

Before sampling the animals were restrained and the teats cleaned, first with dry paper towels followed by cotton wool soaked with chlorhexidine disinfectant. Approximately 1-3 ml of milk from each animal were collected in separate sterile test tubes. Samples were also collected from containers used for milk collection by the owners and milk bowls for the cats, using sterile e-swabs with Amies transport medium. The collected milk samples were transported in a chilled cooler.

3.2 Bacterial Growth and Confirmation

After mixing each milk sample thoroughly using a vortex, 10 µL from every milk sample, were spread onto the separate sections of SELMA PLUS® agar plates with a sterile 10 µL loop. Since an unexpectedly low bacterial growth rate was noted on the first agar plates, 30 µL from the samples was streaked on each section on the remaining SELMA PLUS® agar plates. The swabs were streaked directly onto SELMA PLUS® agar plates. SELMA PLUS® agar plates are divided into four sections, containing cattle blood with esculin, MacConkey, mannitol salt and PGUA-agar (VetBact 2018), respectively. The plates were incubated at 37 °C for 24-48h, with the first growth evaluation after 24h and the 2nd after 48h.

When substantial bacterial growth was detected on the SELMA PLUS® plates, colonies suspected of being *E. coli* and *S. aureus* were isolated and streaked onto blood agar plates before being incubated at 37 °C for 24-48h. On the SELMA PLUS® plates *E. coli* grows on blood agar with greyish opaque colonies 2-3mm in diameter and on the MacConkey agar with similar sized colonies and a red or purple colour (SVA 2017). To differentiate *E. coli* from other gram-negative bacteria, the PGUA-agar is used. *E. coli* colonies on PGUA have a yellow-greenish coloration, unlike other gram-negative bacteria, which stay neutral in colour. *Staphylococcus aureus* grows on the blood agar with yellowish opaque colonies 2-3 mm in diameter, often with a double haemolysis, and on mannitol salt with a yellow coloration.

After 24-48h incubation on blood agar, isolated colonies of suspected *E. coli* were confirmed using a potassium hydroxide (KOH) test and spot indole test. The potassium hydroxide test is used to identify gram negative bacteria by degrading the cell wall causing DNA to be released, which makes the solution viscous (Vetbact 2017c). The spot indole test is mainly used to confirm suspected strains of *E. coli*. It reacts with indole produced by tryptophanases causing the solution to become blue (Vetbact 2017b). *Escherichia coli* is positive in the KOH-test and the spot-indole test (VetBact 2023a).

Isolated colonies of *S. aureus* on blood agar were confirmed using a catalase test and DrySpot™ Staphytect Plus™ test. The catalase test is primarily used on gram positive bacteria and utilizes the ability of catalase, an enzyme produced by some bacteria, to detoxify hydrogen peroxide to water and oxygen, which can be seen as the onset of gas production (Vetbact 2017a). The DrySpot™ Staphytect Plus™ test is used to confirm suspected *S. aureus* strains by detecting a clumping factor and polysaccharides specific to *S. aureus* causing the solution to agglutinate (ThermoFisher Scientific n.d.). *Staphylococcus aureus* is positive in the catalase test and DrySpot™ Staphytect Plus™ test.

3.3 Disk Diffusion

The method for disk diffusion was done according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, as summarized below (EUCAST 2024a).

When confirmed, isolated *E. coli* and *S. aureus* colonies from the blood agar plates were suspended in approximately 5 mL of sterile saline to a density of 0.5 McFarland; a variation of ±0.05 McFarland was accepted. The density was measured using a densitometer.

The inoculum was spread from multiple directions over the entire surface area of one Mueller-Hinton agar plate with a sterile cotton swab. The chosen antibiotic-disks were then evenly applied onto the surface of the agar plates. Antibiotics chosen for this study were cefoxitin, tigecycline, gentamicin, ciprofloxacin and meropenem for *E. coli* and cefoxitin, tigecycline, gentamicin, ciprofloxacin and benzylpenicillin for *S. aureus.* Concentrations for the disks were 30 µg for cefoxitin, 15 µg for tigecycline, 10 µg for gentamicin, 5 µg for ciprofloxacin, 10 µg for meropenem and 1U for benzylpenicillin.

