Prevalence of antibacterial resistance in domestic cats in Masai Mara, Kenya

A One Health perspective

Agnes Diamanti Barredal

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Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme

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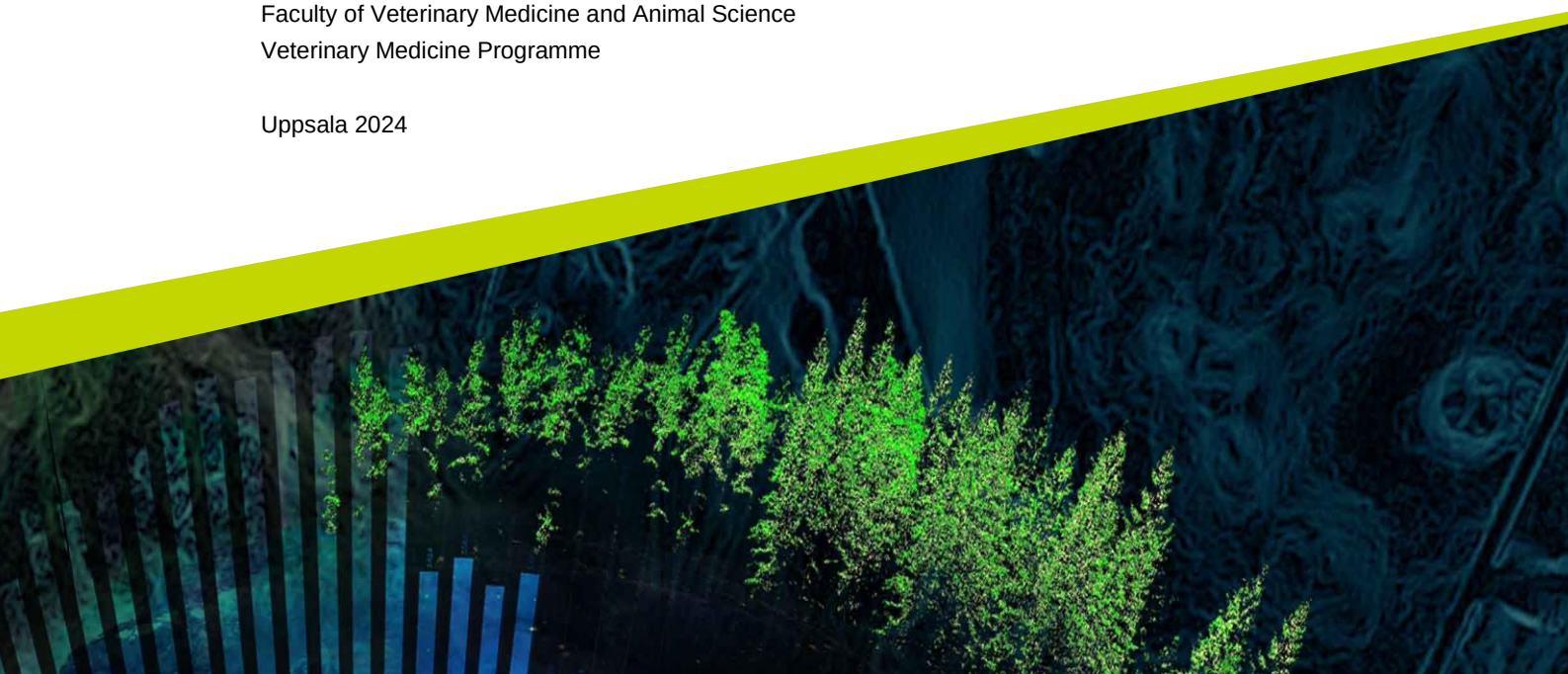

Prevalence of antibacterial resistance in domestic cats in Masai Mara, Kenya - A One Health perspective

Förekomst av antibakteriell resistens hos domesticerade katter i Masai Mara, Kenya - Ett One Health-perspektiv

Agnes Diamanti Barredal

Supervisor:	**Jane Morrell, Swedish University of Agricultural Sciences, Department of Clinical Sciences**
Assistant supervisor:	Therese Hård, Knutenborg Safaripark, Denmark
Examiner:	Eva Axnér, Swedish University of Agricultural Sciences, Department of Clinical Sciences

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Swedish University of Agricultural Sciences
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Abstract

Antibacterial resistance occurs when bacteria change in response to the use of antibiotics. Resistant bacteria and resistant genes can be transmitted between people, but also via animals, food, and the environment. Different bacteria can also spread resistance by exchanging resistance patterns with each other. With increased antibiotic resistance, infections become more difficult or even impossible to cure, which causes great suffering, premature death, and high costs for healthcare.

In Kenya the use of antibiotics is largely unregulated. Antibiotics can be bought without a prescription and broad-spectrum antibiotics are commonly used instead of specific antibiotics, antibacterial resistance is therefore a major and growing problem. With the attendant risk, the resistance could spread to bacteria in the environment and between humans and animals. The incidence of antibacterial resistance in domestic cats could serve as an indicator of the amount of resistance in the environment as well as the likely risk of cats serving as a reservoir of resistance for humans and wild animals.

This study was conducted in the Mararianta district in the nature reserve Masai Mara in Kenya. In Mararianta, domestic cats live in close contact with both human tribes and wild animals and are therefore a possible transmission risk for both disease and antimicrobial resistance for these groups. Antibacterial resistance was studied in rectal bacteria from 40 cats. The collection of samples was done via rectal-swabs, at the time that the cats were presented for castration and vaccination against rabies. The method used for analysing antibacterial resistance was disk diffusion, and the antibiotics used in this study were cefotaxime, tigecycline, meropenem, gentamicin and ciprofloxacin.

The prevalence of antibiotic resistance in all 200 different antibiotic filter patches was 6.5%. The prevalence of cats resistant to at least one type of antibiotics was 22.5% and of these resistant cats 33.3% were resistant to more than one type of antibiotic. In conclusion, antibiotic resistance occurs in the domestic cats in Mararianta, and poses a risk of transmission to both humans and wild animals in the area.

Keywords: Antimicrobial resistance, Antibiotics, Escherichia coli, Domestic cats, One Health, Mararianta, Masai Mara, Kenya

Sammanfattning

Antibakteriell resistens uppstår när bakterier utvecklas som svar på användningen av antibiotika. Resistenta bakterier och resistentgener kan överföras mellan människor, men också via djur, mat och miljön. Olika bakterier kan också sprida resistens mellan varandra genom att utbyta resistensmönster. Med ökad antibiotikaresistens, kommer infektioner bli svårare eller till och med omöjliga att bota, vilket orsakar stort lidande, för tidig död, samt höga kostnader för sjukvården.

I Kenya är användningen av antibiotika i stort sett oreglerad. Antibiotika kan köpas utan recept och bredspektrumantibiotika används ofta i stället för specifika antibiotika. Antibakteriell resistens är således ett stort och växande problem. Med åtföljande risk kan resistensen spridas till bakterier i miljön, och mellan människor och djur. Förekomsten av antibakteriell resistens hos domesticerade katter kan användas som en indikator på mängden resistens i miljön såväl som den sannolika risken för att katter fungerar som resistensreservoar för människor och vilda djur.

