PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF CAMPYLOBACTER SPECIES ISOLATED FROM POULTRY IN MATHIRA, NYERI COUNTY.

Investigator – Edith Kagio Chege

Mentor - Dr Benjamin Swift (RVC)

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RVC Royal Veterinary College University of London

ABSTRACT

Introduction

Antimicrobial resistance is a growing global threat that is increasing animal and human health concerns. Antimicrobial resistance arises when antimicrobial agents fail to effectively kill microorganisms that were previously susceptible to them (Ayukekbong, 2017). The emergence of AMR is attributed to imprudent antimicrobial use arising from inappropriate practices in prescription, misuse, and overuse in both human and animal health (Muloi, 2019)**.** This leads to exposure of animal and human normal flora and pathogens to selection pressure leading to the emergence of antimicrobial resistant strains which can withstand and survive in the presence of antimicrobial agents which would initially kill them. These new strains can be spread from animals to humans and the environment. In low and middle-level countries (LMICs), antibiotics are used for the treatment and prevention (prophylaxis) of infectious diseases in animals and humans. Most of these antibiotics are accessed from pharmacies and agro-veterinary shops over the counter without prescriptions from clinicians ad veterinary professionals and the data on antibiotic use in these countries is scarce (Muloi, 2019).

Campylobacter species are bacterial pathogens recognized as a cause of gastroenteritis in the human population and pose a major public health threat worldwide. There are several species of Campylobacter which include *C. jejuni, C.coli, C. lari* and *C.uppsaliensis,* capable of causing human illness. However, *C. jejuni* and *C.coli* are the most commonly isolated species from poultry and poultry products and cause diarrhoea in patients, mainly children under the age of 1-year, young adults, and immunosuppressed persons. Campylobacter is present in the gut of most animals as normal flora and is transferred to foods from the faeces during slaughter and is acquired through consumption of undercooked poultry and other meat as well as water and unpasteurized milk. Fruits and vegetables can be contaminated by water containing faeces from birds and other animals. Campylobacter can also be transmitted through contact with dog and cat faeces as well as drinking contaminated water. Campylobacter infections are common in low- and middle-income countries as well as developed countries.

Methods

A total of 380 cloacal swabs were collected randomly from 53 farmers in Mathira, Nyeri, Kenya. They were transported in sterile Amies charcoal swabs under a cold chain and stored at 4°C. They were enriched in Preston broth and incubated for 24 h at 42 °C, after which they were streaked onto mCCDA agar and incubated for 48 h at 42 °C under microaerobic conditions generated by Campy Gen™ packs. Typical grey moist, swarming and discrete colonies were identified using MALD-TOF. Antimicrobial susceptibility was carried out with Ampicillin, Tetracycline, Ciprofloxacin, Gentamycin, Erythromycin, and Nalidixic acid by disk diffusion method, and disk diameters were measured and interpreted using the CLSI guidelines.

Results

A total of 271 out of 380 (71.32%) bacteria isolated on culture were *Campylobacter spp*. 190 (50%) were *C. jejuni* while 81 (22%) were *C. coli*.

Resistance to Ampicillin was 40% for *C. coli* and 30% for *C. jejuni* respectively. 54% of *C. coli* isolates were resistant to Tetracycline while 52% were *C. jejuni.*

68% of the C. *coli* were resistant to Ciprofloxacin as compared to *C.jejuni* at 38%. Resistance to Erythromycin for *C. coli* 10% and 17% for C. *jejuni*. 65% of *C. coli* was resistant to Nalidixic acid as compared to 38% of *C. coli*.

Conclusions

The study found a high prevalence of Campylobacters at 71.3% with marked multiple drug resistance to Tetracyclines, Ciprofloxacin, and Nalidixic acid. Resistance to Gentamycin and Erythromycin was markedly low and these antibiotics can be reserved for treatment of human campylobacteriosis.

Strengthening and support of surveillance activities for AMR should be enhanced across human and food animal sectors to establish the extent of emergence and and spread of resistance in Campylobacter.

National and regional laboratories capacity for testing of pathogens of public health importance should be strengthened increased.

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List of Abbreviations

AMP - Ampicillin

AMR - Antimicrobial Resistance

ATCC - American Type Culture Collection

AWERB - Animal Welfare and Ethical Review Board (AWERB)

CCDA - Charcoal Cefoperazone Deoxycholate Agar

CDC - Centers for Disease Control

CIDTs - Culture Independent Diagnostic Techniques

CIP - Ciprofloxacin

CLSI - Clinical Laboratory Standards Institute

DNA - Deoxyribonucleic Acid

ERY - Erythromycin

GBS - Guillain Barre Syndrome

GEN - Gentamycin

IBD – Infectious Bursal Disease

LCL - Low Confidence Level

LMIC - Low-Middle-Income Countries

MALDI -TOF- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight

NA - Nalidixic Acid

NARMS - National Antimicrobial Resistance Monitoring System for Enteric Bacteria

OIE - World Organization for Animal Health

TET - Tetracycline

UCL - Upper Confidence Level

USA - United States of America

WHO - World Health Organization

CHAPTER I: INTRODUCTION

1.1 Introduction

Antimicrobial resistance is a growing global threat that is increasing animal and human health concerns. Antimicrobial resistance arises when antimicrobial agents fail to effectively kill microorganisms that were previously susceptible to them (Ayukekbong, 2017). The emergence of AMR is attributed to imprudent antimicrobial use arising from inappropriate practices in prescription, misuse, and overuse in both human and animal health (Muloi, 2019). This leads to exposure of animal and human normal flora and pathogens to selection pressure leading to the emergence of antimicrobial resistant strains which can withstand and survive in the presence of antimicrobial agents which would initially kill them. These new strains can be spread from animals to humans and the environment. In low and middle-level countries, (LMICs) antibiotics are used for the treatment and prevention (prophylaxis) of infectious diseases in animals and humans. Most of these antibiotics are accessed from pharmacies and agro-veterinary shops over the counter without prescriptions from clinicians ad veterinary professionals and the data on antibiotic use in these countries is scarce (Muloi, 2019). New antibiotics are also not being developed by pharmaceutical companies to replace the already resistant ones even with rising demand for their use. This is due to economic and commercial reasons attributed to high production costs and low sale prices. (O'Neill, 2014)
Campylobacter is a spiral-shaped gram-negative bacillus that poses a major public health threat worldwide. There are several species of Campylobacter which include *C. jejuni, C.coli, C. lari* and *C.uppsaliensis,* capable of causing human illness. However, *C. jejuni* and *C.coli* are the most commonly isolated species from poultry and poultry products and cause diarrhoea that is watery and bloody in patients, mainly children under the age of 1-year, young adults, and immunosuppressed individuals (Carron et al., 2018). Campylobacter occurs in the gut of most animals as normal flora and can be transferred to other organs from intestinal waste during slaughter (Aarts, van Lith, and Jacobs-Reitsma, 1995). Campylobacter infection is zoonotic and is acquired through the consumption of undercooked poultry and other meat as well as water and unpasteurized milk. Foods of animal origin particularly poultry are the major source of *C. jejuni*. Fruits and vegetables can be contaminated by water containing faeces from birds and other animals (Carron et al., 2018). Campylobacter can also be transmitted through contact

with dogs and cats' faeces as well as drinking contaminated water. Campylobacter infections are common in low- and middle-income countries as well as developed countries.

1.2 Background

Campylobacter has been recognized as the major cause of gastroenteritis in humans with WHO recording that almost 1 in 30 persons fall ill and 33 million people get affected annually, although this is generally seen as an underestimate. The Center for Disease Control (CDC) records that 1.5 million infections occur in the USA annually, with some of which going unreported or undiagnosed (Center for Disease Control and Prevention, 2019). It causes diarrheal infections especially in young children below 1 year and young adults of 15-25 years, as well as immunosuppressed individuals (Aarestrup and Engberg, 2001), who are likely to suffer from prolonged usually severe cases of illness.

It has been observed that Campylobacter infections are attributed to the consumption of undercooked or poorly prepared foods of animal origin especially poultry (Corry and Atabay, 2001). Reduction of contamination of raw poultry would greatly reduce the incidences of infection, especially at the farm level and poultry slaughter (Keener et al., 2004) as well as proper handling of food, such as using separate knives and cutting boards during the preparation of food.

There are several species of Campylobacter which include *C. jejuni, C. coli, C. lari,* and *C. uppsaliensis,* capable of causing human illness. However, *C. jejuni* and *C. coli* are the most commonly isolated species from poultry and poultry products. Clinical manifestations of Campylobacter infections include fever, abdominal pain, and bloody or watery diarrhea that occurs 2-5 days after ingestion of contaminated food or water. Other associated disorders include the debilitating neurological disorder Guillain-Barre Syndrome (GBS) and reactive arthritis. (Tandel et al., 2016)

Most cases are self-limiting and resolve within a short period and might not require antimicrobial therapy. However, whenever necessary, macrolides and fluoroquinolones may be used for treatment (CDC, 1983).

Despite Campylobacter being a global public health threat, that has the potential to cause severe gastroenteritis in populations around the world, very little research has been conducted in Kenya, and data on Campylobacter is lacking and risk factors, as well as its importance in public health, is unknown. The first study to be documented in Kenya on Campylobacter found

33% to 44% prevalence from broilers and indigenous chicken farms as well as 60-64% for poultry meat from retailers (Carron M, Chang Y-M, Momanyi K, Akoko J, Kiiru J, Bettridge J, et al, 2018). A study carried out on domestic animals and isolated 51.5% of *Campylobacter* species from faecal and rectal swabs of healthy chicken, showing that poultry and other domesticated animals play a role in the transmission of Campylobacter to humans (Turkson, Lindqvist, Kapperud, 1988),. As mentioned there has been little research outside the Metropolitan areas of Kenya. In Nyeri County, there is no data on Campylobacter, yet the region boasts of increased poultry production and high demand for poultry products including eggs and poultry meat (Carron M, Chang Y-M, Momanyi K, Akoko J, Kiiru J, Bettridge J, et al, 2018). It is on this concern that this research was developed.

1.3 Objectives

1.3.1 General objectives

To assess the prevalence, determine the antimicrobial resistance patterns of *Campylobacter spp.* from poultry in Mathira Sub-County, Nyeri County

1.3.2 Specific objectives

1. To assess the prevalence of *Campylobacter* spp. in poultry Mathira Sub-County of Nyeri County.
2. To determine antimicrobial resistance patterns of Campylobacter species.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter seeks to give an overview of the literature around *Campylobacter spp.* Regarding its prevalence and its antimicrobial resistance patterns. This literature has been derived from previous studies done around the world and includes an overview of how the bacteria spreads, its initial origin, and measures that have been put in place to avoid infections in children and their prevalence in poultry.

2.2 The Campylobacter spp.

The first *Campylobacter* report was made in 1886 by Theodore Escherich describing them as non-culturable and spiral-shaped. Later in 1906 *Campylobacter* was observed in a pregnant sheep in its uterine mucus. In the early and mid-twentieth century, *Campylobacter* was observed in cattle with diarrhea (*Vibrio jejuni),* bovine fetuses, and pigs with diarrhea as *Vibrio coli(* (Vandamme P., Debruyne L., De Brandt E., Falsen E., 2010).