Prepared plates were incubated at 35 °C for 18h. After incubation, plates were examined for resistance. The zone of inhibition was measured using a standard metric ruler from the zone edges across the diameter. The measured zones were noted according to the 2024 EUCAST standard (EUCAST 2024b). If measured zones showed a diameter less than the noted breakpoints, the isolate was categorised as resistant. If the zone of inhibition was measured to be greater than the highest noted breakpoint, the isolate was susceptible i.e., not resistant. If the measured inhibition zone was found to be between two different noted breakpoints, the strain was considered as being susceptible with increased exposure. This means the bacteria are most likely susceptible when exposed to a higher dose of the antibiotic than normally recommended. Reference values for the breakpoints of *Enterobacterales*, including *E. coli*, as published by EUCAST, are shown in Table 2 (EUCAST 2024b).

Table 2. Clinical breakpoints for E. coli *for chosen antibacterial drugs as published by EUCAST (2024b).*

Antibacterial agent	Breakpoint
Cefoxitin	19 mm
Tigecycline	18 mm
Gentamicin	17 mm
Ciprofloxacin	22-25 mm
Meropenem	16-22 mm

Reference values for the breakpoints of *Staphylococcus spp*, including *S. aureus*, as published by EUCAST are shown in Table 3 (EUCAST 2024b).

Table 3. Clinical breakpoints for S. aureus *for chosen antibacterial drugs as published by EUCAST (2024b).*

Antibacterial agent	Breakpoint
Cefoxitin	22 mm
Tigecycline	19 mm
Gentamicin	18 mm
Ciprofloxacin	17 mm
Benzylpenicillin	26 mm

4. Results

The 12 herds that were tested with Californian Mastitis Test (CMT) are shown in Table 4. The total number of sheep and goats in the selected herds ranged from 35 to 95. In total, 130 sheep and goats from 12 different households were tested with the CMT. A total of 28 samples from 20 different sheep or goats with a CMT score of 3 or higher could be obtained from 10 different households. None of the sampled sheep and goats showed signs of clinical mastitis. An overview of the tested herds, approximate herd size, number of animals tested with CMT, and number of total samples collected from each herd is shown in Table 4.

Table 4. Overview of the total herd size, number of animals tested with CMT, and number of total samples collected from 12 households.

Herd	Total herd size (approximate)	Sheep/goats tested with CMT	Samples collected
A	45	7	5
B	95	31	3
C	40	8	2
D	45	18	1
E	40	7	1
F	35	9	1
G	40	8	3
H	65	19	6
I	40	8	5
J	45	6	1
K	40	5	0
L	35	4	0
Total		130	28

4.1 Bacterial Growth

In 20 of the 28 samples, no bacterial growth was seen after 24h and 48h. From samples taken from herd A, 10 µL was spread onto each section on the SELMA PLUS® agar plates. From samples taken from all other herds, 30 µL was spread onto each section. Eight of the 28 (29%) samples had mixed bacterial growth. If colonies suspected of being *E. coli* or *S. aureus* were present, they were isolated and tested with KOH, Spot indole, Catalase and DrySpot™ Staphytect Plus™ tests. None of the isolated colonies matched the criteria for *E. coli* or *S. aureus* and were therefore deemed not to be of interest for this study and labelled as other bacteria. The results of the bacterial growth and chemical testing from the 28 collected samples are shown in Table 5.

Table 5. Results of bacterial growth from 28 milk samples collected from sheep and goats with suspected subclinical mastitis.