Denna studie genomfördes i Mararianta som ligger i naturreservatet Masai Mara i Kenya. I Mararianta lever domesticerade katter i nära kontakt med både människor och vilda djur, och är således en möjlig infektionsrisk för de båda. Antibakteriell resistens analyserades i bakterier från 40 katter. Insamlingen av prover gjordes via rektal-svabbar, och förutom provtagningen så kastrerades katterna samt vaccinerades mot rabies. Metoden som användes för att analysera antibiotikaresistens var diskdiffusion, och de antibiotikasorter som användes var cefotaxim, tigecyklin, meropenem, gentamicin och ciprofloxacin.

Prevalensen av antibiotikaresistens i alla 200 olika antibiotikafilterplåster var 6,5 %. Prevalensen av katter som var resistenta mot minst en typ av antibiotika var 22,5 % och av dessa resistenta katter var 33,3 % resistenta mot mer än en typ av antibiotika. Slutsatserna av denna studie visar att antibiotikaresistens förekommer hos de domesticerade katterna i Mararianta, och att det således finns en risk för spridning till både människor och vilda djur i området.

Nyckelord: Antimikrobiell resistens, Antibiotika, Escherichia coli, Domesticerade Katter, One Health, Mararianta, Masai Mara, Kenya

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Abbreviations

Cip	Ciprofloxacin
Cn	Gentamicin
Ctx	Cefotaxime
E. coli	*Escherichia coli*
EHEC	Enterohemorrhagic *E. coli*
ESBL	Extended Spectrum Beta-Lactamase
ETEC	Enterotoxigenic *E. coli*
Indole	Spot indole test
KOH	Potassium hydroxide test
Mem	Meropenem
Oxidase	Cytochrome c oxidase test
SLU	Swedish University of Agricultural Sciences
Tgc	Tigecycline
WHO	World Health Organization

1. Introduction

Antibacterial resistance is a major and growing problem in Kenya, not only among humans but also among animals. In countries like Kenya antibiotics can be bought without a prescription or a laboratory diagnosis (Muoi *et al.* 2019). Antibiotics could even be prescribed to patients with viral or fungal infections, where they are of no use. In addition, broad-spectrum antibiotics are often prescribed instead of specific antibiotics, making the risk for developing resistance greater. With increased antibacterial resistance, bacterial infections become more difficult or even impossible to cure. This results in great suffering for humans and animals, premature deaths, and high costs for healthcare. According to WHO, antibiotic resistance is one of the biggest threats to the world today and the resistance is rising to dangerously high levels (World Health Organization 2020). With globalization new resistance mechanisms are spreading all over the world, and in countries such as Kenya, where they do not have standard treatment guidelines, resistance is even worse. Globally in 2019, 4.95 million people died suffering from drug-resistant infections, and of these 1.27 million were attributed to antimicrobial resistance (Institute for Health Metrics and Evaluation n.d.). In 2019 in Kenya, 37 300 deaths were associated with antimicrobial resistance.

Cats represent a potential source of transmission of antibacterial resistance to, and between, humans because of their close contact with them (Guardabassi *et al.* 2004). Cats and humans have close contact with each other; they often live inside the household and physical contact is common. In Masai Mara, Kenya, domesticated cats live in close contact with both human tribes and the wild animals that occur in the area. The cats in Masai Mara are in contact with both wildlife and humans, and therefore are a possible disease transmission hazard for both populations. Therefore, the cats in Masai Mara represent a population of interest for study. The aim of this study was to investigate whether antibacterial resistance occurs in the domesticated cats in Masai Mara, using *Escherichia coli* as an indicator bacterium.

2. Literature Review

2.1 Antibiotics

Antibiotics are medicines used to prevent and treat bacterial infections; they either kill the bacteria or prevent it from reproducing (NHS 2022). Some bacterial infections can be cured without using antibiotics, and antibiotics will not work against other types of infection, for example a viral infection. There are different types of antibiotics that work in different ways and are therefore active against different bacteria. Antibiotics can either be bactericidal or bacteriostatic. Bactericidal antibiotics kill the bacteria while bacteriostatic antibiotics prevent the growth of the bacteria (Bernatová *et al*. 2013). In addition, most antibiotics can be classified into different groups (Table 1) according to their mechanism of action:

Table 1. Antibiotic-groups and their mechanism of action

Group:	Effect:	Mechanism:	Other information:
Penicillin (beta-lactam)	Bactericidal effect	Inhibits cell wall synthesis	Most commonly prescribed antibiotics
Cephalosporin (beta-lactam)	Bactericidal effect	Inhibits cell wall synthesis	Example Cefotaxime
Carapenem (beta-lactam)	Bactericidal effect	Inhibits cell wall synthesis	Example Meropenem
Glycopeptide	Slowly bactericidal	Inhibits cell wall synthesis	Often used as a drug of last resort
Aminoglycoside	Bactericidal effect	Inhibits the bacterial protein synthesis	Example Gentamicin
Tetracycline	Bacteriostatic effect	Inhibits the bacterial protein synthesis	Example Tigecycline
Macrolide	Bacteriostatic effect	Inhibits the bacterial protein synthesis	
Lincosamide	Bacteriostatic effect	Inhibits the bacterial protein synthesis	
Oxazolidinone	Mostly bacteriostatic	Inhibits the bacterial protein synthesis	
Chloramphenicol	Mostly bacteriostatic	Inhibits the bacterial protein synthesis	
Streptogramin	Bacteriostatic effect	Inhibits the bacterial protein synthesis	
Sulfonamide	Bacteriostatic effect	Prevents the bacteria to grow and multiplicate	
Ansamycin	Bactericidal effect	Inhibits the synthesis of RNA	Has antibacterial, antiviral and anticancer activities
Fluoroquinolone	Bactericidal effect	Blocks the DNA-replication	Example Ciprofloxacin
Lipopeptide	Bactericidal effect	Disrupts multiple cell membrane functions	

2.1.1 World Health Organization's list of essential medicines

In 2019, WHO published a list of the most essential medicines that are needed for a basic health-care system in the world (World Health Organization 2019). In this list there are several antibiotics, classified into different categories. One group is "access group antibiotics", which have a wide range of activity against common pathogens, and they have low potential of resistance. They are widely available, quality assured and affordable. In this category, antibiotics such as gentamicin and penicillin are to be found. Another group is "watch group antibiotics" which includes antibiotics with higher potential for causing resistance, including the highest priority medicines that are critically important for human medicine. In this category are cefotaxime, ciprofloxacin and meropenem. The last group of antibio-

tics in the list is “reserve group antibiotics”, which includes antibiotics that should be reserved for treatment of suspected or confirmed multi-drug-resistance organism, for example fosfomycin. These should only be used as a last resort.

2.1.2 Antibiotics used in this study

The antibiotics used in this study were cefotaxime, tigecycline, meropenem, gentamicin and ciprofloxacin.

Cefotaxime is a broad-spectrum, beta-lactam antibiotic in the third-generation of cephalosporins (Wikipedia 2023a). Cefotaxime is a bactericidal antibiotic that works by interfering with the synthesis of the peptidoglycan layer of the bacterial cell wall. It is active against gram-negative and gram-positive bacteria including several bacteria with resistance to the classic beta-lactams, such as penicillin. Cefotaxime should be effective against *E. coli.*

Tigecycline is a tetracycline antibiotic developed in response to the growing rate of antibiotic resistance in bacteria such as *E. coli* (Wikipedia 2023f). It is a broad-spectrum antibiotic that is active against both gram-negative and gram-positive bacteria. Tigecycline has a bacteriostatic activity by binding to the 30S-subunit of the ribosome in bacteria inhibiting bacterial protein synthesis.