2.3 Colonization in poultry and transmission routes

The positivity rate of poultry flocks for *Campylobacter spp.* depends on the type of production used. Hendrixson and DiRita, (2004) demonstrated that due to increased environmental exposure, intensively reared birds have fewer positivity rates than organic free-range chicken. Therefore, as (Newell and Wagenaar, 2000) demonstrate, organic and free-range chicken can be colonized with multiple genotypes of *Campylobacter spp*. which appear consistent with the exposure of the chicken to diverse environmental conditions. To reduce the levels of *Campylobacter spp*. contamination, good agricultural practices, poultry processing, and good manufacturing practices need to be applied (Aarts, van Lith Jacobs-Reitsma, 1995). However, control cannot be attained if the sources and transmission routes of infected flocks are not identified. As stated earlier in this section, the presence of *Campylobacter spp*. is prevalent in the gastrointestinal tract in poultry, especially broilers; although according to Cox et al., (2009), it is not well defined on how, when, and to what extent *Campylobacter* is transmitted between poultry and their progeny.

Horizontal transmission has been accepted as the most common method of transmission of *Campylobacter spp*. to broilers. According to (Horrocks et al., 2009), horizontal transmission occurs rapidly when individual birds within a flock are colonized by *Campylobacter spp*. Also, once individual birds are colonized, they are difficult to eliminate as stated by (Carrillo et al.,2004).

Several factors affect the rate of increased colonization and dissemination including; high flock size, litter, wild birds, fecal contact, water supplies, rodents, other animals, and poultry process personnel (Horrocks et al., 2009).

According to (Cox et al., 2009), *Campylobacter spp*. is very susceptible to dehydrated conditions and rarely survives in animal feed because of their usual conditions. Also, (White, Baker, and James, 1997) in an earlier study indicated that the implication in the spread of *Campylobacter spp*. is minimal and the infection mainly characterized with feed is the *Salmonella* spp. Because of the ubiquity presented by *Campylobacter* spp., wild droppings from birds will often contaminate the environment and food for animals. However, Harrison reports that feed can act as a medium for the contact-contact transmission of already established *Campylobacter* spp. in poultry houses.

Vertical transmission of *Campylobacter spp*. from breeding flocks remains undefined by most researchers, in research conducted by(Clark and Bueschkens, 1985), they injected fertile chicken eggs with *C. jejuni* and demonstrated that 11% of the eggs that hatched into chicks showed the presence of the *C. jejuni* in their intestinal tract. Further research by (Lindblom, SjÃrgren, and Kaijser, 1986) showed that even chickens raised in laboratory conditions away from farm conditions continued to be colonized by *C. jejuni.* In another study, a 35% positivity rate was observed for *C. jejuni* in their cecal content in newly hatched chicks suggesting that colonization occurred before their transfer to the farm (Chuma et al., 1994). In a study by (Cox et al., 2009), they demonstrated that following an intra-cloacal inoculation of 1-day old broiler chicks, *C. jejuni* can circulate rapidly to the lymphoid organs and extend their existence in these locations. Additionally, the presence of *Campylobacter* in eggs may infer the possibility of vertical transmission.

2.4 The Prevalence of Campylobacter spp. from poultry

In most countries, poultry meat is a major source of high-quality protein. It is preferred because it is rich in minerals, vitamins, and essential amino acids (Marangoni et al., 2015). Compared to lean roasted beef, lean chicken has a higher protein content and is cheaper than both pork and beef for the same quantity of meat (Sul, Kim, and Kim, 2019). Additionally, chicken meat has been preferred around the world because it has a unique taste and short time of preparation. However, campylobacteriosis has been observed in human populations worldwide due to the high consumption of chicken and its products, both in high-income and low-middle income countries (LMIC). Most cases of campylobacteriosis are thought to occur from handling raw poultry, eating

undercooked or raw poultry, and cross-contamination of raw to already cooked foods (Tauxe et al., 1997). According to (El-Shibiny, Connerton, and Connerton, 2005), if the production system is organic for free-range chicken, *C.coli* becomes the most dominant species. In England, a surveillance study was conducted and the results indicated that in comparison to *C. coli,* the *C. jenuni* species was responsible for approximately 12 times more prevalence in the number of campylobacteriosis cases in humans (Friedman et al., 2000). While this study may suggest otherwise, *C. coli* remains an important source of campylobacteriosis in humans.

Campylobacter spp. is held mainly in the inner tract of a chicken's intestine, especially the colon and the cecum. During processing, leaks and ruptures may cause the contents of the inside of the intestinal tract to be transferred to the skin of the carcass, a process responsible for contamination in many poultry processing industries (Berrang et al., 2001). The survival of the *Campylobacter spp*. is highly favored by the conditions of the skin as they are stored on the skin crevices and channels such that they persist even in storages of up to frozen or 4^{o}C (Simmons and Gibbs, 1979). In support of the latter inference (Scherer et al., 2006) reported, that *Campylobacter spp*. can grow on the skin of a packaged carcass stored at room temperature, which poses a threat to consumers if the chicken is not properly handled or stored. In another study to investigate the survival of the *C. jejuni* species in poultry relative to the effects of environmental temperatures during different seasons, the presence of *Campylobacter spp*. was a concern during the warmer months (Willis and Murray, 1997). In their study, of all the samples tested, approximately 87-97% were positive for *C. jejuni* with the lowest recorded number of positive samples observed at 7% in December and 33% in January. The study also indicated that the variability in the intestinal colonization of *C. jejuni* was substantial across different broiler groups at different ages during the production period.

2.5 The antimicrobial resistance patterns of *Campylobacter spp.* from poultry

In a study conducted in Poland, between 2014 and 2018, antimicrobial resistance of *Campylobacter* showed that the majority of *C. jejuni* and *C. coli* isolates were resistant to Ciprofloxacin (92% and 93.9% respectively) and Nalidixic acid (90.3% and 93.8% respectively., there were no significant statistical differences between these two Campylobacter species to Ciprofloxacin whereas in Nalidixic acid the difference was significant (Maćkiw, et al., 2012). Resistance was observed in Erythromycin at approximately 1.1% and 1.3% in Gentamycin. However, in Poland, between 2017 and 2018, all *C jejuni* identified were susceptible to Erythromycin. *C. coli* isolates were mainly resistant to Ciprofloxacin and represented 93.9% of all

isolates through the study period while Nalidixic acid represented 93.8% of all isolates. However, the isolates in percentages did not have much difference since the values were at 90.5% in 2015 and 97.1% in 2017 for Ciprofloxacin and Nalidixic acid respectively. A relatively higher rate of *C. coli* than *C. jejuni* isolates was resistant to Erythromycin at 6.4% and 1.1% respectively (P= 0.00005) (Maćkiw, et al., 2012). The resistance rates of *C. coli* and *C. jejuni* to Tetracycline and Streptomycin were also identified.

In a study conducted in Tunisia, the antimicrobial resistance patterns were tested against the eight antimicrobial agents as shown in the table below:

Table 1: Antimicrobial resistance patterns in Tunisia

Antimicrobial	*C. jejuni* % (n=91)	*C. coli* % (n=41)	Total % (n=132)
Ampicillin	73.6* (67)	34.1 (14)	61.4 (81)
Amoxicillin/acid clavulanic	52.7 (48)	34.1 (14)	47.0 (62)
Ciprofloxacin	98.9 (90)	100 (41)	99.2 (131)
Nalidixic Acid	57.1* (52)	22.0 (9)	46.2 (61)
Erythromycin	100 (91)	100 (41)	100.0 (132)
Tetracycline	100 (91)	100(41)	100.0 (132)
Chloramphenicol	83.5 (76)	100 (41)	88.6 (117)
Gentamycin	14.3 (13)	9.8 (4)	12.9 (17)

*Significantly higher resistance ($P < 0.05$) of C. *jejuni* compared with *C. coli*.

Source (Gharbi et al., 2018)

2.6 Campylobacter infection in Human Beings

2.6.1 Microbiology

The genus *Campylobacter* is made up of spiral-shaped, curved, Gram-negative bacilli and is derived from the Greek and Latin words “curved rod”. Approximately half of the species of the genus *Campylobacter* affect humans and are a source of disease. *Campylobacter jejuni* and *Campylobacter coli* are the most common species of the genus that are associated with disease in humans. In 2015 in a study by (Aarestrup and Engberg, 2001), the majority of the isolates (88%) were *C. jejuni. C. jejuni* consists of two subspecies that include *C. jejuni sub. spp jejuni* which is the most common isolated cause of *Campylobacter* infection in the United States and *C. jejuni sub. spp doylei*- a less common subspecies. However, other Campylobacter species cause

infections in humans and they include; *C. fetus*, *C. lari*, *C. upsaliensis*, and *Campylobacter hyointestinalis.*

2.6.2 Pathogenesis

Campylobacter infection in humans is facilitated by host factors and multiple existing organisms. Outbreaks, studies from healthy volunteer groups, and observational data from previous cases have resulted in an inference that the inoculum necessary to cause *Campylobacter* enteritis in humans can be approximately 500 organisms, which is a low figure. According to (Harrison et al., 2013) & (Adak et al., 1995), pathogenic organisms flourish and survive if the gastric acid barrier allows and *Campylobacter* is not an exception. As such, patients with low gastric acidity may be at a higher risk of *Campylobacter* infection and this includes those receiving proton pump inhibitors.
In a histological approach, acute mucosal inflammation and edema that is characterized by infiltration of the *lamina propria* and crypt abscess formation cause an infection that is identical to *salmonellosis* (van Spreeuwel et al., 1985). Although the exact mechanisms of infection are not completely conclusive, diverse virulence factors have been identified to include adhesins, plasmids, chemotactic, and flagella factors. According to (Bolton, 2015), early infection is recognized when bacteria, through fimbriae-like filaments, attach to intestinal epithelial cells. Consequently, the colonization is facilitated by the flagella and chemotactic factors in the gastrointestinal tract. *Campylobacter* can evade inborn immune responses which differentiate it from other intestinal pathogens since *Campylobacter* flagellins are not observed to enhance proinflammatory cytokines.

2.6.3 Epidemiology

Data from the United States in 2016 indicated through culture and culture-independent diagnostic tests (CIDTs) was 17.4 for every 100, 000 persons in incidences of *Campylobacter* enteritis confirmed as the major cause of foodborne illness in the United States. Through the Foodborne Diseases Active Surveillance Network, *Campylobacter* has been tracked since 1996 and was categorized as a notable disease in 2015. Typically, infections caused by *Campylobacter* are mild but in 2016, reports showed hospitalization resulting from reported infections represented 20% of the reported infections and a mortality rate of 0.3% which was represented by 26 deaths (Marder et al., 2017). Children younger than 5 years reported the highest incidences of infection in the United States but there is also a notable peak in young adults. During hot periods such as summer, the infections are the highest and are mostly domestically acquired. However international

travellers returning home were detected with *Campylobacter* enteritis. According to (USA Department of Health and Human Services, CDC, 2014) in developing countries, infections are mainly hyperendemic and infections with symptoms occur mainly in young children and infants, who can be infected recurrently. Ensuing infections present asymptomatic characteristics making symptomatic disease rare in adults or older children (Platts-Mills and Kosek, 2014). Also, according to (NARMS, 2019) *Campylobacter* is sporadic and only an approximate 0 caused an outbreak. However, for few cases that have caused an outbreak, they have been associated with contaminated water and milk. At low temperatures, *Campylobacter spp*. thrives for extended periods to 4 weeks). Since *Campylobacter spp*. is abundant in the environment, sanitization is the most important approach to preventing its transmission. In addition, unchlorinated water has been associated with waterborne Campylobacter outbreaks while improper pasteurization and drinking raw milk have been associated with milk-related outbreaks (Jones et al., 1981). As argued by Davis et al., (2016), the difficulty to culture *C. jejuni* may lead to milk-borne outbreaks as the milk passes the associated routine testing even with the presence of the subspecies.