ID	Species	CMT score	KOH	Spot indole	Catalase	DrySpot™ Staphytect™	Isolated bacteria
1A	Sheep	3					No growth
2A	Sheep	3					No growth
3A – L	Sheep	3	–	–	–	–	Other bacteria
3A – R	Sheep	3					No growth
4A	Sheep	3					No growth
1B	Sheep	3	–	–	+	–	Other bacteria
2B	Sheep	3					No growth
3B	Sheep	3					No growth
1C – L	Goat	3					No growth
1C – R	Goat	3					No growth
1D	Sheep	3	–	–	–	–	Other bacteria
1E	Sheep	3					No growth
1F	Sheep	3					No growth
1G	Sheep	4	–	–	–	–	Other bacteria
2G – L	Goat	3	+	–	–	–	Other bacteria
2G – R	Goat	3	–	–	–	–	Other bacteria
1H – L	Goat	3	–	–	+	–	Other bacteria
1H – R	Goat	3					No growth
2H – L	Goat	3					No growth
2H – R	Goat	3					No growth
3H – L	Goat	3	–	–	–	–	Other bacteria
3H – R	Goat	3					No growth
1I – L	Goat	3					No growth
1I – R	Goat	3					No growth
2I – L	Goat	3					No growth
2I – R	Goat	3					No growth
3I	Goat	4					No growth
1J	Sheep	3					No growth

A total of five swabs were collected from milk collecting containers and cat feeding bowls respectively. The results of bacterial growth of the swabs from milk collecting containers are shown in Table 6. All the swabs from milk collecting containers had growth of mixed flora but none had colonies suspected of being *S. aureus* or *E. coli*.

Table 6. Results of bacterial growth from five samples collected from milk collecting containers from five different households.

ID	KOH	Spot indole	Catalase	DrySpot™ Staphytect™	Isolated bacteria
1A		–	–		Other bacteria
1B			+	–	Other bacteria
1C					Other bacteria
1D					Other bacteria
1E					Other bacteria

The results of bacterial growth of the swabs from cat feeding bowls are shown in Table 7. All swabs had growth of mixed flora, and one had colonies suspected of being *E. coli*. One of the suspected colonies was isolated and later confirmed using KOH and Spot indole test.

Table 7. Results of bacterial growth from five samples collected from cat feeding bowls from five different households.

ID	KOH	Spot indole	Catalase	DrySpot™ Staphytect™	Isolated bacteria
1F					No growth
1G		–		–	Other bacteria
1H	+	+			*Escherichia coli*
1I				–	Other bacteria
1J					Other bacteria

4.1.1 Antibacterial Susceptibility Testing

In one of the five swabs taken from cat feeding bowls, *E. coli* could be isolated and tested for antibiotic resistance. The results of the antibacterial susceptibility testing are shown in Table 8. The clinical break points according to EUCAST (2024b) are shown in Table 2[7].

Table 8. The zone of inhibition of the tested antibiotics in isolated E. coli.

ID	Cefoxitin	Tigecycline	Gentamicin	Ciprofloxacin	Meropenem
1H	22mm	19 mm	19 mm	27 mm	29 mm

[7]

Antibacterial agent	Breakpoint
Cefoxitin	19 mm
Tigecycline	18 mm
Gentamicin	17 mm
Ciprofloxacin	22-25 mm
Meropenem	16-22 mm

5. Discussion

The aim of this study was to investigate the prevalence of antibiotic-resistant *E. coli* and *S. aureus* in the milk and the milk chain of sheep and goats in Mararienta, and to gain knowledge about the potential role these animals play in transmitting resistant bacteria to domestic cats and their owners.

It is difficult to draw any definite conclusions since relatively few samples were taken. However, milk appears to be an unlikely cause for the high prevalence of resistant *E. coli* in domestic cats as seen previously (Diamanti Barredal 2024), since the prevalence of subclinical mastitis was low and none of the bacteria isolated were resistant. Regardless, to draw further conclusions more extensive research would be needed.

5.1 Prevalence of Subclinical Mastitis in the Sheep and Goats

From 130 tested sheep and goats, only 20 (15.4%) had a CMT score of 3 or higher, and none had signs of clinical mastitis. Other studies in and around Maasai Mara reported a higher prevalence of subclinical mastitis of approximately 85% in dairy goats, of which 22% were *S. aureus* (Kabui *et al.* 2024). One reason for this could be that the animals are housed differently and allowed to graze during most of the day. In the study by Kabui *et al.* (2024), more than 50% of the farmers practiced zero grazing. The animals in the present study all belonged to the Maasai people, who let the animals graze over large areas (Bekure & ILCA 1991); thus, the animals are standing up or walking during the day. By continuously moving to dry and clean areas, the probability of developing mastitis might be lower. This could, however, vary in different seasons.