Meropenem is a beta-lactam antibiotic active against many gram-negative and gram-positive bacteria, and also anaerobic bacteria (Wikipedia 2023e). Meropenem is active against the family Enterobacteriaceae, to which *E. coli* belongs. Meropenem is a bactericidal antibiotic that inhibits bacterial cell wall synthesis. It is on the list of essential medicines, which classifies meropenem as a critically important antibiotic for human medicine.

Gentamicin has bacteriostatic activity by binding to the 30S-subunit of the bacterial ribosome, which negatively impacts protein synthesis (Wikipedia 2023d). Gentamicin is active against a wide range of bacteria, mostly gram-negative bacteria such as *E. coli.* Gentamicin is classified by WHO as critically important for human medicine.

Ciprofloxacin is a fluoroquinolone antibiotic that is also classified as critically important for human medicine by WHO (Wikipedia 2023b). Ciprofloxacin is a broad-spectrum antibiotic that is active against most bacterial pathogens, being particularly effective against gram-negative bacteria such as *E. coli.* The antibiotics work by inhibiting a type II topoisomerase and topoisomerase IV that are necessary for cell division.

2.2 Antibiotic Resistance

Resistance to antibiotics occur when bacteria change in response to the presence of antibiotics; bacteria can thus become antibiotic resistant (Folkhälsomyndigheten 2023). Resistant bacteria can be transmitted between people, but also via animals, food, and the environment. Different bacteria can also spread antibiotic resistance by exchanging resistance genes with each other. The more antibiotics we use, the faster antibiotic resistance increases. With increased antibiotic resistance, infections become more difficult or even impossible to cure, which causes great suffering, premature death, and high costs for healthcare.

Antibiotic resistance is one of the biggest threats to the world today and is rising to dangerously high levels in all parts of the world (World Health Organization 2020). New resistance mechanisms are spreading globally, threatening our ability to treat common infectious diseases, both in animals and humans. In countries without standard treatment guidelines and where antibiotics can be bought without a prescription, the spread of resistance is even worse.

2.2.1 Resistance mechanisms and transmission

Resistance occur when bacteria develop defence strategies against antibiotics (CDC 2022). The mechanisms by which resistance develops can be different. Bacteria with an outer layer membrane can use the membrane to selectively prevent antibiotic drugs from entering. Other bacteria can get rid of the antibiotics using pumps in their cell walls, to prevent the antibiotics from accumulating in the cell. Bacteria can also destroy the antibiotics with enzymes or change the antibiotic's receptors so that they no longer fit. When people or animals become sick and are treated with antibiotics, bacteria with these resistance mechanisms will survive. The resistant bacteria will multiply and some of the resistant bacteria can also pass their resistance mechanisms directly to other bacteria. Even dead bacteria can pass on genes for antibiotic resistance to other bacteria.

2.2.2 Statistics on Antimicrobial Resistance

Globally in 2019 4.95 million people who died suffered from drug-resistant infections and of these deaths, antimicrobial resistance caused 1.27 million deaths (Institute for Health Metrics and Evaluation n.d.). One in five of these deaths were children under the age of 5 years. In Kenya 2019, 37 300 deaths were associated with antimicrobial resistance. The number of deaths in Kenya associated with antimicrobial resistance is higher than deaths from enteric infections, digestive diseases, neoplasms, maternal and neonatal disorders, diabetes, and kidney diseases and other infectious diseases. The only causes of death more common than antimicrobial resistance in Kenya in 2019 were sexually transmitted infections

including HIV/AIDS, cardiovascular diseases and respiratory infections including tuberculosis. There are five bacterial pathogens to be aware of associated with antimicrobial resistance in Kenya; *Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus* and *Salmonella typhi.* In 2019, *Escherichia coli* was the second most common bacterium associated with antimicrobial resistance deaths in Kenya.

2.3 Escherichia coli

E. coli is a bacterium in the family Enterobacteriaceae (VetBact 2023). It is a gram-negative coliform rod that is a facultative anaerobe. Strains of *E. coli* are divided into different pathotypes depending on which virulence factors they have. The majority of these strains are non-pathogenic intestinal bacteria, belonging to the normal microbiota of the gut in both animals and humans. But some strains of the bacteria are pathogenic, such as Enteropathogenic *E. coli*, EPEC, and Enterotoxigenic *E. coli*, ETEC, which, can cause fever, diarrhea, vomiting, septicemia and endotoxic shock, among other clinical signs, in several animal species.

When people suffer from ETEC, administration of antibiotics has been shown to shorten the course of illness and duration of the disease, although the resistance to commonly used antibiotics is increasing (Wikipedia 2023c). *E. coli* have an outer membrane surrounding the cell wall which provides a barrier to certain antibiotics such as penicillin. The antibiotics of choice are therefore usually fluoroquinolones or azithromycin such as rifaximin.

2.3.1 Extended Spectrum Beta-Lactamase, ESBL

Some bacteria have an enzyme that breaks down many beta-lactam antibiotics, making the bacteria resistant to the traditional penicillins and most cephalosporins (Folkhälsomyndigheten 2016). It is most common in the bacteria *Escherichia coli* and *Klebsiella pneumoniae*. Today, there is only one group of beta-lactam antibiotics to which ESBL-producing bacteria are sensitive. According to the Swedish Public Health Agency there is a clear connection between high antibiotic use and the development and spread of resistance caused by ESBL-producing bacteria. Contagion with ESBL occurs both in society and in healthcare; traveling to countries with a high prevalence incurs a risk for becoming infected.

2.4 One Health

One Health is an integrated approach to optimize the health of people, animals and the environment (World Health Organization 2017). It mobilizes different disciplines and communities of society to work together for a more sustainable solution to a problem. A One Health-perspective is particularly important when it comes to issues such as zoonoses, antimicrobial resistance, water- and food safety. The increase of antimicrobial resistance is due to antimicrobial use and misuse in human, animal and environmental sectors and also the spread of resistance within and between these sectors (McEwen & Collignon 2018). Most of the antibiotics used to treat infections in humans are also used to treat infections in animals, making it important to take a One Health approach when addressing the problem and trying to find a solution.

Of the three domains in One Health, human has the highest prevalence of multi-drug-resistance genes (Robinson *et al*. 2016). Apart from treating infectious diseases, antibiotics make modern medicine possible by allowing surgical procedures, and chemotherapy to treat cancer. On the animal side, the problem is most investigated in livestock, for example resistance among mastitis organisms is well-documented and considered an emerging problem. Even antibiotic resistance in small animal clinics is growing. The third part of One Health, the environment, is the least investigated but still just as important to understand the whole problem. Environmental bacteria serve as a source for antimicrobial resistance genes that can become incorporated into pathogens in people and animals. This phenomenon increases due to the large amount of antibiotic residues that enter the environment from the pharmaceutical industry, from hospitals and from intensive livestock farms. The impact of these factors is most likely to be more pronounced in developing countries with little environmental legislation.

2.5 Antibiotic resistance in animals in low- and middle income countries

The use of antibiotics in veterinary medicine in low- and middle income countries is largely unregulated (Brown *et al.* 2020). In a study about antibiotic-resistant bacteria detected in milk in Nairobi, it was concluded that the lack of regulations concerning the use of veterinary drugs, such as antibiotics, may contribute to the increase of antimicrobial resistance.

In East African countries, including Kenya, laboratory services are not always available, and when they are they are seldom equipped to perform susceptibility testing of bacteria (Wamola 2002). Antibiotics for animals are therefore normally

prescribed based on empirical experience instead of on laboratory diagnosis and identification of the resistance pattern of the bacterium. Antibiotics can even be prescribed to patients with viral or fungal infections, where they are of no use. In addition, broad-spectrum antibiotics are often given instead of specific antibiotics, making the risk for developing resistance greater.