2.6.4 Clinical presentation

Gastroenteritis is the most common illness resulting from *C. jejuni* and *C. coli* infection. The symptoms that may be indicative of the illness in children include diarrhea, abdominal pain, vomiting, and fever. Dehydration is another effect of the illness and therefore patients require a lot of fluids (Karmali and Fleming, 1979). Blood in stools is observed in approximately 50% of the infected children population(Karmali and Fleming, 1979). Prominent fevers and seizures, encephalopathy, and meningismus are also symptoms associated with *Campylobacter spp*. infection (Levy et al., 1986). However, in immunocompromised patients, bacteremia is seen, although rare. *Campylobacter* infections can mimic other gastrointestinal illnesses such as the commonly mistaken identification with intussusception characterized by bloody stools and vomiting without fever. In older children, severe lower quadrant pain without diarrhoea that mimics appendicitis can be caused by acute *Campylobacter* ileocolitis (Puylaert et al., 1989). *Campylobacter* enteritis usually progresses distally from the small bowel, but only colitis and bloody diarrhoea may be rarely seen in patients with severe infection and could be confused with IBD (Castaño-Rodríguez et al., 2017).

2.6.5 Complications

Some of the complications associated with campylobacter infections include septic arthritis, bursitis, soft tissue infections, and osteitis. Acute extra-intestinal complications of enteritis include myocarditis, cholecystitis, septic pseudoaneurysm, pericarditis, and peritonitis in patients with peritoneal dialysis. Erythema nodosum, glomerulonephritis, hemolytic anemia, IgA nephropathy, postinfectious irritable bowel syndrome, and intestinal perforation have all been described as well. After *Campylobacter* enteritis, reactive arthritis can occur over a mean of 11 days but the maximum can extend to 40 days after the start of diarrhea. It is usually asymmetrical and more prevalent in severe patients affected with HLA-B27 phenotype and predominantly affects the knees (Pope et al., 2007). Also, 7% of patients are affected by arthritis but approximately 20% report arthralgia (Pope et al., 2007). Based on culture surveys and serologic studies, *Campylobacter* spp. is the most commonly identified cause of GBS. In the United States, patients who were infected by *Campylobacter* approximately 14 days earlier represent 30-40% of GBS development. It is reported that one in every a thousand patients infected with enteritis associated with Campylobacter is at risk of developing Guillain Barre Syndrome (GBS). (Nachamkin, Allos, and Ho, 1998). Based on histologic analysis inflammatory changes of *Campylobacter* infection ought to assist in differentiating it from the chronic changes in Infectious Bursal Disease (IBD). According to Castaño-Rodríguez et al., (2017), a suggestion has been presented that *Campylobacter* infection can facilitate the development of IBD.

2.6.6 Diagnosis

Unlike other bacterial and viral gastroenteritis, *Campylobacter* enteritis has been observed to be clinically indistinguishable. For instance, for children who present with acute diarrheal illnesses, diagnostic testing is always indicated with or without vomiting or fever. The latter is because knowing the cause does not affect clinical management. (Bonilauri et al., 2016).

Stool culture has been used as the gold standard for the isolation and characterization of *Campylobacter* species despite their difficulty to isolate. Campylobacter grows best on media containing selective antibiotics and in microaerobic conditions with 5% to 10% oxygen, 1% to 10% carbon dioxide, and some hydrogen. *C. jejuni* and *C. coli* grow best at 42°C.

Nucleic acid and amplification tests through the use of CIDTs are on the rise (Marder et al., 2017). The advantage of the latter tests is that they have quicker with shorter turn-around times than conventional culture approaches in diagnostics. Through reverse transcriptase-polymerase chain reaction, 20% to 40% better identification efficiency of *Campylobacter* from stool compared to

culture-based approaches. However, their clinical significance is not always clear because these CIDTs tests identify nucleic acids rather than cultured organisms (Bonilauri et al., 2016). *Campylobacter* isolates pose resistance to tetracyclines and quinolones hence if treatment is required there is a concern for resistance, therefore, rendering cultures necessary even after identification by CIDTs(CDC, 1983). Serological tests are not suitable for the diagnosis of acute *Campylobacter* infection, however, patients with reactive arthritis or GBS with negative stools can be tested.

2.6.7 Resistance

Fluoroquinolones are effective in the treatment of Campylobacter infection but also face resistance patterns thus limiting these agents for indication of Campylobacter infections. According to a report documented in the United States 2014 data, Ciprofloxacin showed marked resistance to *C. jejuni* (27%) and *C. coli* 36% isolated.

CHAPTER 3: METHODOLOGY

3.1 Study area

The study area was Mathira East and West sub-counties of Nyeri County, Kenya. Nyeri County is located in the former Central Province of Kenya, about 150 kilometres north of Nairobi. Nyeri shares its borders with five other counties; Kirinyaga to the east, Nyandarua to the west, Muranga to the south, Laikipia to the north, and Meru to the northeast. The rainfall average lies between 500 mm and 1500 mm during the short and long rain periods making it conducive for its diverse agricultural activity. Nestling between Mount Kenya and the Aberdare ranges, agriculture is the main economic activity in Nyeri. Poultry farming is the third in rank among dairy and beef sectors in terms of revenue generation in the county.

Mathira has six administrative wards namely Konyu, Iria-ni, Konyu, Magutu, Kirimukuyu and Ruguru. Farmers in these wards were identified and random sampling was done. Five cloacal swabs were collected from farmers with a flock below 100 birds, 10 cloacal swabs from farms with 100- 1000, and 20 cloacal swabs from farms with over 1000 birds.

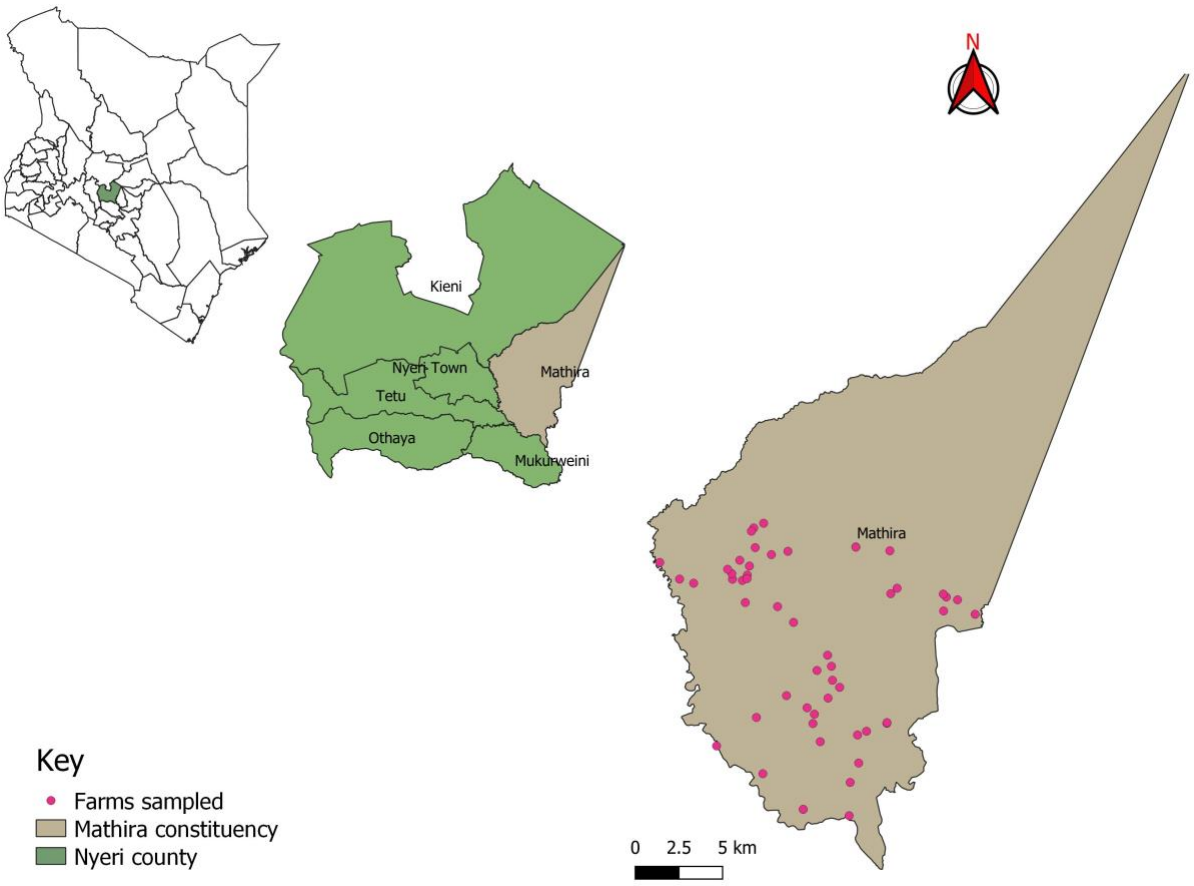

Figure 1: Geographical area where samples were collected covering Mathira constituency in Nyeri county, Kenya

3.2 Sampling criteria

A total of 53 farms recruited for the study were identified by the animal health field personnel attached to the Mathira Sub-County veterinary and livestock production office. Some of these farmers were involved in small-scale poultry production while others reared poultry on a large scale for commercial purposes. The main breeds were broilers, layers, dual-purpose (for both meat and egg production) as well as indigenous (local) chicken.

3.3 Inclusion and Exclusion criteria

Farmers with a flock between 2-3 weeks were included in the study so that by the time of sampling, the birds had acquired the maturity age and were ready for market, to enter the food chain and

hence potentially spreading AMR to humans via food and were also continuously and maximally exposed to antibiotics in the course of production. These farmers formed the sampling frame.
Risk factors associated with Campylobacteriosis were investigated such as the nature of the poultry house's floor and any biosecurity measures undertaken at the farm.

3.4 Study design

A cross-sectional study was carried out and involved the administration of a semi-structured questionnaire. The questionnaire captured the farm location, farmer's biodata, nature of poultry houses, and the antibiotics used at the farm to collect antibiotic use data

3.5 Sample size calculation

Using a 37% prevalence level (Carron M, Chang Y-M, Momanyi K, Akoko J, Kiiru J, Bettridge J, et al, 2018) and Epitools and a level of significance of 0.05, the sample size and frame were calculated as 359

3.6 Sampling Methodology

Cloacal swabs were collected from the birds using sterile Amies cotton swabs in charcoal to prevent them from drying and the toxic effect of oxygen and labeled. The samples were carefully packed in zip lock bags to avoid any possibility of leakage or cross-contamination and transported to the laboratory. Swabs were maintained in the cold chain at 4°C, protecting them from light, extreme temperatures, and desiccation until they reached the laboratory for processing. Upon reaching the laboratory, each cloacal swab was transferred to Preston broth for 24 h at 42°C for enrichment. If samples were not processed on the same day, they were maintained in the laboratory at 4°C and processed before 72 h lapse.