Another observation during the sampling was that the sheep and goats were not kept as conventional dairy animals, where the lambs and kids are kept separate from the ewes and does for long periods. The households that consumed sheep and goats' milk in Mararienta, kept the lambs and kids within the herd and only took the milk that was left in the udder. Thus, the udder is drained continuously which most likely prevents bacterial colonisation.

Another reason for the low prevalence of subclinical mastitis might be the relatively low age of the animals. It was mentioned by the owners that the sheep or goats are often not much older than three to four years of age. One reason for this is that many sheep and goats are sold or slaughtered when they mature. Another possibility is that the animals succumb to diseases or predators as they age. Thus, the average age is kept low. This could also reduce the possibility for subclinical mastitis since older animals are more prone to develop clinical and subclinical mastitis than younger animals (Gustafsson *et al.* 2022).

Since this research was done during the dry season and in a limited area, no conclusions can be drawn about the whole population of sheep and goats in Mararienta. Thus, more research is needed, investigating the presence of mastitis during several seasons and in a larger sample group.

5.2 Antibiotic-Resistant Bacteria in Milk, Milk Collection Containers and Cat Feeding Bowls

Of the 28 collected milk samples, only eight (29%) had bacterial growth and none were of *E. coli* or *S. aureus*. The reason for minimal bacterial growth in the collected samples, even though they had a CMT score of 3 or higher, could be that the bacterial load was too low to detect. Even though the tubes were thoroughly mixed with a vortex, and most agar plates were streaked with 30 µL instead of the usual 10 µL, minimal bacterial growth was noted. This means that the bacterial load that was potentially present in these samples was probably not sufficient to cause mastitis and is probably not a major concern for the transmission of resistance to the cats or humans consuming the milk.

All the swabs taken had some degree of bacterial growth with mixed flora. This was expected since the containers were often left exposed to the elements. Additionally, only in one of the ten swabs taken was *E. coli* found, and no resistance was noted. However, according to the owners, the containers in most homes were routinely cleaned, after each use or when it was visibly dirty, with hot water and soap. This means that no conclusions can be drawn whether the containers pose a risk in transmitting resistant bacteria or not, since the flora and prevalence of *S. aureus* and *E. coli* in the containers could vary over time. However, *E. coli* can be present and found in the receptacles used to contain milk.

5.3 Goats and Sheep as Transmitters of Antibiotic-Resistant *E. coli* and *S. aureus*

As no resistant bacteria of interest were found in the milk, in the milk collecting containers or in the cat feeding bowls, and the prevalence of subclinical mastitis was low, it seems that another cause must be found for the high prevalence of multi-resistant *E. coli* in domestic cats as presented by Diamanti Barredal (2024).

Sick animals were commonly treated with broad spectrum antibiotics, such as oxytetracyclines, without investigating if the cause was a bacterial infection, due to the largely unregulated use of antibiotics in the area and limited availability of veterinary diagnostics. Thus, some animals might be treated with antibiotics unnecessarily. Moreover, if the treated animals did not survive, the meat would be fed to the dogs and cats, even though drug residues would still be present. Therefore, a greater risk might arise of transmitting antibiotic resistance from consuming meat from treated animals rather than by regular consumption of milk. However, to confirm this, more research must be done.

5.4 One Health

Because the sheep and goats live in close contact with other domestic animals, wildlife and the environment, a One Health approach is needed when investigating the risk they pose as transmitters of antibiotic-resistant bacteria.

Since limited bacterial growth and no resistance was found in the milk, milk collection containers and in the cat feeding bowls, consuming the milk from sheep and goats most likely does not pose a risk in transmitting resistant bacteria to humans and domestic cats.

Although no resistant bacteria could be found in the milk or the swabs, resistant bacteria can still be present as commensals on the skin, in the gut or in animals with clinical mastitis. The containers used for milk should, therefore, be cleaned regularly and milk handled with care to minimize the chances of contamination with harmful pathogens.