In a study by Muoi *et al.* (2019) they concluded, as previously described, that selling antibiotics without prescription is not uncommon in low-and middle income countries, such as Kenya, even though WHO recommends a medical prescription. The authors visited 19 veterinary drug stores in the capital of Kenya, Nairobi. There they found that none of the antibiotics were sold on prescription and that the customers often could purchase antibiotics according to their own preference.

The challenges of antimicrobial resistance are particularly high in low- and middle income countries due to the high frequency of infectious diseases together with living conditions which predispose to frequent interactions between people and animals (Caudell *et al.* 2020). Genotypic studies in low-and middle income countries showed evidence of transmission of antimicrobial resistance between animals, humans and the environment. For example, in Uganda and Tanzania, similar resistance patterns were found in isolates from humans and animals. In contrast, genotypic studies from high-income countries, such as the Netherlands, have largely shown distinguishable patterns in livestock and the human population, most likely as a result of limited contact between animals and humans.

2.6 Antibacterial resistance in pets

In a review article made by Guardabassi *et al.* (2004), dogs and cats were considered to represent a potential source of transmission of antimicrobial resistance to humans because of their close contact with them. The relationship between humans and pets has changed through the years and is now closer than ever. Today these animals are often kept inside the households and close physical contact, such as humans petting the animals or the animals licking the humans, occurs at a higher frequency. The close contact together with the fact that the same classes of antimicrobial agents are used in human and small animal medicine creates a risk of transfer of resistant bacteria and/or resistance genes.

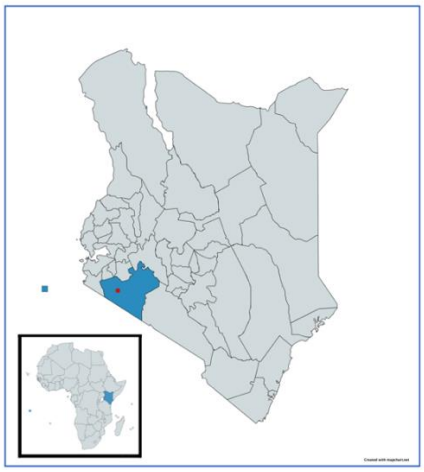
Resistance in *Escherichia coli*, with focus on ESBL, in dogs, cats and their owners in northern Kenya was investigated in a study by Albrechtova *et al.* (2012). The study showed that ESBL-producing *E. coli* isolates were found in 47 out of 216 dogs (22%), 2 out of 50 cats (4%) and 4 out of 23 humans (17%). The isolates with identical genome profiles were detected in animals and humans living in the same

area. The results from this study suggest a spread of resistant bacteria between humans and domesticated carnivores. The authors suggest that the resistance rates in dogs compared to cats could be explained by the different behaviours of the two species. Dogs are probably in closer contact with the households as they eat left-overs from human meals, while cats may prefer hunting in the bush and are therefore not in as close contact with the humans.

2.7 Masai Mara

Masai Mara is a nature reserve in the southwest of Kenya bordering the Serengeti in Tanzania (Wikipedia 2022). Masai Mara is home to several different wild felids; African wildcat, gazelle, cheetahs, lions, servals and leopard along with many other animals such as elephant, giraffe, buffalos, zebras, antelopes etc. It is also home to several human communities. Mara North Conservancy is a private reserve which forms the north-western zone of Masai Mara and is a vital part of the ecosystem in Masai Mara (Mara North Conservancy 2023). The conservancy actively works for a sustainable environment with conservation of wild animals as well as the local communities.

Mararianta district is located in Mara North Conservancy in the national reserve Masai Mara. In Mararianta, wild animals are free to enter the area whenever they want, creating an interface between wild animals, domestic animals, and people. Mararianta district has, in addition, a large population of free-roaming domestic cats.



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

Figure 1. Map of Kenya where Mararianta is marked with red dot. Map done with Mapchart.net by Emilia Schultz (2023).

2.7.1 Cats as transmission hazards

In a review article (Lepczyk *et al.* 2015), cat behaviour was studied in relation to disease risk. The authors reported, among other findings, that domesticated cats that have access to both urban and rural environments, in contact with both wildlife and humans, are a possible disease transmission hazard for several diseases. Because people, domestic cats and wild animals live in close contact with each other in Mararianta, antibiotic resistance can transfer between them, which means that cats, which should not have been treated with antibiotics, can harbour resistant bacteria. Furthermore, this resistance can be transferred to wild animals. The aim of the present study was therefore to investigate whether the cats in Mararianta carry bacteria that are resistant to antibiotics; the results could contribute to the conservation of the wild felids in the reserve as well as improving the welfare of the domestic cats and people in Mararianta.

3. Material and Methods

3.1 Material collection

The samples were collected in the Mararianta district in Mara North Conservancy in the national reserve Masai Mara in Kenya. This area in Masai Mara has a large population of feral and free-roaming cats. Different households in the districts were visited and asked if they owned a cat and wanted to participate in the study; 40 cats were available for the study, both females and males. As the owners were asked if they wanted to participate, no ethical approval was required. The cats were taken to a temporary clinic where they were sedated before the collection of samples via rectal-swabs. In addition to the sampling, the cats were castrated and vaccinated against rabies. The castration and vaccination was done under a project that aimed to prevent the expansion of the population of free-roaming cats in Mararianta, reduce the spread of diseases between wild animals, cats and humans, prevent the genetic mixing between cats and the wild felids, and increase the social status of the cat population. After surgery and sample collection, the cats were released into the same area where they were caught, or returned to their owners.

3.2 Bacterial growth

The faecal samples were taken with an e-swab in the rectum and streaked directly onto a McConkey agar plate. The agar plate was then incubated at 37°C for 24 hours. On McConkey agar, *E. coli* are about 2-4 mm in size and rose-purple in colour (Figure 2).

Figure 2. E. coli on a McConkey-agar. Photo taken by Agnes Diamanti Barredal.

After incubation, identification of *E. coli* was made on the McConkey agar. An *E.coli* colony was transferred from the third smear on the McConkey agar to a blood agar plate and then three smears was made on the blood agar. The blood agar plate was then incubated at 37°C for 24 hours. On blood agar, *E. coli* are medium-sized (2-3 mm), opaque, sticky and grey-white; they have a characteristic smell. Some strains produce a narrow clear haemolysis zone on blood agar (Figure 3).