3.7 Laboratory testing

Pre-enriched cloacal material was then plated in modified charcoal cefoperazone deoxycholate agar-Preston (mCCDA) (CM 0739, Oxoid, Basingstoke, UK). Plates were incubated under microaerobic conditions generated by Campy Gen™ packs at 42°C for 48 h in anaerobic jars. Colonies that were grey, moist, and spreading, with a metallic sheen, and slightly raised and discrete on mCCDA were identified and their morphological characteristics are written down. The colonies were further sub-cultured into Blood agar (CM0055, Oxoid, Basingstoke, UK) enriched with 10% horse blood and incubated at 42°C for 48 h to obtain pure colonies. The colonies were subjected to Gram stain to observe for spiral-shaped rods or seagull appearance. The bacterial

colonies were then identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-ToF Biotyper®) mass spectrometry (Bruker Daltonics GmbH & Co. KG.), an identification system for microorganisms based on mass spectrometry to the species level.

3.8 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method. Distinct *Campylobacter* colonies were suspended in 0.2% sterile normal saline and standardized to 0.5 MacFarland turbidity index using a nephelometer. The cell suspensions were then streaked onto dried Mueller Hinton blood agar plates within 15 minutes of standardization, using a sterile cotton swab. The streaking was repeated two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. The rims of the plate were finally swabbed and the plate was left ajar for 3-5 minutes to allow any excess surface moisture to be absorbed before applying antibiotic-impregnated disks.

Antibiotic disks; Ampicillin (AMP)10ug, Tetracycline (TET)30ug, Ciprofloxacin (CIP)5ug, Gentamycin (CN)10ug, Erythromycin (ERY)15ug and Nalidixic acid (NA)30ug (Oxoid) were dispensed on the agar surface using a disc dispenser or sterile forceps. Gentle pressure was applied to the disks to ensure complete contact with the agar surface. The plates were then incubated in the microaerophilic environment for 24 h at 42°C. After incubation, each plate was observed for the zones of inhibition, with plate lids removed under illuminated light for the different antibiotics. The zones of inhibition were measured using a vernier calliper and recorded. Interpretation of the zones was done using the Clinical Laboratory Standards Institute (CLSI, M100 2021) and (CLSI, M35) editions

Campylobacter jejuni (ATCC 11351) and *Campylobacter coli* (ATCC 11366) were used for quality control during the test runs.

3.9 Dissemination of Results

The results of this study will be disseminated to the Directorate of Veterinary Services, Kenya which is the Beneficiary Institution, the County Department of Veterinary Services in Nyeri, for further dissemination to relevant agencies. The results will also be shared with poultry farmers in the study area.

3.10 Ethical Consideration

All the farmers who participated in the study consented to be interviewed and were assured of the protection of their biodata. The study was approved by Faculty Biosafety, Animal use and Ethics committee, Department of Veterinary Anatomy, University of Nairobi and London School of Hygiene and Tropical Medicine, Animal Welfare and Ethical Review Board (AWERB).

3.11 Funding

The study was funded by the Fleming Fellowship Fund program under the Department of Health and Social Sciences through the London School of Hygiene and Tropical Medicine, the Host Institution. The institutions were not involved in the design, data collection, analysis, and write-up of this report.

CHAPTER 4: RESULTS

4.1 Socio-Demographic characteristics of respondents

The demographic data of the respondents considered during the survey included age, sex, education status, occupation, and gender. During the household survey, a total of 53 farmers were interviewed out of which 62%(33/53) were females while 38%(20/53) were males.

The age of the majority of the interviewed farmers ranged between 30-50 (49%) years followed by farmers who were above 50 years at 42% and 18-to 30 at 9.43 respectively. This showed that farmers in the age gap between 30 and above 50 years are the most engaged in poultry farming and form the most productive population involved in subsistence farming (Table 2).

The majority of the farmers interviewed had attained secondary education (64.15%) followed by tertiary education at 26.4 % while the remainder possessed basic education at 9.43%. The level of understanding is important because it complements the understanding of the respondents in implementing basic farm husbandry practices including biosecurity measures, diagnosis, and drug use among other practices.

Table 2: Socio-Demographic characteristics N=53

Key Variable	**Frequency**	**Proportion%**
Gender		
Female	33	62.26
Male	20	37.74
Occupation		
Farming	42	79.25
Others	11	20.75
Age		
18-30	5	9.43
30-50	25	47.17
Over 50	23	43.4
Education		
Primary	5	9.43
Secondary	34	64.15
Tertiary	14	26.42

4.5 Farm Characteristics and practices

The type of floor for the poultry houses is an important factor to consider because it ensures the health status of birds as a result of easy cleaning and rodent control. Wooden floors are hard to clean and spaces in between the timber can cause toe injuries to the birds. It also traps droppings and bedding material. Concrete floors on the other hand are easy to maintain, clean and disinfect and keep rodents away. Metallic floors are also easy to clean and maintain as they do not trap droppings and keep predators away. They can however cause injuries to birds' feet and legs and may be very cold during the cold weather. Earthen floors are difficult to disinfect since all the organic matter from the surface cannot be removed and affects the antimicrobial action of most disinfectants. The majority of the poultry house floors from the sampled households were made of wood at 39.6% followed by concrete floors at 32.1% and earthen floor houses stood at 26.4%. Only one farmer (1.9%) had caged birds reared in metallic floored houses (Table 4).

Table 3:Poultry house floor type

Floor-type	Frequency	Proportion(%)	Exact 95%LCL	Exact 95%UCL
Concrete	17	32.08	19.92%	46.32%
Earthen	14	26.42	15.26%	40.33%
Metallic	1	1.89	0.05%	10.07%
Wooden	21	39.62	26.45%	54.00%

Key: LCL- lower Confidence Level, ULC- Upper Confidence Level

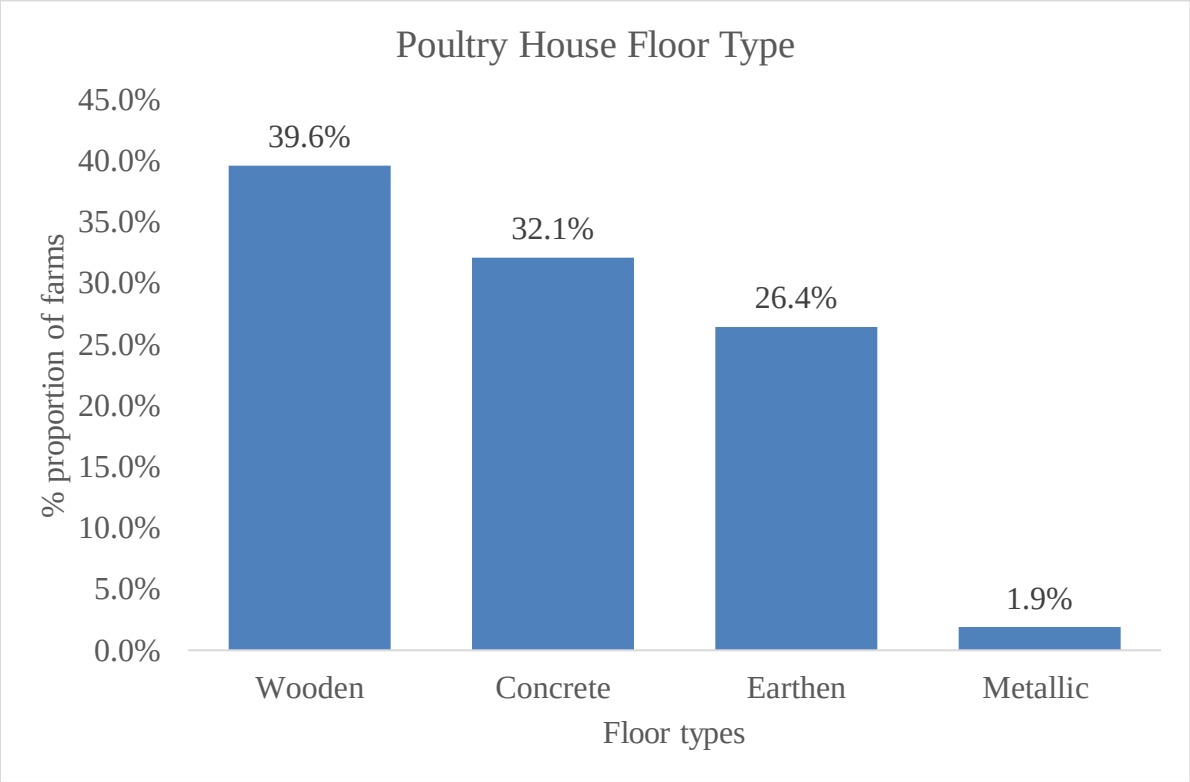

Figure 2 Poultry House Floor Type

4.6 Antibiotics use at the farm

Out of the 53 households visited, 36 (68%) reported having used Tetracycline, 28 (53%) Sulphonamides, 16 (30%) Macrolides, 15 (28%) Aminoglycosides, 10(19%) Polymixins and 5(9%) Fluoroquinolones, respectively. These antibiotics were administered to birds in the last two months before the study commenced. Farmers were able to identify these antibiotics from a pictorial booklet containing different brands of antibiotics.

Of the fifty-three farmers interviewed, 32(60.4 %) reported having used antibiotics on their farms while 21(39.68%) said they had not used antibiotics on their farms, within two months before sampling.