Since meat from animals that have been treated with antibiotics, but succumbed to their illness, are routinely fed to the dogs and cats, it is of interest to discuss the risk this poses in a One Health perspective. As the use of antibiotics is largely unregulated, the chances of drug residues being present, even when seemingly healthy animals are slaughtered, is quite high. Humans are, therefore, also exposed to these residues and are at risk of developing resistance since seemingly no regard is taken to the withdrawal period of the drugs.

In addition, sheep and goats regularly fall prey to predators. Drug residues in the meat then also affect wild animals and could accelerate transmission of resistance. Most wild animals are not often treated with antibiotics *per se*, and

therefore resistance does not affect them directly. However, if resistance develops in their gut bacteria, it can spread over large areas and to other domestic and wild animals. Additionally, endangered species might also be affected and since they are often protected and treated when injured, resistance to common antibiotics might pose a serious threat to their conservation.

To minimize the risk of developing antibiotic resistance, meat from sheep or goats recently treated with antibiotics should not be consumed by dogs, cats, and humans, nor should the carcasses be left for wild predators. Since drug residues can be found in the meat for a long time after treatment, it is recommended not to slaughter treated animals within the withdrawal period, to ensure that the levels of the residues do not exceed the maximum residue limit (MRL), as stated in the product summary of the medicine (EMA n.d.).

To further investigate the prevalence of antibiotic resistance in Mararienta, samples could be collected from the environment, wildlife and humans. This was beyond the scope of the present study, because of limited time and funding, and strictly regulated management of wildlife.

5.5 Selection of Antibiotics and Choice of Method

The antibiotics used in this study were cefoxitin, tigecycline, gentamicin, Ciprofloxacin, meropenem and benzylpenicillin. These antibiotics were chosen to allow comparison with the previous study done by Diamanti Barredal (2024), who used the same antibiotics. These antibiotics were also of interest for their importance in human and animal welfare.

Tigecycline was relevant since it is a broad-spectrum antibiotic used as a last resort to treat infections with multi-drug resistant *Enterobacterales* in humans (Korczak *et al.* 2024). It was therefore of interest in this study to gain a general indication of resistance amongst the isolated strains of *E. coli*. As tigecycline is a tetracycline, it was also suitable for generating an overview of resistance to this group of antibiotics in *S. aureus*.

Since penicillinase production is common in *S. aureus* and MRSA, it was important to investigate the resistance to benzylpenicillin in isolated *S. aureus*. Benzylpenicillin is not effective against gram-negative bacteria; thus, testing susceptibility of isolated *E. coli* in this study was not relevant.

Meropenem was included to obtain a general indication of resistance to carbapenems in isolated *E. coli*. Susceptibility of staphylococci to carbapenem is inferred from cefoxitin susceptibility testing (EUCAST 2024b), hence testing susceptibility to meropenem was not necessary in the isolated *S. aureus*.

Gentamicin and ciprofloxacin were chosen to obtain a general indication of resistance to aminoglycosides and fluoroquinolones, respectively, in isolated strains of *E. coli* and *S. aureus*.

The gold standard for testing for MRSA is identifying the *mecA* gene in *S. aureus* strains by PCR (Fernandes *et al.* 2005). However, this method is expensive and needs elaborate laboratory equipment. Using cefoxitin as a surrogate marker to identify MRSA in disk diffusion, as in this study, is a well-accepted indirect method. Cefoxitin was therefore of interest for detecting resistant strains of *S. aureus* and of *E. coli* producing extended-spectrum beta-lactamases (ESBL).

5.6 Limitations and Bias

This project was done as a master thesis, and time was limited - samples could only be collected over a 10-day period in November 2024. We were only able to sample sheep and goats during the afternoon when the herds came back from grazing, since they at that time had been separated from their lambs long enough for us to obtain some milk. This limited the number of households that could be visited in one day. If more time would have been available, more households and more animals could have been tested to obtain a more reliable representation of the prevalence of subclinical mastitis and antibiotic-resistant bacteria. Changes in prevalence during different seasons could also have been studied.

Another limitation was that only households within walking distance from the accommodation could be visited since transportation was not available. This creates a selection bias in this convenience sample and the collected samples cannot be guaranteed to represent the whole population. Since the number of samples was low and they were only collected from certain households, the precision is low. Thus, the present study is more sensitive to random variation; households with animals that have very good udder health compared to the rest of the population may have been picked.