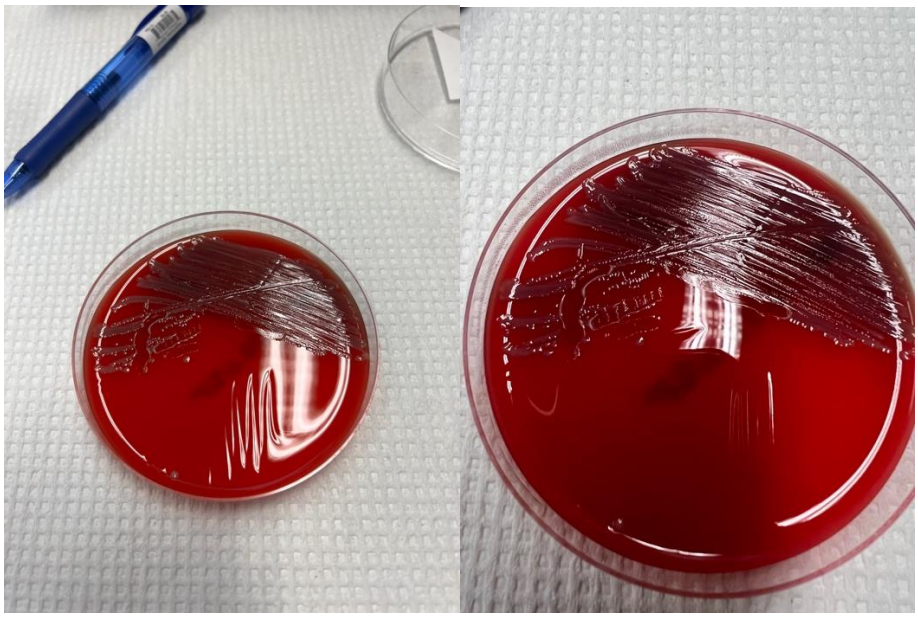


Figure 3. E. coli on a blood-agar. Photo taken by Agnes Diamanti Barredal.

3.3 Typing of bacteria

To check that the identification of the bacteria was correct, a potassium hydroxide (KOH) test, oxidation test and spot indole test were performed. The indicator bacteria for this study, *E. coli*, is KOH positive, oxidase negative and indole positive.

The KOH test identifies gram negative bacteria (VetBact 2017b). Potassium hydroxide dissolves a peptidoglycan layer of the cell walls in gram negative bacteria, releasing intracellular contents, including DNA. The DNA makes the KOH-solution viscous, and it will therefore stick to a plastic loop when touched. Gram negative bacteria, such as *E. coli*, will be affected by KOH because they have a thick peptidoglycan layer in the cell wall, and their DNA will be released.

The oxidation test identifies if the bacterium is aerobic or anaerobic. Cytochrome c oxidase is the terminal enzyme in the respiratory chain possessed by some bacteria (VetBact 2009). Many aerobic bacteria have oxidase, while bacterium such as *E. coli*, which is a facultative anaerobe, lack this enzyme.

The spot indole test analyses whether the bacteria can hydrolyse tryptophan to, among other things, indole (VetBact 2017a). Bacteria that express tryptophanase, which is an enzyme that hydrolyse tryptophan to indole, pyruvic acid and ammonia, give a positive result. *E. coli* is indole positive.

3.4 Disk diffusion

After the bacterium was grown and identified, a suspension was made. Preparation of the inoculum was done using the colony suspension method. Three different bacterial colonies were taken from the blood agar plate, suspended in 1 ml of sterile water, and mixed to a density of 0.5 McFarland. If the density is higher, there will be reduced areas of inhibition, and vice versa. This analysis was made in the field and therefore a densitometer to measure McFarland was not available, instead the density was estimated visually (Figure 4).

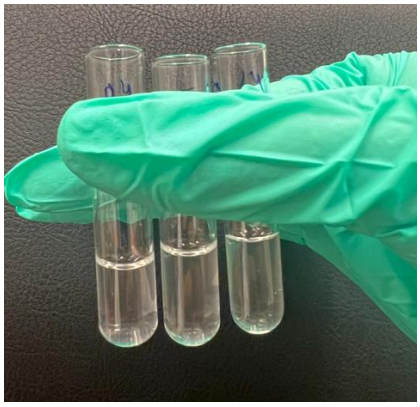


Figure 4. Suspension in the density 0,4, 0,5 and 0,6 McFarland. Photo taken by Agnes Diamanti Barredal.

The method used for analysing antibacterial resistance was disk diffusion. Disk diffusion is a qualitative method where the bacterial strains are first isolated, in this case *E. coli*, and then spread on a Mueller-Hinton agar plate for determining resistance (Sveriges veterinärmedicinska anstalt 2023). Filter patches with a specific concentration of antibiotics are then placed on the agar plate and the antibiotics will diffuse out into the medium. The agar plates are incubated in 37°C for 24 hours and the results can be read as the diameter of the zone of inhabitation around the disc where no colonies grow (Figure 5). The inhibitory concentration can be indirectly estimated by extrapolation from standard curves. The zone diameter breakpoints are calibrated to the bacterial MIC breakpoints that are published by EUCAST (EUCAST 2023b). The test method is indirect.

Figure 5. Disk diffusion on Mueller-Hinton agar. Photo taken by Agnes Diamanti Barredal.

4. Results

In total 40 cats were tested, 11 males and 29 females. Of the 40 cats, 39 of them were owned by the Maasai people in the Mararienta district. One cat was a feral cat captured in Karen Blixen Camp using a trap. It was possible to culture *E. coli* in all samples bar one.

4.1 Inhibition of bacterial growth

The antibiotics used in this study were cefotaxime, tigecycline, meropenem, gentamicin and ciprofloxacin. The reference values for *E. coli* for these antibiotics published by EUCAST are (EUCAST 2023a):

- Cefotaxime, ctx: 25-31 mm.
- Tigecycline, tgc: 20-27 mm.
- Meropenem, mem: 28-35 mm.
- Gentamicin, cn: 19-26 mm.
- Ciprofloxacin, cip: 29-37 mm.

If the bacterium has a zone of inhibition below these reference values, it is counted as resistant to this type of antibiotic.

Table 2. Zone of inhibition for gram positive, oxidase negative and indole positive bacteria where the values highlighted in yellow represent the resistant bacteria

Cat	KOH	Oxidase	Indole	Ctx[1] (mm)	Tgc[2] (mm)	Mem[3] (mm)	Cn[4] (mm)	Cip[5] (mm)
1	+	-	+	10	21	30	10	9
2	+	-	+	27	21	28	23	32
3	+	-	+	27	23	37	24	35
4	+	-	+	26	23	32	19	39
5	+	-	+	9	22	32	21	36
6	+	-	+	26	21	34	23	30
7	+	-	+	31	23	35	24	38
8	+	-	+	30	21	34	23	36
9	+	-	+	26	22	36	24	34
10	+	-	+	33	22	34	23	35

11	+	-	+	26	22	36	23	31
12	+	-	+	31	21	36	25	31
13	+	-	+	8	21	33	25	8
14	+	-	+	9	23	34	23	34
15	+	-	+	29	24	36	23	36
16	+	-	+	32	24	34	31	39
17	+	-	+	31	22	35	23	37
18	+	-	+	32	21	35	25	38
19	+	-	+	32	24	34	25	37
20	+	-	-	31	24	35	24	39
21	+	-	+	30	22	33	24	32
22	+	-	+	26	21	29	22	33
23	+	-	+	31	23	28	25	38
24	+	-	+	32	22	35	22	36
25	+	-	+	30	24	33	25	38
26	+	-	+	30	21	35	21	30
27	+	-	+	30	22	35	22	31
28	+	-	+	8	21	32	21	31
29	+	-	+	30	23	36	21	39
30	+	-	+	25	21	33	22	24
31	+	-	+	29	22	34	21	29
32	+	-	+	30	22	35	23	39
33	+	-	+	28	21	34	21	31
34	+	-	+	31	24	35	20	22
35	+	-	+	32	22	35	26	36
36	+	-	+	12	22	30	23	29
37	+	-	+	30	22	32	24	31
38	+	-	+	11	16	28	21	31
39	+	-	+	31	22	35	22	36
40	+	-	+	27	20	35	22	35

[1] Ctx = cefotaxime
[2] Tgc = tigecycline
[3] Mem = meropenem
[4] Cn = gentamicin
[5] Cip = ciprofloxacin

4.2 Prevalence of antibiotic resistance in Mararianta

Prevalence of antibiotic resistance in all 200 different antibiotic filter patches was 6.5% (13/200). The prevalence of bacterial resistance to at least one type of antibiotics was 22.5% (9/40); of these resistant bacteria, 33.3% (3/9) were resistant to more than one type of antibiotics (Figure 6).