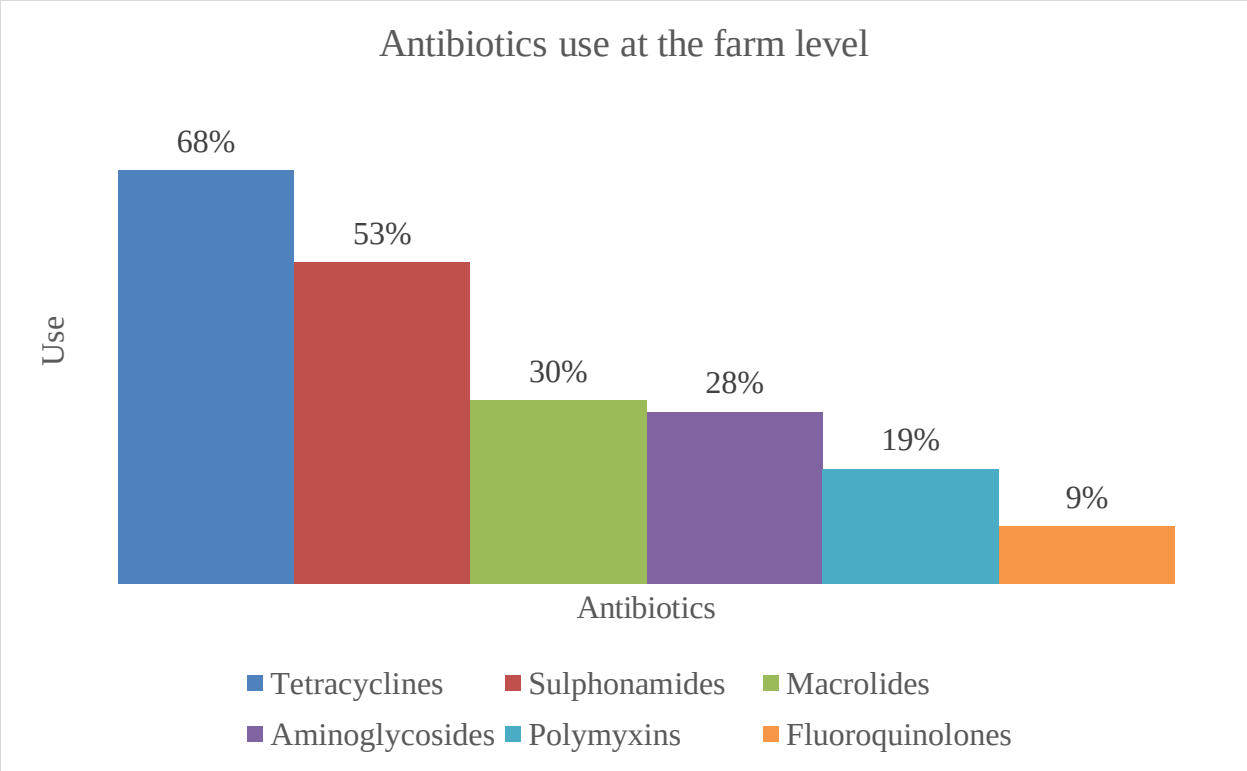

Figure 3: Antibiotics use at the farm level

4.7 Reasons for antibiotic use

Ten out of thirty-two (30.2%) used antibiotics on their farms for prophylaxis while nine (28.3%) used antibiotics for the treatment of infectious diseases on the farms. Thirteen (41.5%) however did not use antibiotics on their farms within two months before sampling.

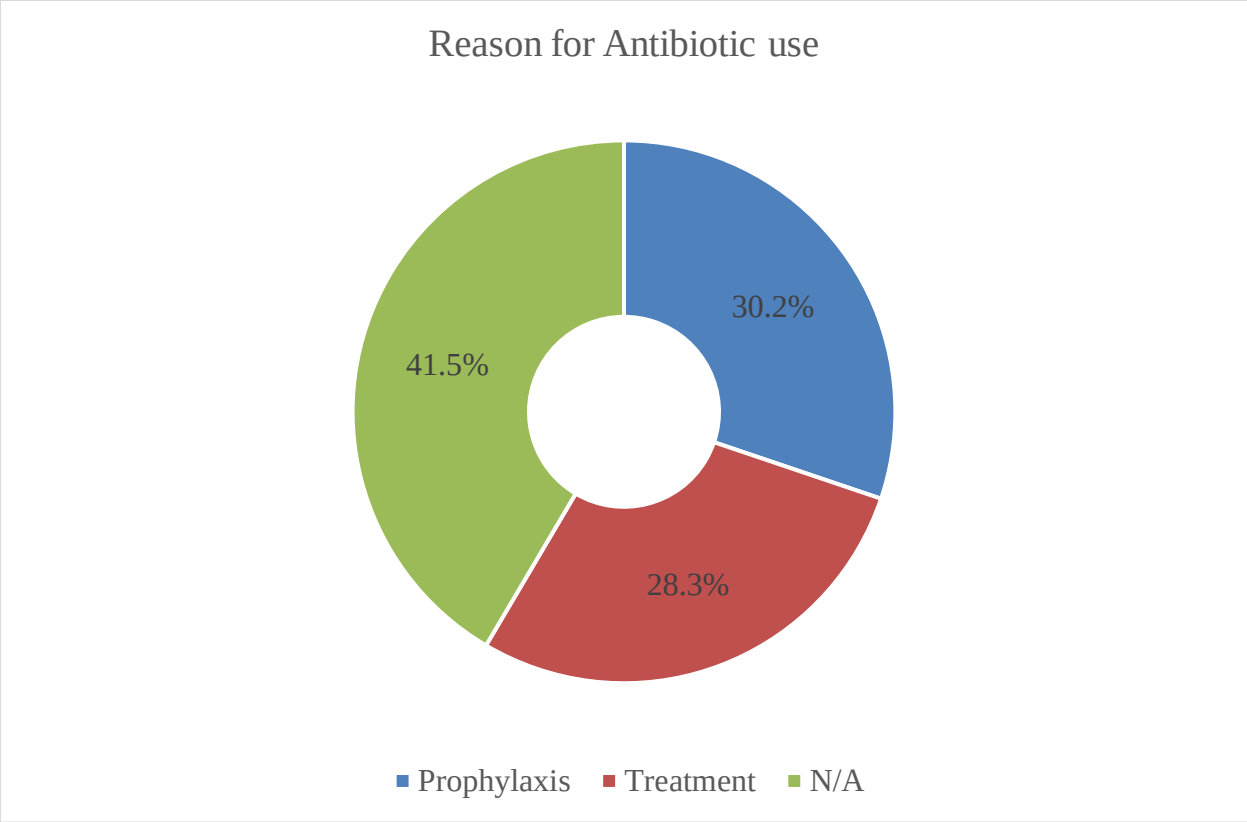

Figure 4: Reason for Antibiotic use

4.9 Laboratory results

4.9.1 Isolate distribution and prevalence of *Campylobacter* species

Cloacal swabs were collected from three hundred and eighty birds. Of these, 65 (17.11%) were from broilers, 155 (40.79%) dual-purpose 35 (9.21%) local chicken and 125 (32.89%) layers, respectively. Out of a total of 380 samples tested for *Campylobacter spp.,* 271 tested positive indicating a prevalence of 71.3% (95% CI; 66.5%-75.6%,) of the cultured bacteria. 190/380(50%; 95% CI; 45%-55%) were *Campylobacter jejuni* while 81/380 (21.32%; 95% CI; 17.5%-25.7%) were *Campylobacter coli.* Distribution per chicken breed was as shown in Table 5 and Fig. 6