5.7 Conclusion

Since no bacteria and no resistance were found in the milk, milk collection containers or in the cat feeding bowls, consuming the milk from sheep and goats most likely does not pose a risk in transmitting resistant bacteria. The animals might, nevertheless, pose a risk in accelerating the development of resistant bacteria if they are slaughtered and consumed shortly after being treated with antibiotics. However, to determine if this hypothesis is true, more research would be needed.

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Popular Science Summary

Antibiotics are drugs used to treat bacterial infections. Antibiotic resistance happens when bacteria are no longer affected or killed by the antibiotics. Resistance can occur naturally, as spontaneous changes in the bacterial DNA or via transmission from one bacterium to another. Resistant bacteria can also be transmitted between humans, animals and the environment via food, direct contact or waste. Antibiotic resistance is a global health problem worsened by the misuse of antibiotics across human, animal, and environmental sectors. Unfortunately, with increased antibacterial resistance, common diseases can become life-threatening.

In Kenya, the use of antibiotics is mostly unregulated and access to veterinary diagnostics is often limited. This is identified as a potential reason for the high occurrence of resistant bacteria in domestic cats in the region of Mararienta in Kenya, as seen in a previous study. It is also known that cats and humans regularly consume milk from livestock, making milk a potential source for transmitting the resistant bacteria.

This study investigates the occurrence of antibiotic-resistance in *Escherichia coli* and *Staphylococcus aureus* in milk from goats and sheep, and in swabs taken from milk containers and feeding bowls for cats. *Escherichia coli* and *Staphylococcus aureus* are two common bacteria responsible for many diseases in both humans and animals. Considering the close relations of humans, animals, and the environment in Mararienta, a One Health perspective was needed. One Health is the combined approach of several sectors to achieve better human, animal and environmental health.

Only milk from animals with an elevated cell content in the milk but with no visible signs of udder disease were targeted. A total of 130 sheep and goats from 12 households were tested to see if inflammation in the udder was present. Twenty-eight milk samples and 5 swabs each from milk containers and feeding bowls were analysed for bacterial growth and resistance to antibiotics. The method used to analyse resistance was disk diffusion, a method where bacteria are isolated and incubated on a special medium to which paper patches infused with the chosen antibiotics were applied. The antibiotics were cefoxitin, tigecycline, ciprofloxacin, gentamicin, meropenem and benzylpenicillin. In the tested samples,

limited bacterial growth was seen and no resistance was detected. Only 15.4% of the tested animals had signs of inflammation in the udder.

Consequently, the low occurrence of animals with inflammation in the udder and minimal bacterial growth in milk samples suggest that milk is an unlikely source of antibiotic resistance transmitted to humans and domestic cats in Mararienta. However, it was noted that the carcasses of diseased livestock treated with antibiotics were often fed to cats and dogs. This means that antibiotic residues might still be present in the treated animal. Continuous exposure to low doses of antibiotics could affect the common gut bacteria of the cats and dogs and could therefore pose a potential risk for the spread of antibacterial resistance.

However, to draw any further conclusions, more research must be done.

Acknowledgements

I would like to express my heartfelt gratitude to everyone who contributed to the successful completion of this thesis.

First and foremost, I extend my deep appreciation to Karen Blixen Camp for giving us the opportunity and facilities to conduct our research. My sincerest thanks to my supervisor, Jane Morrell, and assistant supervisors Therese Hård and Dinah Seligsohn whose guidance, expertise, and encouragement were instrumental throughout this journey. A warm thank you also to our interpreter Jacqueline Noosaron for the generous help, and to David Noosaron and James Nayetuni for their help and allowing us to conduct our research.

A special thanks goes to the Veterinärmedicinska fakultetens stipendiesamfond and Kungliga skogs- och lantbruksakademin for their generous financial support, which made this research possible. Thank you to the Swedish Veterinary Agency, Thermo Fischer Scientific and Next2vet for the generous support and providing the necessary materials.

Lastly, I am profoundly grateful to my friends and family for their encouragement and understanding during this academic journey. Thank you all for believing in me and supporting me every step of the way.

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