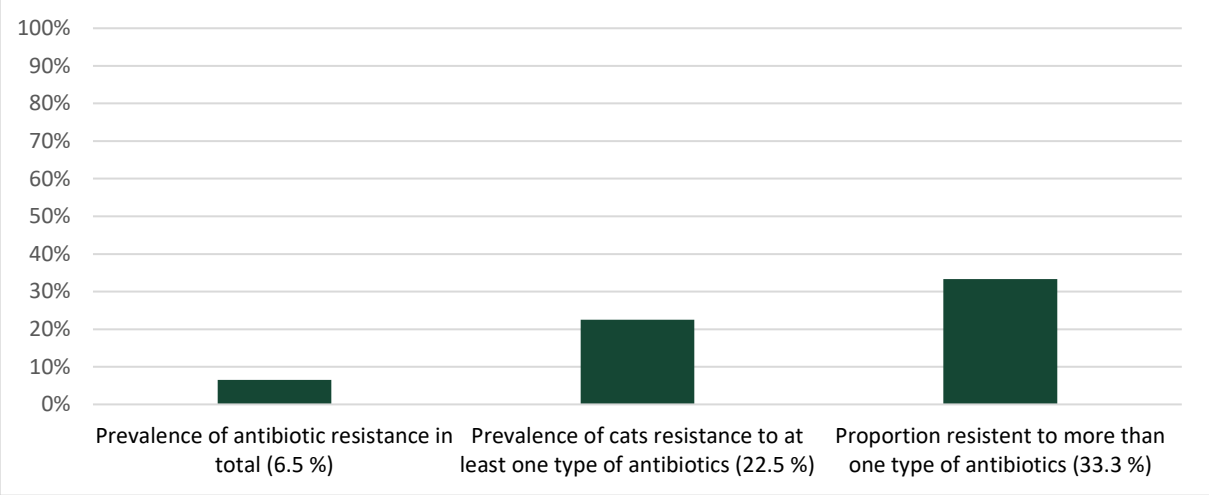


Figure 6. Prevalence of resistant bacteria.

Resistance to the different antibiotics varied (Figure 7).

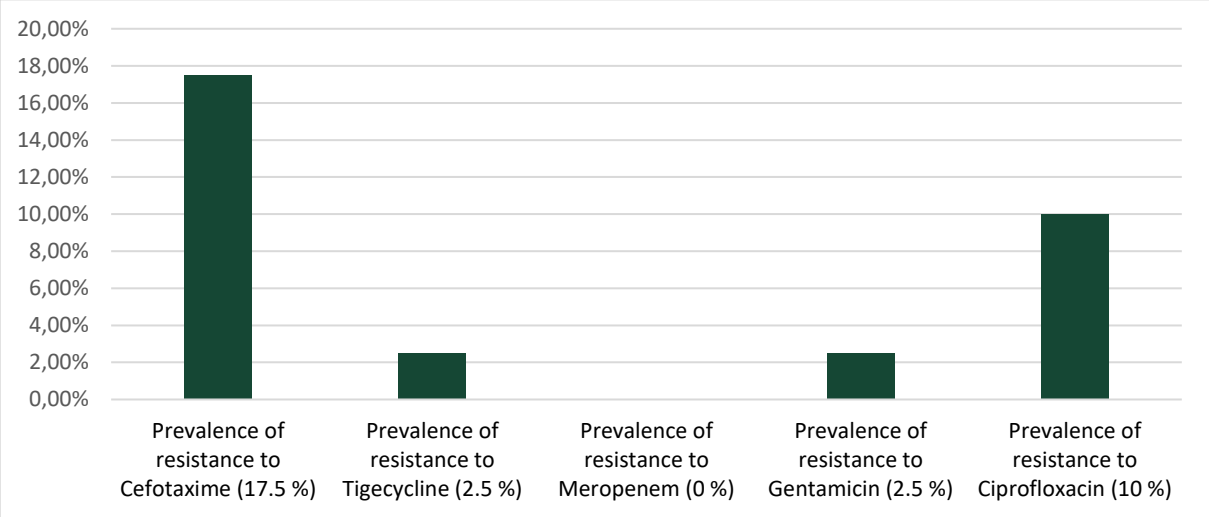


Figure 7. Resistance to the different types of antibiotics.

5. Discussion

The aim of this study was to investigate whether the cats in Mararianta carry antibiotic resistance. The total prevalence of antibiotic resistance in all 200 different antibiotic filter patches was 6.5%, and the proportion of cats that had resistance to at least one type of antibiotics was 22.5%. Of these resistant bacteria, 33.3% were resistant to more than one type of antibiotics. Resistance was more common to some antibiotics than others. The prevalence of resistance to cefotaxime was 17.5% while the prevalence of resistance to meropenem was 0%. Because relatively few cats were sampled in this study, it is difficult to draw conclusions about the entire cat population in Mararianta, but what can be concluded is that antibiotic resistance occurs in the population. Since there is antibiotic resistance in the cat population, there is a risk of infection to both humans and wild animals in the area.

5.1 Prevalence of antibiotic resistance

In this study the prevalence of cats' resistance to at least one type of antibiotics was 9 out of 40 (22.5%). Compared to the study done by Albrechtova *et al*. (2012) that found ESBL-producing *E. coli* in 2 out of 50 cats (4%), the result from this study is very high. However, in the study made by Albrechtova *et al.* only resistance to cefotaxime was tested, while in this study four more antibiotics were tested in addition to cefotaxime. In the Albrechtova *et al.* study they initially used disk diffusion as in the present study, and the ESBL-positive bacteria in the Albrechtova *et al.* study were then sequenced by PCR. The size of the groups also differs; although both studies have small sample-groups, there is a difference between 40 and 50 cats. The fact that both studies have small sample-groups also makes them more sensitive to random variation. The timing of the study may also have an effect; antibiotic resistance has increased in the recent years and therefore the result of this study conducted in 2023 may be higher than in the study published in 2012.

That such a high prevalence of antibiotic resistance was found in this study may relate to the fact that the use of antibiotics is largely unregulated in Kenya. In studies made by Muoi *et al*. (2019), they concluded that selling antibiotics without a prescription is common in low-and middle income countries; antibiotics are often prescribed based on empirical experience instead of laboratory diagnosis (Wamola,

2002). If antibiotics are administered to more people and animals, the risk of developing resistance will increase. Resistance will also increase if broad-spectrum antibiotics are used instead of narrow-spectrum antibiotics and if the antibiotics are used incorrectly. All of these risks increase with more unregulated antibiotic use, which is probably the reason that the prevalence of resistance is so high in Kenya. Another aspect that would have been interesting to investigate further, is whether the prevalence of resistance in cats differs in different countries, and how it relates to different countries' antibiotic use.

5.2 Resistance in different types of antibiotics

The fact that the resistance in this study differs between different types of antibiotics probably depends both on which types of antibiotics are more commonly used in the area, and how easy it is for the bacteria to develop resistance mechanisms against these specific types of antibiotics. The fact that cefotaxime, which is a broad-spectrum antibiotic, has the highest prevalence of resistance in this study may have greater consequences than if a narrow-spectrum antibiotic had the same prevalence. Ciprofloxacin also had a high prevalence in this study. Ciprofloxacin is, like cefotaxime, a broad-spectrum antibiotic. In addition, it is also classified as critically important for human medicine by WHO; resistance against ciprofloxacin can thus have grater consequences for human health.