Table 4:*Campylobacter spp.* distribution per chicken breed

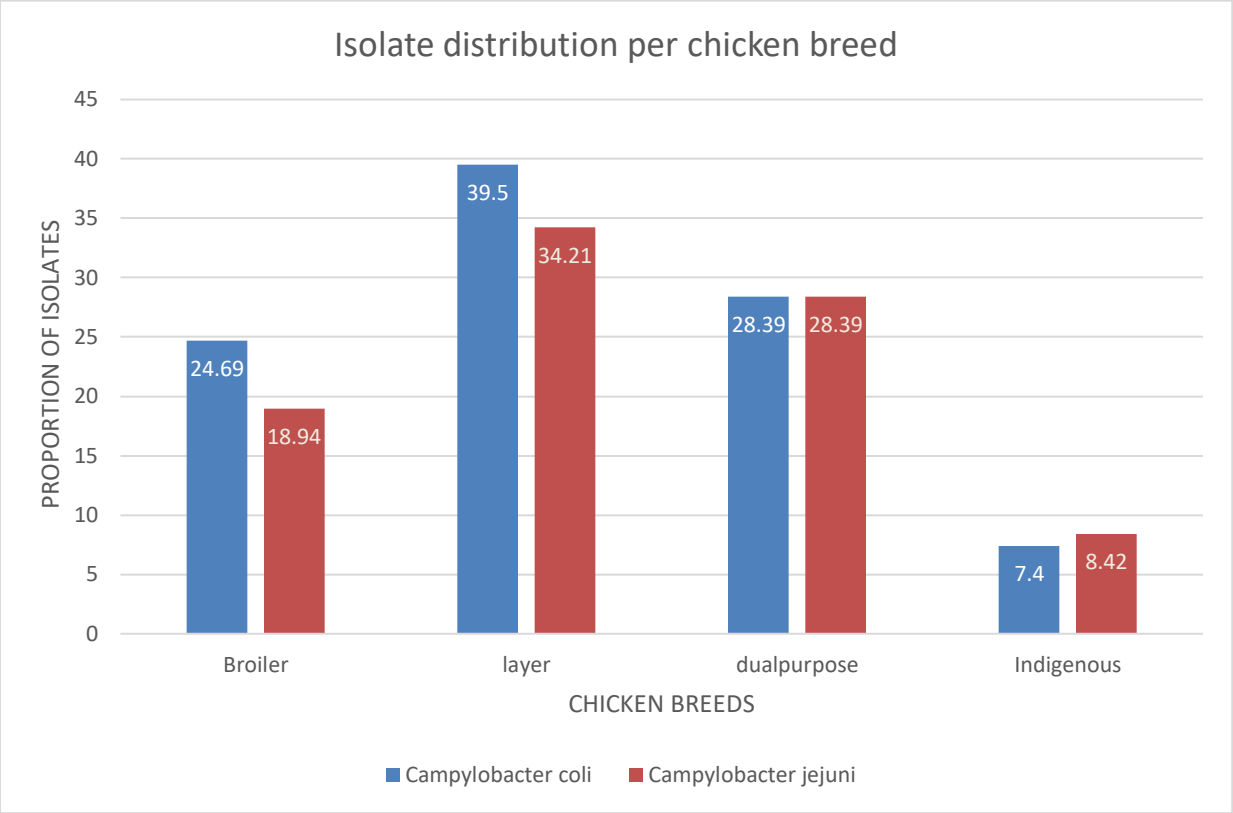

Figure 5: Isolate distribution per chicken breed

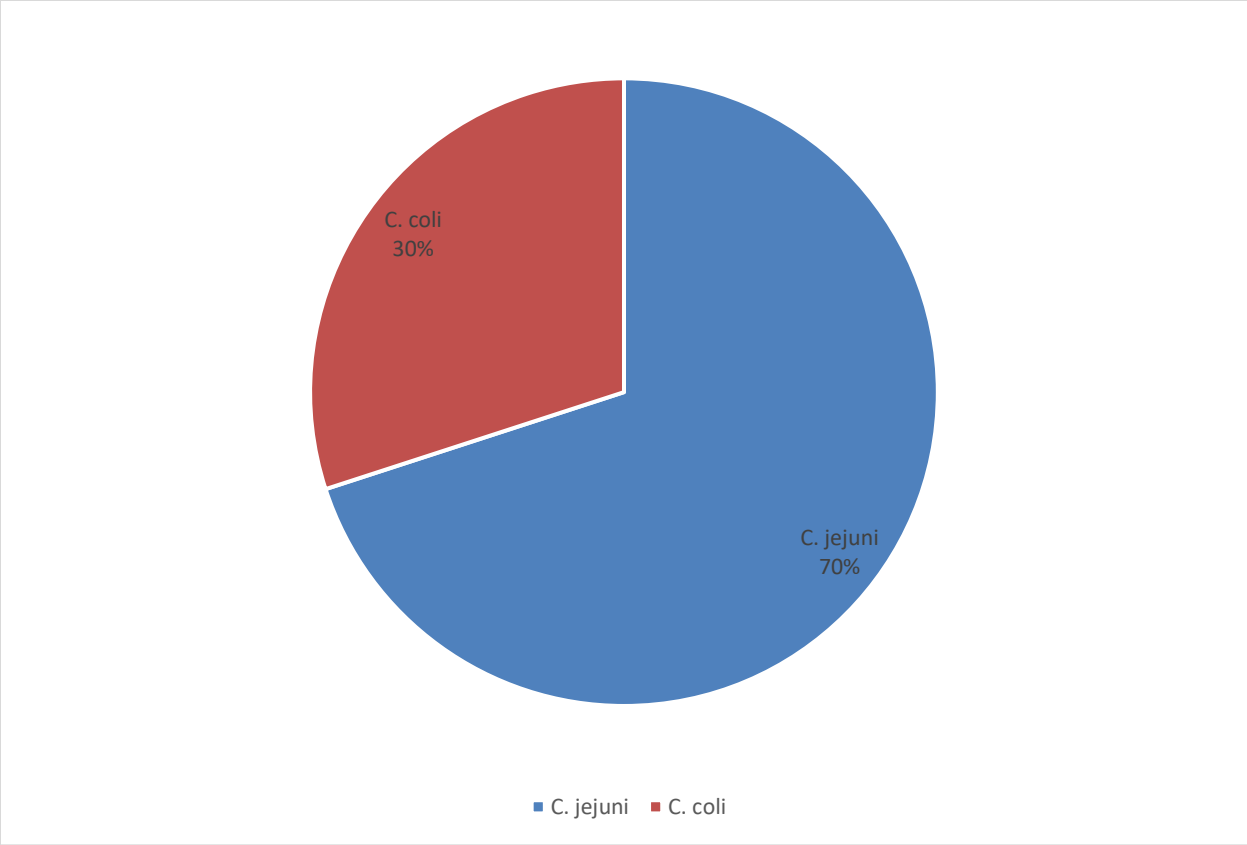

Figure 6: Prevalence of Campylobacter

4.9.2 Antimicrobial resistance

Resistance was found to Ampicillin (*C. coli* 58% and *C. jejuni* 39%), Ciprofloxacin *(C. coli* 69%)and *C. jejuni* 32%), and Nalidixic acid(*C. coli* 69%)and *C. jejuni* 44%)was significantly different with (P=0.0024, P=<0.0001, P=0.0002) respectively.

There was no significant difference in resistance of both isolates to Tetracycline (P=0.6474), Gentamycin(P=0.1500), and Erythromycin (P=0.1500)

Both *Campylobacter coli* and *Campylobacter jejuni* were found to be resistant to multiple antibiotics. *C. coli* showed resistance to Ampicillin (58%), Tetracycline (56%), Ciprofloxacin (69%), and Nalidixic acid (69%) respectively. Low resistance was however recorded for Gentamycin (10%) and Erythromycin (11%) respectively. *Campylobacter jejuni* was found to be resistant to Ampicillin (39%), Tetracycline 59%), Ciprofloxacin (32%), and Nalidixic acid (44%)

respectively. Low resistance was however recorded for Gentamycin(7%) and Erythromycin (18%) respectively.

Table 5:Antimicrobial-resistant profiles (%) of *Campylobacter spp*

Antimicrobial Resistance(%)						
	Antibiotics					
Bacteria	AMP	TET	CIP	GEN	ERY	NAL
C. coli	58	56	69	10	11	69
C.jejuni	39	59	32	7	18	44

Key: AMP-Ampicillin**, TET-**Tetracycline**, CIP-**Ciprofloxacin**, GEN-**Gentamycin, **ERY-**Erythromycin**, NAL-**Nalidixic acid.

Table 6:Chi-square comparison of *C. jejuni* and *C.coli*

Key Variable	Proportion 1 (*C.coli*)	Proportion 2 (*C. jejuni*)	Difference	95% CI	Chi-squared	P-value
Ampicillin	58%(47/81)	39%(74/190)	20%	7.0297%-32.1036%	9.186	0.0024
Tetracycline	56%(46/81)	59%(112/190)	3%	-9.5078%-15.7666%	0.209	0.6474
Ciprofloxacin	69%(55/81)	32%(61/190)	37%	24.2173%-47.9553%	31.592	<0.0001
Gentamycin	10%(8/81)	7%(14/190)	3%	-3.6272%-11.9111%	0.700	0.4027
Erythromycin	11%(9/81)	18%(34/190)	7%	-2.9014%-14.9498%	2.073	0.1500
Nalidixic acid	69%(55/81)	44%(84/190)	25%	12.1218%-36.3371%	14.157	0.0002

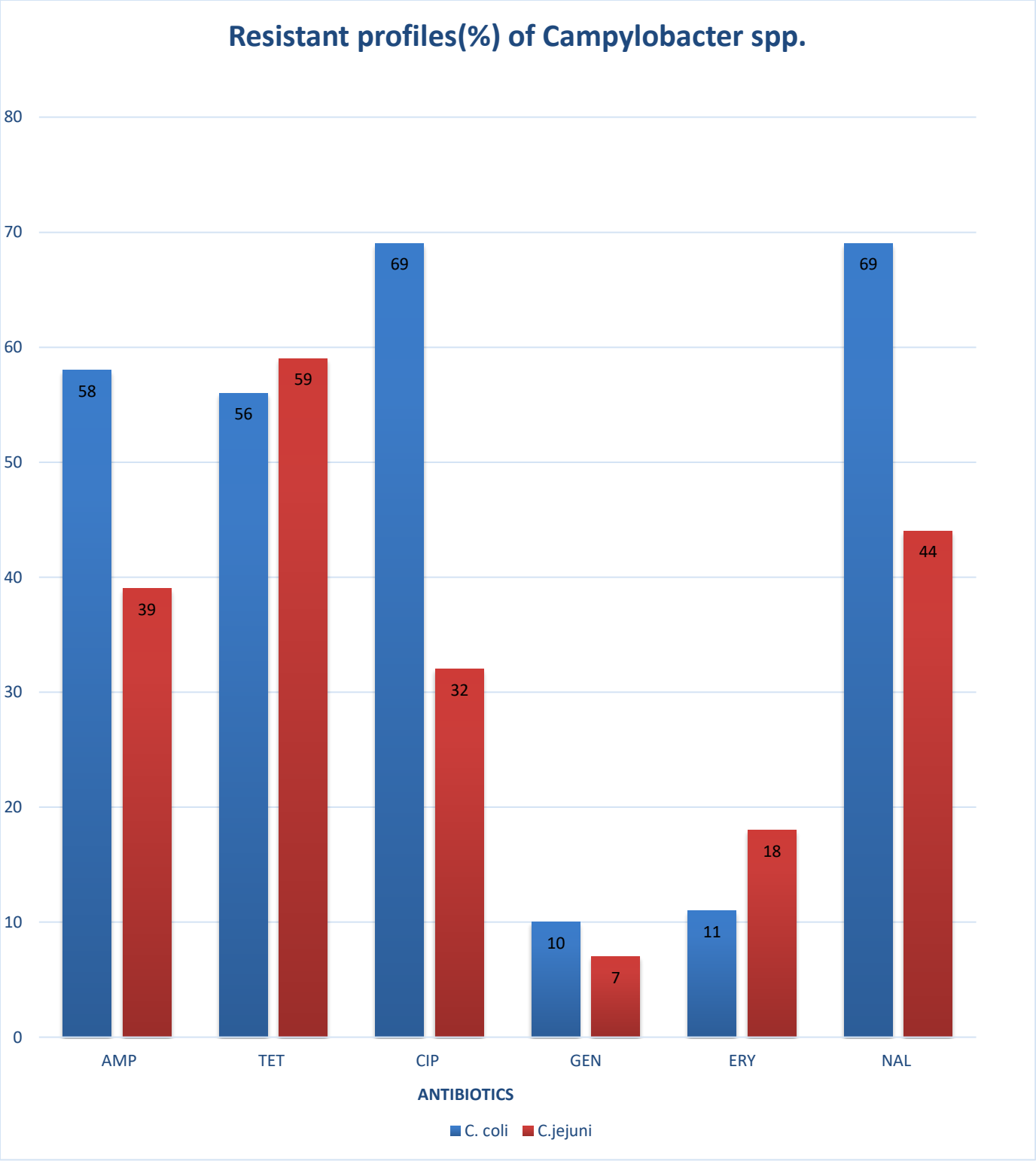

Figure 7:Resistant profiles (%) of *Campylobacter spp*.

CHAPTER 5: DISCUSSIONS

5.1 Summary of the findings

Three hundred and eighty cloacal swabs were collected from birds in fifty-three households in Mathira, Nyeri County. The overall prevalence of *Campylobacter spp.* identified from the cultured samples was found to be 71.3%. *Campylobacter jejuni* was the highest isolated species with a prevalence of 70% followed by *Campylobacter coli* with a prevalence of 30%.In a study done in Nairobi Kenya, the prevalence of *C.jejuni* was found to be 59% and *C.coli* 36% (Carron M, Chang Y-M, Momanyi K, Akoko J, Kiiru J, Bettridge J, et al, 2018) in raw chicken that was sampled from butcheries. This was lower for *C.jejuni* but slightly higher for *C.coli* as compared to this study.

A study conducted by (Ouko, 2021) on diarrheal patients found an overall prevalence of *Campylobacters* to be 11.6% while the prevalence of singular isolates *C.jejuni* and *C.coli* was 89.2% and 10.8% respectively. Patients in the study who had exposure to poultry, and pets and consumed poultry meat were found with higher risk levels of infection to *Campylobacters.* Poultry has been regarded as the greatest source of transmission of *Campylobacter* to the environment, water, and food from which humans and other animals acquire the bacteria.

This study found that there is a high prevalence of *Campylobacter spp* in Mathira county in Nyeri, Kenya caused by several factors including the use of antibiotics farm and biosecurity levels. Farmers in the study site were found to administer antibiotics for either treatment or prophylaxis especially when they lose their birds to infections or hear of disease outbreaks and death of birds from neighbouring farmers. Among antibiotics commonly used at the farm level, tetracyclines and sulphonamides were the most administered at 68% and 53% respectively. Laboratory results showed high resistance to Tetracyclines by both *C. jejuni* and *C.coli* at 59% and 56% respectively. There was, however, no significant difference in resistance of both isolates to Tetracycline (P=0.6474). High resistance to Tetracycline may be attributed to the fact that Tetracycline is commonly used by farmers as a therapeutic as well as a prophylactic agent. Uses of Tetracycline over long periods may lead to the emergence of tetracycline-resistant Campylobacter species which can spread the resistant genes to other ani, mals especially food-producing animals. Human beings would then acquire these tetracycline-resistant *Campylobacters* through improper handling and consumption of poorly processed and undercooked animal products. Resistance to

Ciprofloxacin and Nalidixic acid was found to be high at 69% though they have been administered less at the farm level by 9% of the farmers interviewed. Resistance of Campylobacter to fluoroquinolones is due to mutations to DNA gyrase and DNA topoisomerase, enzymes responsible for DNA replication and transcription. Selection pressure in the presence of fluoroquinolones leads to rapid resistance in *Campylobacter* as a result of selection for mutations in DNA gyrase. Due to the Presence of fluoroquinolone-resisCampylobacter*acter* in food animals and animal products, the -lasting persistence poses a public health threat as was described in a study by (Fratamico, 2010). Use of fluoroquinolones at the farms for therapeutic and prophylactic in poultry with *Campylobacters adds* to rapid selection for resistance and these resistant bacteria persist until the time of slaughter hence consumers are e used to the risk of infections by fluoroquinolone-resistant *CaCampylobacter*hen they handle or ingest raw or improperly cooked poupoultryat (Fratamico, 2010). RResistanceof *Campylobacters* isolated from this study to Erythromycin, a macrolide was found to be 11% for *C. coli* and 18% for *C.jejuni.* The use at the farm level was at 30%. This resistance level was low compared to other antibiotics tested. A study by (Amera Gibreel, 2006) demonstrated that the use of macrolides in veterinary medicine has contributed to the resistance trends making macrolides become a major public health concern. This is due to the administration of macrolides as growth promoters in sub-lethal concentrations to food animals over a long which exerts selective pressure for resistance to both animal and human pathogens and commensals. In a study conducted in Poland between 2014 and 2018, the rate of resistance of *C. coli* and *C. jejuni* to Erythromycin was recorded at 6.4% and 1.1% respectively (P= 0.00005) (Maćkiw, et al., 2012), lower than demonstrated in this study. A review conducted by (Kathariou, 2017) recorded that fluoroquinolone resistance to *Campylobacter* in human beings has led to the consideration of macrolides such as Erythromycin and Azithromycin as drugs of choice for the treatment of human campylobacter infections.