5.3 Risk of infection spreading

To evaluate how big the risk of infection spreading between different animals and humans, resistance genes need to be sequenced. In the study made by Albrechtova *et al.* (2012) in northern Kenya, they found out that the same ESBL-producing *E. coli* isolates were found in dogs, cats and their owners. Similar results were found in a study by Caudell *et al.* (2020). In that study, evidence of transmission of antimicrobial resistance between animals, humans and the environment was seen in low- and middle income countries. This may also indicate a spread of infection between humans and the domesticated animals in Mararianta, but to be sure of this, further research is required.

5.4 One Health

How resistance has been transmitted to bacteria in these cats is debatable. The cats have probably not been treated with antibiotics, but rather acquired the resistance from humans, other animals or the environment, making this a One-Health problem.

Possibly the cats in this study are more tame than other cats in the area, which is why they were easy to capture. This could mean that they live closer to humans and therefore have been infected with more antibiotic resistance than other cats. The cats could have been infected both through the close contact between themselves and their owners, but also through, for example, being fed left-overs from humans. In order to evaluate whether the antibiotic resistance in the cats was transmitted from humans, it would have been interesting to sequence both the cats' resistance genes and the humans' resistance genes. However, this was beyond the scope of the present study.

Another possible source of infection for these cats is via other animals. It is unlikely that the wild animals in the area have been treated with antibiotics. However, the domesticated dogs in the area may have been treated with antibiotics as various projects are often held for dogs, where they are, among other things, castrated and vaccinated. In these projects, there is a chance that the dogs have been treated with antibiotics, and thus they may have developed antibiotic resistance. The dogs and the cats in Mararianta not only live in close contact with people but also with each other. The cats are therefor also a source of infection for the dogs.

The cats could also have contracted the infection from the environment. Because Kenya is a country with little environmental legislation, antibiotic-resistant bacteria can enter the environment from the pharmaceutical industry, from hospitals or from livestock farms. In Mararianta there are many cattle- as well as sheep- and goat farms. These animals could have been treated with antibiotics and thus infect the cats with antibiotic resistant both via the environment and if the cats eat food containing these animals. There are also hospitals in the area and thus there is a risk that bacteria that carry resistance may also have come from the environment.

It is important to have a One Health approach both when looking at the causes of infection and at the consequences. That cats harbour bacteria that carry antibiotic resistance means that they can spread the resistance to other animals, people, and the environment. Most of the antibiotics used to treat infections in humans are also used to treat infections in animals, which means that resistance to these has greater consequences for the human population (McEwen & Collignon 2018).

5.5 Limitations and bias

The study is a master's project and was therefore limited in several ways. It was only possible to sample 40 cats in the time available. If we had had access to more cats, more samples could have been taken and thus more reliable conclusions could have been drawn. The cats were taken to the clinic by a veterinary assistant who,

with the help of the Maasai, caught their cats. Cats that were difficult to catch were not included in the study, which creates a selection bias in this convenience sample. A random selection in the Mararianta cat population would have been very difficult as there is no record of the number of cats, or who owns them, or how to access them. This selection bias means that we cannot guarantee that the sample is representative of the entire population. Since it is a relatively small sample, the precision is low. The study is thus more sensitive to random variation; we may have caught all the cats in the area that harbour bacteria that are resistant to antibiotics or may have sampled only those with low antimicrobial resistance in their bacteria.

5.6 Source of error

The assays were made in the field and therefor a McFarland densitometer to measure density was not available. Instead, we trained to differentiate the density visually. If the samples were actually of higher density, there would be reduced areas of inhibition around the disks, and vice versa. The fact that we had no measuring tool for this can therefore cause misleading results. To reduce this source of error, both I and two of my colleagues practiced detecting differences in the density when we were in Sweden and had access to a densitometer, as well as using pictures of different densities to support the determination of the density.

Twenty of the tests were made without blood agar due to contamination of the agar plates with maggots. Without the blood agar it was harder to ensure that we had the correct bacteria, *E. coli*. This can lead to mixed flora being grown on Mueller-Hinton agar in some cases. Since we were in a nature reserve, without access to a laboratory, we could not obtain new agar plates. Instead, we had to visually select *E. coli* from the McConkey-agar, using pictures and descriptions of the bacterium on McConkey-agar as a guide. In all samples except one, cat number 20, *E. coli* could be cultured. Although cat number 20 had bacteria that looked like *E. coli*, the spot indole test was negative instead of positive, suggesting that it was another gram negative anaerobic bacterium rather than *E. coli*. It was decided to include this cat in the study even though the characteristics of the cultured bacteria did not concur with the criteria for *E. coli*, since antibacterial resistance can be studied on other gram negative anaerobic bacteria as well.

6. Conclusions

From this study we can conclude that antibiotic resistance occurs in the domestic cats in Mararianta, and poses a risk of infection to both human and wild animal populations in this area.

References

Albrechtova, K., Dolejska, M., Cizek, A., Tausova, D., Klimes, J., Bebora, L. & Literak, I. (2012). Dogs of nomadic pastoralists in Northern Kenya are reservoirs of plasmid mediated cephalosporin- and quinolone-resistant Escherichia coli, including pandemic clone B2-O25-ST131. *Antimicrobial Agents and Chemotherapy*, 56 (7), 4013–4017. https://doi.org/10.1128/AAC.05859-11

Bernatová, S., Samek, O., Pilát, Z., Šerý, M., Ježek, J., Jákl, P., Šiler, M., Krzyžánek, V., Zemánek, P., Holá, V., Dvořáčková, M. & Růžička, F. (2013). Following the mechanisms of bacteriostatic versus bactericidal action using Raman spectroscopy. *Molecules*, 18 (11), 13188–13199. https://doi.org/10.3390/molecules181113188

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

Caudell, M.A., Dorado-Garcia, A., Eckford, S., Creese, C., Byarugaba, D.K., Afakye, K., Chansa-Kabali, T., Fasina, F.O., Kabali, E., Kiambi, S., Kimani, T., Mainda, G., Mangesho, P.E., Chimpangu, F., Dube, K., Kikimoto, B.B., Koka, E., Mugara, T., Rubegwa, B. & Swiswa, S. (2020). Towards a bottom-up understanding of antimicrobial use and resistance on the farm: A knowledge, attitudes, and practices survey across livestock systems in five African countries. *PLOS ONE*, 15 (1), e0220274. https://doi.org/10.1371/journal.pone.0220274

CDC (2022). *How Antimicrobial Resistance Happens.* Centers of Disease Control and Prevention. https://www.cdc.gov/drugresistance/about/how-resistance-happens.html [2023-09-14]

EUCAST (2023a). *Clinical breakpoints - breakpoints and guidance*. The European Committee on Antimicrobial Susceptibility Testing. https://www.eucast.org/clinical_breakpoints [2023-09-13]

EUCAST (2023b). *Disk diffusion methodology.* The European Committee on Antimicrobial Susceptibility Testing. https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology [2023-09-13]

Folkhälsomyndigheten (2023). *Antibiotika och antibiotikaresistens.* https://www.folkhalsomyndigheten.se/smittskydd-beredskap/antibiotika-och-antibiotikaresistens/ [2023-09-13]