This study also established that farmers (81%) accessed antibiotics from agro-vet outlets without prescrifromn from veterinary professionals and animal health service providers. Antibiotics bought from these outlets included Tetracyclines (Oxytetracycline), Aminoglycosides(Streptomycin),macrolides(tylosin and erythromycin),polymixins(colistin), fluoroquinolones(enfloxacin) and sulphonamides. Some of these antibiotics are classified as critical for use in human medicine by the World Health Organization hence posing a health threat to the public. Most of the farmers also self-medicated their birds on observing sick ones without consultation and advice

from professionals hence lacking technical backup and knowledge on antibiotics. This showed lack of prudent use of antimicrobials at the farm level, which is a driver for antimicrobial resistance(AMR)

5.2 *Campylobacter spp.* prevalence

Campylobacter spp. is the etiology for human campylobacteriosis in many countries due to its prevalence and effect on human beings following the consumption of infected poultry or other related flocks. Based on different categories of the isolated *Campylobacter*, it is evident that there are different rates in farms concerned with the taken samples. In other studies, it is evident that there is a high identification of *Campylobacter spp.* during rainy days than on sunny days (Hansson et al., 2007). While this was not a focus of this research, it is important evidence that would otherwise guide the farm practices during rainy periods. The latter demonstrates that campylobacter occurs in a widespread manner within environments and there exist several vectors that facilitate the transmission of the pathogen to humans.

Evidence also shows that the existence of the chicken reservoir serves as the main agent of transmission for *Campylobacter* to humans. Existing studies indicate that chickens especially broilers provide natural environments for holding *Campylobacter* species affecting humans. The broiler chicken carries high numbers of bacteria in their cecum up to the slaughter period resulting in the contamination of carcasses in the abattoir as indicated by the authors(Inglis et al., 2021)

As a result, the infections caused by campylobacteriosis in humans remain high. Therefore, evidence shows that a focus on *Campylobacter* ecology and epidemiology in chickens would facilitate the identification of effective rearing strategies where the zoonotic pathogen can be persistent and be introduced in a flock. According to (Mota-Gutierrez et al., 2022), a focus on the latter facilitates the ability to develop intervention strategies that would effectively lower the number of colonized flocks and carcasses that are contaminated. These would effectively minimize the number of *Campylobacteriosis* cases related to chicken transmission (Abd El-Hack et al., 2021). However, farmer's practices also affect the manner through which rearing is conducted in terms of hygiene (Agunos et al., 2014) & (Shane, 1992). As seen from the results in this study, most the farmers use wooden floors for their chickens and this is a high contributor as seen from this evidence. Also, according to Narvaez-Bravo et al., (2017) in the context of zoonotic pathogens, *campylobacter* is fundamental in its presence in livestock and can cause illnesses in humans mainly through raw and undercooked meat consumption.

Bacterial population dynamics are also fundamental aspects of the regulations imposed by governments on poultry farms and slaughter houses a Brazilian study, the authors presented minimized *C.jejuni*-positive chilled chicken carcasses between periods. Following a risk assessment conducted quantitatively where a prediction was made that in poultry colonization, a 2-$\log_{10}$ reduction would reduce human infections 30-fold (Melo et al., 2019). Therefore, significant efforts have primarily maintained focus on minimizing loads imposed by *Campylobacter* in poultry production to retail. However, regardless of the lower prevalence of *C.jejuni* in chicken carcasses, research shows that particular effects from the environment may result in virulent and diverse strains, and this harbors a high potential to cause severe infections in humans (Melo et al., 2019). Thus, as is evident in such research, the transmission of Campylobacter bacteria can mutate and this would result in different kinds of pathogenesis in human beings if they get infected, thus the need for enhanced control., There is a need to reduce the campylobacter loads in poultry production to retail. Generally, there is a need to implement improved approaches to monitoring and control of Campylobacter during the assessment of not only the prevalence but of the evolutionary changes within these bacterial populations.

The chicken intestine is of particular interest in the hosting of *Campylobacter* in poultry since the intestines provide optimal temperatures, low oxygen levels, and high nutrition (Burnham and Hendrixson, 2018). However, a contradiction to this in the transmission of the bacteria during processing and slaughtering is that the bacteria are exposed to high oxygen levels and this includes their preservation(Oh, McMullen and Jeon, 2015). The latter evidence indicates that there is a hyper-aerotolerant *Campylobacter* prevalent in raw chicken meat. This applies to Arcobacter which is similar to Campylobacter but characterized by animals and humans, the latter is frequently isolated in poultry as described by Vandamme et al., (1992) & Snelling et al., (2006). In the pediatric setting, evidence indicates the contribution of *Campylobacter spp*. as a cause of diarrheal diseases is an issue that has necessitated the need for the establishment of *Campylobacter*-associated investigations to be carried out. In a survey carried out by Vries et al., (2018), the diversity provided by genomic activities of *Campylobacter spp*. showed that it was isolated from humans and poultry and was highly related implying that zoonotic transmission was likely as described by Hermans et al., (2012). In the findings of Vries et al., (2018), a high positivity for gyrA-T86I, tetO, and blaOXA-61 carried all these resistance determinants from both the human and poultry isolates. Relating this to the current study, it is evident that zoonotic transmission is

likely a significant contributor to the high prevalence levels, therefore the risk imposed on humans is high.

From a regulatory perspective, the government or other relevant bodies must implement effective food safety management practices. Evidence from China indicates a high-risk score for *Campylobacter jejuni* in chicken and this has shown the need to implement essential efforts in the control of the risk imposed by *Campylobacter jejuni* in chicken and the concern mainly encompasses chicken breeding mechanisms and the preparation processes (Wang, Guo, and Li, 2013). This evidence is applicable in the current study based on the findings which indicate high levels of prevalence of both *C. coli* and *C. jejuni* from the isolates. Therefore, regulatory measures are fundamental in curbing the issue which was observed on the farms and contrasted with laboratory results.

Another factor for consideration in this study is the comprehension of the epidemiological and ecology of *Campylobacter* in poultry in the Kenyan context. This comprehension while showing significant improvement worldwide is slow in Kenya. Evidence from Chen et al., (2006) indicates that 40 cells can facilitate a successful infectious dose in chickens. From the study, it is evident that horizontal transmission is the main source of *Campylobacter spp.* infection in poultry. In their presence in the environment, *Campylobacter* is considered ubiquitous and thus can be transferred into the poultry farm in different ways. Climatic factors according to Sibanda et al., (2018) are highly potent alongside routine flock management and can affect the transmission. The authors reckon that there ought to be increased biosecurity to facilitate *Campylobacter* contamination and should be of paramount importance especially during periods when flocks are thinned or during summer periods (an important factor for investigation as was not considered in this study). As evidenced by Sibanda et al., (2018), the high prevalence of *Campylobacter* in flocks and human cases is affected by low levels of biosecurity.

5.3 Antimicrobial resistance

The main antibiotics investigated in the study in Mathira included; Ampicillin, Tetracycline, Ciprofloxacin, Gentamycin, Erythromycin, and Nalidixic acid. From the results, it is evident that both *C.coli* and *C.jejuni* exhibited high multi-drug resistance especially as observed in Ampicillin, Tetracycline, Ciprofloxacin, and Nalidixic acid. Evidence from previous literature indicates that in poultry, there are multidrug resistances to these or more classes of antimicrobials as presented by (Li et al., 2017). The authors present this evidence for *C.coli and C.jejuni* in the Chinese

context. In the investigation, live bird markets show the latter prevalence and patterns and this indicated the need to implement measures for interventions that are efficient to ensure high levels of control and monitoring of *Campylobacter* species for contamination and the dissemination of antimicrobial resistance among *Campylobacter spp.* in the production process of poultry. This is evident in the case of Mathira in Nyeri country as indicated from the results where most of the poultry is sold in open-air markets and fast-food outlets for both slaughtered and live birds.

The prevalence of multi-drug resistance in different types of birds has been observed in many investigations on AMR in poultry around the world. A study by Varga et al., (2019) indicates a high prevalence in turkey, chicken, and duck, and the outcomes were unexpectedly low in-game birds when assessed based on their E.coli isolates. In their recommendation, they suggest a judicious approach to antimicrobial use to ensure the limitation of multidrug resistance bacteria emergence.

As observed from this research, the majority of the farmers engaging in poultry farming are for food production purposes. According to other evidence by Van Boeckel et al., (2019), this approach concurs with the notion that poultry is the fastest growing per capita and most abundant livestock and is among the flock with common sources of multi-resistant bacteria (Elwinger et al., 2016). The situation in Kenya is that it is rapidly growing its economy and has attained a middle-income country status therefore, there is an increased demand for animal product sources. The intensification of agriculture is therefore at its core and this has caused the increased emergence of antimicrobial resistance and thus increase ng the general resistance. Therefore, based on the observed evidence above from this research, an effective systems framework is required to facilitate the reduction of the burden caused by bacterial resistance among humans, animals, and the environment. Worldwide, standards for national veterinary services are lacking in meeting international standards as evidenced by Forman et al., (2012) with Kenya not being an exception. Therefore, there is a need for access to trained veterinary services to ensure the improvement of diagnostic capability, treatment, and antibiotic prescriptions for poultry use.

As advocated for by the World Organization for Animal Health (OIE), veterinary systems that are effective are essential in economies that are stabilizing, improving food security and food safety, and making attempts to minimize the exposure of AMR, and other pathogenic organisms and species (World Health Organization, 2015). The effectiveness of veterinary governance does not only reduce AMR burdens but also at the same time improves other infectious diseases as

presented by (Thornton, 2010); (Thanner, Drissner, and Walsh, 2016) & (Tang et al., 2017). In Kenya, alongside other nations in the world, there is the implementation of veterinary services in overseeing animal production, slaughter, food processing, product distribution, retail inspection, and foodborne and occupational diseases (Leach et al., 2017) & (Goutard et al., 2015). Based on the results observed in this study, there need to be stronger veterinary services capacities in low- and middle-income countries concerning food animal systems, a major recommendation in this research.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The prevalence of *Campylobacter spp*. in Nyeri, Kenya as demonstrated by this study is high and resistance to most commonly used antibiotics at the farm level is noted. This poses a risk to public health as most antibiotics that are classified as critical for use in human medicine are easily accessible to farmers over the counter. Farmers who are the primary caregivers to the livestock under their watch have been established to carry out self-medication on these animals without technical help from professionals and lack knowledge on antimicrobial resistance and antimicrobial use.