Folkhälsomyndigheten (2016). *Sjukdomsinformation om bakterier med Extended Spectrum Beta-Lactamase (ESBL).* https://www.folkhalsomyndigheten.se/smittskydd-beredskap/smittsamma-sjukdomar/extended-spectrum-beta-lactamase-esbl/ [2023-09-14]

Guardabassi, L., Schwarz, S. & Lloyd, D.H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria: Review. *Journal of Antimicrobial Chemotherapy*, 54 (2), 321–332. https://doi.org/10.1093/jac/dkh332

Institute for Health Metrics and Evaluation (n.d). *The burden of antimicrobial resistance (AMR) in Kenya.* https://www.healthdata.org/sites/default/files/files/Projects/GRAM/Kenya_0.pdf [2023-09-13]

Lepczyk, C.A., Lohr, C.A. & Duffy, D.C. (2015). A review of cat behavior in relation to disease risk and management options. *Applied Animal Behaviour Science*, 173, 29–39. https://doi.org/10.1016/j.applanim.2015.07.002

Mara North Conservancy (2023). *ABOUT US.* http://maranorth.org/about-us/ [2023-10-06]

McEwen, S.A. & Collignon, P.J. (2018). Antimicrobial resistance: a One Health perspective. *Microbiology Spectrum*, 6 (2), 10.1128/microbiolspec.arba-0009–2017. https://doi.org/10.1128/microbiolspec.arba-0009-2017

Moore, D. (2023). *Antibiotic Classification & Mechanism.* https://www.orthobullets.com/basic-science/9059/antibiotic-classification-and-mechanism [2023-11-01]

Muloi, D., Fèvre, E.M., Bettridge, J., Rono, R., Ong'are, D., Hassell, J.M., Karani, M.K., Muinde, P., van Bunnik, B., Street, A., Chase-Topping, M., Pedersen, A.B., Ward, M.J. & Woolhouse, M. (2019). A cross-sectional survey of practices and knowledge among antibiotic retailers in Nairobi, Kenya. *Journal of Global Health*, 9 (2), 020412. https://doi.org/10.7189/jogh.09.020412

NHS (2022). *Antibiotics.* https://www.nhs.uk/conditions/antibiotics/ [2023-11-01]

Robinson, T.P., Bu, D.P., Carrique-Mas, J., Fèvre, E.M., Gilbert, M., Grace, D., Hay, S.I., Jiwakanon, J., Kakkar, M., Kariuki, S., Laxminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T.P. & Woolhouse, M.E.J. (2016). Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110 (7), 377–380. https://doi.org/10.1093/trstmh/trw048

Schultz, E. (2023). *The occurrence of FeLV, FIV and FeCoV in free-roaming cats in Mara North Conservancy, Kenya: a possible threat to wild felids?* (Independent project) Swedish University of Agricultural Sciences. Veterinary Medicine Programme. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-18619

Sveriges veterinärmedicinska anstalt (2023). *Laboratoriemetoder för resistensbestämning.* https://www.sva.se/amnesomraden/antibiotika/laboratoriediagnostik/laboratoriemetoder-for-resistensbestamning/ [2023-09-13]

VetBact (2023). *Escherichia coli.* https://www.vetbact.org/?artid=68 [2023-09-13]

VetBact (2017a). *Indol-test.* https://www.vetbact.org/index.php?displayextinfo=35&vbsearchstring=spot%20indole [2023-10-06]

VetBact (2017b). *Kaliumhydroxidtest.* https://www.vetbact.org/index.php?displayextinfo=117 [2023-10-06]

VetBact (2009). *Katalas/Oxidas.* https://www.vetbact.org/index.php?displayextinfo=23&vbsearchstring=oxidase%20test [2023-10-06]

Wamola, I.A. (2002). Minimising antibiotic resistance to Staphylococcus aureus in developing countries. *East African Medical Journal*, 79 (11), 574–579. https://doi.org/10.4314/eamj.v79i11.8801

Wikipedia (2023a). *Cefotaxime.* https://en.wikipedia.org/w/index.php?title=Cefotaxime&oldid=1166093495 [2023-10-19]

Wikipedia (2023b). *Ciprofloxacin.* https://en.wikipedia.org/w/index.php?title=Ciprofloxacin&oldid=1179641066 [2023-10-19]

Wikipedia (2023c). *Escherichia coli.* https://en.wikipedia.org/w/index.php?title=Escherichia_coli&oldid=1172793607 [2023-09-13]

Wikipedia (2023d). *Gentamicin.* https://en.wikipedia.org/w/index.php?title=Gentamicin&oldid=1173828673 [2023-10-19]

Wikipedia (2022). *Masai Mara viltreservat.* https://sv.wikipedia.org/w/index.php?title=Masai_Mara_viltreservat&oldid=50511292 [2023-09-14]

Wikipedia (2023e). *Meropenem.* https://en.wikipedia.org/w/index.php?title=Meropenem&oldid=1170191361 [2023-10-19]

Wikipedia (2023f). *Tigecycline.* https://en.wikipedia.org/w/index.php?title=Tigecycline&oldid=1173596427 [2023-10-19]

World Health Organization (2020). *Antibiotic resistance.* https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance [2023-09-13]

World Health Organization (2017). *One Health*. https://www.who.int/news-room/questions-and-answers/item/one-health [2023-09-15]

World Health Organization (2019). *World Health Organization Model List of Essential Medicines.* https://iris.who.int/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?sequence=1&isAllowed=y [2023-10-19]

Popular science summary

Antibacterial resistance occurs when bacteria change in response to the use of antibiotics. Resistant bacteria can be transmitted between people, but also via animals, food and the environment. Different bacteria can also spread resistance by exchanging resistance genes with each other. With increased antibiotic resistance, infections become more difficult or even impossible to cure, which causes great suffering, premature death, and high costs for healthcare. Antibiotic resistance is one of the biggest threats to the world today and is rising to dangerously high levels.

In countries such as Kenya, antibiotic use is largely unregulated. Selling antibiotics without a prescription is common and they may even be prescribed to patients with viral or fungal infections, where they are of no use. In addition, broad-spectrum antibiotics, i.e. antibiotics that work against many different types of bacteria, are often sold instead of specific antibiotics, making the risk for developing resistance even bigger.

Cats represent a potential source of transmission of antibacterial resistance to, and between, humans because of their close contact with them. In Masai Mara, Kenya, domesticated cats live in close contact with both human tribes and the wild animals that occur in the area. Therefore, they are a possible disease transmission hazard for both humans and wild animals. The aim of this study was to investigate whether antibacterial resistance occurs in the domesticated cats in Masai Mara, where *Escherichia coli* was used as an indicator bacterium.

In total 40 cats were available for in the study, both males and females. The collection of samples was done via rectal-swab when the cats were presented for vaccination and castration. The method used for analysing antibacterial resistance was disk diffusion, a method where the bacterium is cultured and then filter patches with different antibiotics are used to determine the resistance. The antibiotics used in this study were cefotaxime, tigecycline, meropenem, gentamicin and Ciprofloxacin.

The prevalence of antibiotic resistance in all 200 different antibiotic filter patches was 6.5%. The prevalence of cats resistant to at least one type of antibiotics was

22.5% and of these resistant cats 33.3% was resistant to more than one type of antibiotics. Resistance was more common to some antibiotics than other. For example, the prevalence of resistance to cefotaxime was 17.5% while the prevalence of resistance to meropenem was 0%. In conclusion, antibiotic resistance occurs in the domestic cats in Mararianta, and poses a risk of infection to both human and wild animal populations in this area.

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