The occurrence of *Campylobacter spp.* based on the above research indicates a high relationship between the practices at the farm level and the enforcement of standards of safety and biosecurity implemented by the Kenyan government. Also, it is observed that the poultry farmers in the study do not adhere to high hygiene levels in the food processing procedures which are carried out on daily basis. Horizontal contamination has been observed as a major issue that is causing high levels of *Campylobacter spp.* prevalence in Nyeri, Kenya.

Based on the results observed in this study, there need to be stronger veterinary services capacities in low- and middle-income countries concerning food animal systems. Therefore, there is a need for access to trained veterinary services to ensure the improvement of diagnostic capability, treatment, and antibiotic prescriptions for poultry use.

High levels of multi-drug resistances observed in both *C. coli* and *C. jejuni* isolates call for a judicious approach to antimicrobial use to ensure the limitation of multidrug resistance bacteria emergence. This is applicable and based on the farm practice should be emphasized to poultry farmers in Kenya. Efforts that aim at reducing the colonization of chickens with Campylobacter need to be addressed to prevent their spread to human beings and the environment hence curbing antimicrobial resistance,

6.2 Recommendations

A recommendation concerning several factors that may contribute to the occurrence of *Campylobacter spp*. in poultry requires changes in skill and knowledge base harboured by farmers in the poultry rearing industry. According to Hansson et al., (2018), this study recommended that future studies focus on improving the knowledge of the true number of infected humans, enhance the methods of pathogenicity to minimize infections to humans, encourage more rigorous training

to prevent the transmission of *Campylobacter* from raw to ready-to-eat food, creation of effective antimicrobial agents against *Campylobacter spp.*, improve the comprehension of the transmission routes of *Campylobacter spp.* to chicken. Also, from a technological perspective, the antimicrobials that are more effective in the treatment of people infected with campylobacter must be fundamental. The formulation of effective preventive measures at the farm level is fundamental. In Kenya, from the study, the researcher's experience and based on the data collected in Mathira constituency in Nyeri, was that the existing systems for which farmers approach treatment of their poultry are significantly lacking from a procedural perspective. Because of this, there is a high need for rigorous surveillance, if the prevalence of Campylobacter infections is to be minimized. Also, due to the nature of hygiene, there is a need to increase the existing biosecurity in Kenyan farms. The food handlers in Kenya appear to have shortages in knowledge on good food hygiene practices when handling poultry products. The regulatory framework in Kenya ought to enhance the laws on drug prescription in Kenya. Other recommendations include; capacity building for labs involved in surveillance in terms of human resources, equipment, reagents, and supplies, and promotion of stewardship activities in hospitals and the agriculture sector.

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APPENDICES

Appendix 1: Questionnaire

Questionnaire for Campylobacter study

Date…….

Location…….

GPS co-ordinates…….

Biodata

1. Name …… Tel. No……..
2. Age 18-30 30-50 Over 50
3. Education level Primary Secondary Tertiary
4. Occupation

Farm details/experience

5. Duration of poultry rearing 0-5 5-10 Over
6. Type of birds Broiler Layers Dual purpose
7. Other animals on the farm…….
8. Poultry house floor type
   Earthen Concrete Wooden
9. Which poultry diseases have you ever encountered on the farm……?
10. Do you request services of an animal health service provider when birds are sick
    Yes No
11. If yes, do you call a government officer or a private service provider? ……
12. Which disease control measures do you undertake on your farm?
    Use of footbaths
    Use of cleaning and disinfecting agents
    Control of access to poultry house
    Vaccination
    Administration of drugs
    Other (specify)

Antibiotic usage

13. Why do you use antibiotics

    When told by a vet

    When they see a sick animal

    When they hear about sick animals in the neighboring farms

14. What clinical signs of sickness will make you use an antibiotic**?**

Weight loss

Diarrhoea

15. Where do you source your antibiotics from?
    Friends and neighbors
    Agro-vet
    County government
    Animal Health service provider
    Other: ……..
16. On the drugs shown, how many times you have used them in birds in the last two months.
    • Alternatively ask to see the packaging of commonly used drugs and classify them according to the list below or provide images of the antibiotics commonly retailed in the area
    • For drugs used, please indicate the reason for use: multiple options for each drug possible
    Antibiotics Prevent disease Treat sick animal/Bird

	Treatment	Prophylaxis
a. Tetracyclines (Example, Oxytetracycline Hydrochloride)		
b. Sulphonamides (Example Sulfadimidine)		
c. Polymyxins (Example Colistin)		
d. Fluoroquinolones (Example enrofloxacin)		
e. Macrolides (Examples erythromycin, Tylosin)		
f. Aminoglycosides (Examples Gentamycin)		
g. Other antibiotics (specify) g. Antibiotics intended for human consumption		

17. How do you dispose of unused or expired antibiotics (s)?
    Select one that applies from the list
    □ Keep for later use
    □ Assist neighbour
    □ Burn
    □ Bury
    □ Other……..

Appendix 2: Ethical approval 1

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/298

Ms. Edith Kagio Chege,
Veterinary Laboratories,
Nairobi
19/05/2021

Dear Edith,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

An investigation of the antimicrobial resistance patterns of campylobacter species isolated from broiler chickens.

We refer to your proposal submitted to our committee for review and your application letter dated 17th April 2021. We have reviewed your application for ethical clearance for the study.

The number of chicken, sample collection and processing protocol meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that a KVB registered veterinary surgeon will supervise the study.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D

Chairperson, Biosafety, Animal Use and Ethics Committee,

Faculty of Veterinary Medicine,

University of Nairobi

Appendix 3: Ethical approval 2

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Keppel St. London WC1E 7HT United Kingdom
Switchboard: +44 (0)207 7636 8636

Animal Welfare and Ethical Review Board (AWERB)

Form Ext-3

Approval for research using animals at an external site

TO: Ms Edith Kagio Chege, Veterinary Laboratories, Nairobi, Kenya, LSHTM/RVC

Date: 7th June 2021

Dear Edith,

Study Title: An investigation of the antimicrobial resistance patterns of Campylobacter species isolated from broiler chickens

LSHTM AWERB reference: 2021-11

Thank you for submitting your application to perform research involving animals at an external site. The AWERB has considered the information you have provided including any amendments to your original application.

Confirmation of ethical opinion:

On behalf of the AWERB, I am pleased to confirm a favourable opinion for the above research on the basis described in the application form, protocols, and supporting documents.

The AWERB now agrees for you to begin this research subject to i) any Specific Conditions stated below and ii) the Standard Conditions described in Annex A.

This approval is valid until **1st September 2021.**

Conditions of the favourable opinion:

Approval is dependent on any human or other local ethical approval having been received, where relevant.

Approved documents:

The final list of documents reviewed and approved by the AWERB is as follows:

• EDITH AWERB Review External overseas site Form Ext 2 v1.3 BS EC dated 07/06/2021

• Sampling Procedure for cloacal swabs in chicken, 25/05/2021

The Named Investigator (NI) or delegate is responsible for informing the AWERB of any subsequent changes to the application. These must be submitted to the AWERB for review using an Amendment Form (Form Ext-4).

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The NI or delegate is also required to notify the AWERB of any SOP violations, incidents, or near-misses relating to animal welfare during the project by submitting Form Ext-5: External Site Incident report form.

At the end of the study, the NI or delegate must notify the AWERB using Form Ext-7 Project Completion.

All forms are available on request from the AWERB Chair and should be submitted to

AWERB@lshtm.ac.uk

Good luck with the project. Please do not hesitate to get in contact if you need any further ongoing advice or support.

Yours sincerely,

Dr. H Helmby
Chair, LSHTM AWERB

London School of Hygiene & Tropical Medicine

Keppel St. London WC1E 7HT United Kingdom
Switchboard: +44 (0)207 7636 8636

LSHTM AWERB

Annex A Standard Conditions for Approved research projects involving research using animals at External Sites

1. The individual named in the application for LSHTM AWERB approval of research involving animals at an External Site (=Named Individual (NI)) is responsible for the overall implementation of the program of work specified and for ensuring that the program of work is carried out in compliance with the conditions of this Authorisation.
2. The NI shall ensure that the appropriate level of supervision and training is provided for all others carrying out procedures listed under the authority of this LSHTM approval.
3. no work will be performed without specific authorisation by the relevant local Institutional Animal Care and Use Committee of the External Site country.
4. all routine husbandry and experimental work must adhere to the SOPs submitted to and authorised by the LSHTM AWERB.
5. any change in SOP must be submitted for amendment and approval by LSHTM AWERB prior to use.
6. unauthorised deviation from these protocols will invalidate the LSHTMs Approval to Work.
7. Any adverse event or near -miss concerning animal welfare must be acted upon as soon as possible on site by competent individuals, including a named local veterinary surgeon, and reported to the LSHTM AWERB within 7 days using the External site Incident Report Form.

8. The NI shall ensure that details of the programme of work and any SOPs specified in the approval, and any additional conditions imposed on those procedures, are known

to i) all individuals performing those procedures; ii) the person/persons responsible for day to day care of the animals iii) the local named veterinary surgeon responsible for the care and welfare of animals in the Project.

9. The NI shall ensure that the approved procedures specified here are designed so as to result in the death of as few animals as possible; and to reduce to the minimum possible the duration and intensity of suffering caused to those animals that die and, as far as possible, ensure a painless death.
10. The NI shall ensure that the authorized procedures applied as part of the programme of work specified in this authorization are those which to the greatest extent use the minimum number of animals; involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm; cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results.
11. The NI shall ensure that these procedures are not applied to an animal if the procedure may cause the animal severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated.
12. The NI shall ensure that where a procedure is being applied to an animal, any unnecessary pain, suffering, distress or lasting harm that is being caused to the animal shall be stopped.

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13. If the application of regulated procedures to animals taken from the wild is authorized in this license the holder shall ensure— (a) that animals taken from the wild are captured by a competent person using a method which does not cause the animal avoidable pain, suffering, distress or lasting harm; and (b) that an animal taken from the wild which is found to be injured or in poor health is not subjected to a regulated procedure unless and until it has been examined by a veterinary surgeon or other competent person; and action has been taken to minimize the suffering of the animal.
14. The NI shall maintain a contemporaneous record of all animals on which procedures have been carried out under the authority of the LSHTM. This record shall show the procedures used and the names of individuals who have carried out the procedures.
15. Any scientific report you publish using data obtained from studies performed under this Approval must be prepared according to the ARRIVE (Animal Research: Reporting of in vivo Experiments) Guidelines provided by NC3Rs UK (for information see www.nc3rs.org.uk).
16. All draft manuscripts in preparation for public dissemination of information obtained under this Project must be submitted to LSHTM AWERB for review prior to submission to the journal. A decision for approval to submit for publication (or refusal to submit) will be provided to the authors within 3 weeks after submission to AWERB.